1995

[1]Benzothiophene-based potentiators of 5-hydroxytryptamine

Zemin Wu
University of Wollongong

[1]BENZOTHIOPHENE-BASED

POTENTIATORS OF 5-HYDROXYTRYPTAMINE

A thesis submitted in fulfilment
of the requirement for the award of the degree

of

DOCTOR OF PHILOSOPHY

from

THE UNIVERSITY OF WOLLONGONG

By

ZEMIN WU, B.Sc., M.Sc.

Department of Chemistry

March 1995

For my parents

DECLARATION

This is to certify that the work described in this thesis has not been submitted for a higher degree at any other university or institution.

Zemin Wu

ACKNOWLEDGMENT

I would like to thank Professor John Bremner for giving me the opportunity to do this PhD, and also for his excellent supervision and encouragement throughout this project.

I am grateful to my fellow students and postdoctoral fellows in the department of chemistry and particularly in the medicinal chemistry research group for their advice, help and friendship.

Thanks are due to the other members of the department of chemistry for their assistance and support. In particular, I would like to express gratitude to Dr John Korth for his friendship and for running most of the mass spectra.

Thanks are also due to Ms Winnie Lau of the Department of Pharmacology, Monash University, Australia, for conducting all the pharmacological tests, and to Associate Professor J. O'Neil (Monash University) for overall assistance and guidance with the pharmacology. Assistance from Dr Renate Griffith and Dr Anneliese Palmer with the molecular modelling program is gratefully acknowledged.

Special thanks must go to Dr Joanne Jamie, who spent a lot of time with great patience to read through the manuscript of this thesis and made numerous suggestions.

Finally I wish to thank the Australian Research Council for providing the scholarship for this study.

Abbreviations

The following abbreviations are used throughout this thesis:

BH_3	borane
CAMM	computer-aided molecular modelling
CI	chemical ionisation
CNS	central nervous system
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
EI	electron impact
eq.	equivalent
EWG	electron withdrawing group
FAB	fast atom bombardment
HOBT	1-hydroxybenzotriazole
LAH	lithium aluminium hydride
MAO	monoamine oxidase
m-CPBA	*m*-chloroperoxybenzoic acid
NA	noradrenaline
$NaBH_4$	sodium borohydride
NaH	sodium hydride
SAR	structure-activity relationship
SSRI	selective serotonin reuptake inhibitor
TCA	tricyclic antidepressant
THF	tetrahydrofuran

Abstract

The general aim of this work was to develop 5-hydroxytryptamine potentiators based on [1]benzothiophene-containing compounds.

The synthetic work for this project comprised three parts. The first part (Chapter 2) deals with the preparation of new 3-chloro-[1]benzothiophene-2-amide derivatives and amide reduced analogues. The amides were prepared in moderate to good yields by reaction of 3-chloro-[1]benzothiophene-2-carbonyl chloride 21 with various amino acids or other amines. Preparation of the corresponding amines was attempted by three approaches. Direct reduction of the amides with various reducing agents was not successful. With the second approach, the reductive-amination of 3-chloro-[1]benzothiophene-2-carbaldehyde 32 with glycinate or sarcosinate afforded the methylene amines. Nucleophilic substitution of 3-chloro-[1]benzothien-2-ylmethyl tosylate 33 with glycinate or sarcosinate proved to be a better procedure to prepare the methylene amines.

The second part (Chapter 3) involved modification of the other [1]benzothiophene ring substituents. A number of substituents were placed at positions 5 and 6 respectively in order to increase the compounds' activity. Most of the work, however, centred on modification of the 3-position by introducing H, Cl, Br, F, OCH_3, OCH_2CH_3 and OH groups to determine whether there is any interaction between the 3-substituent and the amide nitrogen NH and the effect of this on the serotonin potentiation activity. The ring sulfur of N-[3-chloro-[1]benzothien-2-oyl]glycine 19 was also oxidised with *m*-chloroperoxybenzoic acid to the corresponding sulfoxide 122.

In the third part (Chapter 4), a series of [1]benzothiophene-fused oxaza medium ring derivatives 142, 160, 161 and 162 were successfully synthesised. Both aromatic and aliphatic nucleophilic substitutions were investigated to prepare the [1]benzothieno[1,4]oxazepine-5[H]-one derivative 142, and as a result a convenient and efficient method of preparing medium ring-containing [1]benzothiophenes was established, in which 8-, 9-, and 10-membered ring systems were prepared. This methodology is potentially generalisable to other fused heterocyclic ring systems.

Molecular modelling was utilised in conjunction with pharmacological tests to help rationalise the structural modifications to improve the compounds' activity. The global minimum conformations were determined using molecular mechanics with Biosym Insight/Discover software. Comprehensive conformational analyses and molecular superimpositions were conducted. The pharmacological testing of the [1]benzothiophene derivatives was carried out by others at Monash University. More than ten, mostly achiral compounds including the 7-membered ring compound 142, were found to possess moderate 5-HT potentiation activity in the rat cardiovascular model. The pharmacological screening indicated that the most likely mechanism for the potentiation was *via* 5-HT uptake inhibition. Such inhibitiors are of great interest as antidepressants.

Based on the pharmacological tests and the molecular modelling results, a pharmacophore including the [1]benzothiophene ring with appropriate substituents, an amide nitrogen with a specific distance from the fused ring and a specific intramolecular hydrogen bonding interaction was proposed for activity of the [1]benzothiophene-based 5-HT potentiators.

CONTENTS

LIST OF FIGURES

LIST OF SCHEMES

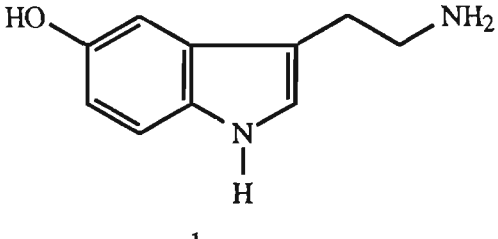
LIST OF TABLES

Chapter 1

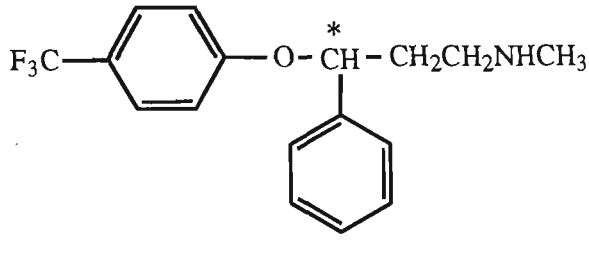
General Introduction

1.1 Introduction

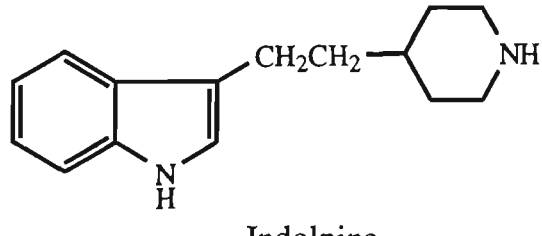
Research on serotonin 1 (5-hydroxytryptamine, 5-HT) has attracted extensive attention since the early 1970's due to its close association with the central nervous system (CNS) and human behaviour[1, 2]. Research on various 5-HT receptor antagonists and agonists, together with selective 5-HT uptake inhibitors has been of major interest to scientists. Serotonin uptake inhibitors are of interest, since they can be used clinically as antidepressants. Examples are fluoxetine[3] (1975) and indolpine[4] (1977), which are effective antidepressants with limited side effects. We were interested in the design, synthesis and structural development of new structural types as selective 5-HT uptake inhibitors, as well as 5-HT_3 antagonists.



1



Fluoxetine

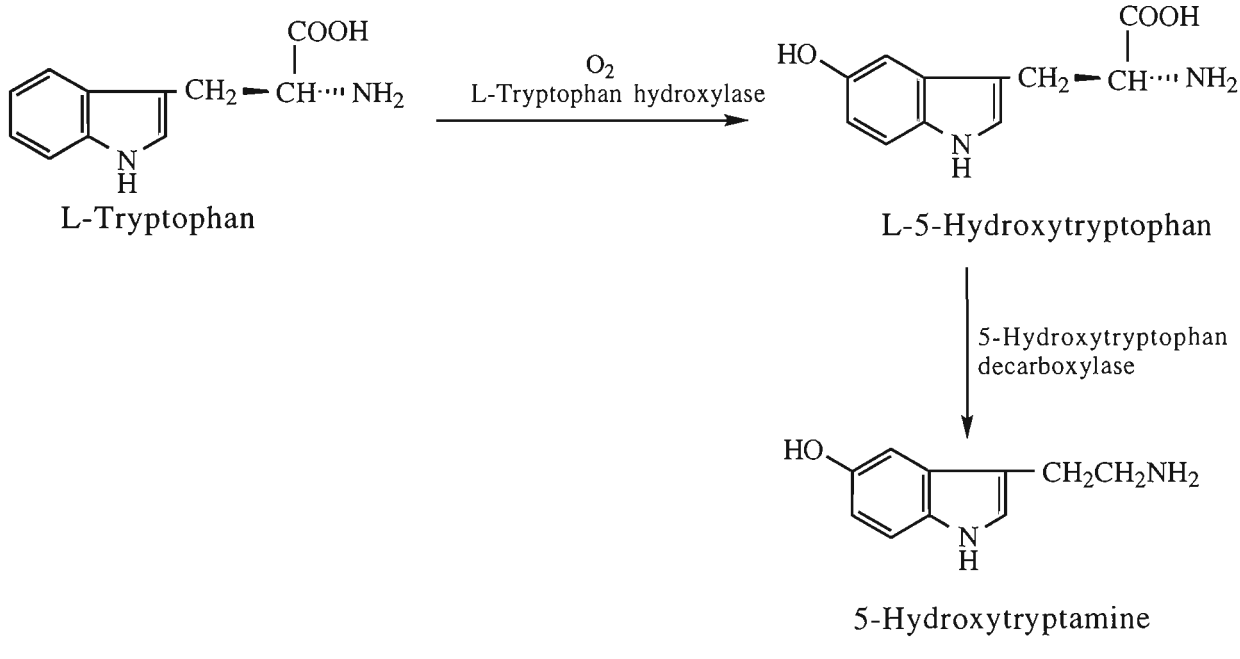


Indolpine

1.2 Serotonin and its history

Serotonin 1, an indoleamine, was first isolated from blood in 1948 and was shortly thereafter identified as 5-hydroxytryptamine (5-HT). Subsequent research revealed that this agent was also present in the central nervous system of a variety of animal species[5]. The levels of serotonin in the CNS only represent about 1-2% of the total amount found in the body[6].

Serotonin can not cross the blood-brain barrier (BBB), and consequently all the neuronal serotonin is formed by a two-step process involving hydroxylation of the essential amino acid L-tryptophan by tryptophan hydroxylase to L-5-hydroxytryptophan (5-HTP) as a rate-limiting step, followed by decarboxylation of 5-hydroxytryptophan to serotonin (Scheme 1.1).

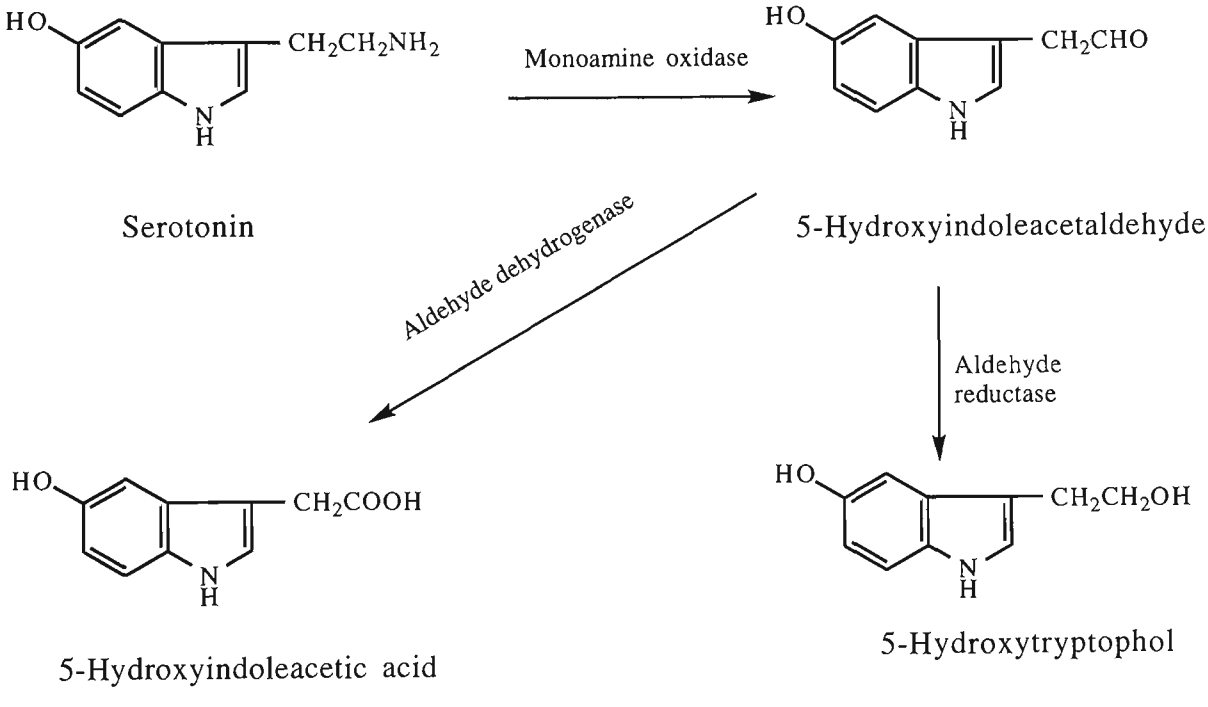


Scheme 1.1

Serotonin is believed to be synthesised in both the nucleus and the terminals of nerve cells, though the latter site is probably more important in

the short term regulation of serotonin synthesis. Serotonin formed in the nucleus is transported to the terminals of the dendrites and axon. The serotonin is released by a calcium ion-dependent process from the readily available pool of serotonin stored in vesicles within which serotonin is protected from being metabolised by mitochondrial monoamine oxidase (MAO), which is an enzyme widely distributed throughout the body and primarily located within the brain[7]. After its release, serotonin is inactivated mainly by re-uptake into serotonergic nerve terminals. Once inside the serotonergic neurone, the transmitter is subsequently either re-stored in the vesicles or metabolised by MAO.

MAO deaminates 5-HT to give 5-hydroxyindole-3-acetaldehyde. This is further metabolised primarily by aldehyde dehydrogenase to 5-hydroxyindoleacetic acid, or in small amounts by aldehyde reductase to 5-hydroxytryptophol[8] (Scheme 1.2).



Scheme 1.2

Within the brain and spinal cord, serotonin acts as an important neurotransmitter involved in a variety of physiological and behavioural functions. These range from the control of sleep and wakefulness, to feeding, thermoregulation, cardiovascular function, emesis, sexual and psychotic behaviour[1].

Fluorescence histochemical methods have been used to visualise catechol and indoleamines *in situ* in the brain, and research results have indicated that serotonin is localised within specific neuronal pathways, the cell bodies being found in the midbrain and brain stem raphe nuclei[9]. Since the 1970's, there has been a growing understanding of the control of serotonin metabolism in nerve endings. On the basis of drug development, information regarding the role of serotonin pathways in the control of physiological and behavioural functions has expanded dramatically. This has led to an understanding of the structure-activity relationship (SAR) for 5-HT activity and to the development of novel 5-HT related agents, particularly of the new generation of antidepressant drugs.

1.3 Serotonin receptors

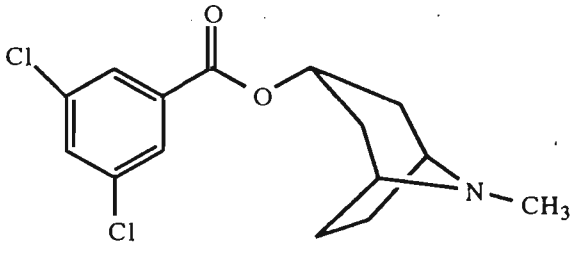
As a neurotransmitter, serotonin transmits signals to the adjacent nerve cells or the adjacent effector organs, hence giving rise to the observed response. To pass the signal on, the neurotransmitters have to combine with the appropriate receptor. It is the act of combining the neurotransmitter with its receptor which initiates the mechanism that ultimately leads to the observed biological response.

A receptor is a discrete region of a post- or pre-synaptic membrane that can selectively bind molecules of specific structures. Receptors are often regarded as enzymes, both in terms of their functions and kinetics. Unlike enzymes, however, the neurotransmitter in this case remains unchanged as a part of the reaction sequence. Instead, the receptors themselves are shifted from an inactive to an active state[10]. The pharmacological action of 5-HT can be attributed to interactions of its indole ring and alkylamino side chain with 5-HT receptors. Experiments have demonstrated that 5-HT interacts with various membrane receptors. It was recognised as early as 1957 that multiple types of 5-HT receptors might exist within the same tissue[11]. Currently, 5-HT receptors have been classified into seven major groups on the basis of their pharmacological and physiological responses: 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7. The 5-HT_1, 5-HT_2 and 5-HT_5 receptors have been further subdivided into 5-HT_{1A-F}, 5-HT_{2A-C}, 5-HT_{5A} and 5-HT_{5B} sites respectively[12]. These 5-HT receptors differ in regional location and function, which makes consequent development of numerous compounds with relative specificity for one or more of these receptor subtypes possible.

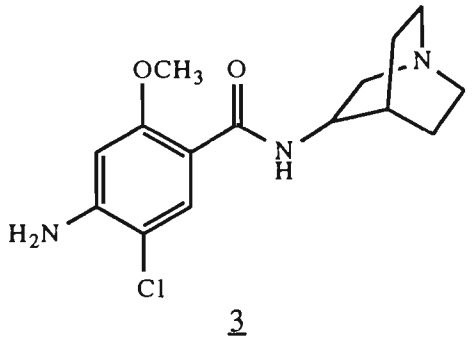
1.4 5-HT_3 receptor and its antagonists

Receptor antagonists are substances that bind to the receptor and prevent other substances that initiate a response in the neuroeffector tissue from doing so, but are inactive themselves[10]. Although there is considerable published material on 5-HT_1, 5-HT_2 and 5-HT_4 receptor antagonists, the 5-HT_3 receptor has attracted the most attention and the understanding of this receptor has increased dramatically over the last decade as a result of the discovery and widespread availability of potent and selective antagonists[13].

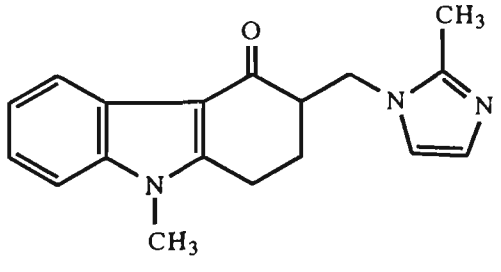
The 5-HT_3 receptor subtype was originally thought to be confined to the periphery of the nervous system. However, more recent animal behavioural studies and binding studies on animal and human brain tissues have suggested a role for this receptor subtype within the CNS[14]. Several highly selective antagonists of the 5-HT_3 receptor have been described and a few of them are the subject of clinical studies. Antagonists such as MDL-72222 2 and zacopride 3 have been used successfully to study the 5-HT_3 receptor distribution and the effects they may exhibit on the physiological process, hence assisting in establishing a complete receptor profile[15]. In particular, antagonists including ondansetron 4, granisetron 5 and ICS 205-930 6 have been shown to be effective in the control of cancer chemotherapy-induced emesis, and are now marketed for this therapeutic indication[16, 17].



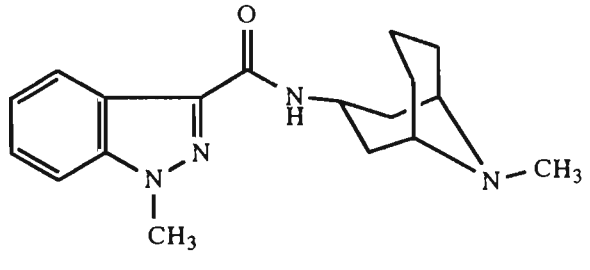
2



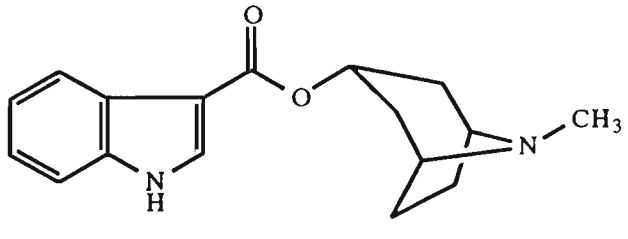
3



4

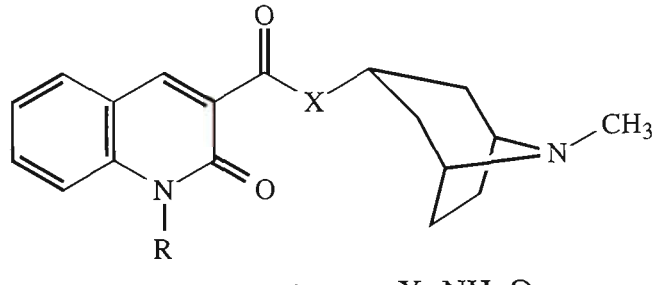


5



6

Many of the currently reported 5-HT_3 antagonists are aromatic amides or esters of appropriate bicyclic amines or alcohols, suggesting that the coplanarity between an aromatic ring and a carbonyl moiety is an important factor for high affinity toward the 5-HT_3 receptors[14, 18, 19]. This pharmacophoric element has been refined by Swain and coworkers[18] by consideration of the possible hydrogen-bonding interactions available. For example, in the case of compounds 3, 4, and 6, the linking group is capable of accepting one or two hydrogen bonds. It was postulated that it might be of advantage to design ligands that would offer the opportunity of participating in two hydrogen bonding interactions. Recently, however, Hayashi and associates[20] have reported that the minimum energy conformation of the antagonist 7 does not possess such coplanarity. This result questions whether the coplanarity is necessary.



7 X=NH, O

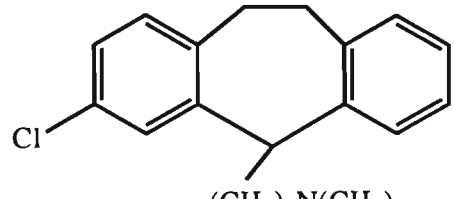
1.5 5-HT uptake inhibitors and antidepressants

After its release from nerve terminals and completion of its activation of the receptor, 5-HT is either transported out of the synaptic cleft back into the nerve terminal *via* the action of a specific membrane transported protein, or stored in storage vesicles, or metabolised enzymatically. An uptake inhibitor prevents the neuronal re-uptake process, causing the neurotransmitter to accumulate in the synaptic cleft and therefore prolongs the action of postsynaptic-receptor stimulation[21].

5-HT uptake inhibition can be demonstrated *ex vivo* by measuring serotonin uptake *in vivo* in brain slices or synaptosomes prepared from animals treated with the uptake inhibitor *in vivo*. Alternatively, the inhibition of the 5-HT uptake carrier on blood platelets can be demonstrated by measuring uptake *in vitro* after administration of the uptake inhibitor *in vivo*. 5-HT becomes depleted from blood platelets after continued administration of a 5-HT uptake inhibitor, since uptake is a means by which platelets acquire serotonin. Serotonin concentration in the synaptic cleft, however, is expected to increase with administration of a 5-HT uptake inhibitor since it can not be removed from the synaptic cleft by the membrane uptake carrier. Although the technology for measuring serotonin concentration directly in the synaptic cleft does not exist currently, several experimental approaches have documented increased extracellular concentration of serotonin. The most extensive evidence for increased serotonin concentration in the synaptic cleft and increased activation of synaptic receptors for serotonin after uptake inhibition comes from the numerous and varied functional effects that have been produced by 5-HT uptake inhibitors. These functional effects include neurochemical, neuroendocrine and behavioural effects.

Serotonin uptake inhibitors are used, or have shown promise for use, in treating numerous psychiatric illnesses and other conditions such as depression, obsessive-compulsive disorder, panic disorder, obesity, bulimia and alcoholism[21]. A great deal of work, however, has been focused on searching for and the development of 5-HT uptake inhibitors as effective antidepressants with high selectivity and/or less side effects than the tricyclic antidepressants[22, 23]. The tricyclic antidepressants (TCAs), now called classical antidepressants, have long been thought to owe their therapeutic efficacy to the inhibition of monoamine uptake, particularly the uptake of serotonin and noradrenaline[24].

Most of these drugs are relatively nonselective in their uptake inhibition. They do not modify the function of 5-HT-containing neurones[25], with the exception of clomipramine 8. This compound is the most potent 5-HT uptake inhibitor of the tricyclic antidepressant drugs and was used clinically at an early stage as a more selective inhibitor of serotonin, but has since been barred from use due to serious side effects[26].



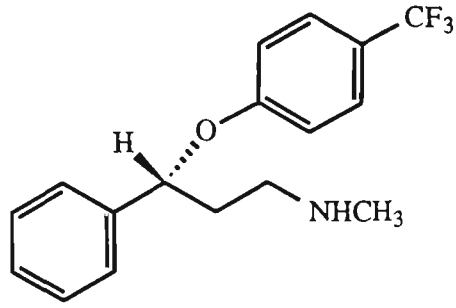
8

As effective antidepressants with fewer side effects and better tolerance than those of the tricyclic antidepressants, the 5-HT uptake inhibitors are required to be not only more selective in their specificity of uptake inhibition, but also in their lack of affinity for neurotransmitter receptors. Many compounds have been described as selective inhibitors of 5-HT uptake relative to their effects on the uptake of dopamine (DA) or norepinephrine, which led to the successful development of selective serotonin reuptake inhibitors (SSRIs) as antidepressants[27, 28, 29]. All selective 5-HT uptake inhibitors so far tested have been found to be effective in major depression[30]. Since most of these drugs belong to different chemical families and the only common property they share is their capacity to inhibit the 5-HT uptake carrier, it is indisputable that they exert their therapeutic effect primarily *via* the 5-HT system[31]. It is known that although the SSRIs inhibit the 5-HT transporter within minutes, they exert their full antidepressant effect only after a few weeks of treatment. Since the SSRIs do not enhance overall brain levels of 5-HT, adaptive changes that could account for the enhanced 5-HT-mediated

neurotransmission underlie their therapeutic effect after their long-term administration. Neurons containing 5-HT also have terminal autoreceptors, which inhibit 5-HT release when activated by 5-HT in the synaptic cleft. Following two or three weeks' treatment with an SSRI, these autoreceptors are desensitised, as is the case for their cell-body counterparts. This desensitisation therefore allows more 5-HT to be released per impulse reaching the terminals. More recently, sustained blockage of the 5-HT carrier was shown to produce a desensitisation of the 5-HT uptake process, most likely resulting from a decreased number of carrier sites. This modification could account for the enhancement of the electrically evoked release of [^{3}H]5-HT from preloaded slices in the guinea-pig frontal cortex after a three-week treatment with an SSRI, because the terminal 5-HT autoreceptor remains normosensitive in this brain region. These adaptive changes of 5-HT-containing neurones can thus explain the delayed enhancement of the 5-HT-mediated transmission mentioned previously, which is completely consistent with the clinical onset of action of the SSRIs in major depression.

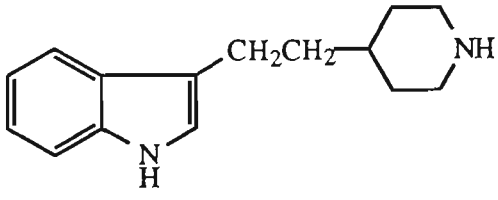
A major breakthrough in antidepressant research has been the introduction of fluoxetine 9 (as its hydrochloride salt; PROZAC), which is considered to be one of the most exciting therapeutic agents of the new generation of SSRIs. After its introduction, more than $US 100 million of sales were recorded within a year[23, 32]. Apart from being an effective antidepressant, fluoxetine has also been active against a wide range of symptoms like anxiety, alcoholism, chronic pain, obesity and bulimia[33]. Although both R- and S-isomers of fluoxetine are pharmacologically active and currently its racemate is in clinical use, experiments have demonstrated the (R)-enantiomer is slightly more potent than the (S)-enantiomer as a 5-HT uptake inhibitor in rat cortical

synaptosomes[34]. Enantiomerically pure isomers have been synthesised by several research groups[32, 35, 36].

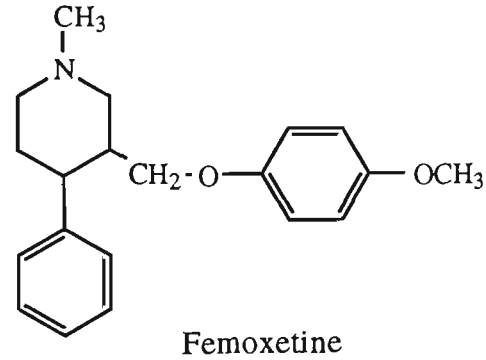


9 (R)-Fluoxetine

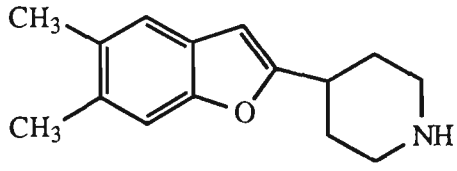
In contrast to the tricyclic antidepressants (TCAs), which structurally resemble each other, the new generation of SSRI antidepressants bear little similarity structurally to each other. A few compounds in use as SSRI antidepressants are shown below.



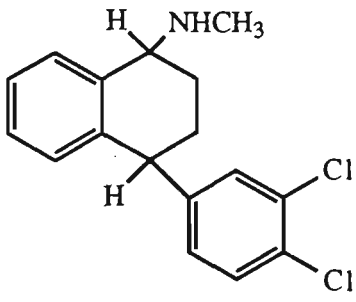
Indolpine



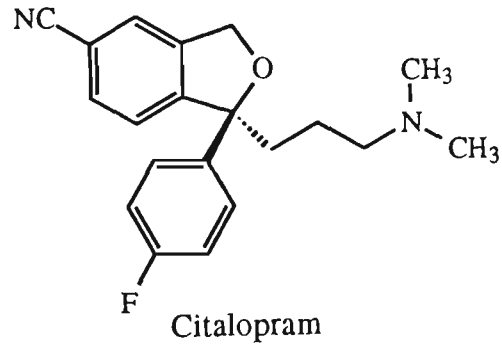
Femoxetine



CGP/6085A



Sertraline



Citalopram

The SSRIs have a delayed clinical onset of action presumably owing to adaptative neuronal changes or cognitive factors and/or as yet unknown neurobiological phenomena. It has been suggested that combining the SSRIs with other antidepressants would promote an enhanced 5-HT release occurring sooner in the course of the treatment[31]. It was found that when a 5-HT_{1A} receptor antagonist is co-administrated with an SSRI, 5-HT release is potentiated in microdialysis experiments carried out in rat brains[37]. Recently, more rapid antidepressant effects resulting from the inhibition of the 5-HT uptake by fluoxetine in combination with the tricyclic antidepressant drug desipramine could provide an opportunity to overcome the delay of onset of action of SSRI antidepressant drugs[38].

Although the side-effects profile is perhaps the most important area in which the SSIRs differ from and represent a significant improvement over earlier antidepressants, it by no means suggests the former do not cause any side effects. It has been recognised that the SSRIs produce nausea, dizziness and nervousness as typical side effects, but they show no pronounced sedative, anticholinergic or cardiovascular effects characteristic of the TCAs[23]. The most common type of adverse effect is gastrointestinal disturbance[39]. In addition, although the SSRIs appear to be safer than the TCAs in their effects on cardiac rhythm, they are not completely safe in this respect. Studies with citalopram in cats have indicated that it has a tendency to produce the same type of cardiac side-effects as the TCAs, though not as powerfully[40]. New agents thus need to be developed to address these problems.

1.6 Computer-aided molecular modelling

Molecular modelling has become a well-established research area in modern medicinal chemistry due to software development and the need for more rational approaches to drug design. Molecular modelling systems provide powerful tools for building, visualising, analysing and storing models of complex molecular systems. These can help interpret the structure-activity relationships crucial for determining the development of new drugs. The goal of molecular modelling is not limited only to providing structure-activity insights, but should also help to suggest new experiments, i.e. new structures tailored to have the desired biological activity[41]. Although molecular modelling can not produce quantitative predictions of activity of compounds, except under very special circumstances, it can provide valuable qualitative guidelines in the design of new structures.

Computer-aided molecular modelling (CAMM) is a powerful tool used by researchers to help explain and/or predict a variety of molecular properties. Drug design is an area of research that is closely associated with CAMM nowadays. With the ever-increasing cost and complexity of structures to be synthesised and evaluated in the laboratory, CAMM plays an important role in assessing drug lead possibilities[42].

Modern applications of CAMM within the pharmaceutical industry fall into two broad classes. In the first class the three dimensional structure of the target enzyme or receptor is known. In this case, CAMM becomes an extremely useful tool as interactions between the substrate and enzyme are easily visualised and calculated. A new or modified substrate can be proposed from these calculations. Unfortunately, there is only very limited knowledge of

receptor structures available, which greatly narrows the scope of use of this technology. The second class of modelling is one in which drug design is based on calculations on the known drug molecules and on the knowledge of the properties of the drugs or ligands.

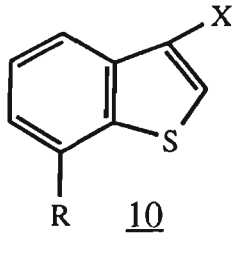
The three-dimensional structure-activity relationship between the 5-HT receptor and antagonists has been reported[42, 43, 44, 46]. However, very little concerning the three-dimensional structure-activity relationship between the 5-HT receptor the and 5-HT uptake inhibitors has appeared[22]. Most recently, a molecular modelling study on the known SSRIs, citalopram, fluoxetine, paroxetine and sertraline has been given by Gundertofte and coworkers[47], suggesting a common pharmacophore including both an aromatic ring of the substrates and an amine.

In this project, CAMM was employed in two ways: i) to help predict the activity of compounds synthesised or to be prepared and ii) in combination with pharmacological screening results of the [1]benzothiophene derivatives to establish their structure-activity relationship as 5-HT uptake potentiators. The prediction of activity was based on the conformational analysis. A large proportion of molecular modelling is associated with study of conformational flexibility of drug molecules. This is due to the assumption that molecules must adopt certain shapes before "being docked" into their site of action and that this shape or conformation must be energetically favourable. The global minimum energy conformation can be searched by molecular dynamics or by systematic searching. Establishment of structure-activity relationships is done by studying molecular similarity, which can be a key feature in the discovery of new drugs. The recognition of matching properties in structurally different

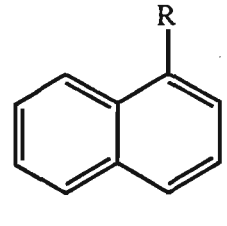
molecules can often lead to hypotheses from which novel structures can be designed or a known molecule can be revised prior to synthesis.

1.7 Background work on [1]benzothiophene derivatives

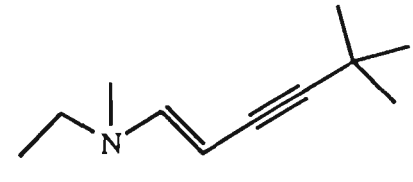
One approach to develop new pharmacologically active compounds is to look at heterocyclic analogues of active compounds, for example [1]benzothiophenes in place of indoles. There are a few published results on [1]benzothiophene derivatives in serotonin related research. As pharmacologically active agents, most references on the [1]benzothiophene derivatives appear in the form of patents. Some [1]benzothiophene derivatives interact with the 5-HT system, as reported by Pinder and colleagues[48], who undertook a comparative pharmacological study on [1]benzofuran, [1]benzothiophene and indene isosteres of 5-HT. Recently, Kawakuboh[49] has reported that some (R)-isomers of [1]benzothiophene derivatives can bind selectively to the 5-HT_{1A} receptor, whereas the (S)-isomers can not. Nussbaumer[50] reported that [1]benzothiophene derivatives of general formula 10 showed significantly enhanced activity against the fungus *Candida albicans* and the compounds can be used not only as potential equivalents of the naphthalene derivatives 11 in bioactive compounds, but also as a tool to selectively modify biological activities. Boyle[51] described some 2,3-dihydro-[1]benzothiophene-3-carboxylic acid derivatives, which were evaluated for analgesic activity, but with low gastric irritancy.



10

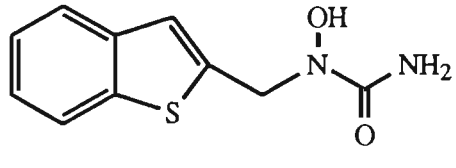


11

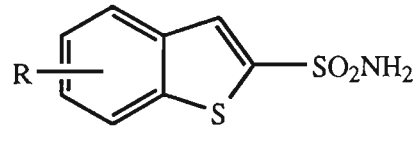


X= Cl, CHO, CH_3S

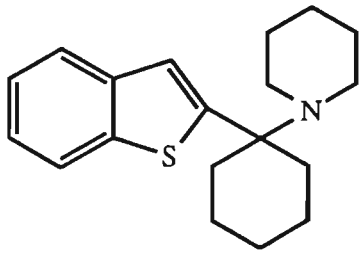
Several papers on [1]benzothiophene derivatives as serotonin or dopamine uptake inhibitors and related inhibitors have appeared in the literature recently. Carter[52] has reported that zileuton 12 is an orally effective inhibitor of 5-lipoxygenase in rats and dogs, and is effective in inhibiting leukotriene-dependent inflammation in mice and inflammatory cell influx in rats. It was found by He and coworkers[53] that some [1]benzothiophene-containing amines (for example BTCP) are instrumental in inhibiting dopamine uptake. Benzothiophenesulfonamide derivatives of the type 13 have been selected by Graham[54] for clinical evaluation to investigate their potential utility as topically active inhibitors of ocular carbonic anhydrase. Such agents are of use in the treatment of glaucoma. Three novel series of [1]benzothiophene-2 derivatives bearing various hydrazone, hydrazine and 1,3,4-oxadiazole moieties (14, 15, and 16) have been reported by Aboulwara[55] as MAO inhibitors and hence potential antidepressants. Novel sulfur-containing indole derivatives, for example 17, have been reported by Malleron[56] to be potent and selective serotonin uptake inhibitors. Of these, compound 13 has been shown to be as active as fluoxetine in *in vivo* tests.



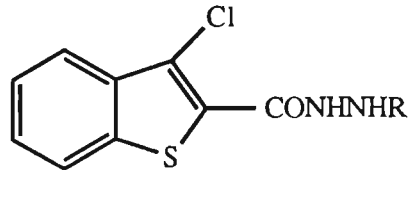
12



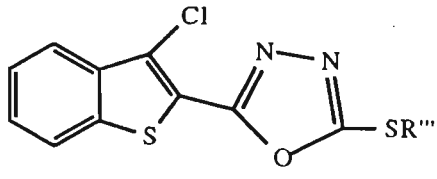
13 R = 4- or 6-OCH_3, OH et al



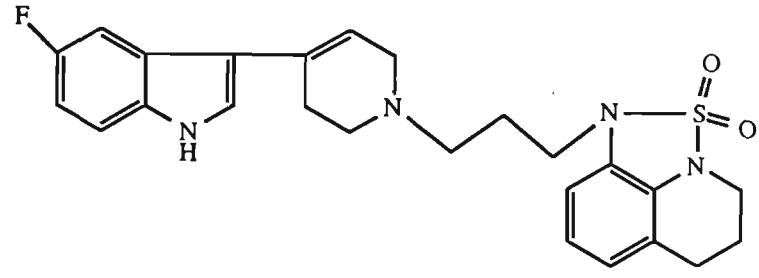
BTCP



14 R=H; 15 R=CHR'R"



16 R'''= alkyl

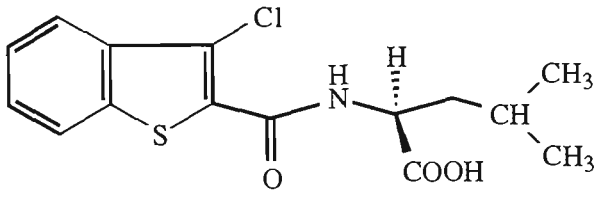


17

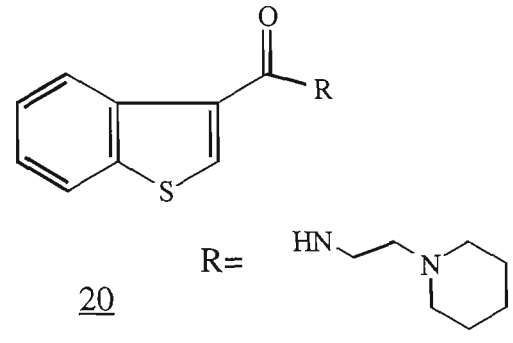
The replacement of the indolic nitrogen of 5-HT by sulfur to give the [1]benzothiophene analogue has been demonstrated to significantly increase the lipid solubility[57] of the molecule. This may make [1]benzothiophene derivatives more likely to penetrate the blood-brain barrier than their indole analogues, which is significant in terms of developing new CNS-active agents.

The discovery of the [1]benzothiophene derivative of L-Leucine 18 by Browne[58] in 1989 in the Department of Chemistry at the University of Tasmania and preliminary pharmacological testing results obtained on 18 at Monash University indicated that there might be some interaction between the compound and 5-HT_3 receptors. As part of an earlier project to develop new 5-HT_3 antagonists, the compound N-[3-chloro-[1]benzothien-2-oyl]glycine 19 was made and tested by Rao[59]. Interestingly, it showed a moderate potentiation of 5-HT uptake activity at the 5-HT_3 receptor in the rat cardiovascular model. Preliminary pharmacological studies indicated that the potentiation is most likely due to 5-HT uptake inhibition. Frankcombe[60] furthered the work by preparing 3-substituted [1]benzothiophene derivatives of the type 20 derived from *N*-acylation of amino acids, and screening them for both 5-HT_3 receptor activity and 5-HT potentiation. One compound (20, R=*N*-(2-aminoethyl)-piperidine) was found to exhibit 5-HT_3 agonist activity and a few compounds (for example, 20, R=glycine) showed moderate 5-HT potentiation. The results of both Rao and Frankcombe hence encouraged a search for further novel

[1]benzothiophene derivatives that could interact with the 5-HT system, particularly with respect to increased 5-HT potentiation activity.



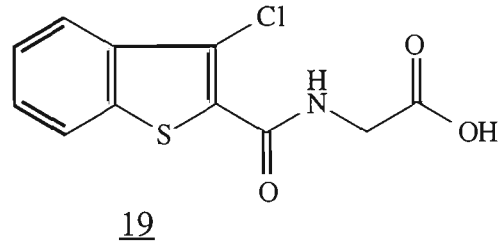
18



20

N-(2-aminoethyl)-piperidine

R= Glycine $NHCH_2COOH$

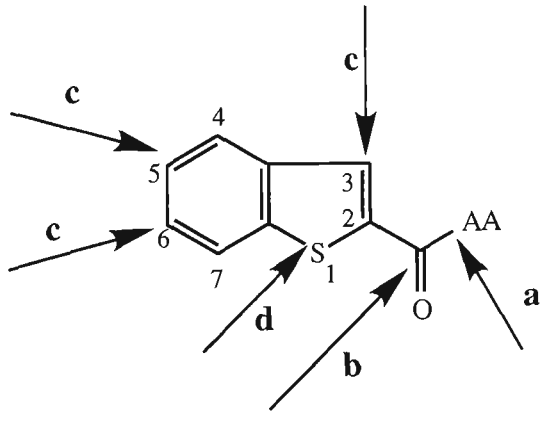


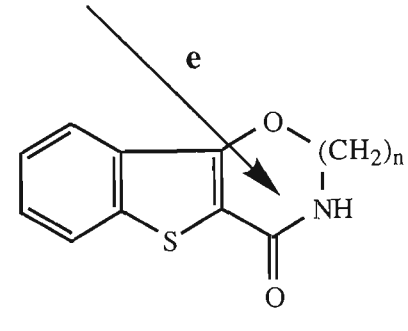
19

1.8 Aims of the project

The aims of this project were thus:

1. To investigate the synthesis of a number of [1]benzothiophene-containing derivatives of amino acids and analogues based on the structural modifications of the glycine derivative 19 as shown in Scheme 1.3.





a. preparation of various amino acid derivatives

b. reduction of the carbonyl functionality

c. incorporation of substituents at the positions 3, 5 and 6.

d. oxidation of the sulfur

e. preparation of the cyclized derivatives

Scheme 1.3 Proposed structural modifications of the [1]benzothiophene-based 5-HT potentiators

2. To use these structurally novel leads to develop more active compounds in terms of 5-HT potentiation based on i) molecular modelling, ii) design, iii) synthesis, and iv) structure-activity evaluation.

3. To investigate a feasible route to prepare a range of medium ring-containing [1]benzothiophene analogues and to study their activities.

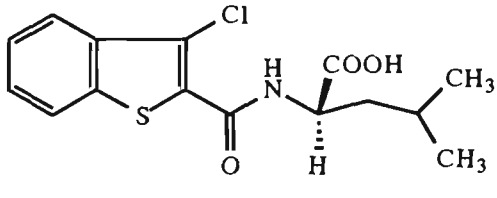
4. To propose a hypothesis to explain the structure-activity correlation based on the modelling results and pharmacological test results.

Chapter 2

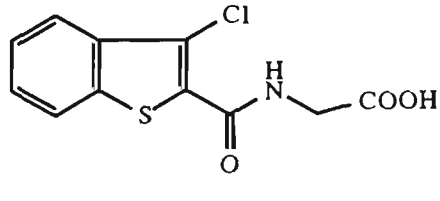
Synthesis of 2-Substituted 3-Chloro-[1]benzothiophene Derivatives

2.1 Introduction

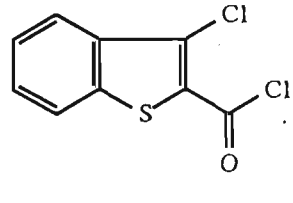
The research on 3-chloro-[1]benzothiophene-2-carboxylic acid derivatives of amino acids as 5-HT potentiators was initiated by the discovery of the *N*-acylated derivative 18 of L-leucine. This prompted a further study carried out by Rao, and, as a result, the 3-chloro-[1]benzothien-2-oyl derivative 19 of glycine was found to be a moderately active potentiator of 5-HT action, probably by 5-HT-uptake inhibition[59]. On the basis of these results and the need to increase their activity as 5-HT uptake inhibitors, it was decided that a wider range of amino acids needed to be incorporated to afford analogues for screening. These analogues were anticipated to be obtained by reaction of the 3-chloro-[1]benzothiophene-2-carbonyl chloride 21 with various amino acids.



18



19

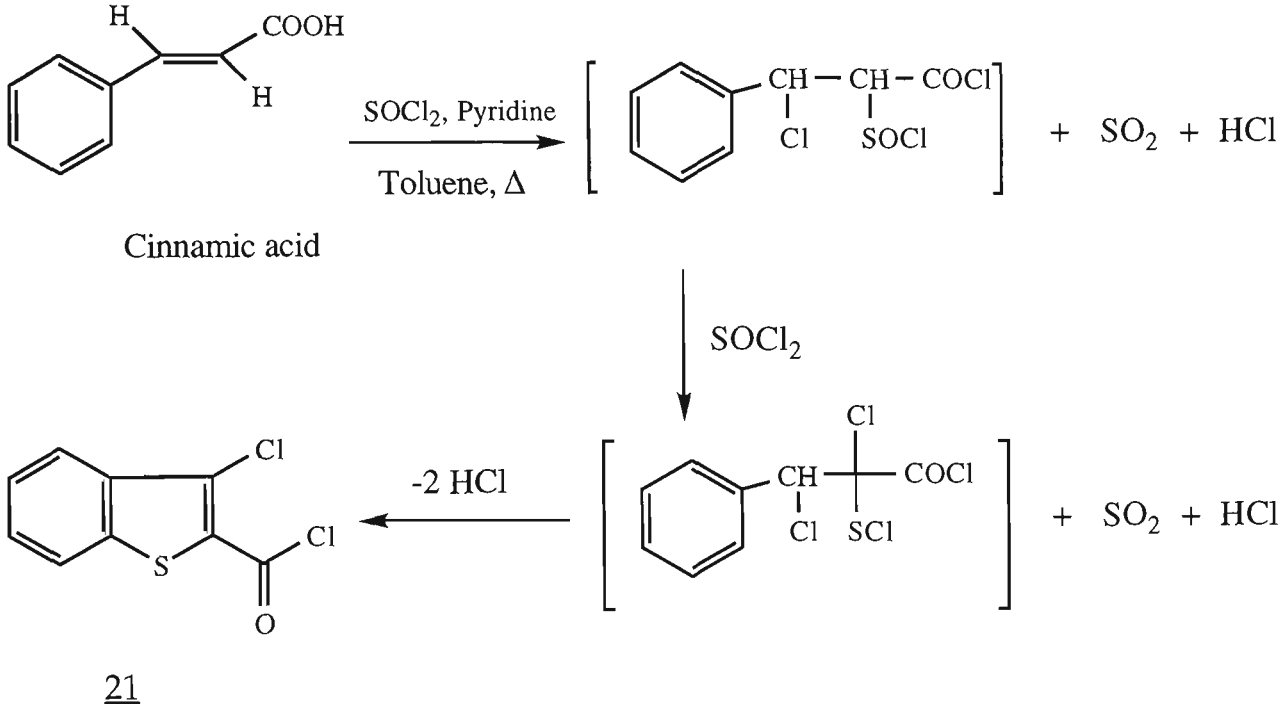


21

2.2 Synthesis of 3-chloro-[1]benzothiophene-2 amide derivatives

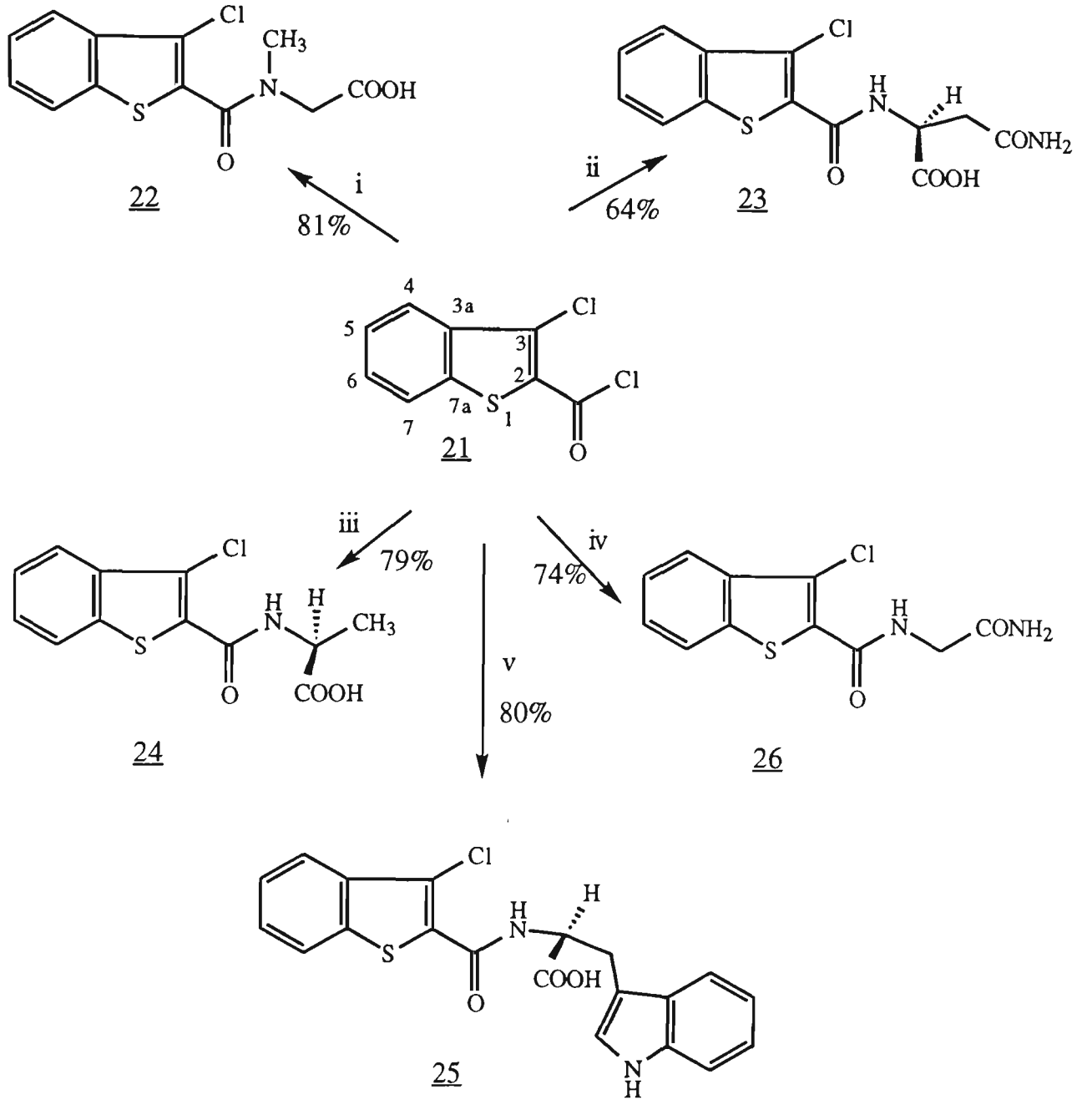
The acid chloride 21 was synthesised according to Wright's method[61] from cinnamic acid, but with slight modifications to improve yields. Following the literature conditions with chlorobenzene as solvent, the acid chloride was obtained in 35% yield. Using toluene as solvent rather than chlorobenzene significantly improved the yield to 64%. One of the possible

pathways for the formation of 21, as proposed by Higa and coworkers[62], is shown in Scheme 2.1.



Scheme 2.1

The general procedure for the preparation of the amide derivatives involved reaction of an amino acid dissolved in dilute aqueous sodium hydroxide solution with the acid chloride 21, followed by acidification of the reaction mixture with hydrochloric acid to precipitate the products. The dilute sodium hydroxide solution was used to both dissolve the amino acid and to release the amino groups from the zwitterionic forms. In all cases, however, a small amount of the acid chloride 21 was hydrolysed to the corresponding carboxylic acid. This acid could be removed by recrystallization of the crude product. The amide of sarcosine 22, L-asparagine 23, L-alanine 24, L-tryptophan 25 and glycinamide 26 were prepared in moderate to good yields (Scheme 2.2).

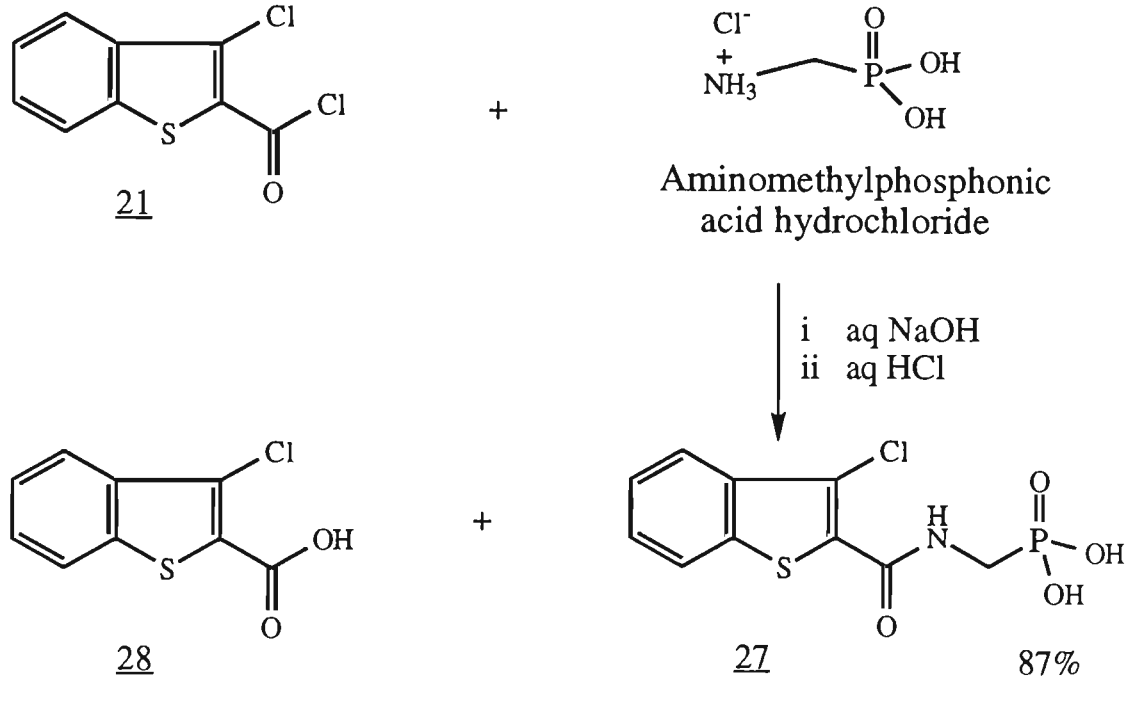


Reagents and conditions: i Sarcosine/aq NaOH ii L-Asparagine/aq NaOH iii L-Alanine/aq NaOH iv Glycinamide/aq NaOH v L-Tryptophan/aq NaOH

Scheme 2.2

This reaction sequence was extended to the preparation of the analogous phosphonic acid 27 by reaction of aminomethylphosphonic acid with the acid chloride 21. The work-up of the reaction, however, was difficult because the phosphonic acid derivative 27 was found to be very water soluble. The

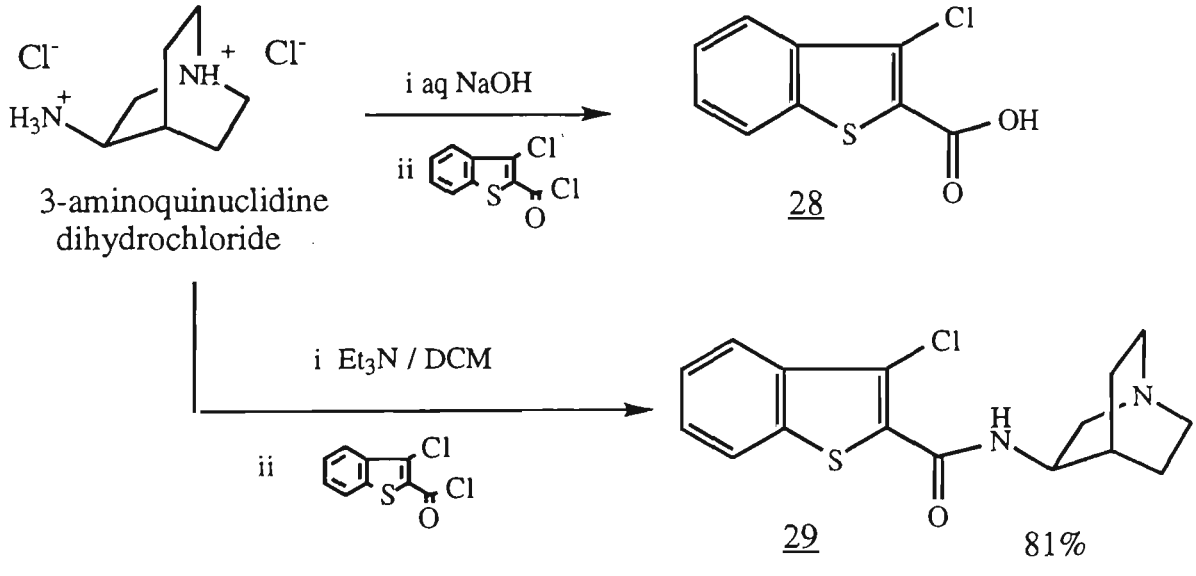
acidified (HCl) reaction mixture was evaporated to give a mixture of solids, which contained a small amount of the acid 28. This acid was removed by washing the mixture with diethyl ether. The sodium chloride formed during the reaction was not removed (Scheme 2.3).



Scheme 2.3

As the quinuclidine amide 3 (*cf* Section 1.3) has been reported to be a 5-HT_3 antagonist[18], it was of interest to see whether the [1]benzothiophene derivative of quinuclidine 29 would also show a similar activity. The synthesis of compound 29 was thus investigated. Attempts to prepare 29 by reaction of a basic solution of 3-aminoquinuclidine dihydrochloride with the acid chloride 21 failed to give the desired product. Instead, all the acid chloride was hydrolysed to the carboxylic acid 28. To overcome this problem, triethylamine was used instead of aqueous sodium hydroxide as the base and the reaction was carried out in dichloromethane. This reaction proceeded smoothly, giving the product 29 in good yield. It is believed that in the case of aqueous sodium hydroxide solution, the 3-aminoquinuclidine generated was insoluble in

aqueous solution therefore unable to attack the acid chloride 21, which was then exclusively hydrolysed by the sodium hydroxide to the acid 28. In the case of triethylamine as base and dichloromethane as solvent, it is most likely that the triethylamine slowly deprotonated the 3-aminoquinuclidine dihydrochloride to give off the free amine, which then dissolved in dichloromethane where it readily reacted with the acid chloride 21 (Scheme 2.4).

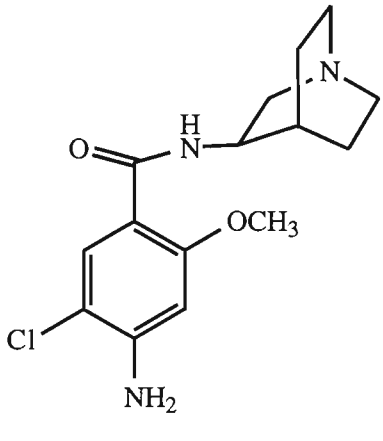


Scheme 2.4

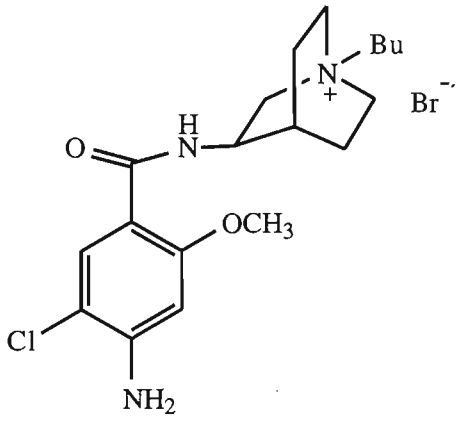
The structures of all the amides were confirmed spectroscopically. In the ^1H NMR spectra, the protons 4, 7 and 5, 6 appeared as multiplets from 7.8 to 7.6 ppm and from 7.5 to 7.2 ppm respectively[63]. The IR absoption bands for the amide carbonyls were around 1630 cm^{-1}, in agreement with those of aromatic amides[64]. All the mass spectra showed chlorine-isotope-containing molecular ion peaks, confirming the presence of one chlorine atom.

2.3 Preliminary pharmacological testing and molecular modelling results of the amide derivatives

A comprehensive discussion of the pharmacological tests and molecular modelling is given in Chapter 5, but a brief mention of the results is given here. The investigation of the 5-HT potentiation activity of the [1]benzothiophene-2 amide derivatives was carried out in the Department of Pharmacology at Monash University, by Associate Professor J. O'Neil and Ms W. Lau. A cardiovascular screen in rats was used. In comparison with the glycine derivative 19, which demonstrated a moderate 5-HT potentiation in preliminarily pharmacological testing, compounds 22, 23, 25, and 27 appeared to be inactive. The amides 24 and 26 enhanced 5-HT induced bradycardic responses, but to a lesser extent than compound 19. The [1]benzothiophene-2 quinuclidine amide 29 was found to be a potent 5-HT_3 antagonist, as expected, since the quinuclidine amide 3 has been reported to be a 5-HT_3 antagonist[18]. Interestingly, its butyl ammonium salt 30 has shown 5-HT_4 agonist activity[65]. The butyl ammonium salt of 29 was likely to show 5-HT_4 agonist activity, but this was not followed up due to work on the 5-HT potentiators.



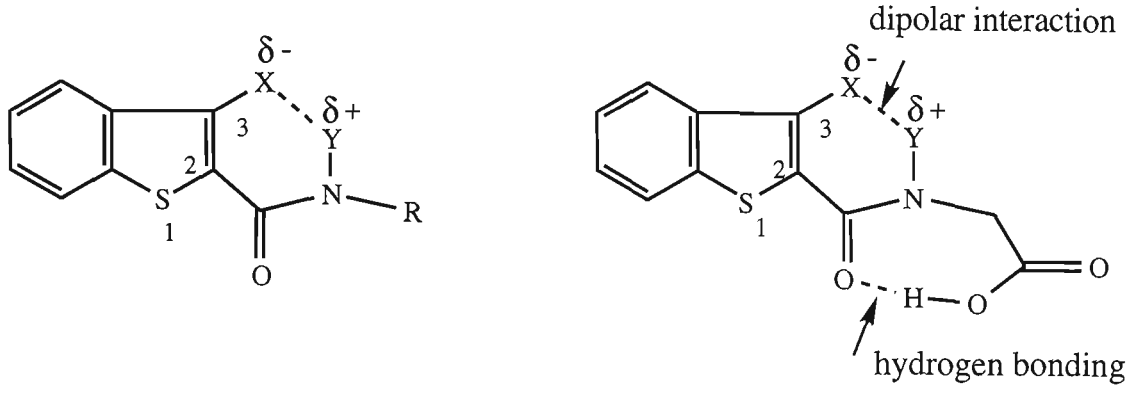
3



30

Molecular modelling results suggested that there might be a dipolar interaction between the 3-substituents and substituents on the nitrogen atom of the amides or nitrogen itself. A conformation with a minium energy for the glycine derivative 19 showed a specific hydrogen bonding between the carbonyl

oxygen of the amide and the hydroxyl group of the carboxylic acid (Scheme 2.5). However, whether this hydrogen bonding was effective in differentiating a compound being active or inactive as 5-HT potentiator remained uncertain and needed further results to confirm.



Scheme 2.5

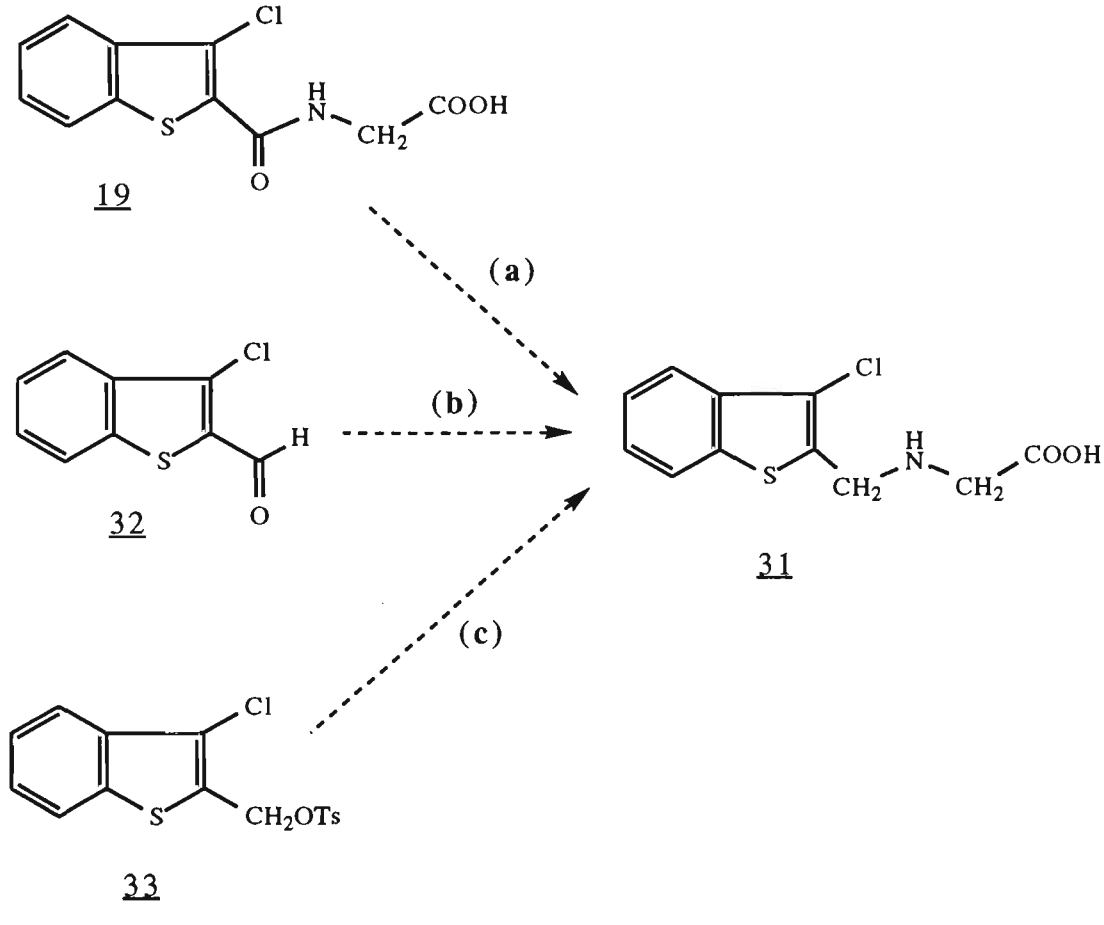
2.4 *N*-[1]Benzothienylmethyl amino acid derivatives

2.4.1 Introduction

As mentioned above, molecular modelling studies suggested an interaction between the 3-substituents and the substituents on nitrogen or nitrogen itself in the 2-substituents. Furthermore, several researchers have suggested the coplanarity of the aromatic ring and carbonyl moiety of an amide or ester could be an important factor for the high affinity of some 5-HT_3 antagonists toward the 5-HT_3 receptors[14, 18, 19]. It was thus of interest to investigate the synthesis and pharmacological activity of methylene derivatives. The transformation of the amide carbonyl functionality to methylene group would remove coplanarity and also affect the dipolar interaction.

methylene group would remove coplanarity and also affect the dipolar interaction.

As the glycine derivative 19 showed a promising 5-HT potentiation activity, its methylene derivative 31 was investigated first. Three possible approaches were considered to prepare 31, as shown in Scheme 2.6.



Scheme 2.6

In path (a), the glycine derivative 19, easily prepared from the acid chloride 21 and glycine, was to be reduced directly to the corresponding methylene compound with the retention of other functionalities such as the 3-substituted halide and the carboxylic acid. In path (b), the aldehyde 32 and glycine or its derivative, were expected to undergo a reductive-amination

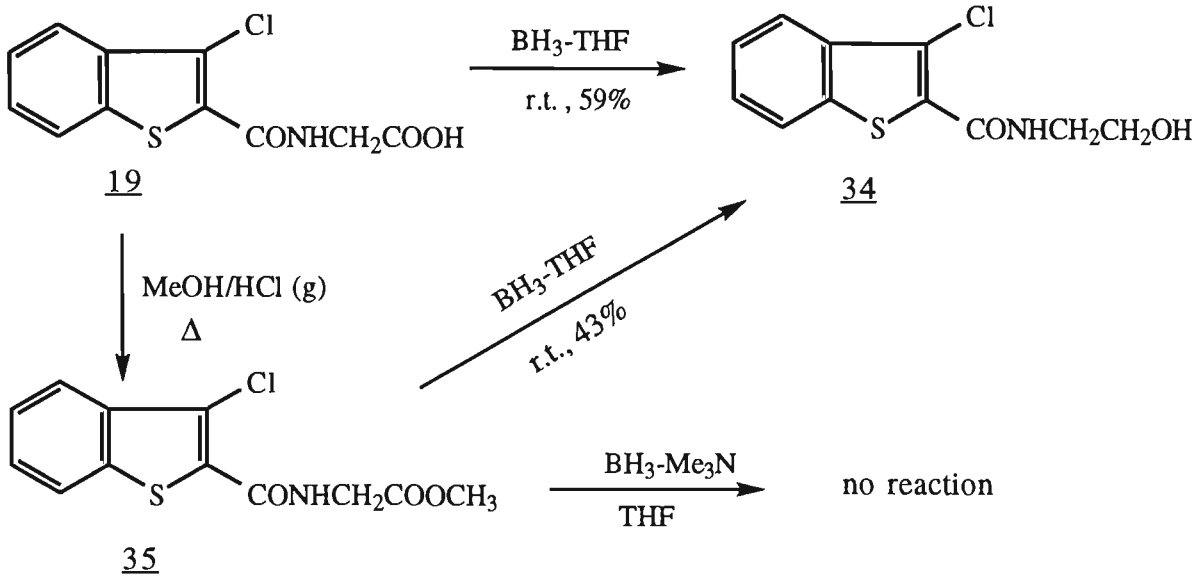
reaction to give the desired methylene compound. Finally, path (c) involved a nucleophilic substitution between the tosylate 33 and glycine or its derivative. Direct reduction of the carbonyl group of 19 was first investigated, as it appeared to be the simplest and most direct route.

2.4.2 Reduction of the amides

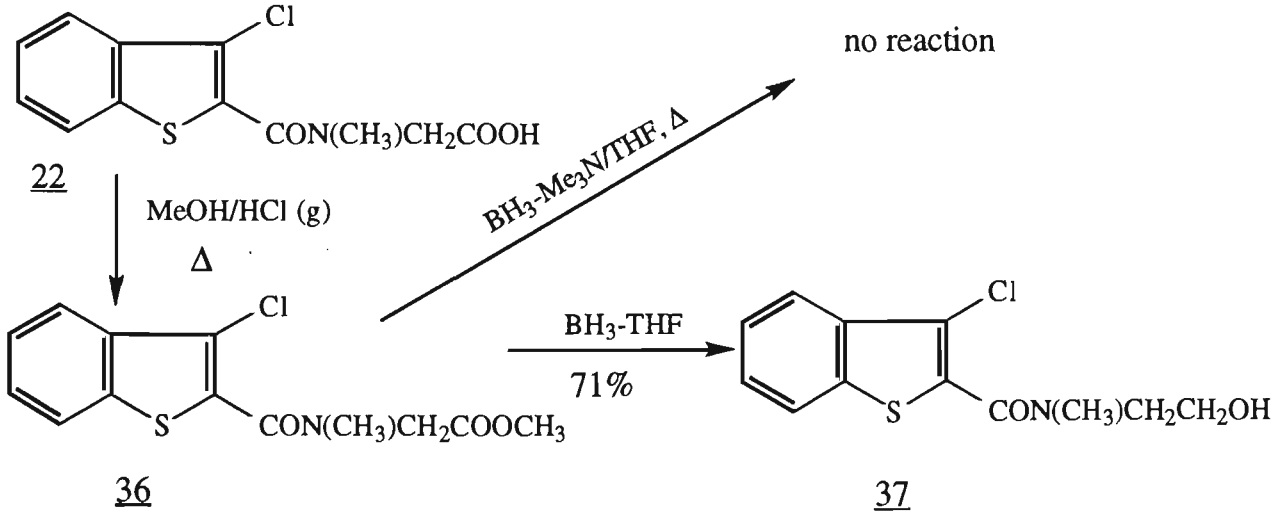
There is an enormous literature on the reduction of amides[66]. Catalytic hydrogenation can be used to reduce amides to amines, but elevated temperatures and high pressures are usually required, which sometimes limits its utility[67]. Amides may also be reduced indirectly to yield amines *via* dissolving metal reductions of imino chlorides, thioamides or related species[68, 69]. Amides are, however, more frequently and efficiently reduced to amines using hydride reagents, and the commonly used reagents in this category are borane and its derivatives, lithium aluminium hydride and its derivatives[70].

Borane complexes, for example BH_3-THF and BH_3-SMe_2, are often used to reduce amides because they are chemoselective, i.e. less reactive groups including alkyl or aryl halides, epoxides, esters and nitro compounds are easily tolerated[71, 72]. Brown[73, 74] has reported that both aromatic and aliphatic amides (primary, secondary and tertiary) can be reduced to amines with BH_3-THF or BH_3-SMe_2 complexes in good to excellent yields. Nevertheless, treatment of the amide 19 with BH_3-THF complex at room temperature afforded the alcohol 34, with no reduction of the amide functionality. This reaction occurred when a stoichiometric amount or an excess of borane was employed. It has been reported that in some cases esters are more resistant towards borane-THF complex[75]. The acid 19 was therefore converted to its methyl ester 35 by refluxing in acidified methanol. Treatment of the ester with

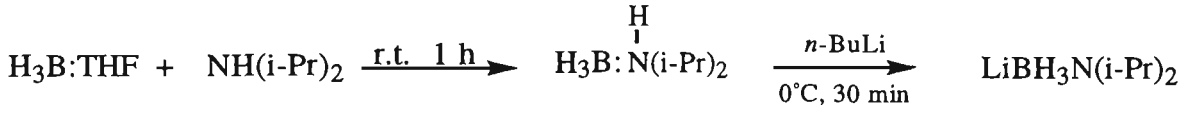
BH_3-THF, however, failed to give the desired amine 31. Instead, the ester was also converted to the alcohol 34 in fair yield.

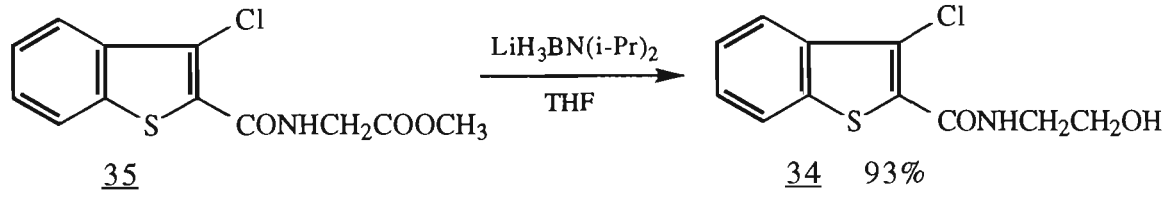


The tertiary amide ester 36 was also treated with borane-THF complex, but similarly, the alcohol 37 was obtained, and again the amide functional group remained unchanged. Borane-trimethylamine complex was also employed to reduce the amide functionality in both esters 35 and 36, but no reaction occurred even after reflux, consistent with its lower reducing capability[76].

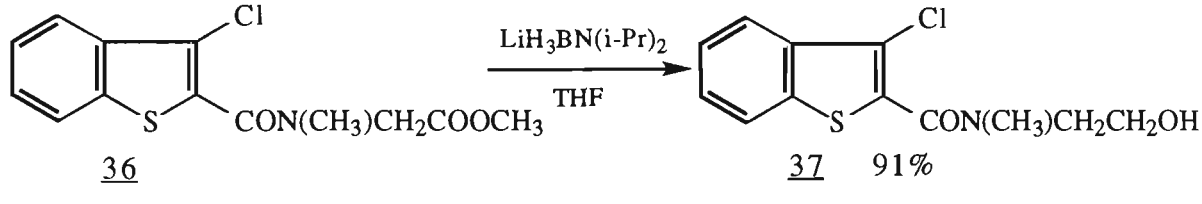


Fisher and coworkers[77] have reported that lithium diisopropylaminoborohydride, prepared *in situ* from the reaction of butyl lithium and borane in the presence of diisopropylamine, is a powerful and stable reducing agent, which can reduce both aliphatic and aromatic amides to the corresponding amines. Treatment of the amides 35 and 36 with $LiBH_3N(i\text{-}Pr)_2$, however, gave the corresponding alcohols again, with no amide reduction.





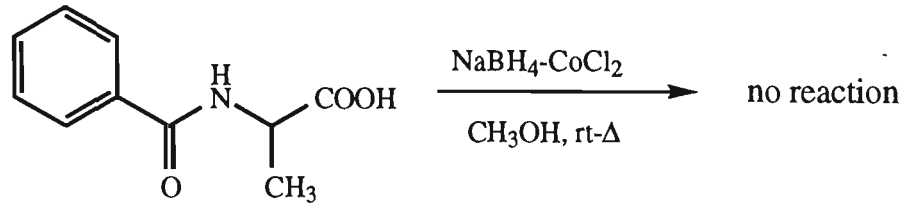
35 34 93%



36 37 91%

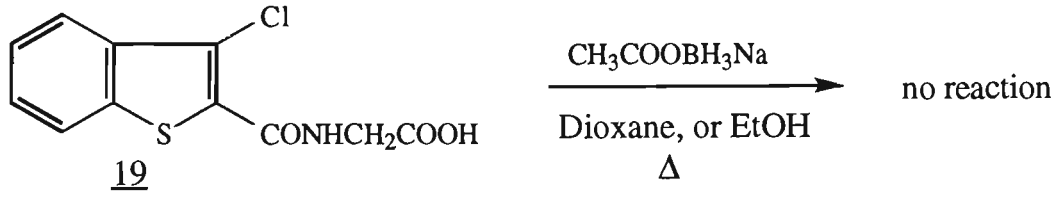
In most cases sodium borohydride alone will not reduce amides, however, sulfur-, oxygen- and nitrogen- substituted sodium borohydrides[78-82] or mixtures of sodium borohydride with inorganic salts including $CoCl_2$, $NiCl_2$, $TiCl_4$ and $AlCl_3$ have been reported[83, 84] to be more reactive than sodium borohydride itself, hence able to reduce amides. Satoh and associates[84] found that treatment of primary aromatic amides or secondary aromatic amides in methanol or dioxan with sodium borohydride in the presence of cobalt (II) chloride hexahydrate afforded the corresponding amines. They also discovered this system did not reduce a carboxylic acid. These conditions were

applied to the model amide 38, prepared from benzoyl chloride and DL-alanine, but no reaction took place, even after a few hours reflux.



38

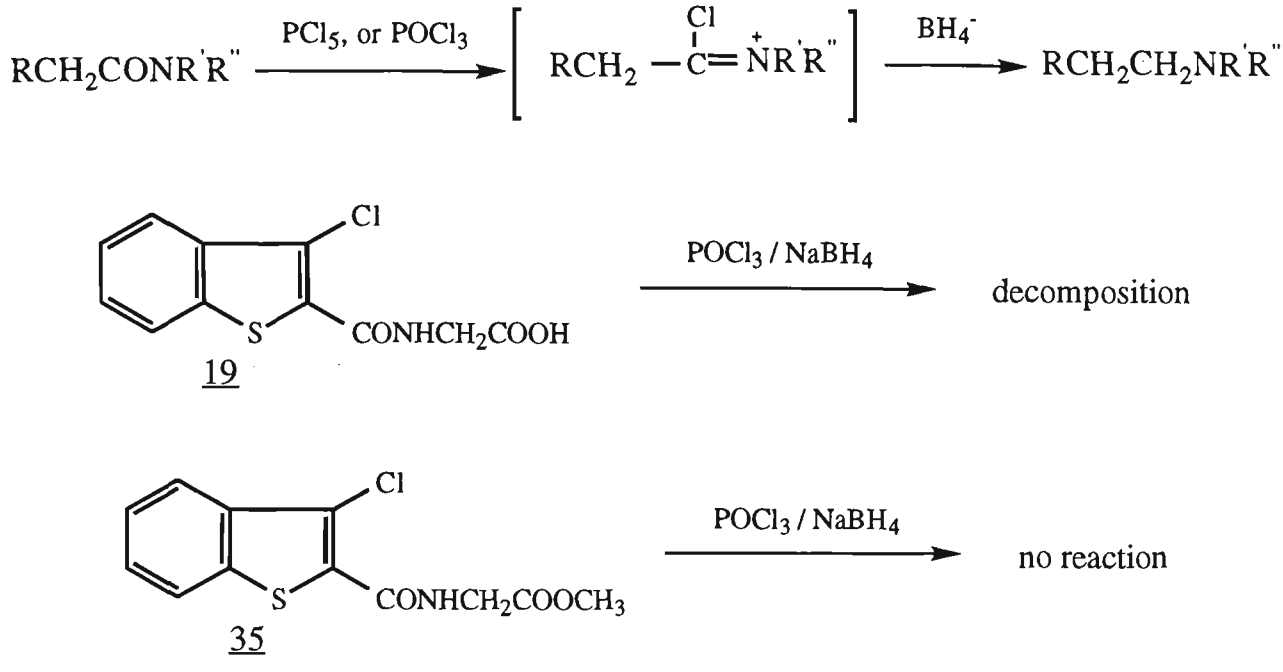
Umino and associates[82] have reported that sodium acyloxyborohydrides, readily available from reaction between sodium borohydride and certain carboxylic acids, exhibit greater reactivity than sodium borohydride itself in the reduction of amides to give amines in good yields. Exposure of the amide derivative 19 to sodium acetoxyborohydride in dioxan or ethanol, after reflux, however, gave no detectable reaction.



19

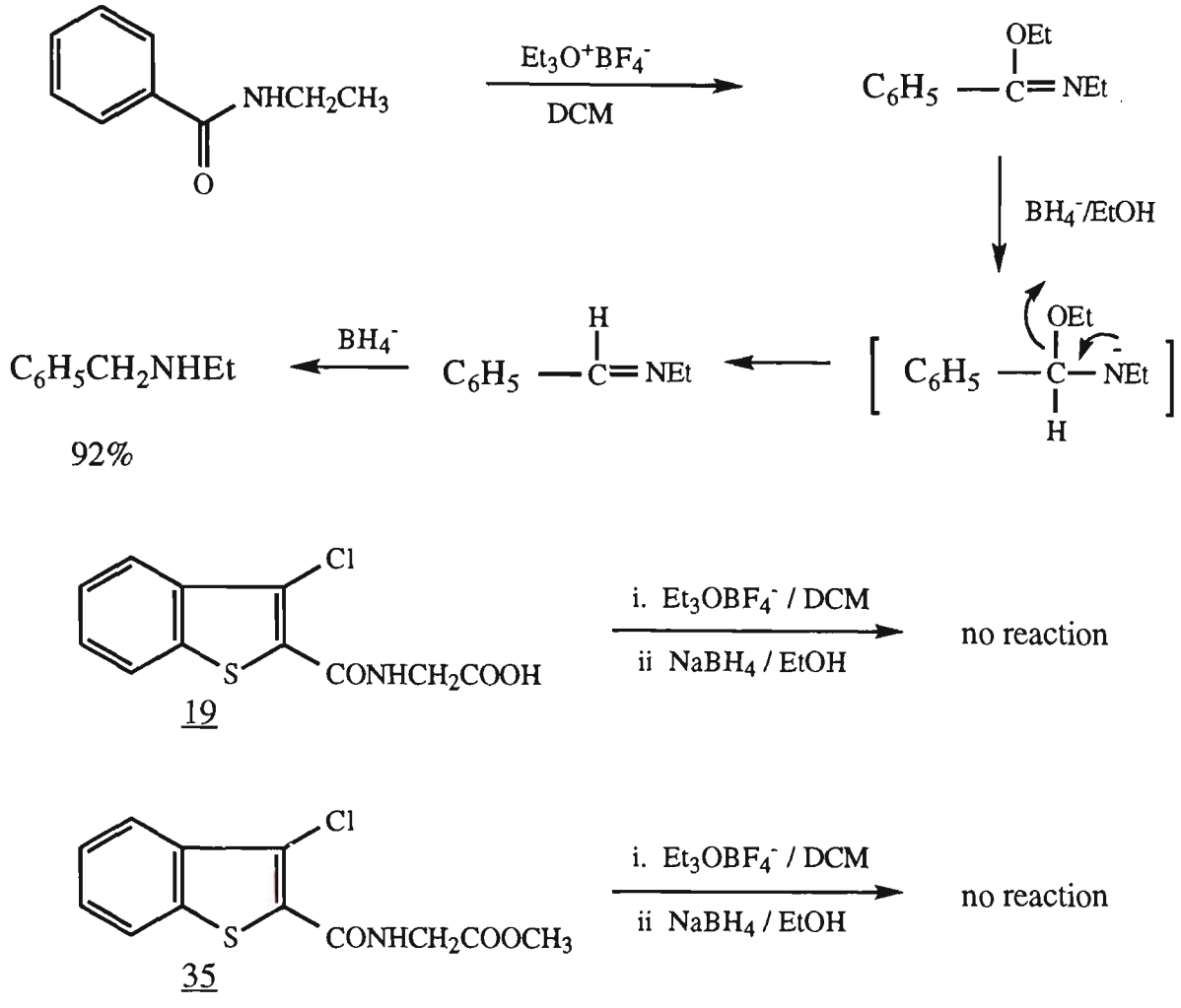
It has been shown that the reduction of amides to the corresponding amines with sodium borohydride in the presence of phosphorus oxychloride or phosphorus pentachloride is a practical and convenient procedure. Rahman[69] and Kuehne[85] have suggested that phosphorus oxychloride or phosphorus pentachloride contribute to the success of the reduction by reacting with the amides to form iminium salts as intermediates, which are then smoothly reduced by sodium borohydride to the amines (Scheme 2.7). Treatment of the amide 19 with phosphorus oxychloride-sodium borohydride under the literature conditions resulted in decomposition of the starting

material. The methyl ester 35 remained unreactive towards phosphorus oxychloride-sodium borohydride or phosphorus oxychloride-sodium borohydride-pyridine.



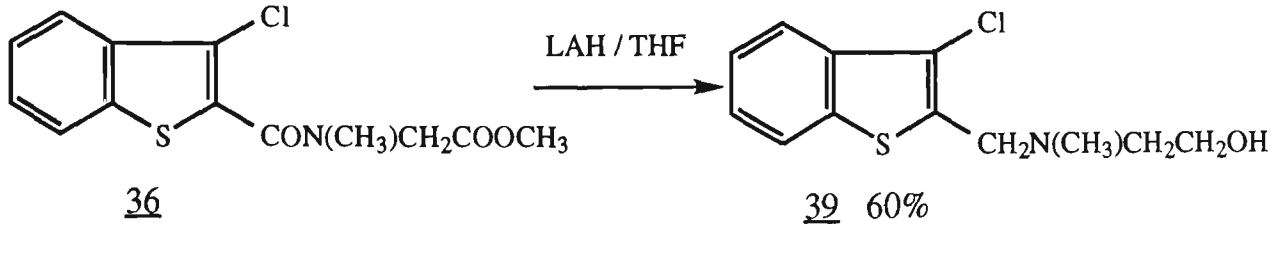
Scheme 2.7

Borch[86] has proposed that triethyloxonium fluoroborate reacts with amides such as *N*-ethyl benzamide in dichloromethane to form stable iminoether fluoroborates, which can be reduced to amines by sodium borohydride (Scheme 2.8). Unfortunately, treatment of both the amide acid 19 and its methyl ester 35 in dichloromethane with triethyloxonium fluoroborate, followed by replacement of the dichloromethane by ethanol and exposure to sodium borohydride, did not result in any reaction. Whether the low reactivity of the amide derivatives towards the aforementioned reducing agents is associated with the steric effect of both the 3-chloro and the sulfur atom is not clear.



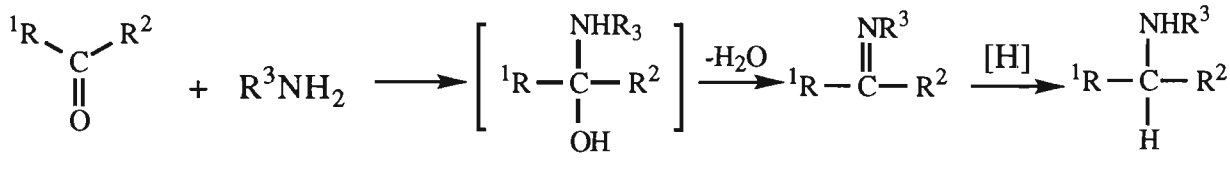
Scheme 2.8

Although lithium aluminium hydride and its analogues are well known reagents for the reduction of amides to amines, the non-selective nature of these reagents precludes their use with certain other reducible groups present[86, 87]. This limitation is illustrated below. The methyl ester amide 36 was reduced by lithium aluminium hydride to the amino alcohol 39 in fair yield.



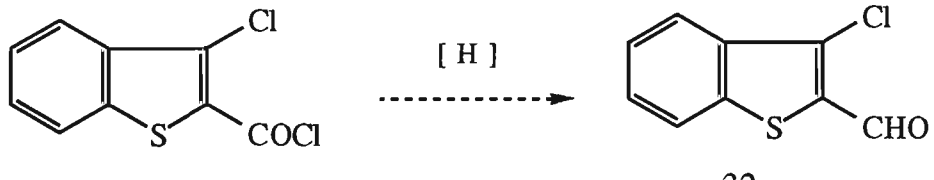
2.4.3 Reductive amination to prepare the methylene compounds

Reductive-amination involving an aldehyde or a ketone and an amine is a frequently used method to prepare substituted amines[88]. The reaction can be regarded generally as occurring in two main stages, i.e. i) the carbonyl group is converted to an imine intermediate; ii) the imine is then reduced to the corresponding amine (Scheme 2.9).



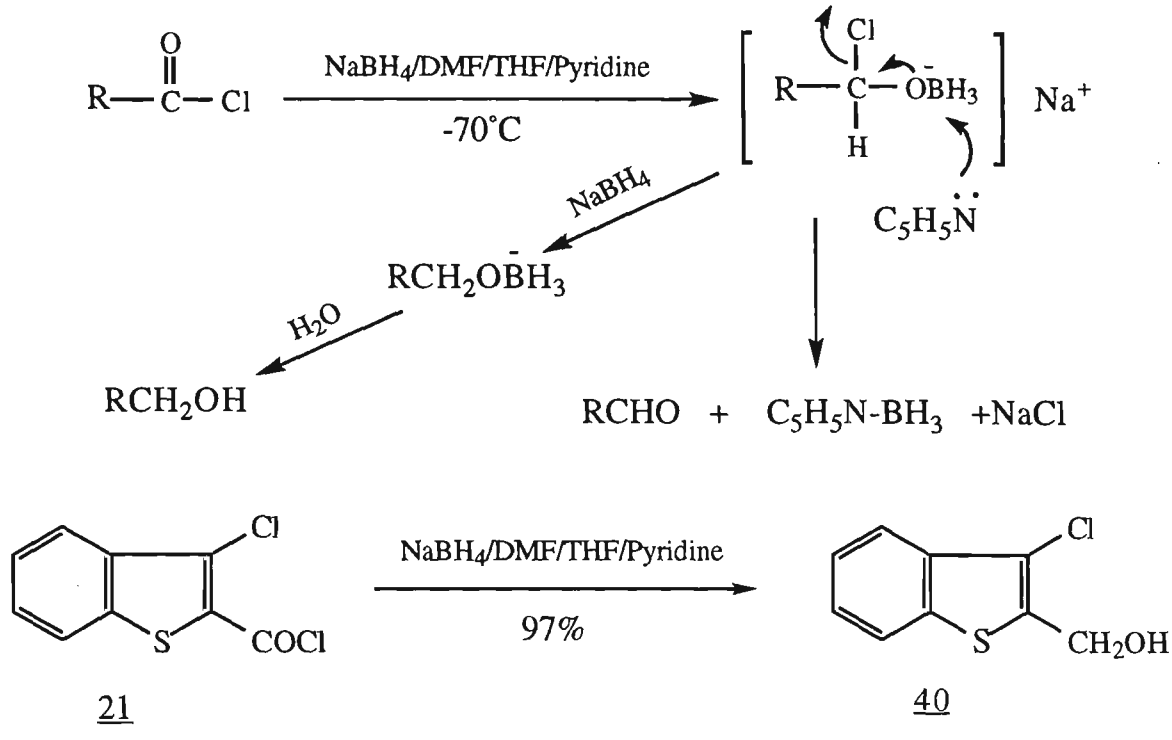
Scheme 2.9

In this project, aldehyde 32 was chosen for the reductive-amination reaction. It was anticipated that the aldehyde could be obtained directly from reduction of the acid chloride 21.



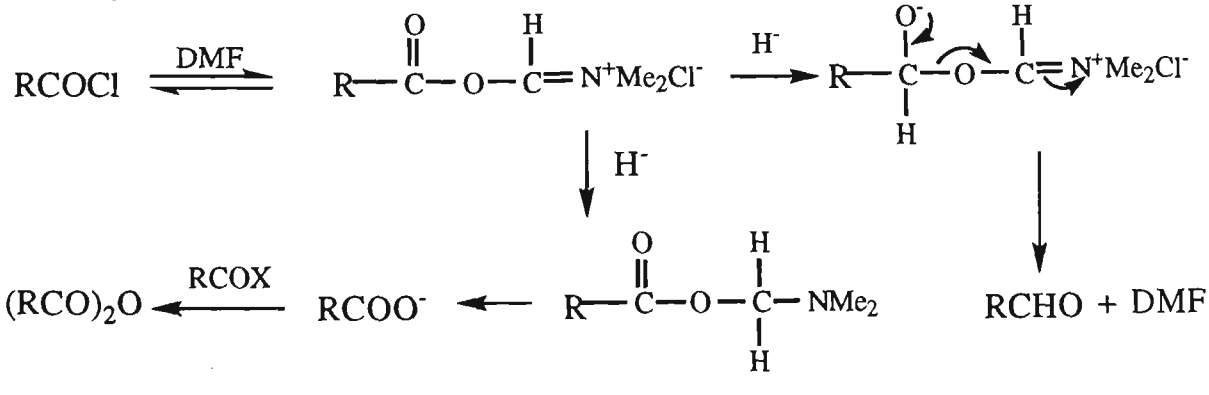
21 32

Babler[89, 90] has found that both aliphatic and aromatic acid chlorides can be reduced in good yield to the corresponding aldehydes by sodium borohydride in a THF-DMF mixture containing pyridine, with only a small amount of the overreduction products. It is suggested that pyridine plays a role in preventing further reduction of aldehydes. This is illustrated in Scheme 2.10. The application of Babler's conditions to the acid chloride 21 led exclusively to the alcohol 40 in excellent yield.

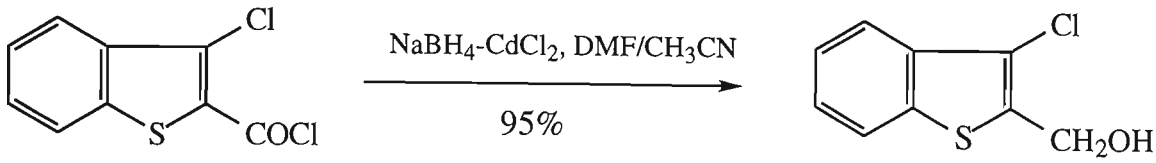


Scheme 2.10

Entwistle[91] has investigated the reduction of acid chlorides with sodium borohydride in DMF in the presence of metallic ions including Cu^{2+}, Cd^{2+} and Zn^{2+}. He found that aldehydes were the predominant products. The proposed mechanism for the reduction involves a DMF complex, as shown in Scheme 2.11. Treatment of the acid chloride 21 with sodium borohydride in DMF in the presence of cadmium chloride, however, gave the alcohol 40 again in excellent yield.

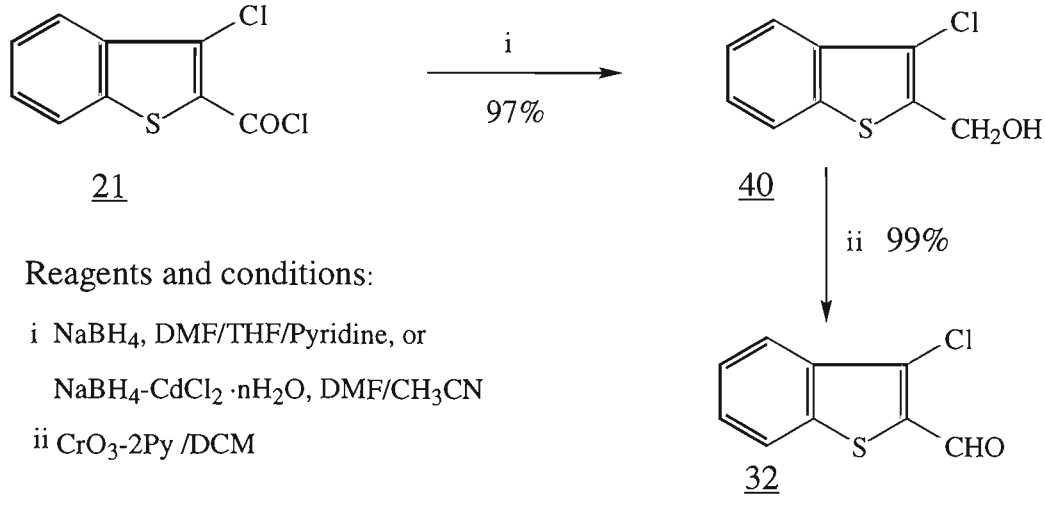


Scheme 2.11



21 40

No further effort was made to directly reduce the acid chloride to the aldehyde 32, since it was found that the alcohol 40 could be oxidised to the aldehyde by dipyridine-chromium (IV) oxide[92] (Collins's reagent) almost quantitatively. The overall yield of the reduction-oxidation was well above 80%, which was better than normally expected by direct conversion of an acid chloride into an aldehyde.



21 40 32

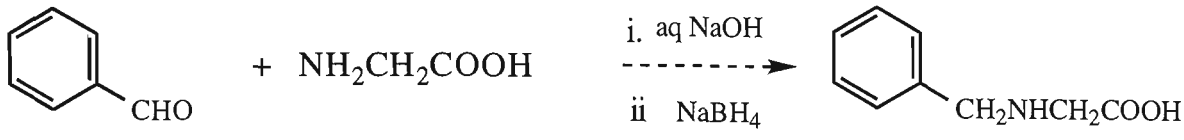
Reagents and conditions:

i $NaBH_4$, DMF/THF/Pyridine, or
$NaBH_4$-$CdCl_2 \cdot nH_2O$, DMF/CH_3CN

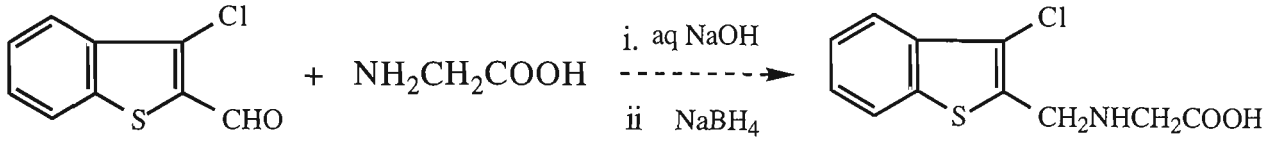
ii CrO_3-2Py /DCM

Quitt[93] has reported preparation of *N*-phenyl glycine by reductive-amination of benzaldehyde with glycine. Attempts to prepare the acid 31 from the aldehyde 32 and glycine using Quitt's reductive-amination conditions, however, were unsuccessful. Instead, the aldehyde was fully reduced to the alcohol 40, implying that the poor solubility of the aldehyde in aqueous solution might have prevented the water soluble sodium glycinate attacking it. Addition of THF in an attempt to increase the solubility of the aldehyde, and

hence induce the condensation between the aldehyde and sodium glycinate failed to stop reduction of the aldehyde 32.

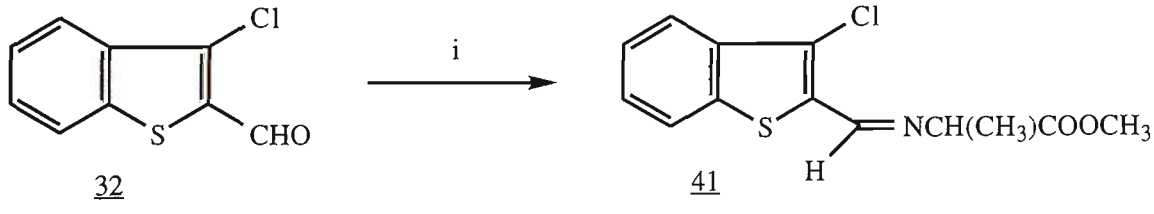


N-phenyl glycine



32 31

With the failure of the one pot procedure, conversion of the aldehyde 32 to the corresponding imine and subsequent reduction was investigated. Several papers involving the condensation of amino esters or their hydrochloride salts with aldehydes or ketones to prepare imines have been published[94, 95]. Bey and Vevert[95] have shown that the condensation of the methyl ester of alanine hydrochloride with benzaldehyde in the presence of triethylamine produces the corresponding imine in high yield. Following this procedure the aldehyde 32 and methyl ester of alanine hydrochloride in benzene containing triethylamine was refluxed to afford the imine 41.

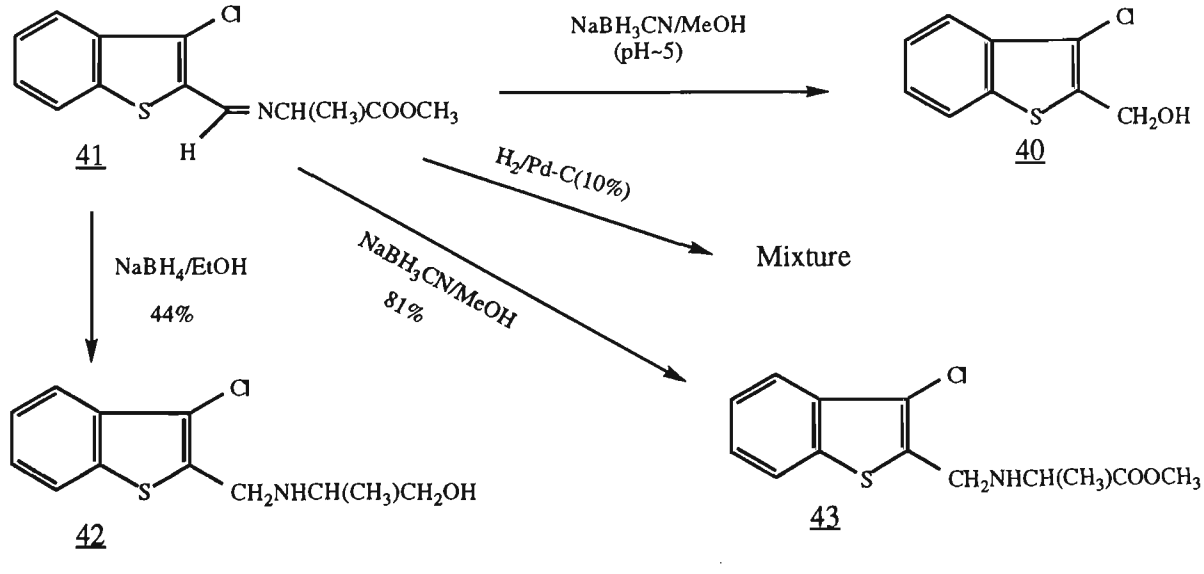


32 41

i Methyl alaninate hydrochloride, Et_3N/benzene, reflux, 80%

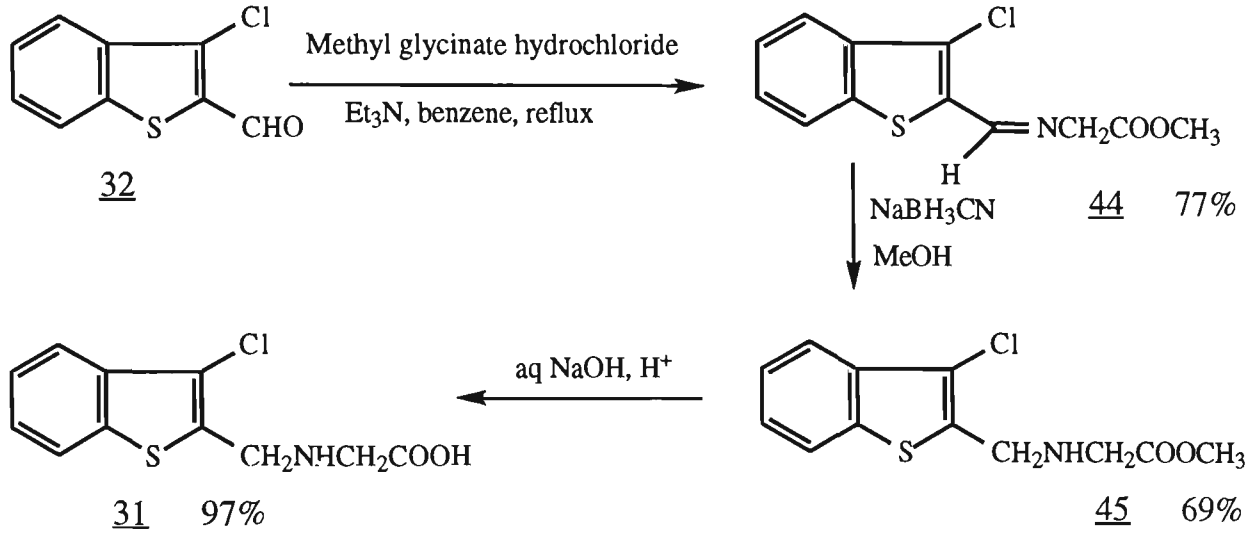
Imines can be reduced to the corresponding amines under various conditions[96]. Among them, catalytic hydrogenation is a favoured method as it

uses mild conditions, and is particularly suitable for systems containing other functional groups that could be reduced by other reducing agents[97]. Surprisingly, exposure of a solution of the imine 41 in absolute ethanol, in the presence of a catalytic amount of 10% Pd-C to a stream of hydrogen, led to mixtures. Apart from catalytic hydrogenation, metal hydride reagents can also be used to reduce imines to amines. The imine 41 was treated with sodium borohydride in ethanol, but was converted to the amide alcohol 42, suggesting that sodium borohydride as a reducing agent here was too powerful for the ester containing imine. Sodium cyanoborohydride has been reported as a mild and excellent agent to selectively reduce imines[98, 99]. Treatment of the imine 41 in methanol under acidic conditions[99] with sodium cyanoborohydride afforded the alcohol 40 as the sole product. This presumably arose from reduction of the hydrolysis product of the imine 41. The imine 41 was then successfully converted in good yield to the amino ester 43 using sodium cyanoborohydride in methanol.



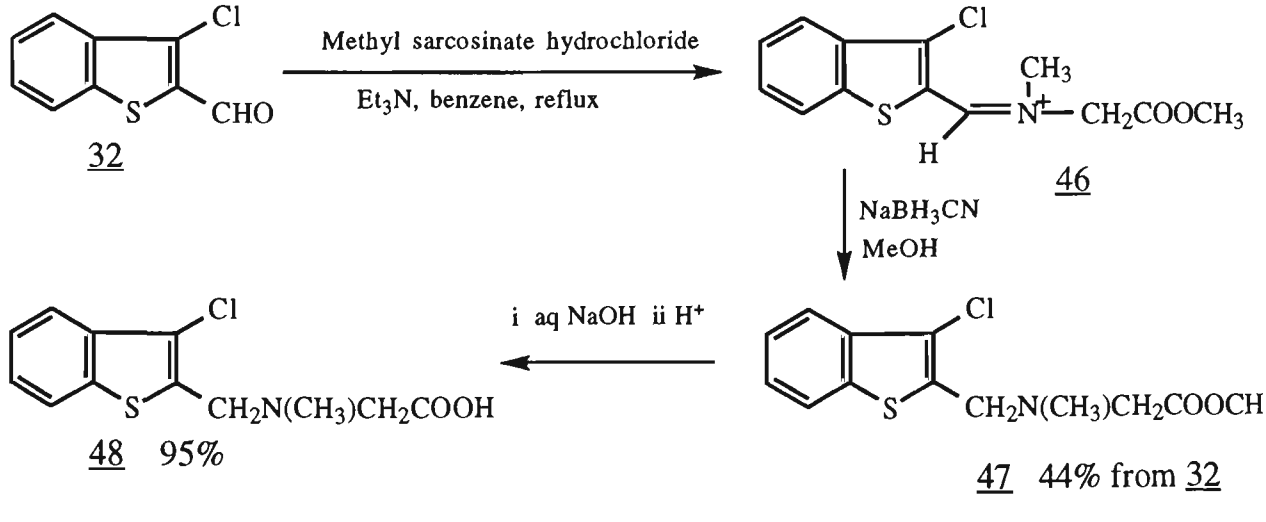
The imine 44 was prepared similarly from condensation of the aldehyde 32 and methyl glycinate hydrochloride. It was reduced in fair yield to the

amino ester 45, which was then hydrolysed to the required amino acid 31 (Scheme 2.12).



Scheme 2.12

The iminium salt 46, derived from methyl sarcosine hydrochloride and the aldehyde 32, was also reduced to the tertiary amino ester 47 using sodium cyanoborohydride in methanol in 37% overall yield from the aldehyde. The low yield was due to the incomplete formation of the iminium salt in the first step. Compound 47 was hydrolysed under basic conditions to give the acid 48 in excellent yield, after acidification of the reaction mixtures (Scheme 2.13).

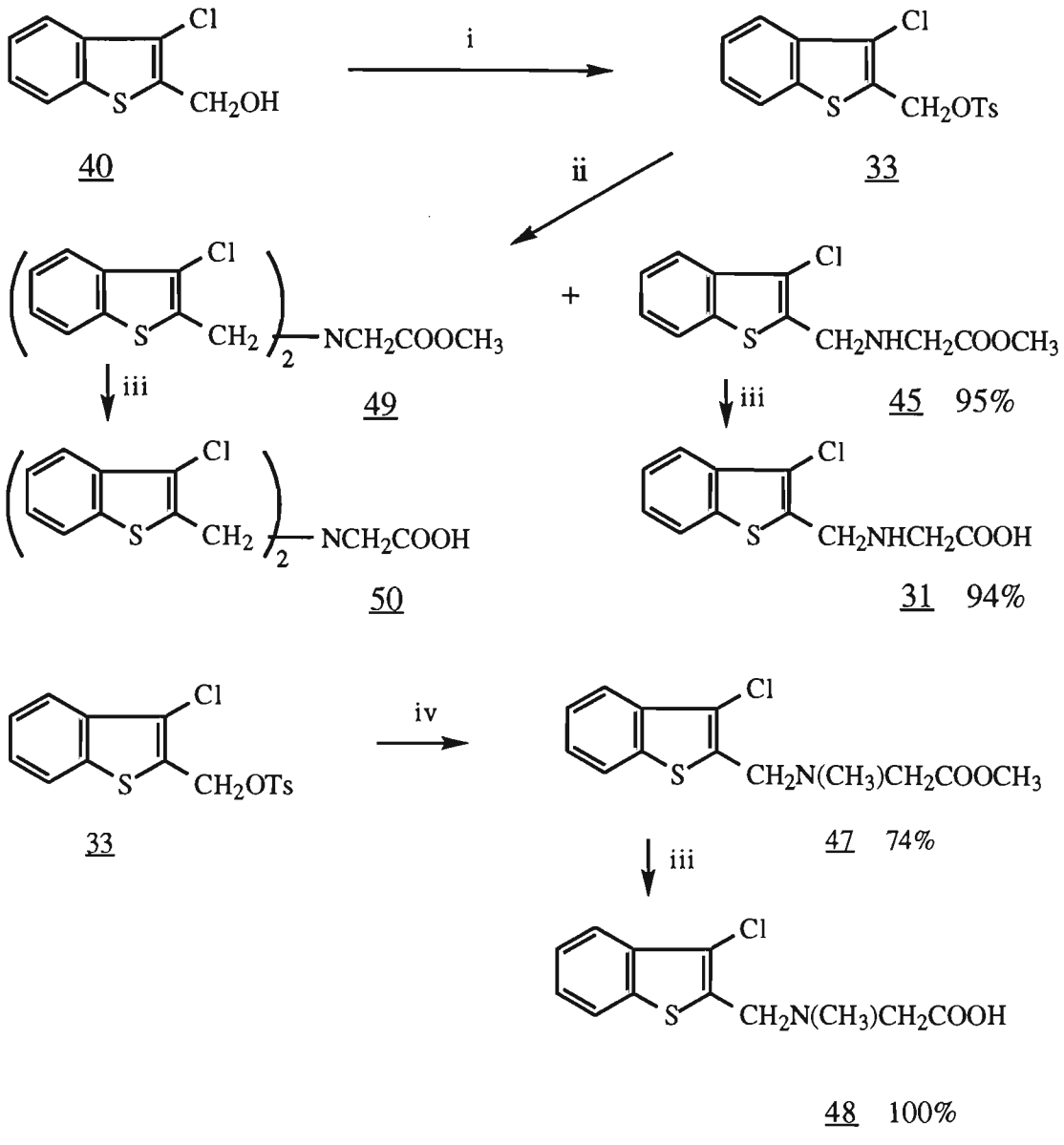


Scheme 2.13

2.4.4 Nucleophilic substitution approach to prepare the methylene derivatives

Another approach to synthesise the amines was to use nucleophilic substitution involving an appropriate nucleophile and a substrate with a good leaving group. It was expected that the amino acid 31 could be prepared from reaction of the tosylate 33 with glycine or its derivatives. As glycine would be soluble only in aqueous solution, in which the tosylate would not, glycine was first converted to its methyl ester. The tosylate 33 was prepared in high yield by treatment of the alcohol 40 in dichloromethane with tosyl chloride in the presence of triethylamine. By refluxing a mixture of the tosylate and methyl glycinate in dichloromethane, the desired amino ester 45 was obtained in excellent yield, together with a little of the tertiary amine 49 generated from dialkylation. Following these procedure, the amino ester 47 was obtained from the tosylate and methyl sarcosinate hydrochloride as the sole product. The amino esters 45, 47 and 49 were hydrolysed to the corresponding amino acids 31, 48 and 50, respectively, in excellent yields (Scheme 2.14).

The above amino acids were then subjected to pharmacological testing. The amino acid 31 displayed enhanced 5-HT induced bradycardic responses, but to a lesser extent than its analogue, the amide derivative 19. The tertiary amino acid 48 and 50 showed no 5-HT potentiation.



Reagents and Conditions: i $TsCl/Et_3N/DCM$ ii Methyl glycinate/DCM, reflux iii aq NaOH i. Methyl sarcosinate/DCM, reflux

Scheme 2.14

2.5 Conclusion

A number of 3-chloro-[1]benzothiophene-2 amide derivatives were prepared in moderate to good yields by reaction of the 3-chloro acid chloride 21 with various amino acids or other amine derivatives. The preliminary pharmacological test results demonstrated that the amides 24 and 26 were

moderate 5-HT potentiators and the quinuclidine amide derivative 29 was a 5-HT_3 antagonist. Subsequent molecular modelling studies on these amides suggested a pharmacophoric element involving a specific intramolecular hydrogen bonding between the amide carbonyl oxygen and the hydroxyl group of the carboxylic acid for activity.

Various approaches were investigated for the preparation of the methylene derivatives 31 and 48. Direct reduction of amide derivatives with a range of reducing agents proved to be unsuccessful. Reductive-amination of the aldehyde 32 with glycinate or sarcosinate provided the desired methylene compounds. A more efficient method, however, involving nucleophilic substitution of the tosylate 33 by glycinate or sarcocinate was developed to prepare the methylene analogues. The methylene amino acid 31 exhibited moderate 5-HT potentiation activity, while its *N*-methylated derivative 48 only showed activity at high doses.

Chapter 3

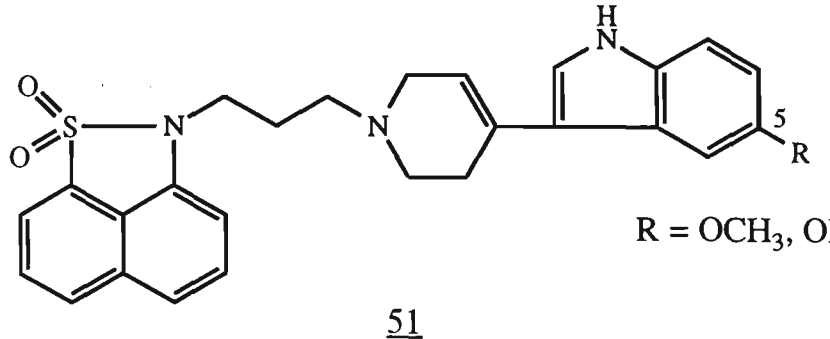
Modifications of the [1]Benzothiophene Substituents

3.1 Introduction

In the foregoing chapter, a number of the [1]benzothiophene-2 amide derivatives were made and their pharmacological activities were investigated. To further develop the project, it was of interest to investigate the effect of structural modification of other [1]benzothiophene ring substituents on activity. The structural modification involved incorporating the substituents into positions 3, 5, and 6 respectively, and changing the oxidation state of the ring sulfur atom. Such structural modifications were expected not only to be of assistance in the search for a compound with higher 5-HT potentiation activity, but also to supply more information on structure-activity relationships.

3.2 The 5- and 6-substituted [1]benzothiophene analogues

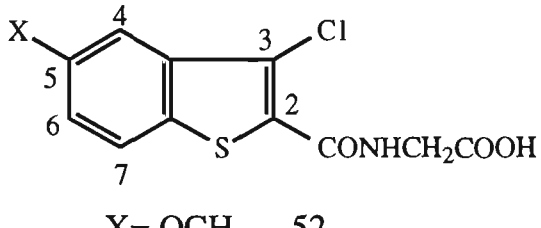
Malleron and coworkers[56] have investigated indole derivatives of the type 51 as potent and selective 5-HT uptake inhibitors. They found that the activity of the compounds with 5-methoxy or 5-hydroxy substituents increased by 50 fold and 125 times, respectively, compared to the 5-unsubstituted compound. The 5-fluoro substituent, however, had virtually no effect on the activity.



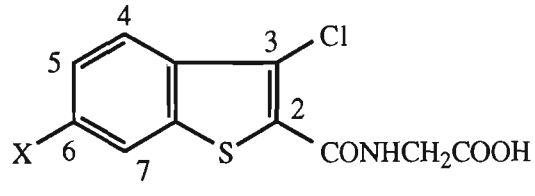
$R = OCH_3$, OH, F

51

Based on these results, it was hoped that the introduction of a 5-methoxy or 5-hydroxy functionality into the [1]benzothiophene ring system would also enhance activity. It was also of interest to investigate the effect of methoxy, hydroxy substituents in the C-6 position, and the fluoro group in the 5- or 6-position on activity particularly for the glycine 3-chloro-2-amide derivatives (Scheme 3.1).



X= OCH_3, 52
X= F, 54
X= OH, 56

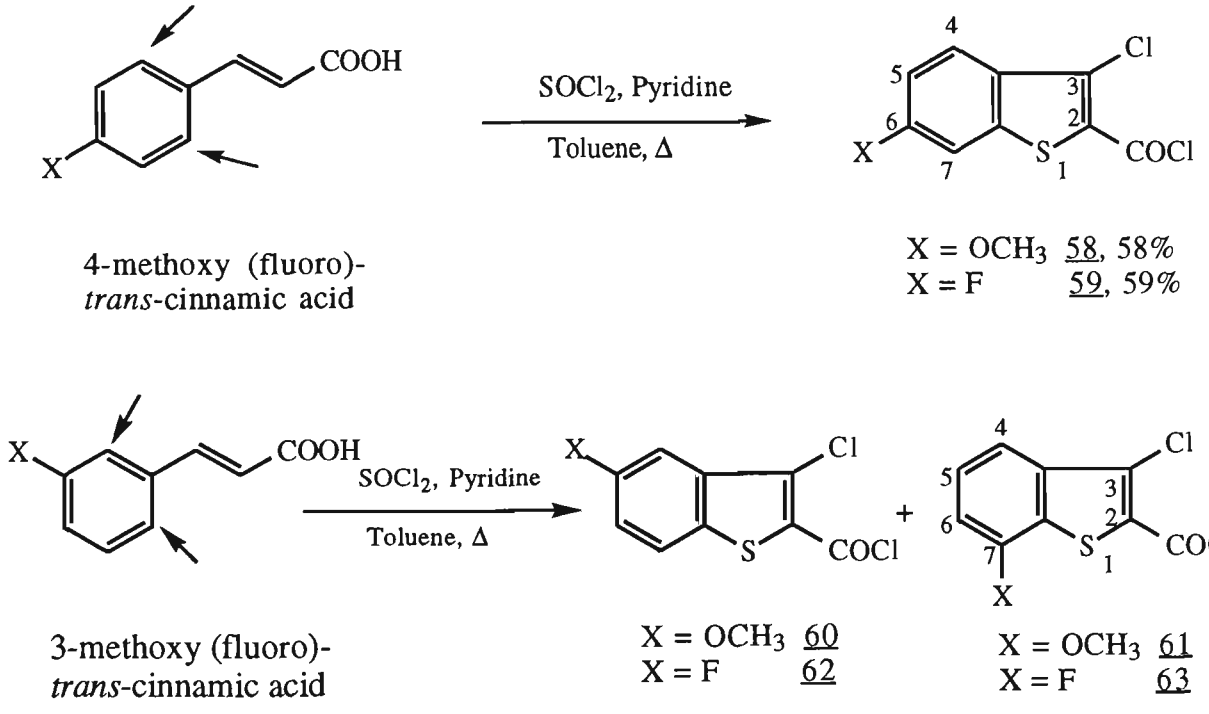


X= OCH_3, 53
X= F, 55
X= OH, 57

Scheme 3.1

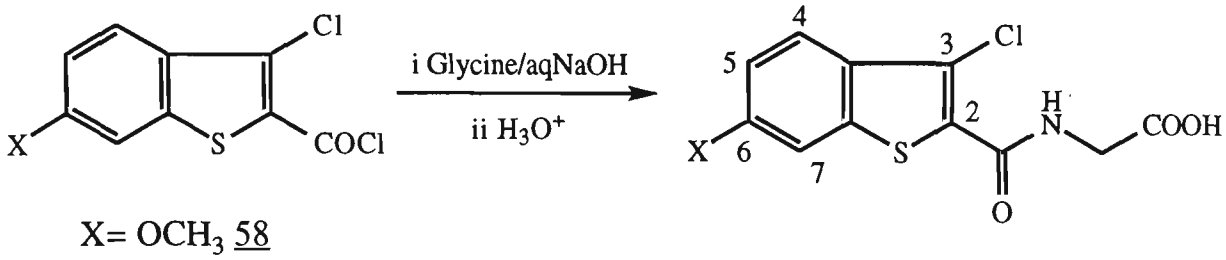
In order to prepare the 5- and 6-substituted glycine derivatives, it was necessary to obtain the appropriate acid chloride precursors. According to the proposed mechanism (*cf* Scheme 2.1) for the formation of the acid chloride 21 from cinnamic acid, the 6-substituted acid chlorides should be readily prepared from 4-substituted cinnamic acids. Treatment of *trans*-4-methoxycinnamic acid or *trans*-4-fluorocinnamic acid with thionyl chloride

in the presence of pyridine afforded the 6-methoxy acid chloride 58 and the 6-fluoro acid chloride 59 respectively, in moderate yields. To obtain the 5-substituted acid chlorides, 3-substituted *trans*-cinnamic acids were employed. The 5-methoxy acid chloride 60 and the 5-fluoro acid chloride 62, however, were isolated as mixtures with their 7-substituted isomers 61 and 63 respectively (Scheme 3.2). The 5-substituted isomers were formed predominantly owing to less steric hindrance in the *para*-position. The regioisomers were not able to be separated and were used as mixtures in the acylation step.



Scheme 3.2

The 6-methoxy and 6-fluoro glycine derivatives 53 and 55 were prepared in moderate to good yield using the acid chloride 58 and 59 according to the standard procedure described in Section 2.2.

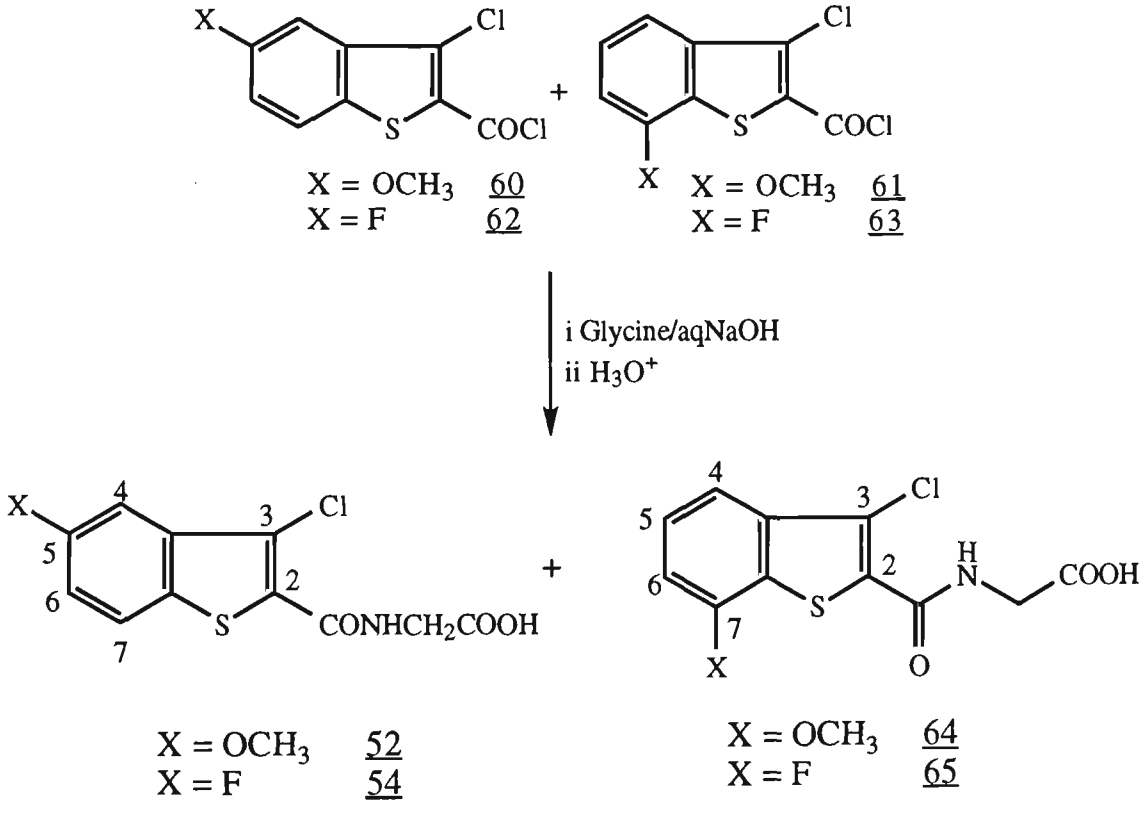


X= OCH_3 58

X= F 59

X = OCH_3 53, 77%
X = F 55, 91%

The preparation of the 5-substituted glycine derivatives 52 and 64, however, was complicated by the presence of their 7-substituted derivatives 64 and 65. Recrystallization of the mixture of 5- and 7-methoxy glycine derivatives 52 and 64 from methanol afforded the 5-methoxy isomer 52 in 54% yield. Nevertheless, the 5- and 7-fluoro glycine derivatives 54 and 65 derived from the reaction of a mixture of the 5-fluoro acid chloride 62 and the 7-fluoro acid chloride 63 with glycine, were not separable either by recrystallization or by chromatography.



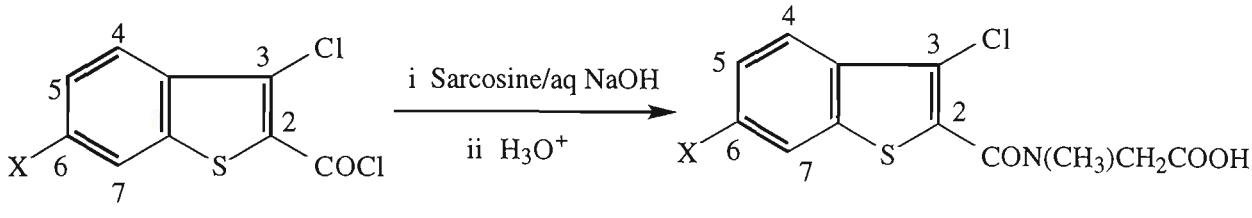
X = OCH_3 60
X = F 62

X = OCH_3 61
X = F 63

X = OCH_3 52
X = F 54

X = OCH_3 64
X = F 65

The *N*-methylated glycine derivatives 66 and 67 were also prepared in excellent yields by reaction of a solution of sarcosine in sodium hydroxide with the 6-methoxy acid chloride 53 and 6-fluoro acid chloride 55 respectively.



X= OCH_3, 58
X= F, 59

X= OCH_3, 66 91%
X= F, 67 95%

The ^1H NMR spectrum of the 6-methoxy glycine derivative 53 displayed a doublet of doublets at 7.13 ppm corresponding to H-5 due to both *ortho* H-4 (J 8.8 Hz) and *meta* H-7 (J 2.4 Hz) couplings, and two doublets at 7.77 ppm and 7.45 ppm corresponding to H-4 and H-7 respectively (Figure 3.1a). The 5-methoxy glycine derivative 52 showed similar coupling patterns, but the chemical shifts of H-4 and H-7 changed considerably (Figure 3.1b), due to the electronic effect of the methoxy group. The incorporation of fluorine resulted in more complicated coupling patterns due to its coupling with all aromatic protons, as shown in Figure 3.1c. It was also found that the N-methylated glycine derivative 67 had slightly more complicated coupling patterns (Figure 3.1d).

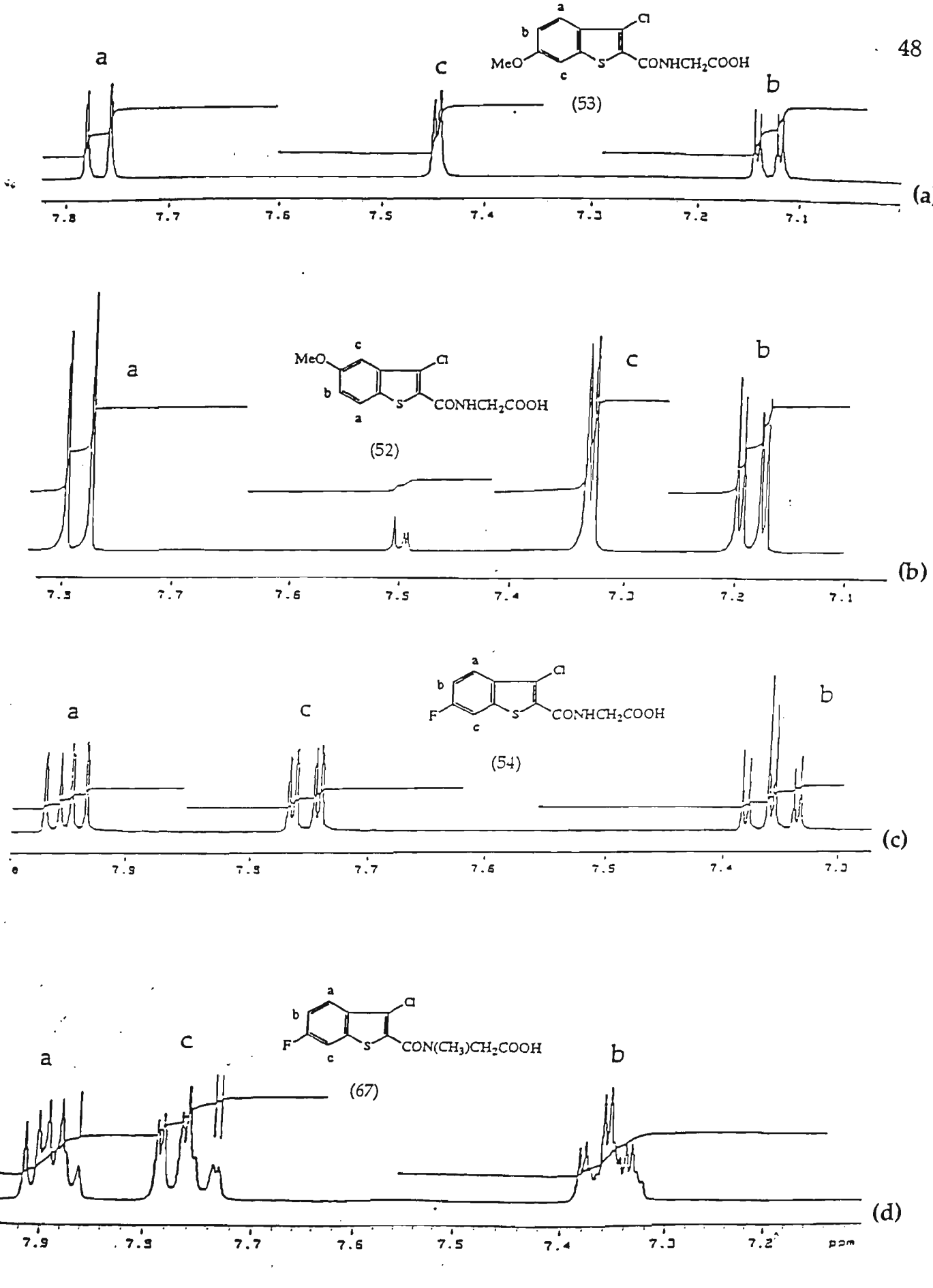
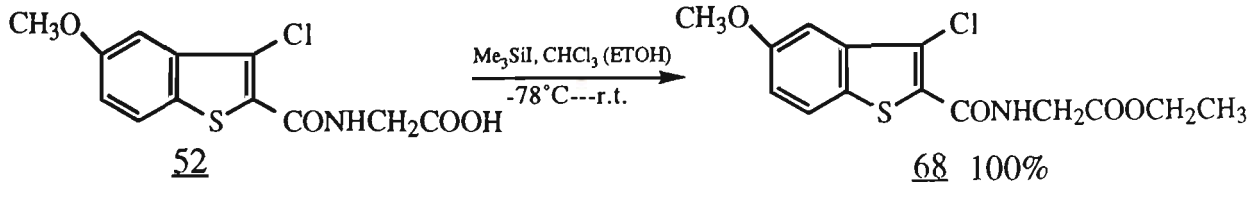


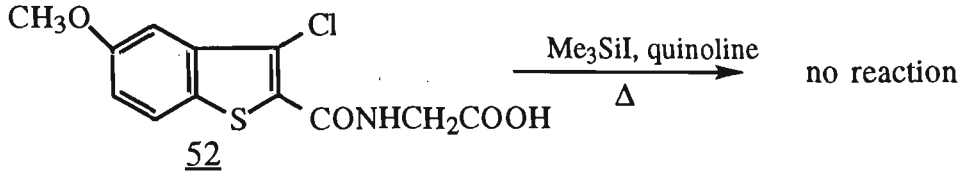
Figure 3.1 ^{1}H NMR aromatic proton patterns of the 5- and 6-substituted [1]benzothiophene derivatives 53, 52, 54 and 67

To obtain the 5- and 6-substituted hydroxy compounds 56 and 57, it was anticipated that direct demethylation of the corresponding methoxy derivatives should be possible. A number of demethylation procedures were attempted and are discussed below. Iodotrimethylsilane[100] has been reported as a mild and selective demethylating agent both in aliphatic and aromatic systems. Treatment of the 5-methoxy compound 52 in unpurified chloroform with an excess of iodotrimethylsilane surprisingly led to an ethyl ester 68 quantitatively, but with no demethylation of the 5-methoxy group. The ester was most probably formed by the acid-catalysed esterification of the acid 52 with ethanol present in unpurified chloroform, as it was found that no ester was obtained when Al_2O_3-filtered chloroform was used as the reaction solvent. Harsher conditions, such as using quinoline[101] as a solvent at 180°C was also applied, but no reaction was observed.

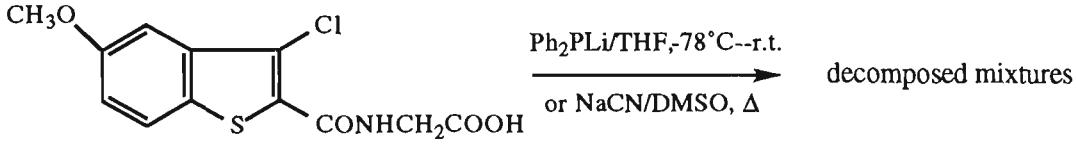
Reaction of 52 with lithium diphenylphosphide[102] resulted in complex product mixtures. This was probably due to the instability of other functionalities present in the molecule towards the strong lithium base. Sodium cyanide[103] in dimethyl sulfoxide at high temperature decomposed the 5-methoxy compound 52, suggesting that the conditions were too vigorous. In contrast, when Newman's method[104], involving sodium sulfide in *N*-methyl pyrrolidone (NMP) at high temperature was tried on 52, the presence of the demethylated compound 69 was indicated by mass spectroscopic analysis of the reaction mixture, but the difficulty of removing the very high boiling point solvent significantly limited the use of this method.



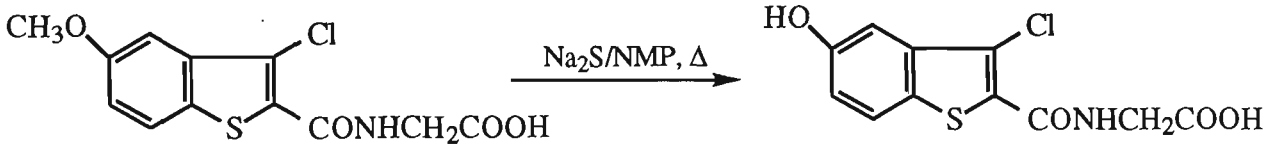
52 68 100%



52

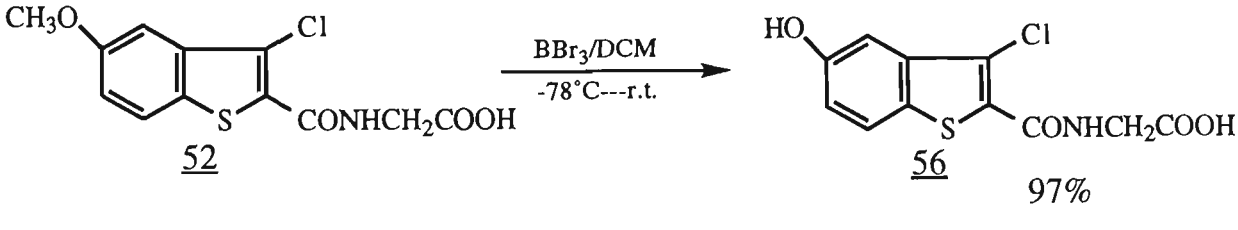


52

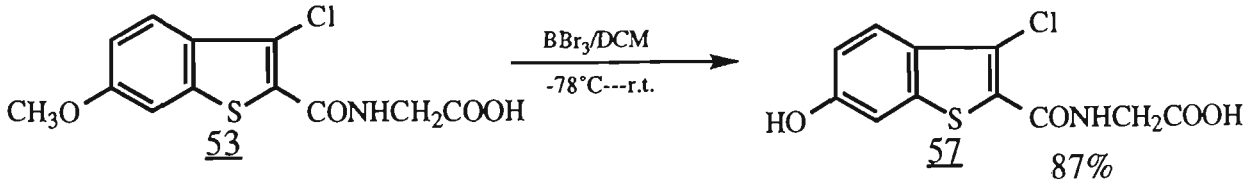


52 56

Finally, following McOmie's procedure[105], the methyl ethers of 52 and 53 were smoothly cleaved by boron tribromide to give the desired 5- and 6-hydroxy compounds 56 and 57, respectively, in good yields. Both the hydroxy compounds were very water soluble, so a modified work-up procedure had to be adopted involving filtering the reaction mixtures through a short column of silica gel, followed by flushing with a large amount of ethyl acetate.



52 56 97%

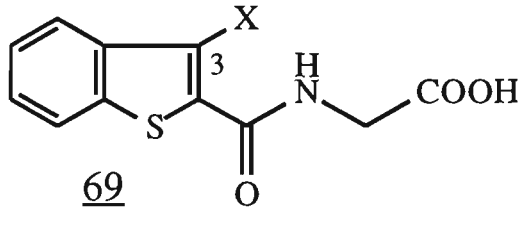


53 57 87%

The pharmacological test results indicated that both the 5- and 6-substituents acted as blocking groups. As a result both the methoxy and fluoro compounds showed no 5-HT potentiation, though some activity was observed for the 5- and 6- hydroxy analogues. The *N*-methylated derivatives, however, displayed no 5-HT potentiation activity.

3.3 The 3-substituted [1]benzothiophene analogues

According to the hypothesis that the interaction between the 3-substituent and the substituent on the nitrogen in the lower chain might play a role in determining the 5-HT potentiation activity of the compounds (*cf* Section 2.3), two factors should be taken into consideration in choosing substituents. They were the steric size and electronegativity of the substituents. Hence, hydrogen, fluorine, bromine, methoxy, ethoxy and hydroxy were to be incorporated into the 3-position for the investigation.

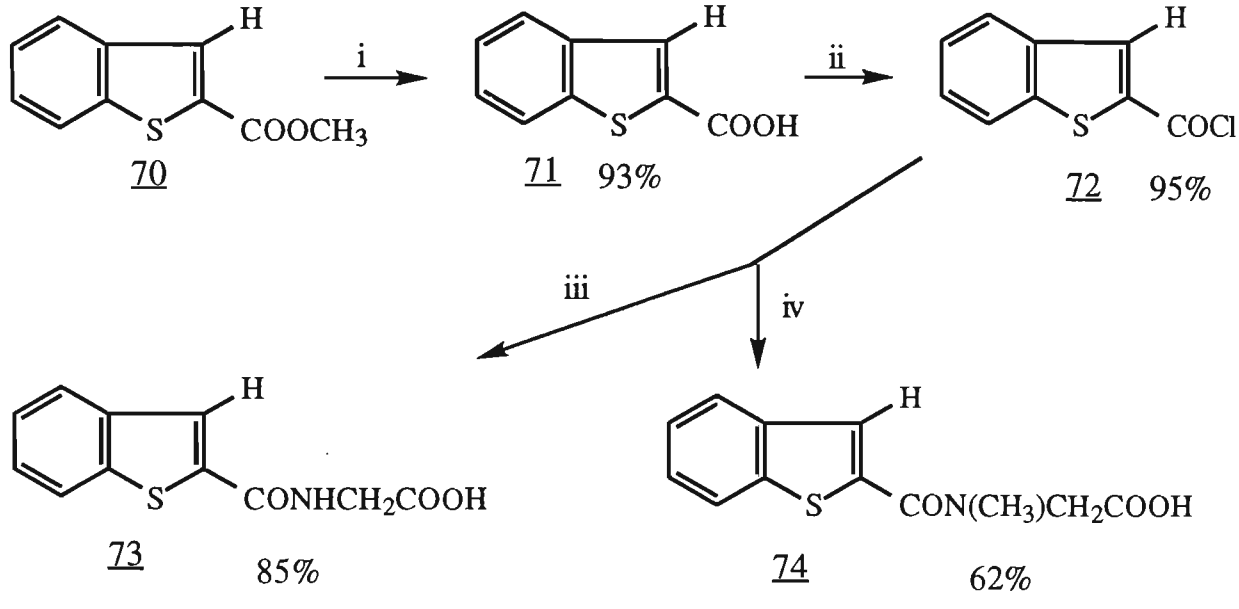


69 X= H, F, Br, OCH_3, OCH_2CH_3, OH

3.3.1 3-Unsubstituted-[1]benzothiophene analogues

The synthesis of the 3-unsubstituted [1]benzothiophene derivatives 73 and 74 was achieved by standard reactions (Scheme 3.3). Methyl [1]benzothiophene-2-carboxylate 70, commercially available or readily prepared from *o*-nitrobenzaldehyde and methyl thioglycolate according to Beck[106], was converted to the acid 71 by basic hydrolysis followed by acidification. The acid 71 was then transformed to the acid chloride 72 by

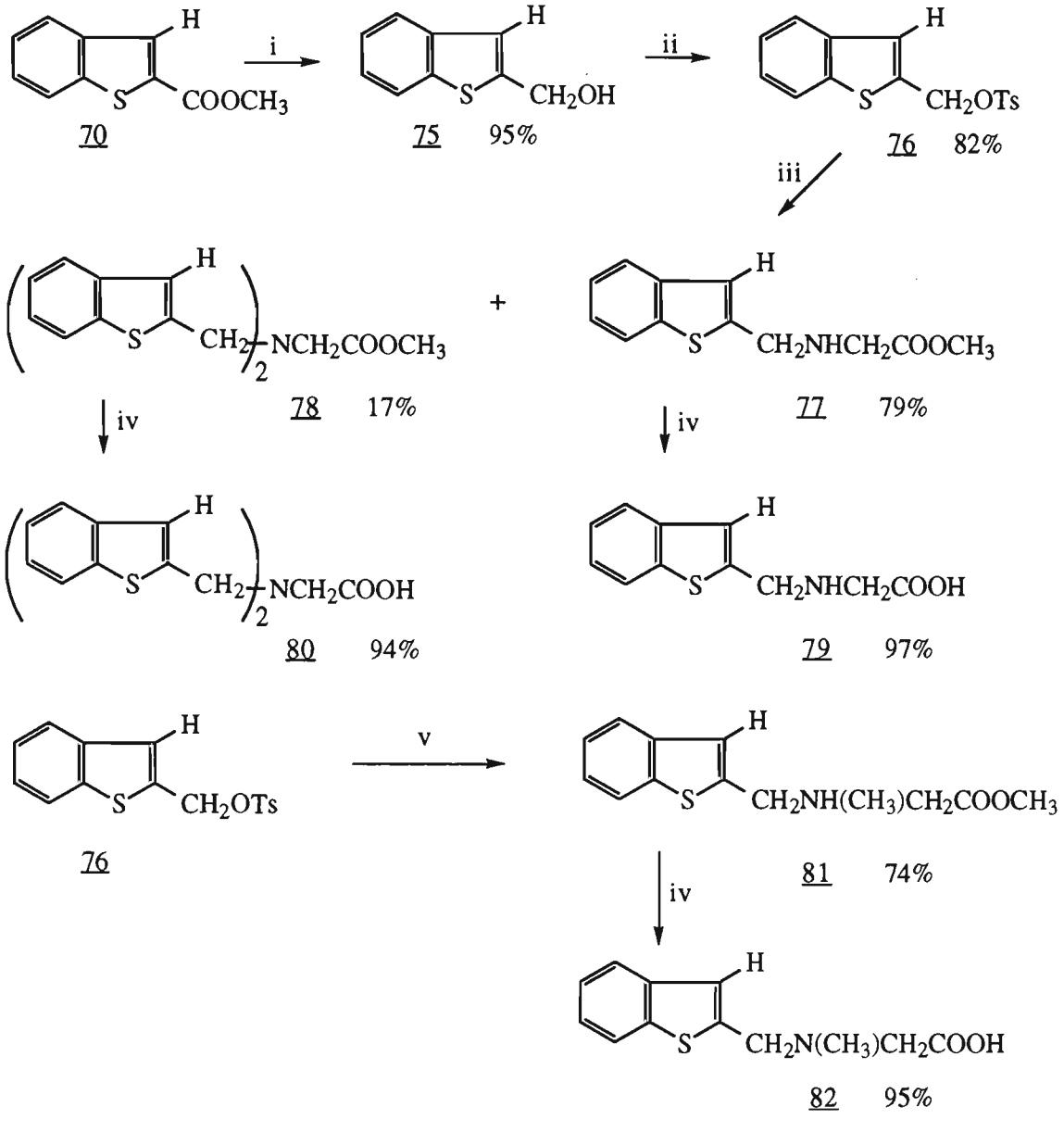
refluxing with thionyl chloride. Treatment of the acid chloride 72 with glycine or sarcosine in aqueous sodium hydroxide, followed by acidic precipitation afforded N-([1][benzothien-2-oyl)glycine 73 and N-([1][benzothien-2-oyl)sarcosine 74 respectively.



i NaOH solution, H^+ ii $SOCl_2$ iii Glycine/NaOH, H^+ iv Sarcosine/NaOH, H^+

Scheme 3.3

To synthesise the corresponding amine analogues of 73 and 74, the nucleophilic substitution method described in Section 2.4.4 was adopted (Scheme 3.4). The methyl ester 70 was converted to the tosylate 76 by a standard sequence. Refluxing a mixture of the tosylate 76 and methyl glycinate hydrochloride in dichloromethane in the presence of triethylamine afforded the desired amino ester 77, together with the tertiary amine 78 as a side product. Likewise the amino ester 81 was obtained from the tosylate and methyl sarcosinate hydrochloride. All the amino esters were finally converted to the corresponding amino acids by hydrolysis (Scheme 3.4).



i. $NaBH_4$, EtOH ii. $TsCl/Et_3N$, DCM iii. Methyl glycinate iv. NaOH solution, H^+
v. Methyl sarcosinate

Scheme 3.4

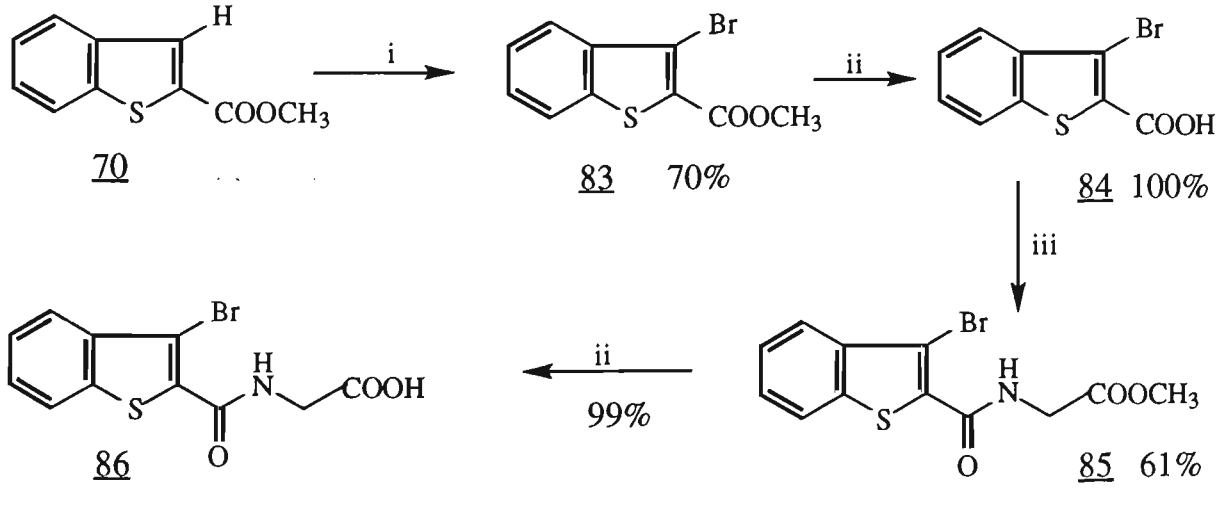
In the pharmacological testing, unlike the *N*-methylated 3-chloro amide 22, the *N*-methylated amide 74 showed a higher activity than its unmethylated analogue 73, while the *N*-methylated amino acid 82 had lower activity than its unmethylated analogue 79 with respect to potentiation of action of 5-HT.

3.3.2 3-Bromo and 3-fluoro-[1]benzothiophene analogues

Since the 3-chlorinated glycine derivatives 19 exhibited moderate potentiation of 5-HT inhibition, it was of interest to investigate the effect of other 3-halo substituents on activity of the glycine derivatives.

3.3.2.1 3-Bromo-[1]benzothiophene analogues

Following the literature procedure of selective bromination of the [1]benzothiophene system[107], the 3-bromo ester 83 was prepared in moderate yield by treatment of the methyl ester 70 in acetic acid with bromine in the presence of anhydrous sodium acetate. Hydrolysis of the 3-bromo ester gave the acid 84. The acid was then coupled with methyl glycinate in the presence of 1, 3-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole[108] to afford the amide ester 85 in moderate yield. Basic hydrolysis gave the corresponding acid 86 (Scheme 3.5), which on pharmacological testing displayed a low degree of 5-HT potentiation.

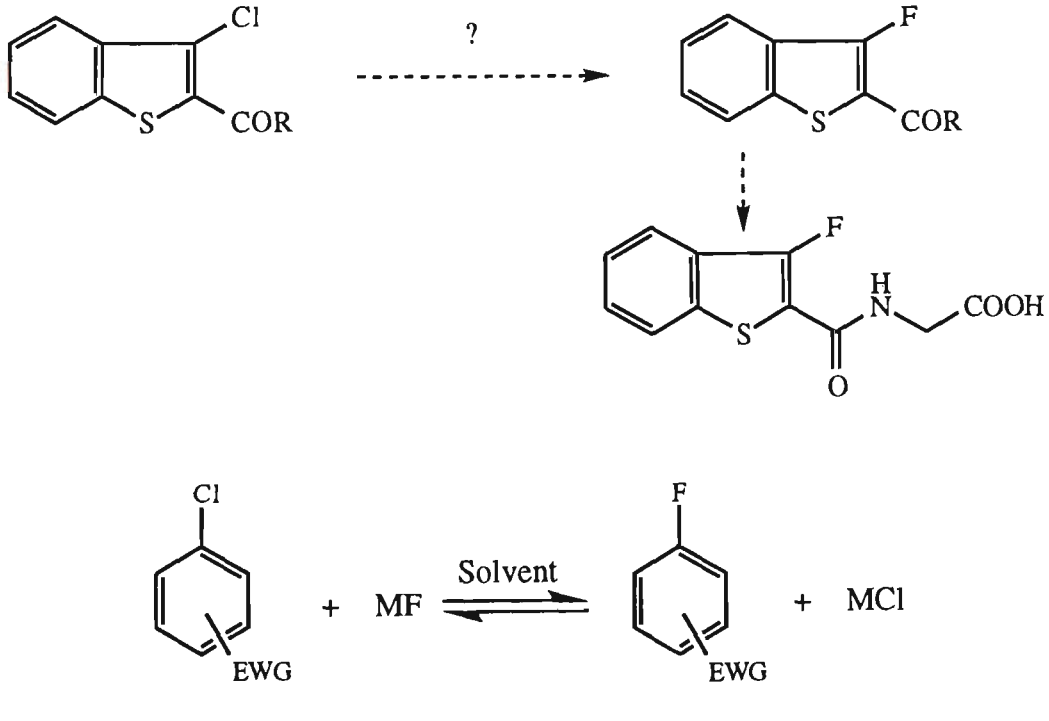


i Br_2/NaOAc/HOAc ii aq NaOH/MeOH, H^+ iii DCC/HOBT/Methyl glycinate/DMF

Scheme 3.5

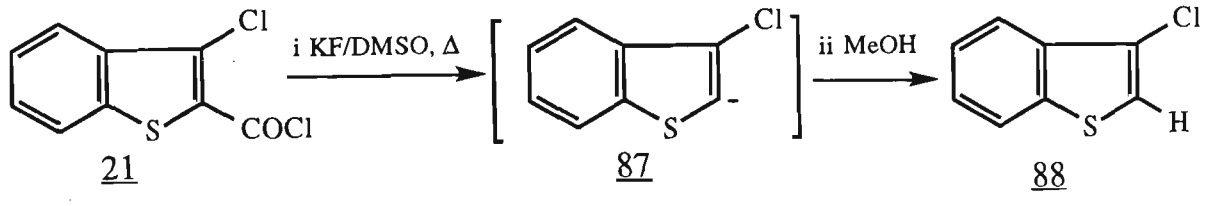
3.3.2.2 3-Fluoro-[1]benzothiophene analogues

One of the methods to introduce the fluoro group into the C-3 position is a direct replacement of chlorine by fluorine through a nucleophilic substitution on the aromatic ring. This would be an ideal choice, due to easy access of the 3-chloro compounds. Though the activity order of leaving groups in aromatic nucleophilic substitution is F>Cl>Br>I, it is still possible to replace chlorine group with fluoride ions in an activated aromatic ring under vigorous conditions [109, 110]. Conditions in favour of such halogen exchange, as summarised by Dolby-Glover[111], are the presence of strong electron withdrawing groups (EWG) on the aromatic ring, and the use of non-nucleophilic high boiling point solvents including dimethylformamide, dimethyl sulfoxide, and N-methylpyrrolidone. Also, the use of an excess of fluoride salt drives the equilibrium of the halogen exchange in the desired direction[110] (Scheme 3.6).



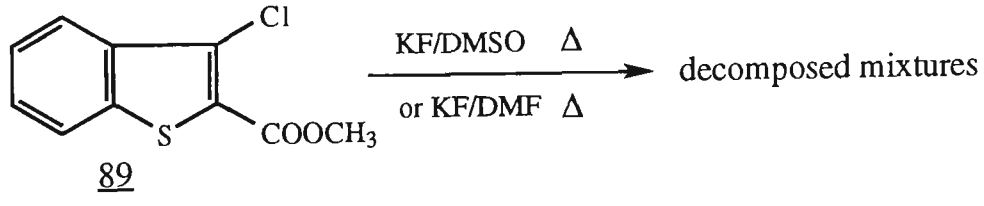
Scheme 3.6

It was anticipated that compound 21 with the acid chloride viewed as an EWG would undergo Cl-F exchange. Refluxing a mixture of the acid chloride 21 and potassium fluoride in dimethyl sulfoxide for two hours, followed by quenching with methanol, however, resulted in complete conversion to 3-chloro-[1]benzothiophene 88. This was possibly formed *via* the intermediate 87, generated from decarbonylation of the acid chloride.

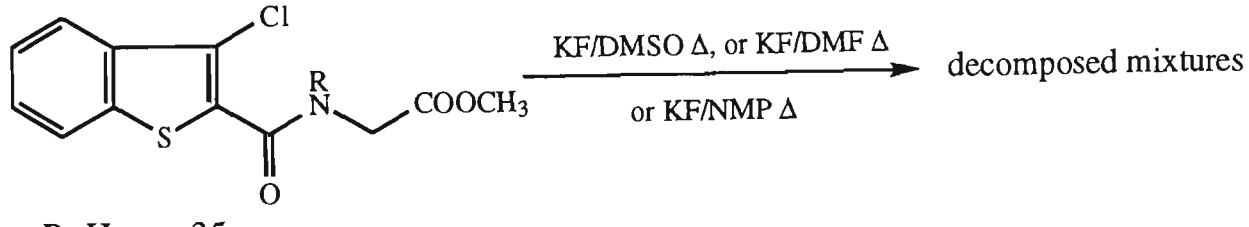


21 87 88

Under the same conditions, however, the methyl ester 89 was completely decomposed. Similarly, the amides 35 and 36, after being exposed to KF/DMSO or KF/DMF or KF/NMP at high temperature, gave none of the required 3-fluoro derivative.



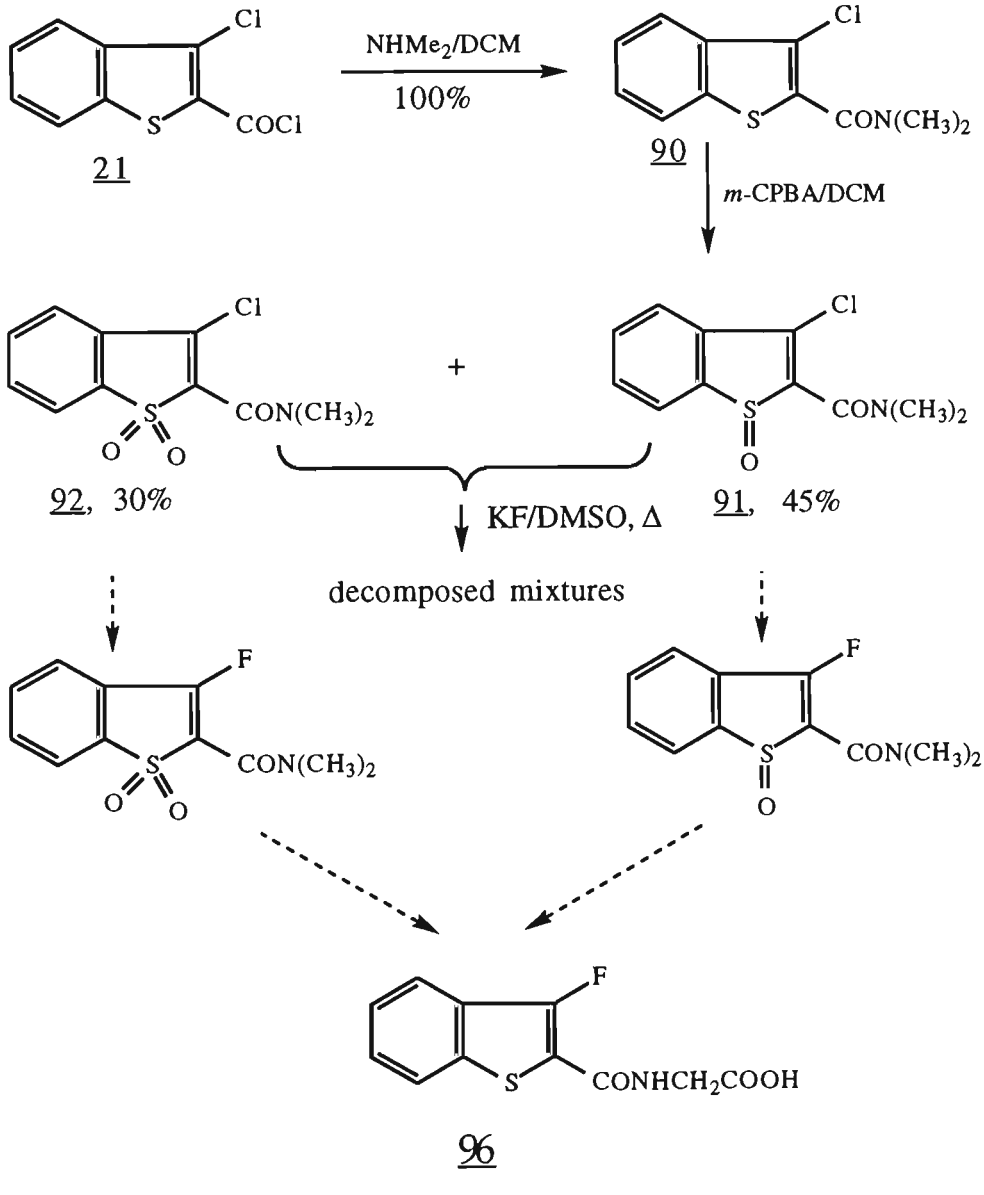
89



R=H, 35
R=CH_3, 36

In view of the above results, it was believed that the [1]benzothiophene ring needed to be more activated to accommodate the F-Cl exchange. As a consequence, the synthesis of the corresponding sulfone and sulfoxide was examined. To minimise any side reactions, the acid chloride 21 was

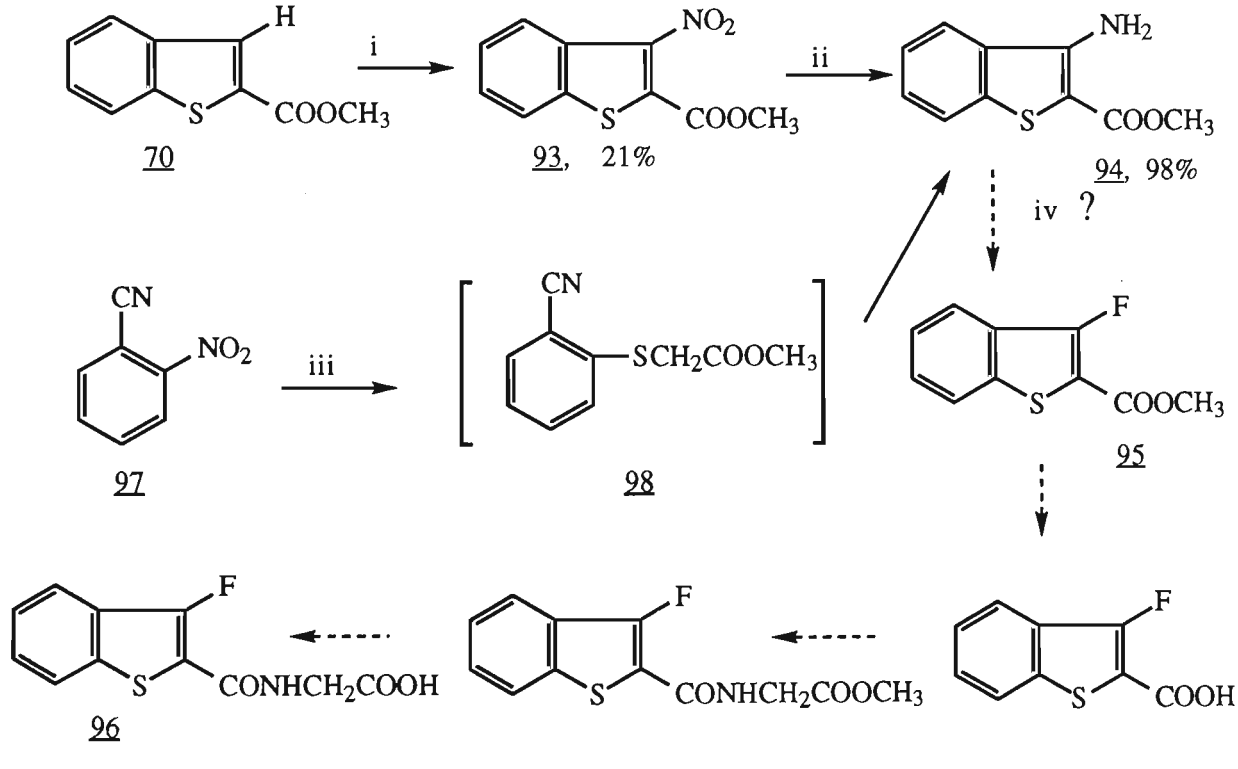
transformed to the tertiary amide 90 by reaction with dimethylamine. The amide was then oxidised with *m*-CPBA to give a mixture of the sulfoxide 91 and sulfone 92. Attempts to fluorinate the sulfoxide 91 and sulfone 92 with KF/DMSO at high temperature gave material which was difficult to characterise (Scheme 3.7). No further work involving Cl-F exchange was conducted.



Scheme 3.7

The next approach involved introducing indirectly the fluoride *via* a diazonium salt, since the inclusion of the fluorine into an aromatic ring by

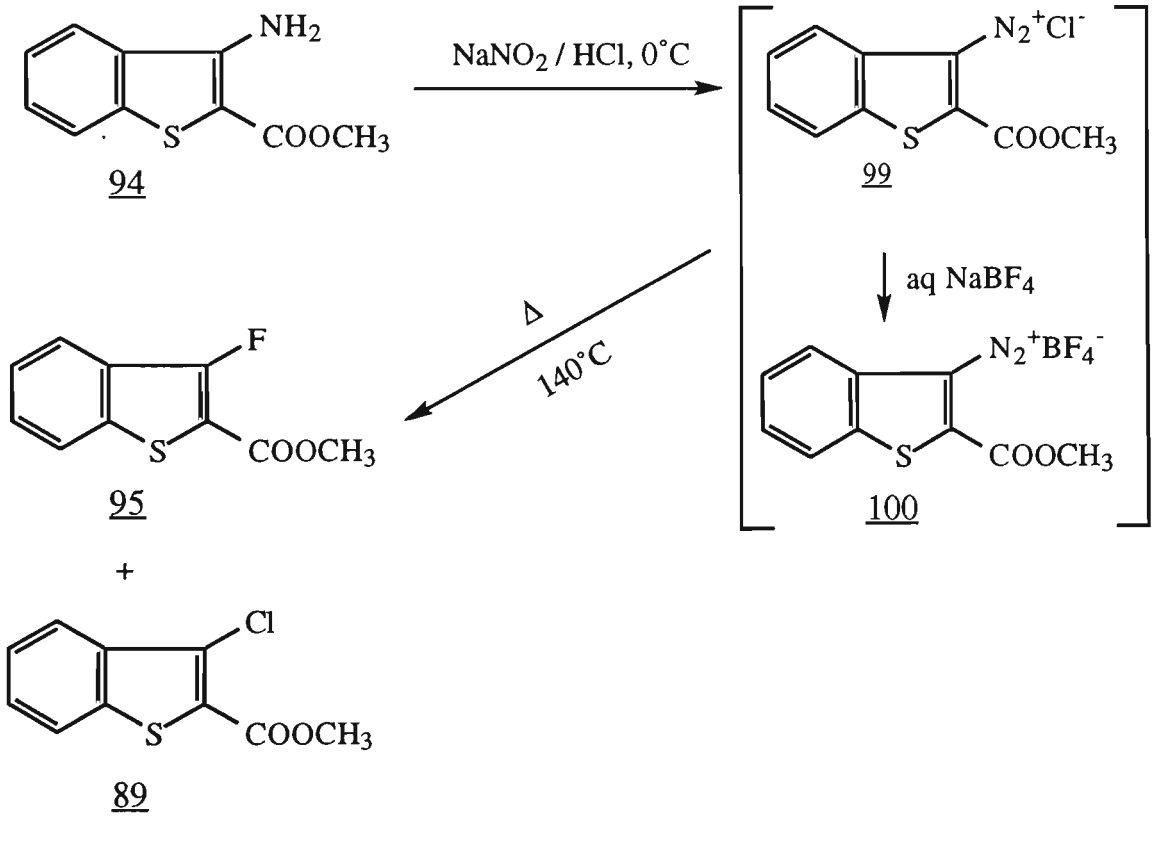
heating a diazonium tetrafluoroborate has been well known[112]. Our intention was to use this reaction to convert the 3-amino ester 94 to the 3-fluoro ester 95, from which the desired 3-fluoro-[1]benzothiophene derivative 96 could be prepared (Scheme 3.8). The commercially available ester 70 was nitrated in the 3-position using a mixture of concentrated nitric acid and acetic acid to give the desired nitro compound 93, albeit in low yield (21%). The nitro compound was reduced by tin (II) chloride[113] to the amine 94 in excellent yield. On a large scale, the amine was more easily and efficiently prepared according to Beck's method[106], which involved replacing the active nitro group of *o*-nitrobenzonitrile 97 by a substituted thiolate group and subsequent base-catalysed intramolecular cyclization of the intermediate 98 (Scheme 3.8).

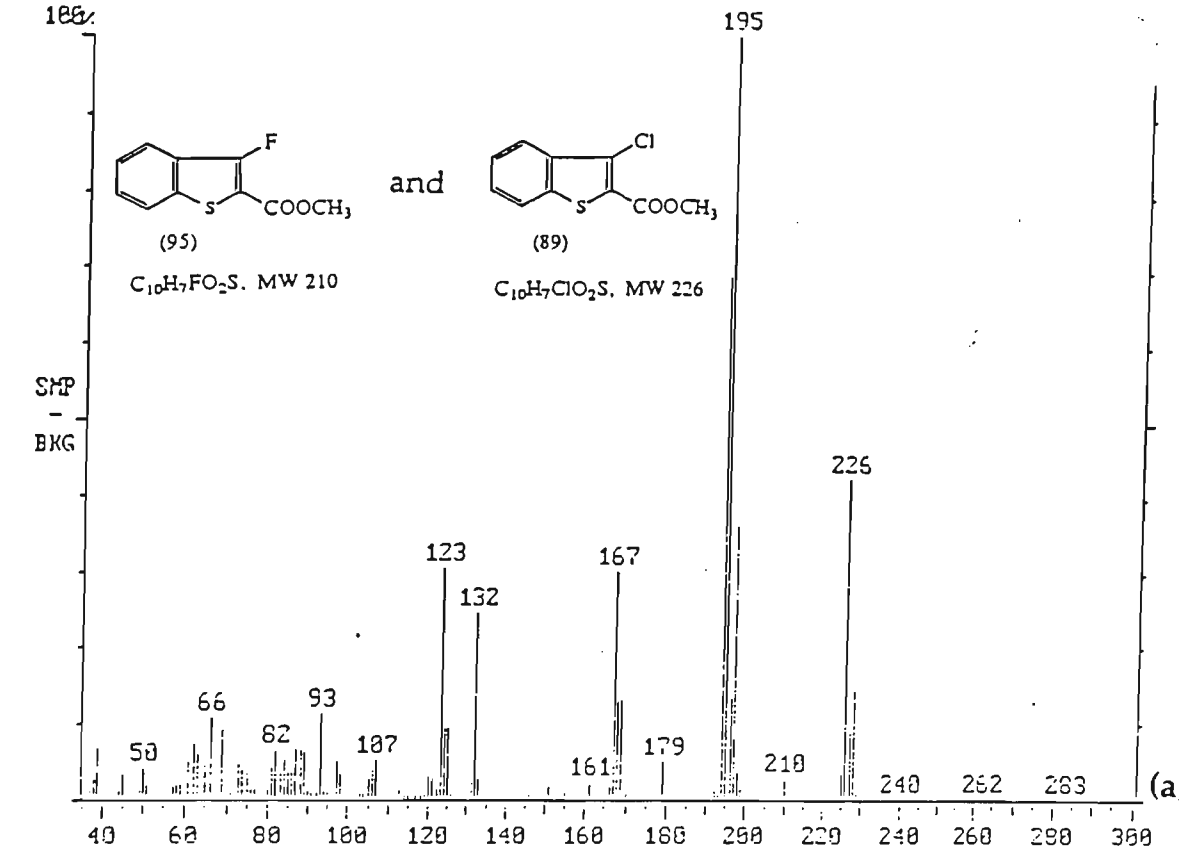


i Concentrated HNO_3/HOAC Δ ii $SnCl_2$/HCl/MeOH iii $HSCH_2COOCH_3$/KOH, aq DMF iv $NaNO_2$, HCl, $NaBF_4$ or HPF_6, then Δ

Scheme 3.8

The amine 94 was treated with nitrous acid, followed by the addition of a solution of sodium tetrafluoroborate[114]. Subsequent heating of the reaction mixture at 140°C for 20 mins, followed by work-up gave, unexpectedly, an inseparable mixture consisting of the dominant 3-chloro ester 89 and a little of the 3-fluoro ester 95, as indicated by mass spectroscopic analysis (Figure 3.2a). The formation of the 3-chloro ester 89 was most likely due to thermal decomposition of the diazonium chloride 99, which was expected to be converted to the diazonium tetrafluoroborate 100. To prevent the formation of the 3-chloro ester, sulfuric acid was used instead of hydrochloric acid, but very little of the 3-fluoro compound 95, confirmed by mass spectroscopic analysis (Figure 3.2b), was obtained (yield<1%,).





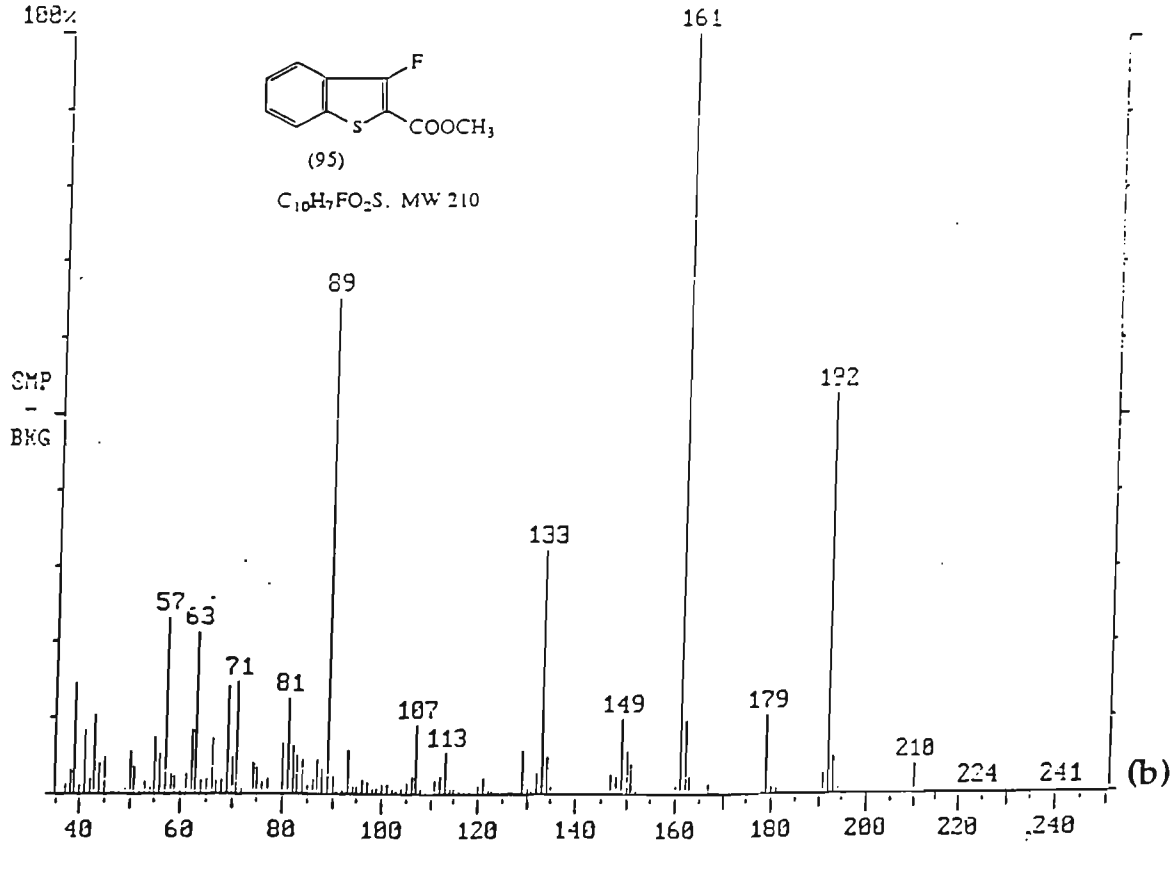
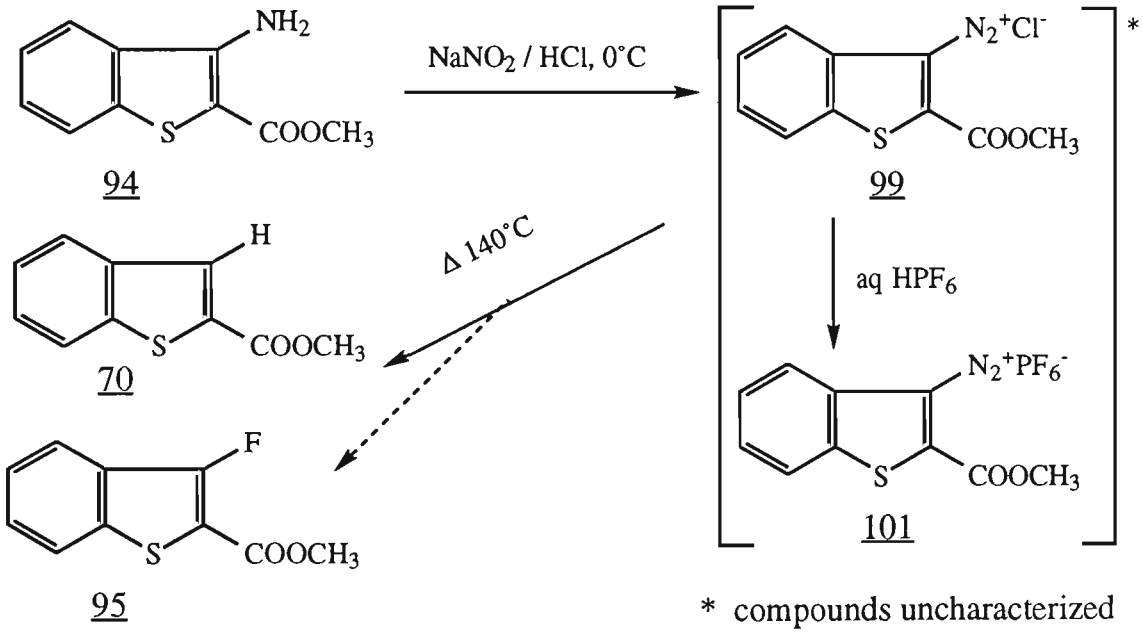


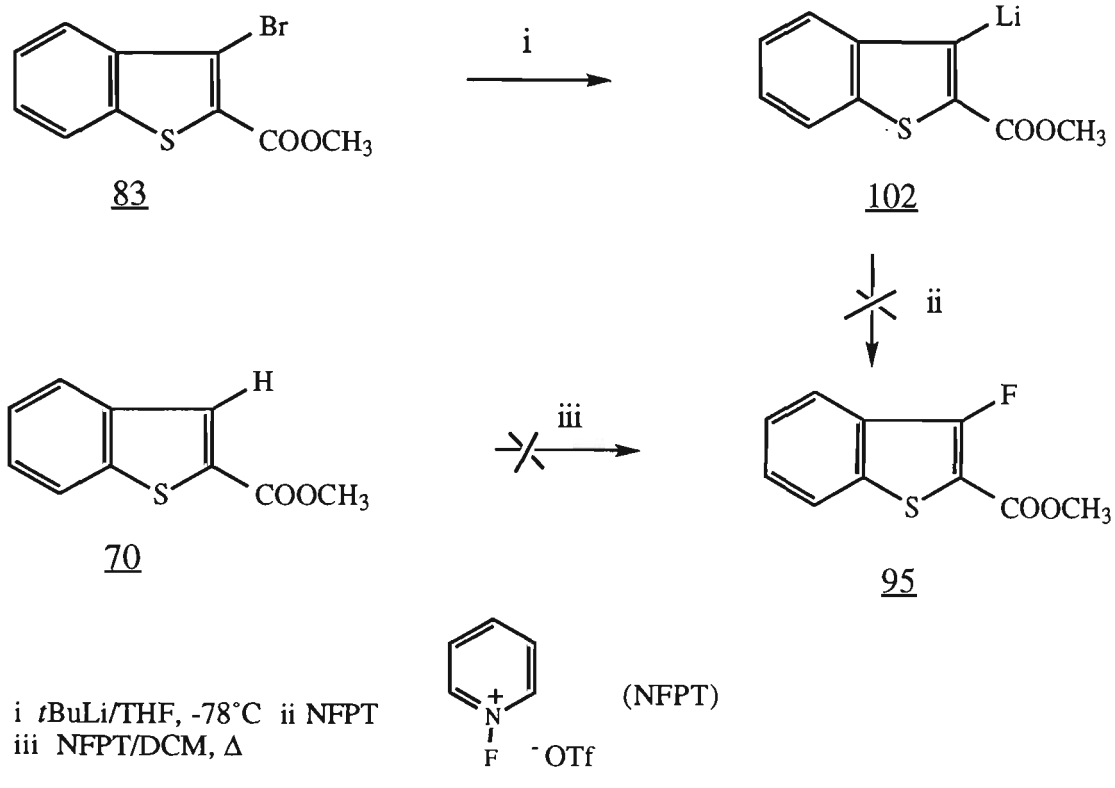
Figure 3.2 EI mass spectra of the 3-fluoro and 3-chloro compounds 95 and 89.

Rutherford and coworkers[115] have reported that the use of hexafluorophosphoric acid rather than sodium tetrafluoroborate or tetrafluoroboric acid in the Schiemann reaction in most cases improved yields significantly. Heating the hexafluorophosphate diazonium salt 101, generated from the amine 94 by sequential treatment with nitrous acid, then hexafluorophosphoric acid, however, afforded a substantial amount of methyl ester 70, with no formation of the 3-fluoro compound 95. The methyl ester 70 was presumably formed through thermally induced radical reactions of the hexafluorophosphate diazonium salt 101.



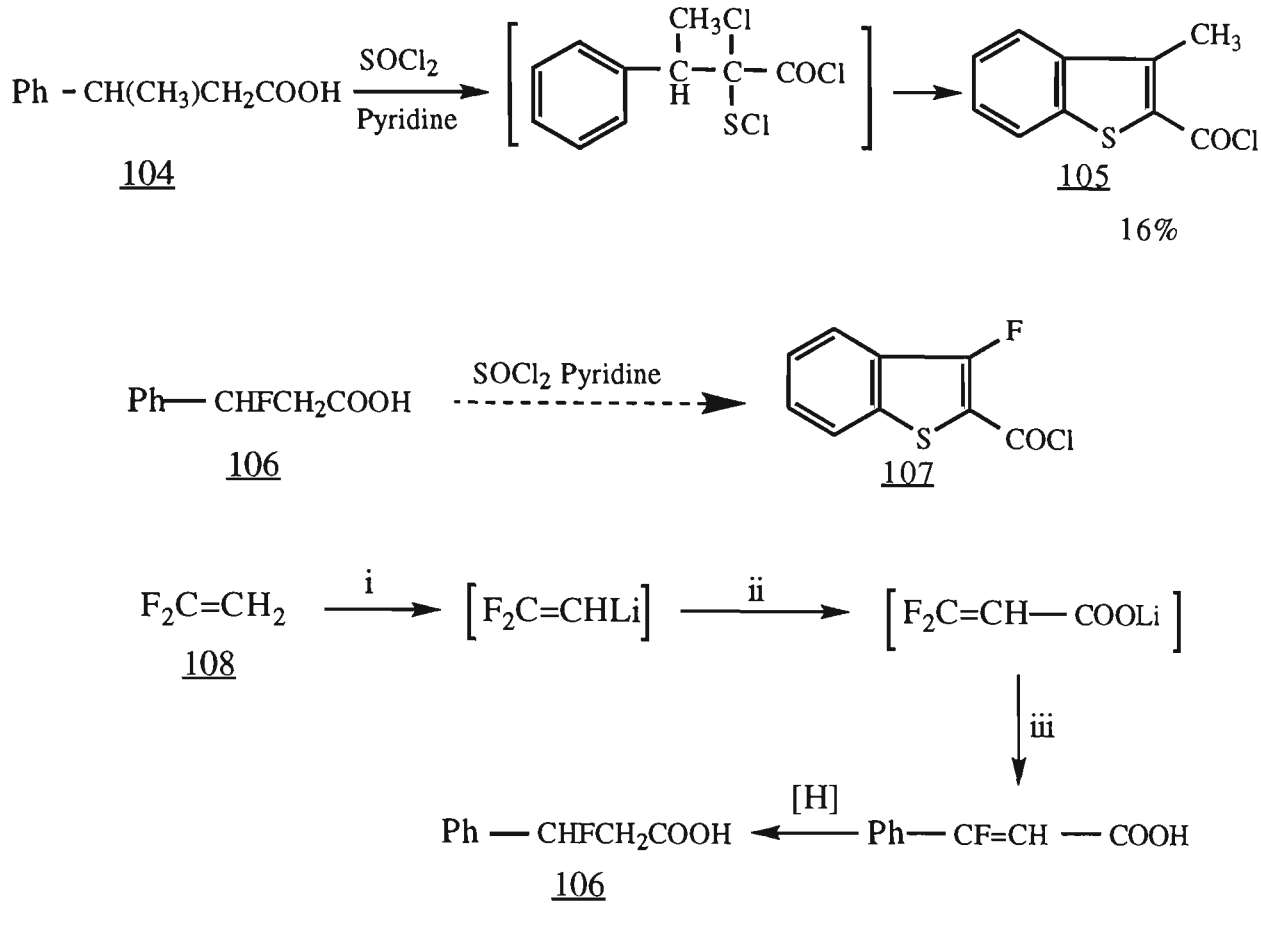
Introduction of fluorine into organic compounds by electrophilic fluorination with various fluorinating agents such as CF_3OF, $FClO_3$, CF_3COOF, or CH_3COOF have been reported[116-119], but is of limited utility because of the explosiveness and toxicity of the reagents. Umemoto and associates[120], however, have reported that *N*-fluoropyridinium triflate and its derivatives are mild and selective electrophilic fluorinating agents for a variety of organic compounds. An electron-rich substrate such as a carbanion is required to trap the fluorine. Unfortunately, reaction of the lithium reagent

102, derived from reaction of the 3-bromo ester 83 with ^{t}BuLi, with *N*-fluoropyridinium triflate did not result in fluorination. No reaction was observed on refluxing a mixture of the methyl ester 70 and *N*-fluoropyridinium triflate in dichloromethane, most likely due to the deactivation of the carboxylate (Scheme 3.9).



Scheme 3.9

The final approach considered was to include the fluorine into the precursor of the benzothiophene derivative 107. It has been shown that the 3-methyl acid chloride 105[61] could be formed upon treatment of 3-methyl-3-phenylpropanoic acid 104 with thionyl chloride in the presence of pyridine. This suggested that under the same conditions 3-fluoro-3-phenylpropanoic 106 might also lead to the desired 3-fluoro-[1]benzothiophene-2-carbonyl chloride 107. The precursor[121] 106, however, could not be prepared in this work due to the inaccessibility of 108, therefore this approach was abandoned.



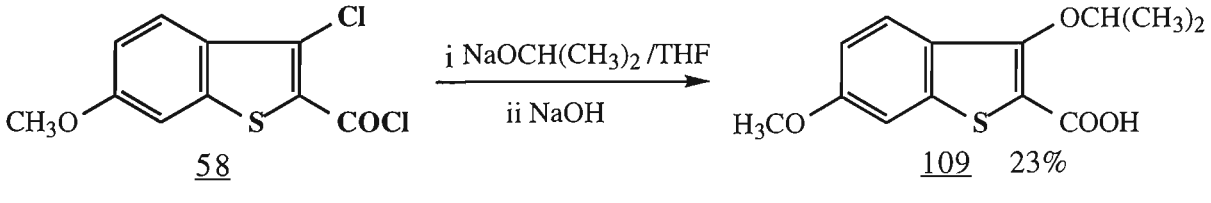
i Sec-C_4H_9Li, -115°C ii CO_2/THF, ether, -105°C iii a PhMgBr/ether b H_3O^+

3.3.3 3-Oxygenated [1]benzothiophene analogues

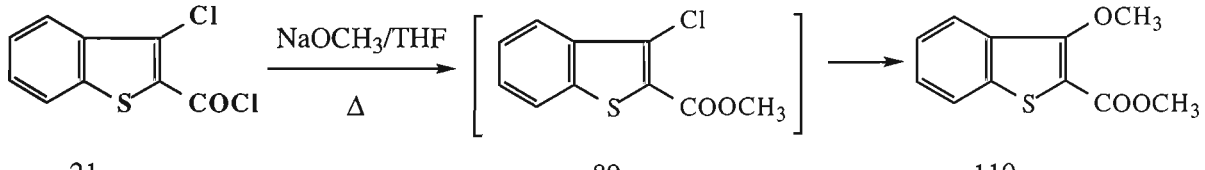
Preliminarily molecular modelling results showed evidence for hydrogen bonding between the oxygen of 3-methoxy, 3-ethoxy or 3-hydroxy groups and the proton of the amide in position 2. This structural characteristic was expected not only to affect the potential activity of those compounds, but also to be helpful in supplying more evidence in evaluating the structure-activity relationships. The synthesis of the 3-methoxy, 3-ethoxy or 3-hydroxy [1]benzothiophene derivatives was thus investigated.

There has not been a great deal of work reported on how to introduce oxygen-containing substituents into the 3-position of [1]benzothiophenes.

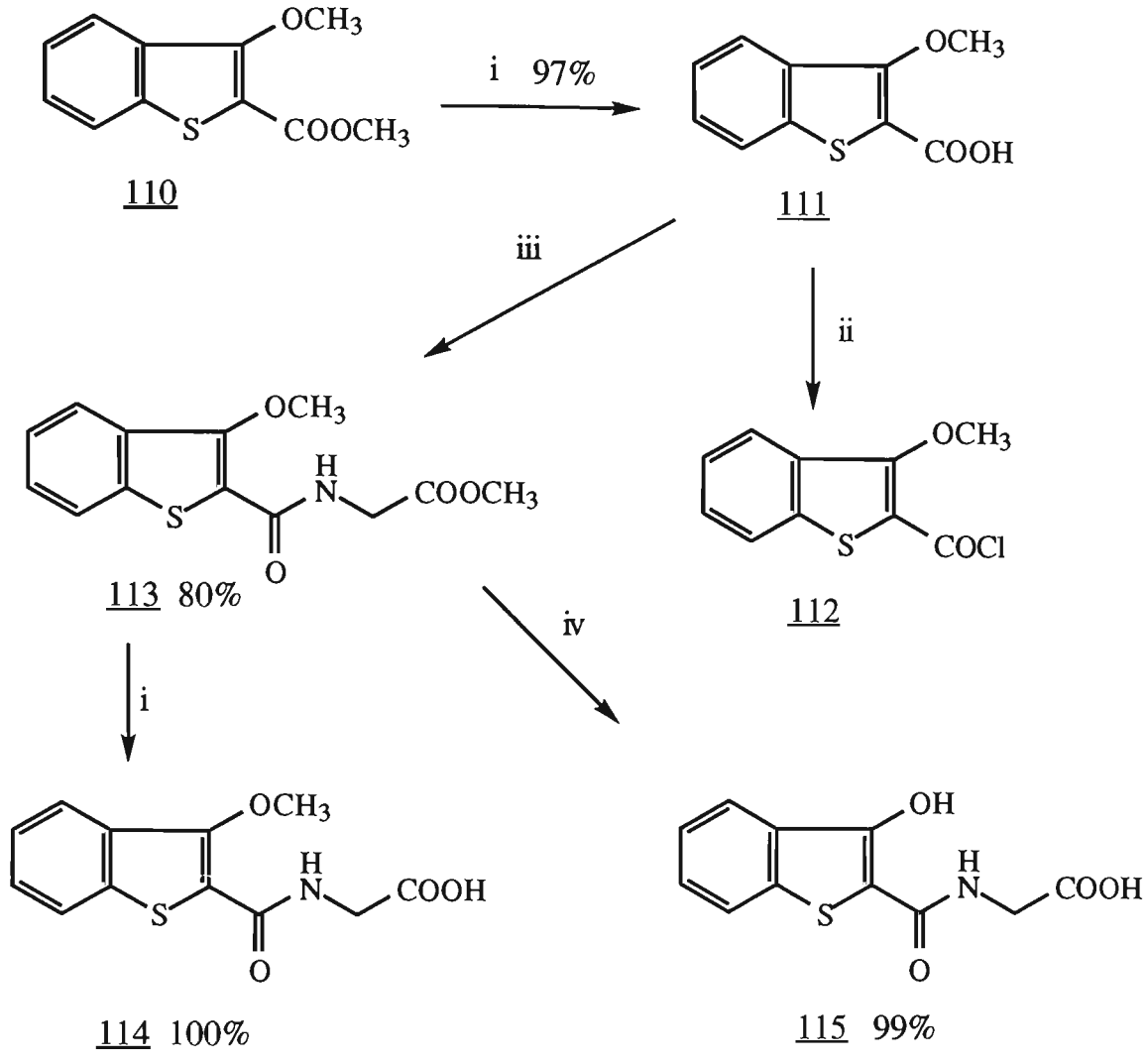
Direct substitution of the 3-chloro group of the acid chloride 58 by an isopropoxy anion, followed by basic hydrolysis to form the 3-isopropoxy compound 109 though in low yield (23%), has been reported by Connor and coworkers[122]. Using this methodology, the 3-chloro acid chloride 21, after being refluxed with sodium methoxide in THF for a few hours, was converted to the 3-methoxy compound 110 in very good yield. From t.l.c. analysis it was apparent that the reaction proceeded *via* two stages, i.e. firstly the 3-chloro ester 89 was formed, and in the second stage the ester was converted to the 3-methoxy compound (Scheme 3.10). The 3-methoxy acid 111, derived from the methyl ester, could not be converted to its acid chloride 112 by the use of thionyl chloride or phosphorus pentachloride, presumably due to the acid sensitivity of the 3-enol ether-like functionality. Thus, the acid 111 was directly coupled with methyl glycinate in DMF in the presence of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole to give the 3-methoxy amide ester 113. The ester was subsequently hydrolysed to the corresponding acid 114. Treatment of the 3-methoxy amide ester 114 with boron tribromide gave a single product 115 in which both 3-methyl ether and methyl ester of 113 were cleaved (Scheme 3.10).



58 109 23%



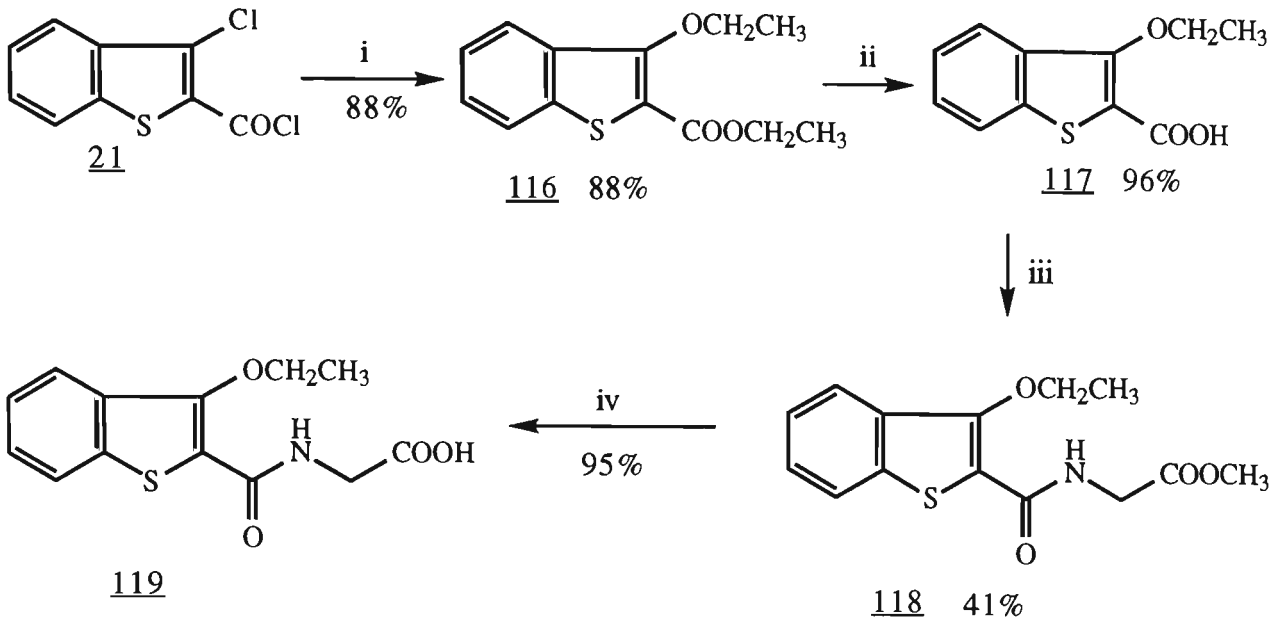
21 89 110



Reagents and conditions: i aq NaOH /CH_3OH, H^+ ii $SOCl_2$ or PCl_5, Δ
iii DCC/HOBT/Methyl glycinate/DMF iv BBr_3/DCM/-78°C to r.t.

Scheme 3.10

The preparation of the 3-ethoxy glycine derivative 119 was achieved using the same procedure as above (Scheme 3.11). The pharmacological test results showed that the hydroxy compound 115 had higher activity than its methoxy analogue 114, while the ethoxy compound 119 was inactive.



Reagents and conditions: i NaOEt/THF, Δ ii aq NaOH/EtOH, H^+

iii DCC/HOBT/Methyl glycinate iv aq NaOH/MeOH, H^+

Scheme 3.11

Interestingly, the inclusion of different substituents on the C-3 position affected significantly the coupling patterns of the other aromatic protons in the ^1H NMR spectra. In the 3-unsubstituted glycine derivative 79, H-4 and H-7 were slightly resolved and H-5& 6 unresolved (Figure 3.3c) in the 400 MHz NMR spectrum. The 3-chlorinated glycine derivative 19 had the least resolution of the aromatic protons (Figure 3.3a), while in contrast, its 3-brominated isomer 86 displayed a dramatically different pattern, with all four aromatic protons clearly separated (Figure 3.3b). The introduction of methoxy or ethoxy into the 3-position did not have any appreciable effect on the coupling patterns of the aromatic protons, however, a good resolution of the aromatic protons was observed for the 3-hydroxy compound 115 (Figure 3.3d, f and e).

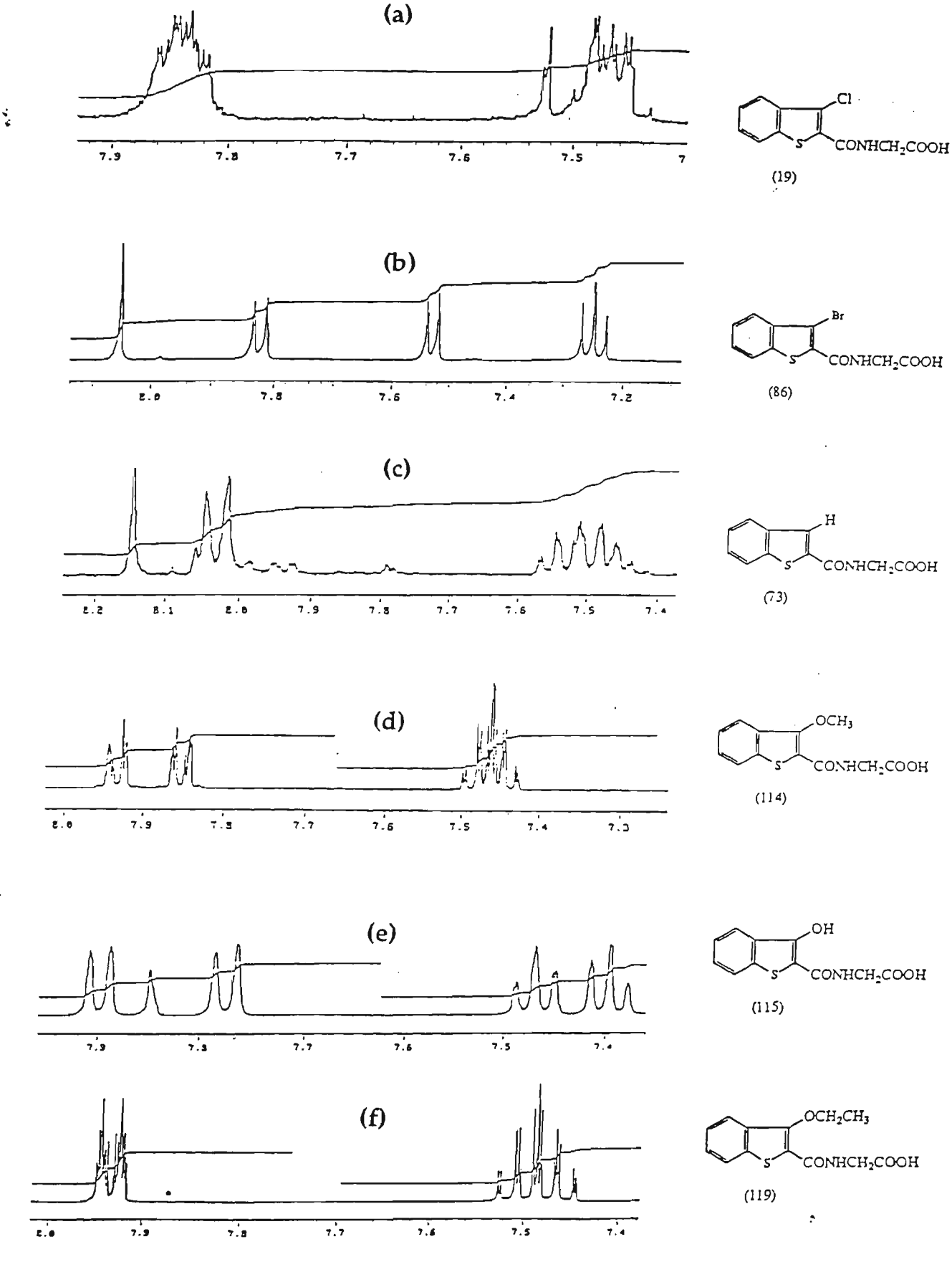
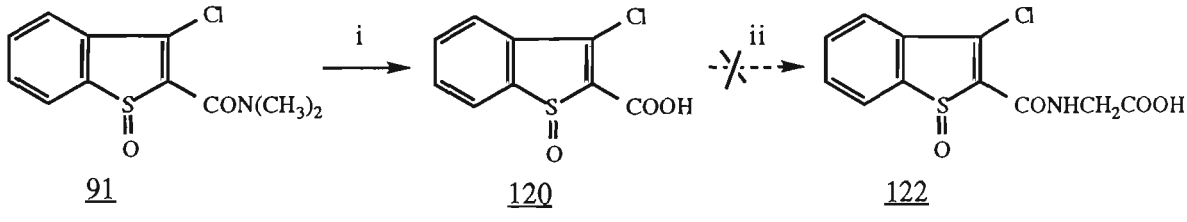


Figure 3.3 ^{1}H NMR aromatic proton patters of [1]benzothiophenes with different 3-substituents

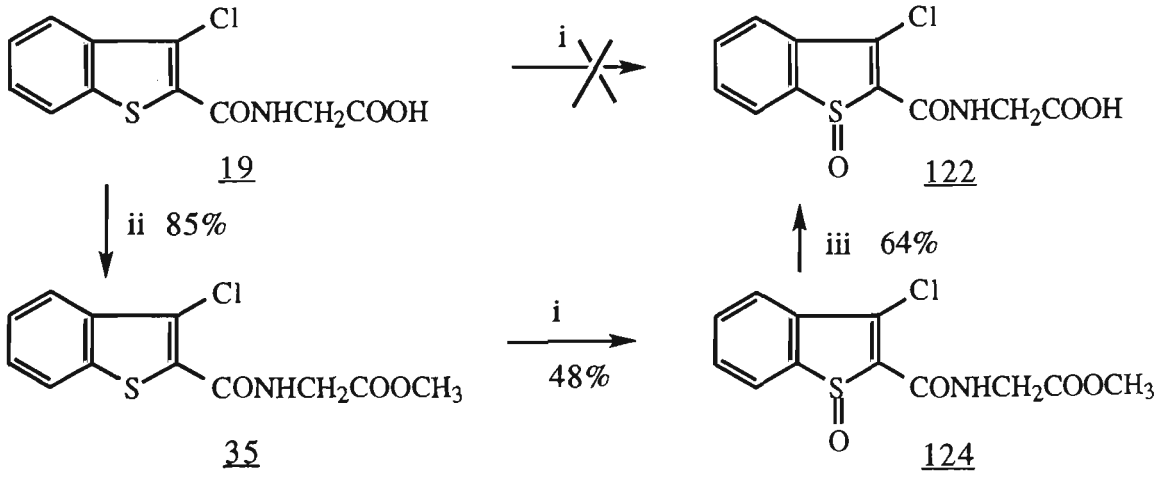
3.4 A [1]benzothiophene sulfoxide derivative

To prepare the sulfoxide derivative of [1]benzothiophene 122, the previously obtained sulfoxide 91 amide was employed as the starting material. The sulfoxide was hydrolysed to give the acid 120. Surprisingly the acid failed to couple with methyl glycinate in DMF in the presence of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole. Direct oxidation of the 3-chloro glycine derivative 19 in dichloromethane with *m*-CPBA was also unsuccessful. The 3-chloro glycinate derivative 35, however, was oxidised to give the sulfoxide 124 in low yield, along with a little of the sulfone. The sulfoxide amide was hydrolysed to the water soluble acid 122 (Scheme 3.12), which showed a lower potency with respect to 5-HT potentiation in comparison with its sulfur analogue 19.



91 120 122

i aq NaOH/MeOH, H^+ ii DCC/HOBT/DMF, Methyl glycinate



i *m*CPBA/DCM ii MeOH/HCl (g)/Δ iii aq NaOH/MeOH, H^+

Scheme 3.12

3.5 Conclusion

The incorporation of methoxy, hydroxy and fluoro groups into the 5- and 6-positions respectively of the [1]benzothiophene ring derivatives was investigated. The 6-substituted methoxy and fluoro derivatives were easily prepared from the corresponding acid chloride derived from appropriately 4-substituted *trans*-cinnamic acids. Likewise, the preparation of the 5-substituted methoxy and fluoro derivatives was conducted using a similar procedure and the 5-methoxy derivative was separated from the 7-substituted isomer by recrystalization. The separation of the 5-fluoro analogue from its 7-substituted isomer, however, proved to be very difficult. The 5- and 6-hydroxy compounds were readily prepared by demethylation of the corresponding methoxy compound with boron tribromide. The fluoro- and methoxy-substituted glycine derivatives did not show 5-HT potentiation activity, while the hydroxy derivative was slightly active.

The introduction of hydrogen, bromo, fluoro, methoxy, ethoxy and hydroxy in the 3-position of the [1]benzothiophene ring was investigated. The 3-unsubstituted derivatives were obtained in good yields using methyl [1]benzothiophene-2-carboxylate 70 as the starting material. Selective bromination of the ester 70 at the 3-position, followed by other transformations, provided the 3-bromo amide 86. The oxygenated groups at position-3 were introduced by direct substitution of the chloro group of the acid chloride 21 with sodium alkoxide. The introduction of the fluoro group to the 3-position was attempted using Cl-F exchange, diazonium salt formation and electrophilic fluorination pathways, but only the diazonium salt route afforded any of this derivative in very low yield. The oxidation of the ring sulfur was achieved by oxidising the 3-chloro methyl ester 35 with *m*-

CPBA. The 3-hydroxy, 3-unsubstituted and sulfoxide derivatives displayed moderate 5-HT potentiation, while the remaining compounds showed little activity.

Molecular modelling results indicated all substituted glycine amide derivatives had the specific intramolecular hydrogen bonding between the amide carbonyl oxygen and the hydroxyl group of the carboxylic acid. An extra hydrogen bonding interaction was observed between the 3-oxygen and the amide functional group in 3-oxygenated compounds. The substituents at the 5- and 6-positions most likely acted as blocking groups, hindering interaction with the 5-HT transporter.

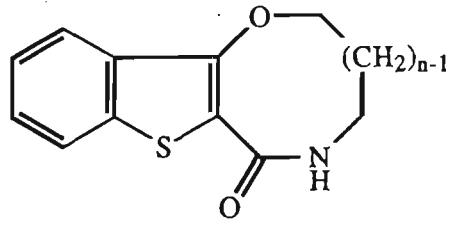
Chapter 4

Synthesis of Medium Ring-Containing [1]Benzothiophene Derivatives

4.1 Introduction

In Chapters 2 and 3, the synthesis of various acyclic [1]benzothiophene derivatives was presented. Some of them showed interesting pharmacological activity. In this Chapter, the synthesis of more restrained cyclic analogues will be discussed. It was hoped that these ring systems would show useful pharmacological activity, and therefore help in evaluating the structure-activity relationships of the benzothiophene derivatives as potentiators of the 5-HT effect at the 5-HT_3 receptor.

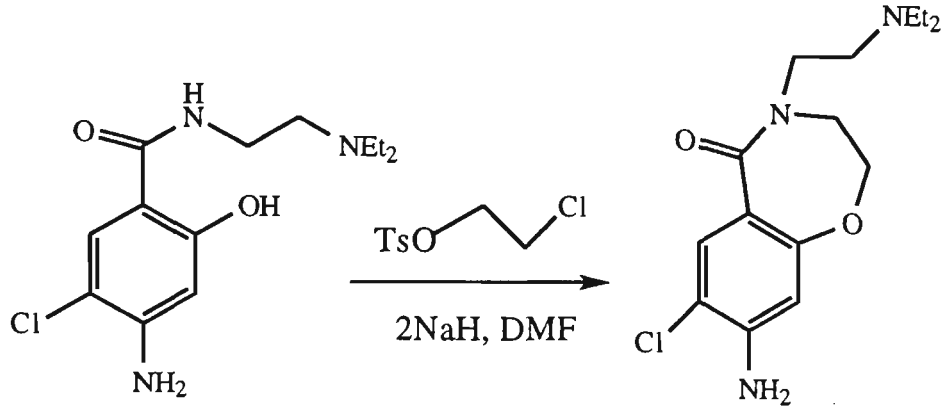
The target cyclic compounds chosen were the oxaza fused system with seven to ten membered medium rings, of which only the eight membered ring skeleton had been accessed previously[123]. The target compounds contained both an electronegative group at the 3-position and lactam functionality at the 2-position of [1]benzothiophene nucleus.



n = 1, 2, 3, 4.

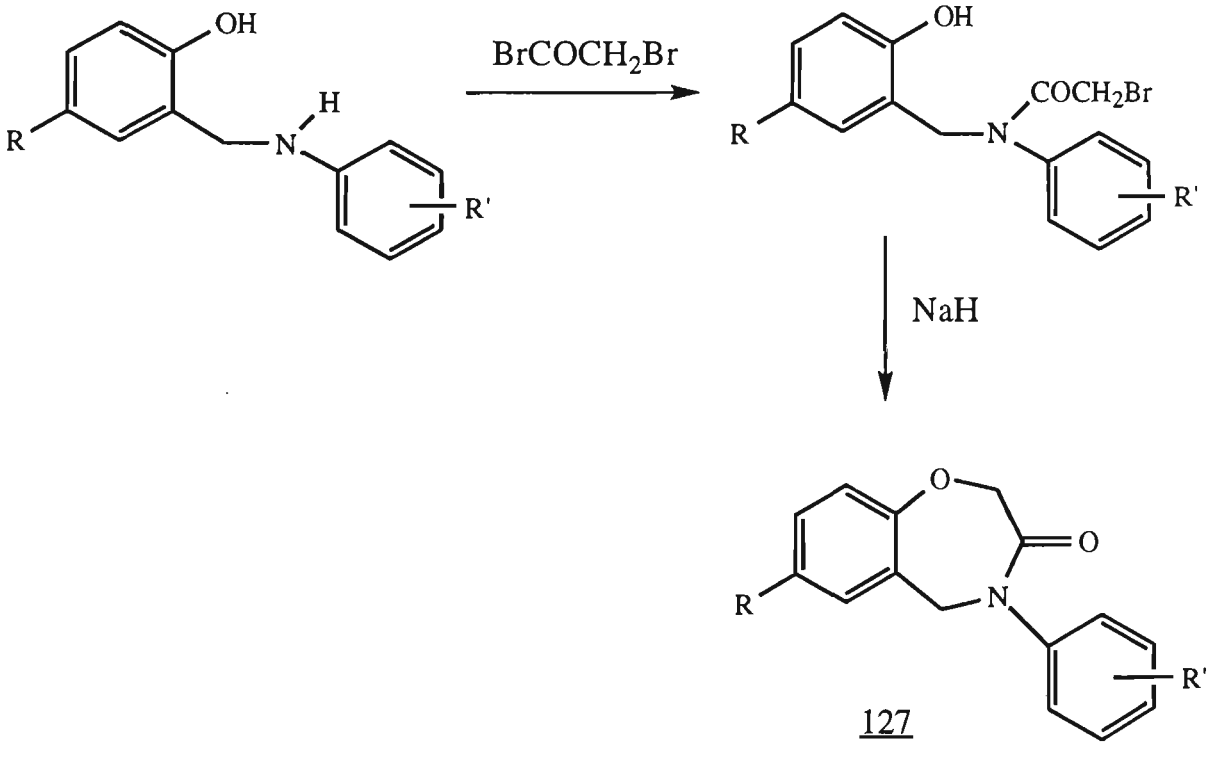
There have been some reports in the literature concerning the synthesis of amide-containing medium-sized ring systems. Monkovic[124] has reported

that treatment of the amide 125 with 2-chloroethyl tosylate in DMF in the presence of sodium hydride gave the 7-membered ring compound 126, *via* O-alkylation and subsequent intramolecular aromatic nucleophilic substitution.



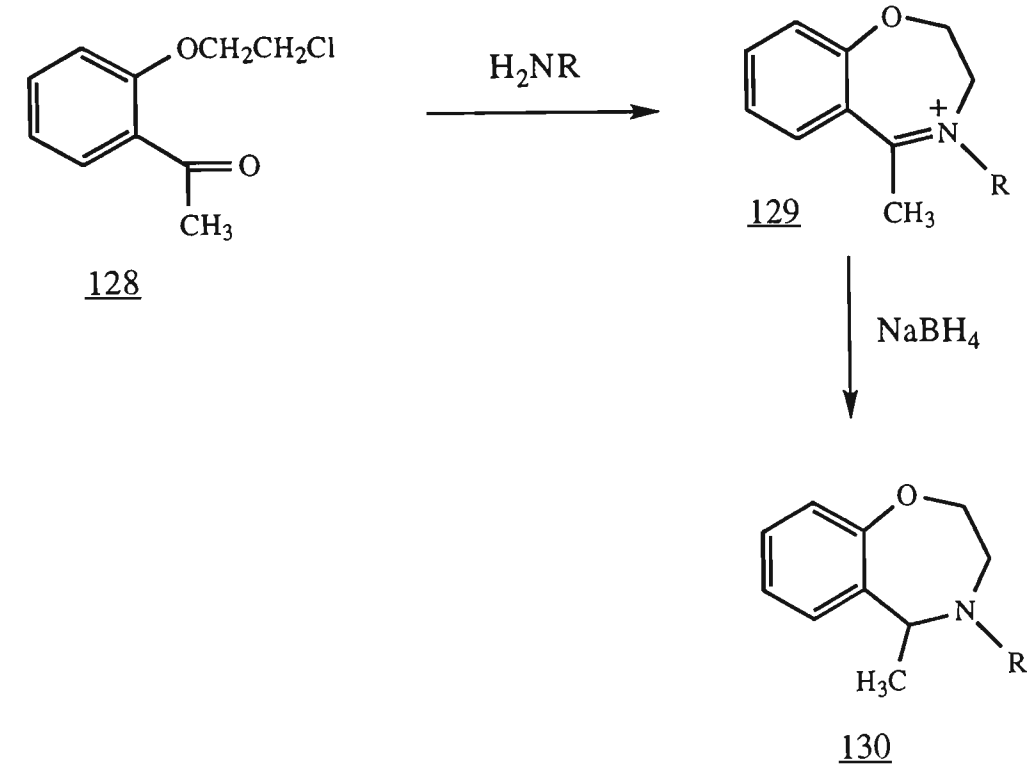
125 126

The seven-membered ring derivatives 127 have been prepared in a similar manner by Derieg[125] using an intramolecular aromatic nucleophilic substitution process.

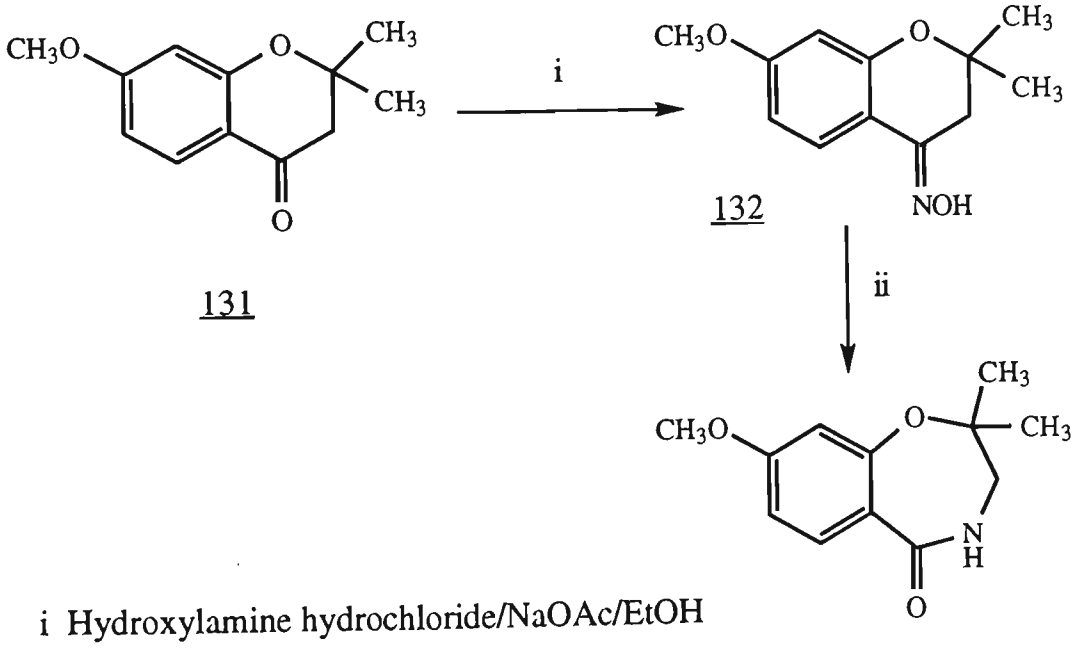


127

Schenker[126] found that the 1,4-benzoxazepine 130 could be prepared from the ketone 128 through the iminium ion 129, while Levai[127] converted the ketone 131 to the 1,5-benzoxazepine-4(5*H*)-one derivative 133 *via* rearrangement of the oxime 132.



128 129 130

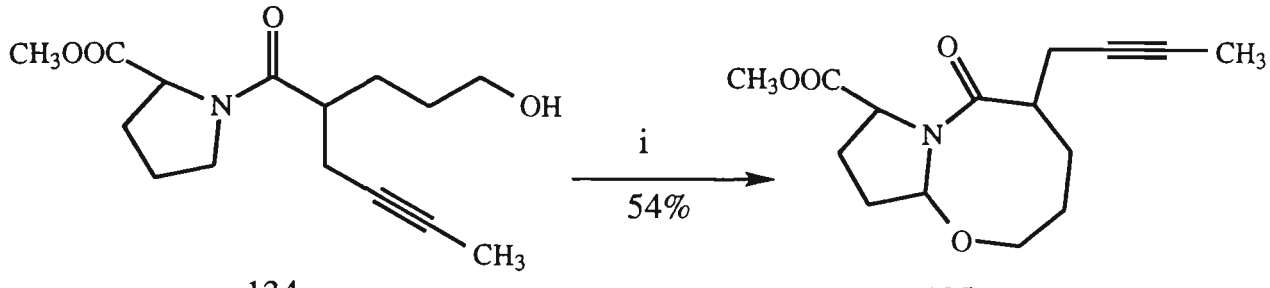


131 132 133

i Hydroxylamine hydrochloride/NaOAc/EtOH

ii Polyphosphoric acid, 50°C

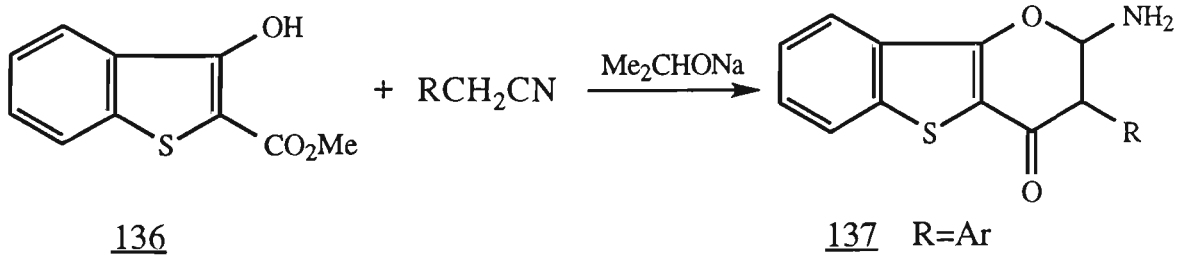
Wong[128] found that electrolysis of the hydroxy amide 134 gave the cyclized compound 135 in 54% yield.



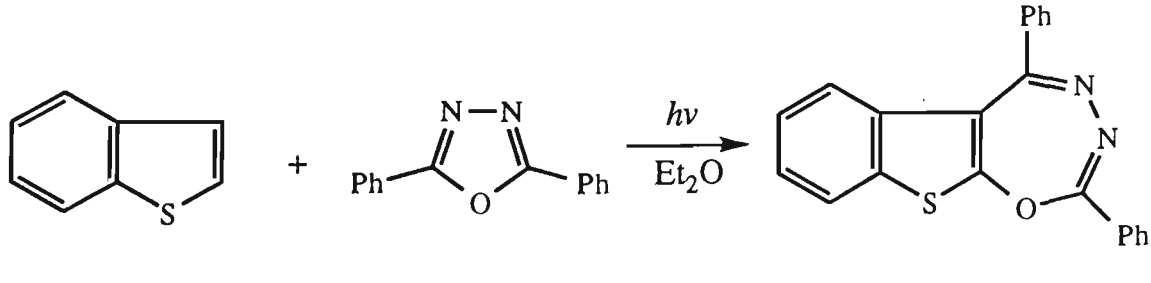
134 135

i Carbon anode, 1 M Et_4NBF_4, 10% MeOH/CH_3CN, 5 eq. K_2CO_3, divided cell, 15 mA, 3.18F/mole

Several researchers have reported the synthesis of fused medium-sized ring-containing benzothiophene derivatives that are structurally close to the target cyclic compounds. Volovenko[129] has prepared the six-membered ring compound 137 by cyclocondensation of the benzothiophene carboxylate 136 with RCH_2CN catalysed by sodium isopropoxide, while Kojio[130] has reported the synthesis of the oxadiazepine 140 by irradiation of a mixture of the benzothiophene 138 and the 2,5-diphenyloxadizole 139.

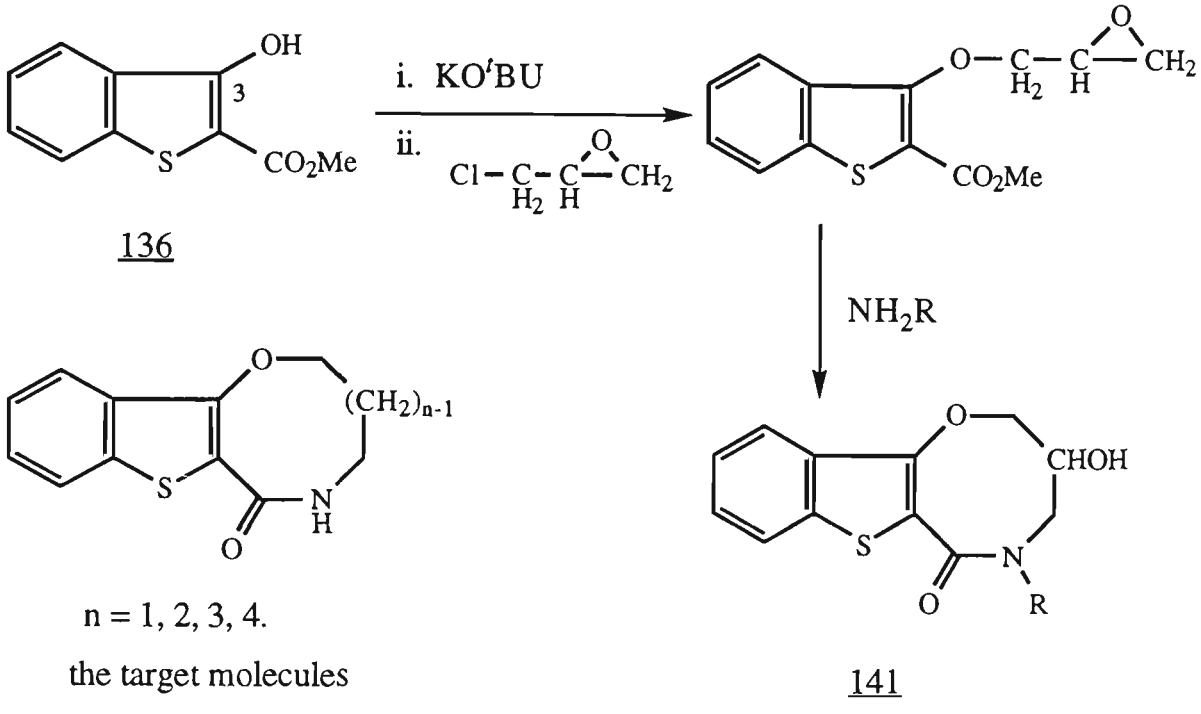


136 137 R=Ar



138 139 140

Conde and coworkers[123] have reported that alkylation of the 3-hydroxy compound 136, followed by subsequent amidation and ring closure, afforded the eight membered ring compound 141. This is structurally the closest to the target cyclic compounds (n=2).

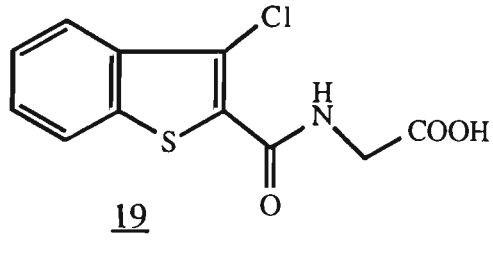


136

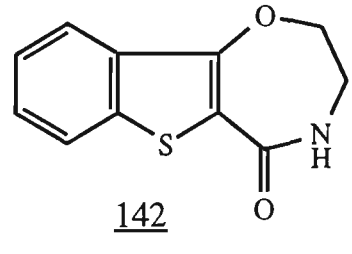
141

4.2 Synthesis of the seven membered ring-containing benzothiophene derivative 142

Since the glycine derivative 19 exhibited a moderate potentiation of the action of 5-HT at the 5-HT$_3$ receptor, its analogue, the 7-membered ring-containing benzothiophene derivative 142 was expected to show some potentiation. As it was the simplest structurally of the target molecules, its synthesis was investigated first.

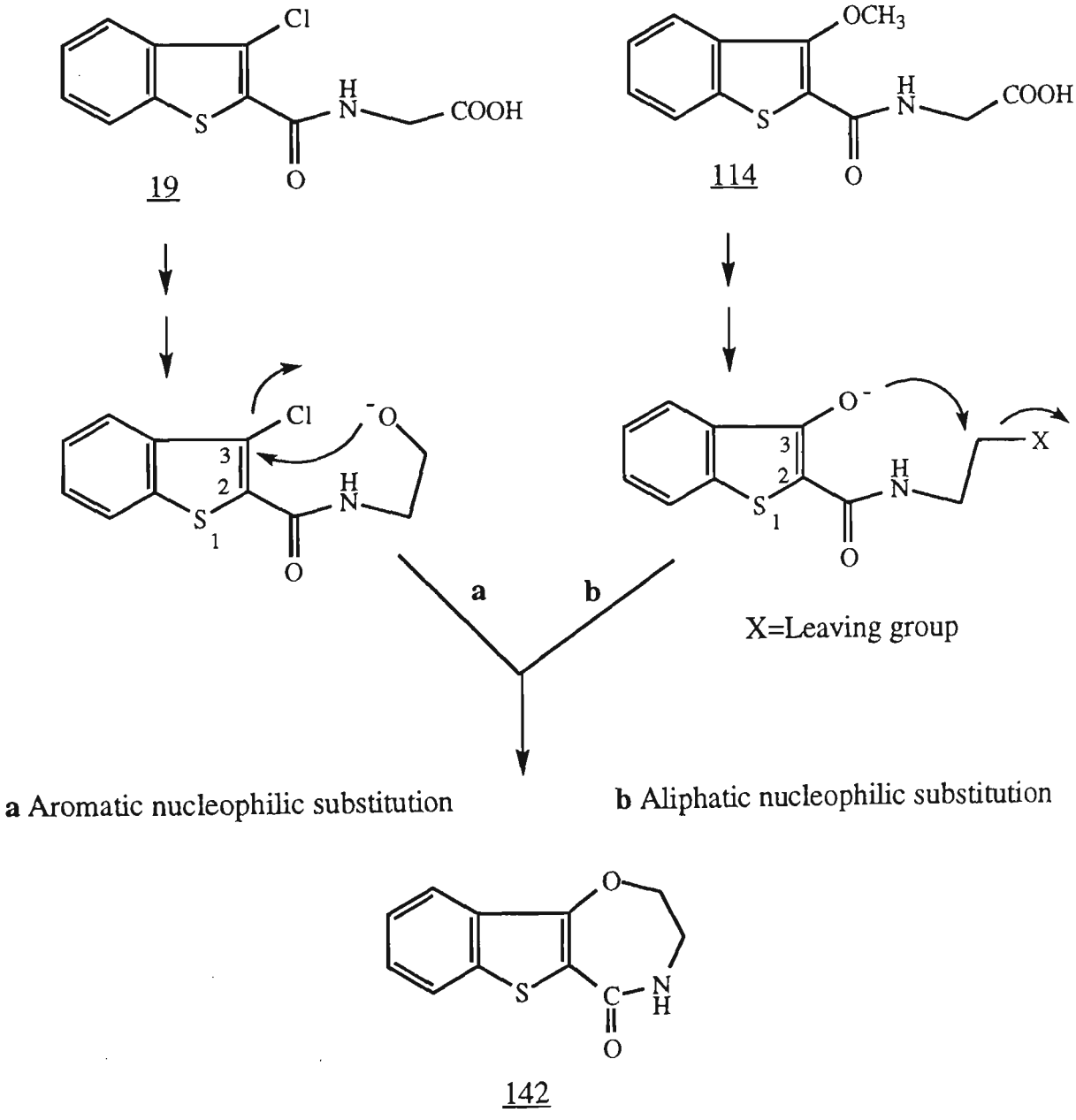


19



142

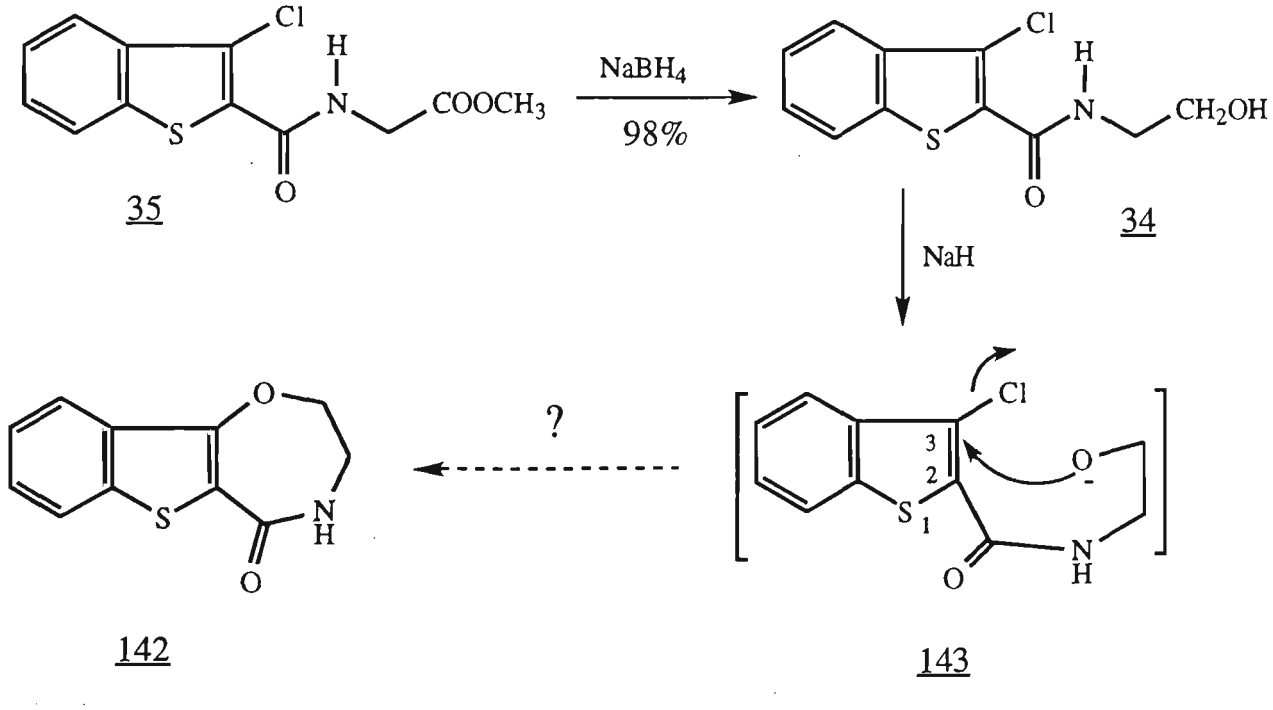
It was envisaged that compound 142 could be prepared readily by intramolecular displacement of a leaving group by an nucleophilic oxygen anion. This could be done by reduction of the carboxylic acid functionality of 19 ,followed by intramolecular aromatic substitution (route **a**); or by demethylation of the 3-methoxy group of the 3-methoxy glycine derivative 114, followed by intramolecular aliphatic nucleophilic substitution (route **b**), (Scheme 4.1)



Scheme 4.1

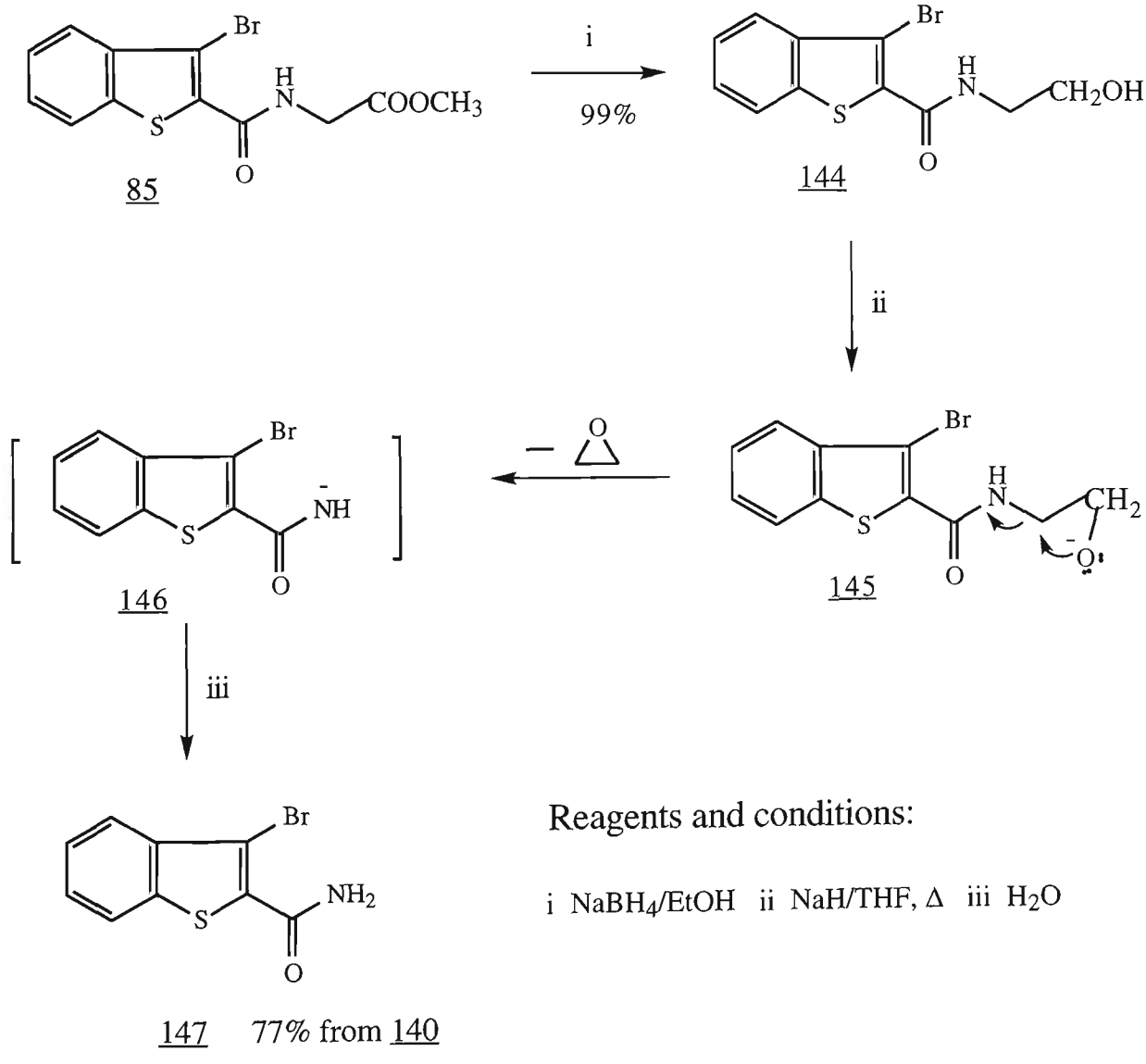
4.2.1 Aromatic nucleophilic substitution approach to the compound 142

In Chapter 4, it was shown that the acid chloride 21 was readily converted to the 3-methoxy ester 110 or 3-ethoxy ester 116 in excellent yields by refluxing with sodium methoxide or sodium ethoxide in THF respectively. It was hoped that such a substitution using a cyclic precursor would also occur at an intramolecular level to give the desired cyclized compound 142. The 3-chloro alcohol 34, prepared in excellent yield by reduction of the glycinate derivative 35 with sodium borohydride in ethanol, was expected to generate the oxygen anion 143 upon treatment with sodium hydride. The anion 143 was anticipated to undergo an aromatic nucleophilic attack *via* addition-elimination at the 3-position to give the cyclized compound 142 (Scheme 4.2). This initial addition would be a favoured 7-endo-trig process, although some conformational rigidity would be imposed by the amide group.



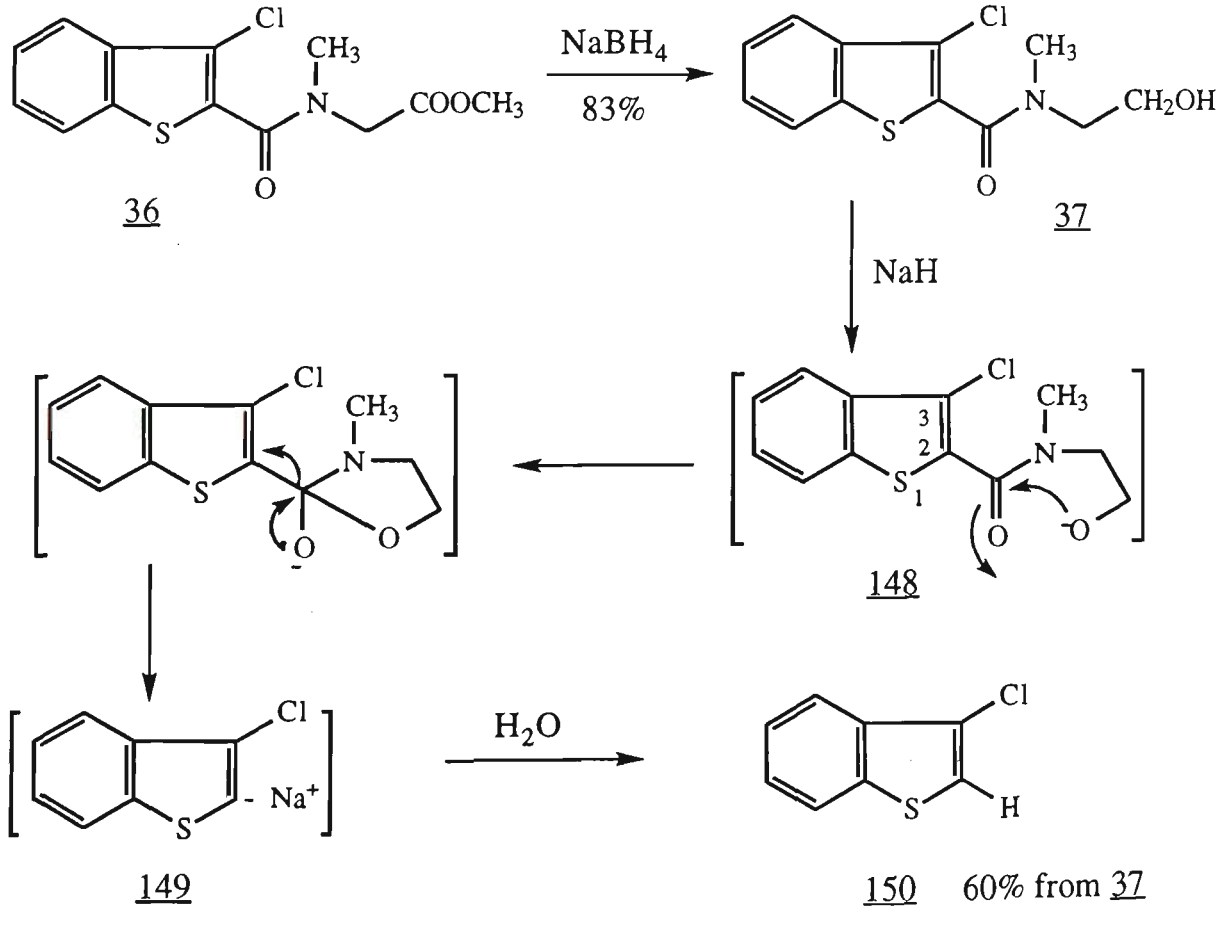
Scheme 4.2

Treatment of the 3-chloro amide alcohol 34 in THF with an excess of sodium hydride, followed by refluxing the mixture, failed to initiate the reaction. Due to easy accessibility of the 3-bromo amide alcohol 144, these conditions were also applied to the bromo compound. Surprisingly, after two hours reflux, the alcohol 144 was converted to the 3-bromo amide 147 in 77% yield. It was assumed that this arose from the oxygen anion 145 attacking the amide-attached sp^3 carbon rather than the aromatic carbon bearing bromo group, to give an intermediate amide anion 146, which upon aqueous work-up protonated to give the amide 147 (Scheme 4.3).



Scheme 4.3

Although the difference in reactivity between the 3-chloro amide alcohol 34 and the 3-bromo amide alcohol 144 towards sodium hydride in THF under the same conditions was difficult to explain, a possible interference from some amide anion formation may have prevented the oxygen anions from attacking the aromatic carbon. To address this problem, the tertiary amide alcohol 37, prepared by reduction of the sarcosinate derivative 36 with sodium borohydride in ethanol, was chosen as the ring closure precursor. After being treated with sodium hydride in THF and then refluxed for two hours, the amide alcohol 37 was converted to 3-chloro-[1]benzothiophene 150. It was believed that this resulted from a nucleophilic attack of the oxygen anion 148 on the amide carbonyl, followed by elimination to give a carbanion intermediate 149, which was protonated upon work-up (Scheme 4.4). No evidence for attack on C3 by the alkoxide ion was obtained.

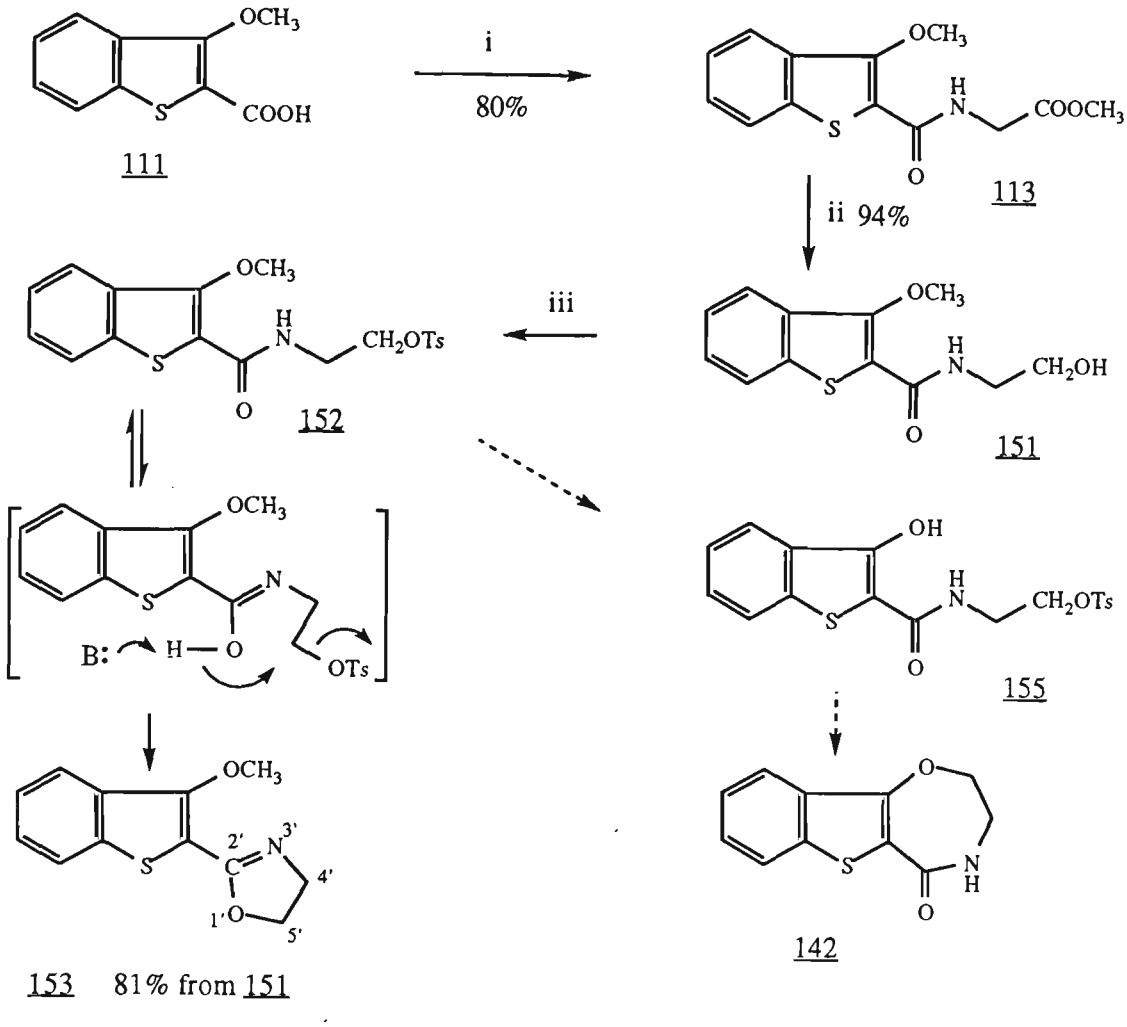


36 37 148 149 150 60% from 37

Scheme 4.4

4.2.2 Aliphatic nucleophilic substitution approach to 142

In Section 3.3 it was noted that the 3-methyl ether 113 could be easily cleaved by the use of boron tribromide in dichloromethane to give the 3-hydroxy compound 115, in which the 3-hydroxy group in basic conditions should be a good nucleophile. To prepare the cyclized compound 142 *via* an aliphatic nucleophilic substitution, it was necessary to have a good leaving group such as a tosylate in the aliphatic chain. Thus, the synthesis of the tosylate 155 was investigated. The 3-methoxy glycinate derivative 113 was reduced to the corresponding amide alcohol 151 in good yield with sodium borohydride. Treatment of the alcohol in dichloromethane at room temperature with triethylamine as a base for 8 hours gave only a small amount of the tosylate 152 together with unreacted starting material. Longer reaction times resulted in some of the tosylate being converted to the 4,5-dihydrooxazole derivative 153, though no starting material was left. Attempts to isolate the tosylate from the mixture was unsuccessful. Moreover, it was found that upon heating the reaction mixture overnight, only the 4,5-dihydrooxazole derivative 153 was obtained. It is likely that the 4,5-dihydrooxazole derivative was formed from an intramolecular nucleophilic substitution as shown in Scheme 4.5. This type of intramolecular nucleophilic displacement of an enolizable ketone or amide functionality has been reported to prepare cyclized compounds[131].

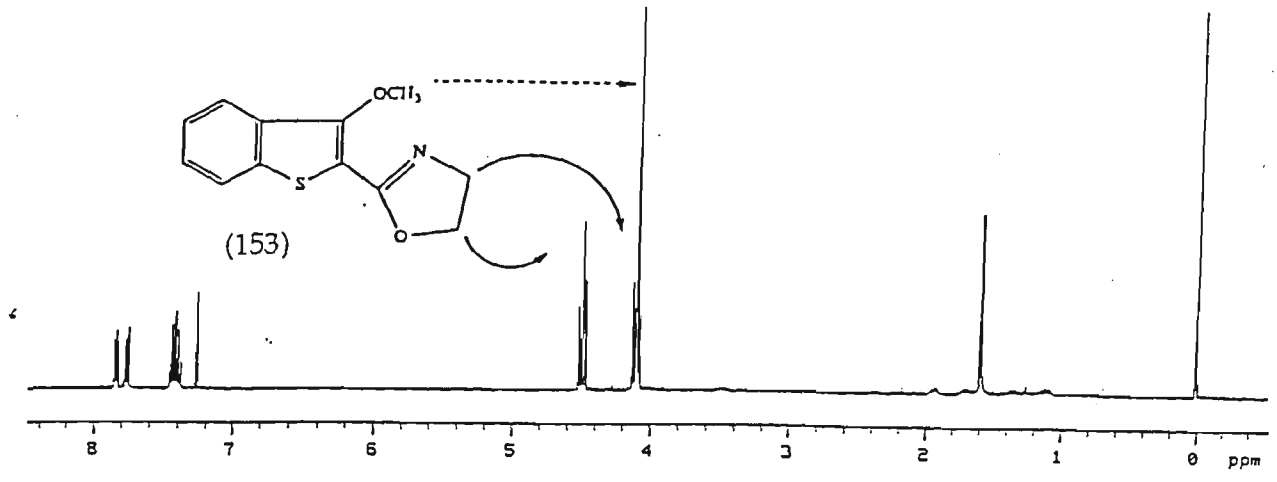


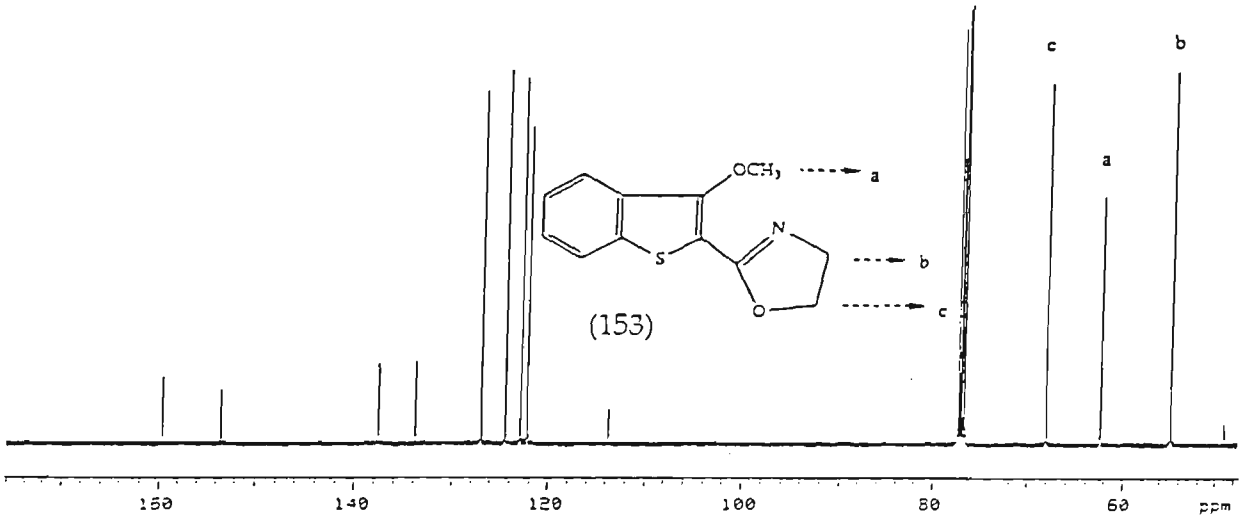
Reagents and conditions:

i DCC/HOBT/Methyl glycinate/DMF ii $NaBH_4$/EtOH iii $TsCl/Et_3N$/DCM

Scheme 4.5

The ^1H NMR spectrum of 153 showed two groups of triplets at 4.49 ppm and 4.10 ppm corresponding to the 5'-methylene and 4'-the methylene respectively. Its ^{13}C NMR spectrum had signals at 68 ppm and 55 ppm corresponding to C5' and C4' respectively. These chemical shifts are consistent with the data for 4,5-dihydrooxazole derivatives[132]. Furthermore, in the IR absorption spectrum a band at 1634 cm^{-1} consistent with a C=N stretch, was observed, while in the ES^+ mass spectrum an MH^+ base peak was observed at *m/z* 234 (Figure 4.1).





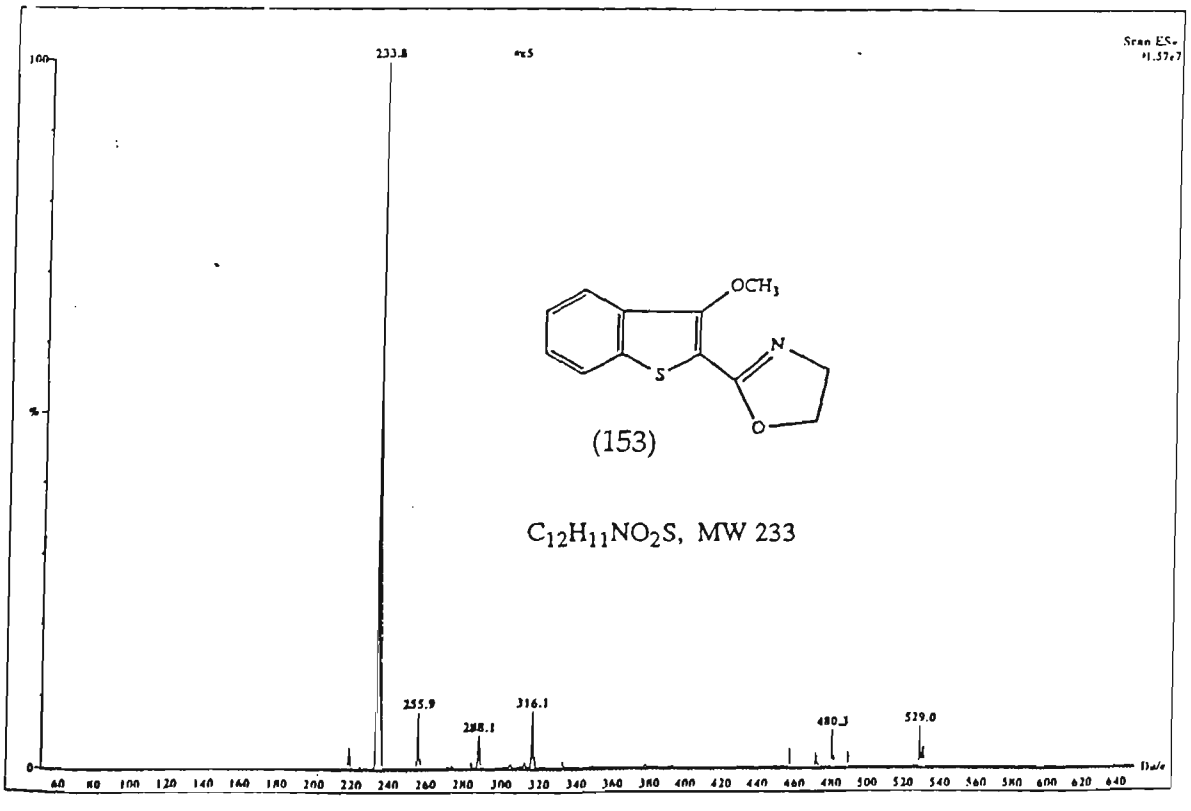
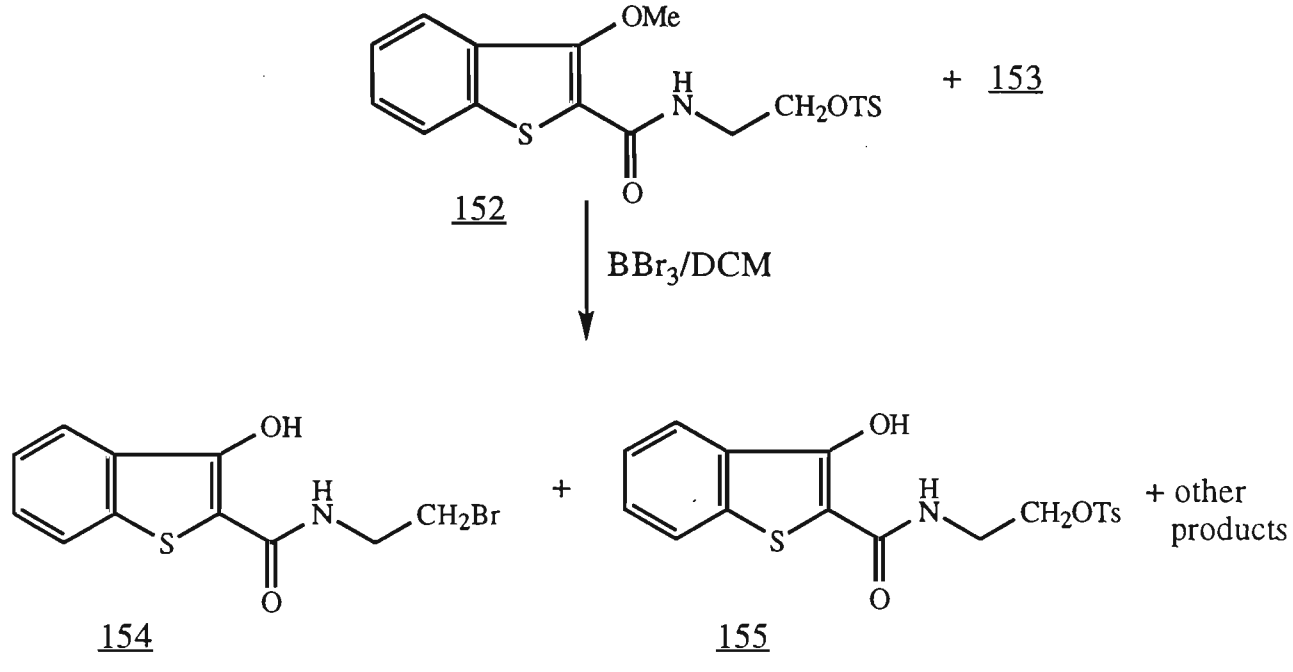
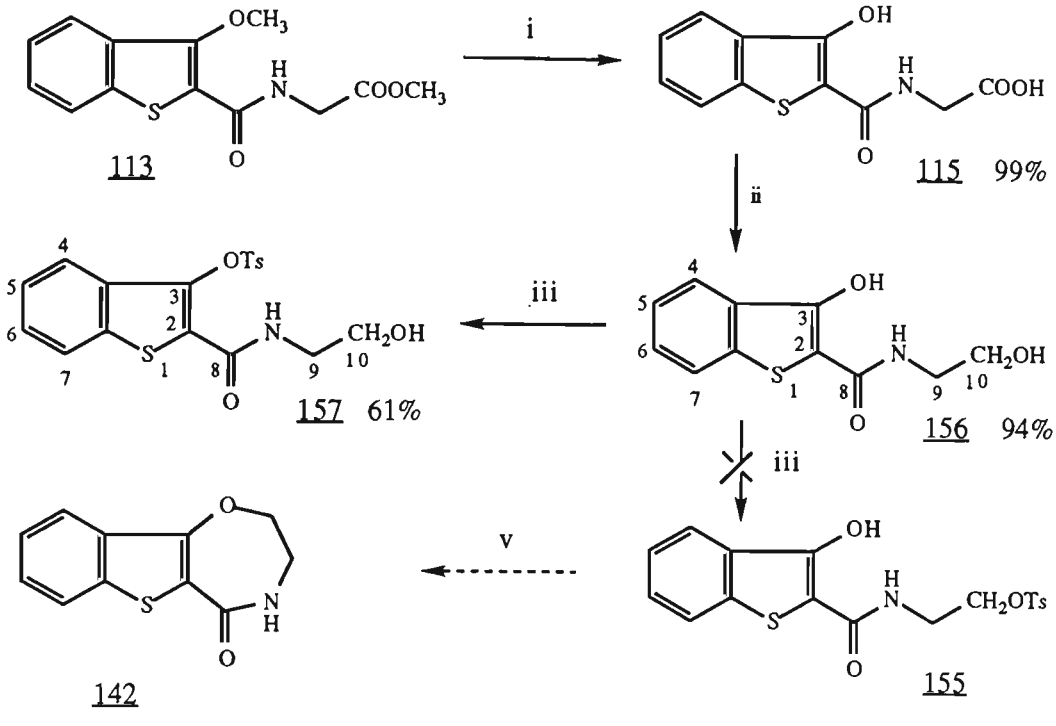


Figure 4.1 ^{1}H and ^{13}C NMR spectra and ES+ mass spectrum of compound 153

Treatment of the crude tosylate 152 with boron tribromide gave several products, as indicated by t.l.c. analysis. They were believed to be mixtures of the 3-hydroxy tosylate 155 and the 3-hydroxy bromide 154, together with derivatives of the 4,5-dihydrooxazole. These products were not separated.



Due to the lack of efficiency in conversion of the 3-methoxy alcohol 151 to the 3-hydroxy tosylate 155, an alternative route involving initial demethylation of 113 and subsequent tosylation was investigated (Scheme 4.6). The 3-methoxy compound 113 was transformed into the 3-hydroxy acid 115 in excellent yield with boron tribromide. The acid was then reduced to the diol 156 with BH_3-THF. Tosylation of the diol was expected to give selectively the 3-hydroxy tosylate 155. Surprisingly, after stirring for four hours with an excess of tosyl chloride in dichloromethane containing triethylamine as a base, the diol was completely converted to the 3-tosylated amide alcohol 157 (Scheme 4.6).

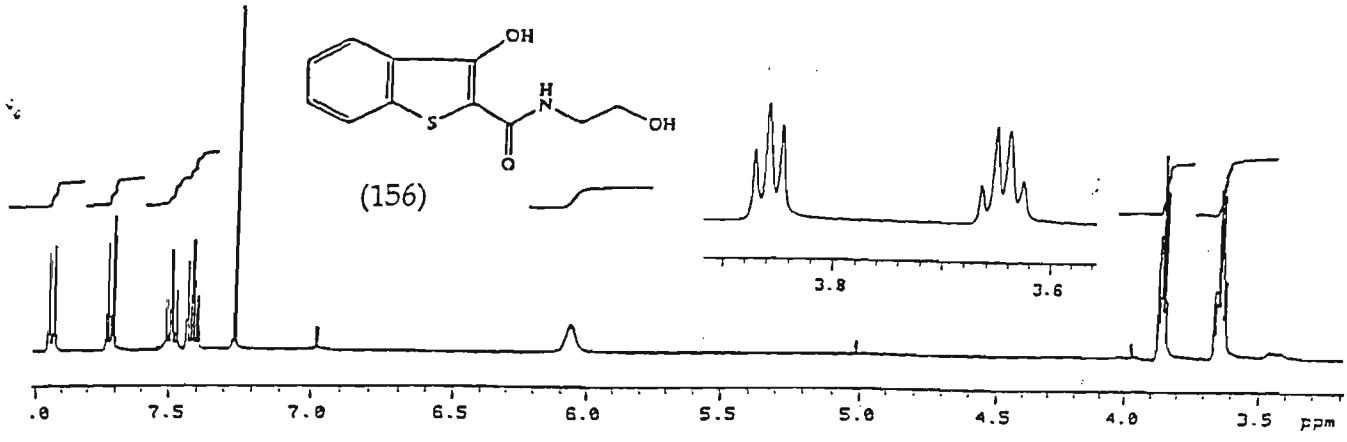


Reagents and conditions:

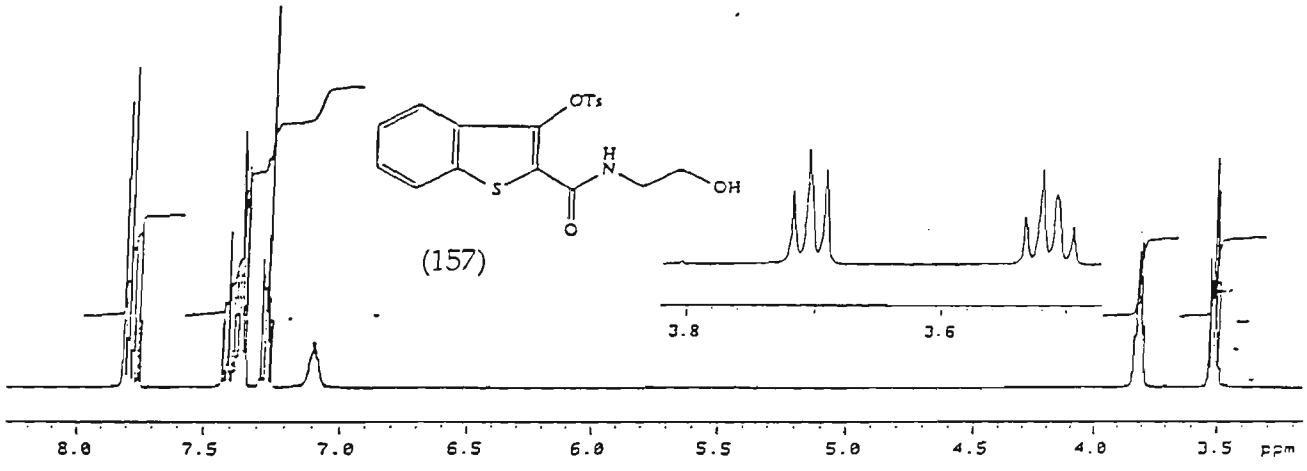
i BBr_3/DCM, -78°C to r.t. ii BH_3/THF iii TsCl/Et_3N/DCM iv. NaH/THF

Scheme 4.6

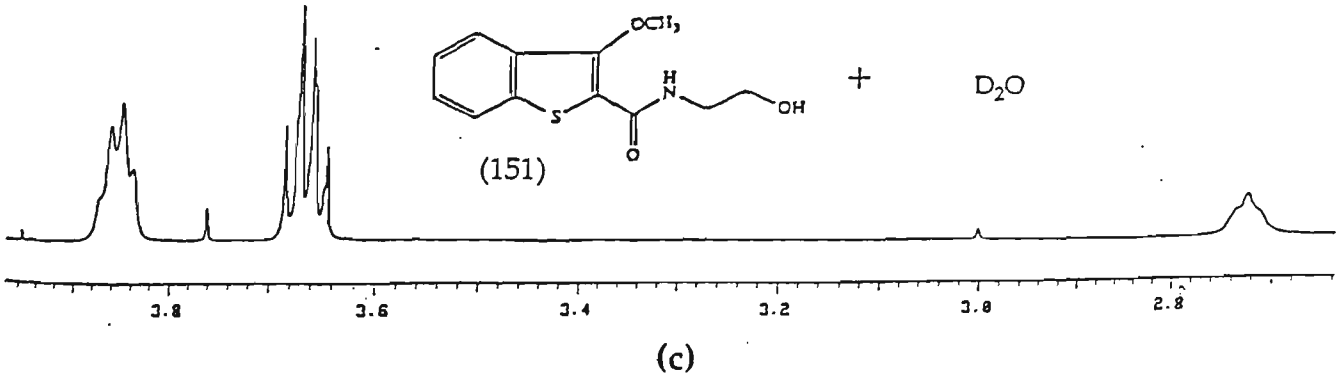
The ^1H NMR spectrum of the 3-tosylate 157 displayed a doublet of triplets at 3.51 ppm and a triplet at 3.81 ppm corresponding to the protons on C9 and C10 respectively. They were identical to the 9-methylene and the 10-methylene proton signals of the starting material 156 (Figure 4.2a and b). In addition, the fact that tosylation of the 3-hydroxy amide alcohol 149 only took four hours to complete, whereas it needed much longer for the 3-methoxy amide alcohol 151 proved the 3-hydroxy group was preferably tosylated in this particular case, though tosylation of phenolic-like hydroxy groups normally need stronger conditions[133]. It is worth noting that the triplet peak of the primary hydroxy group of the amide alcohol 151 did not disappear after deuterium oxide exchange, implying the hydroxy group was probably quite stable, supporting the unexpected sluggishness of its tosylation (Figure 4.2c).



(a)



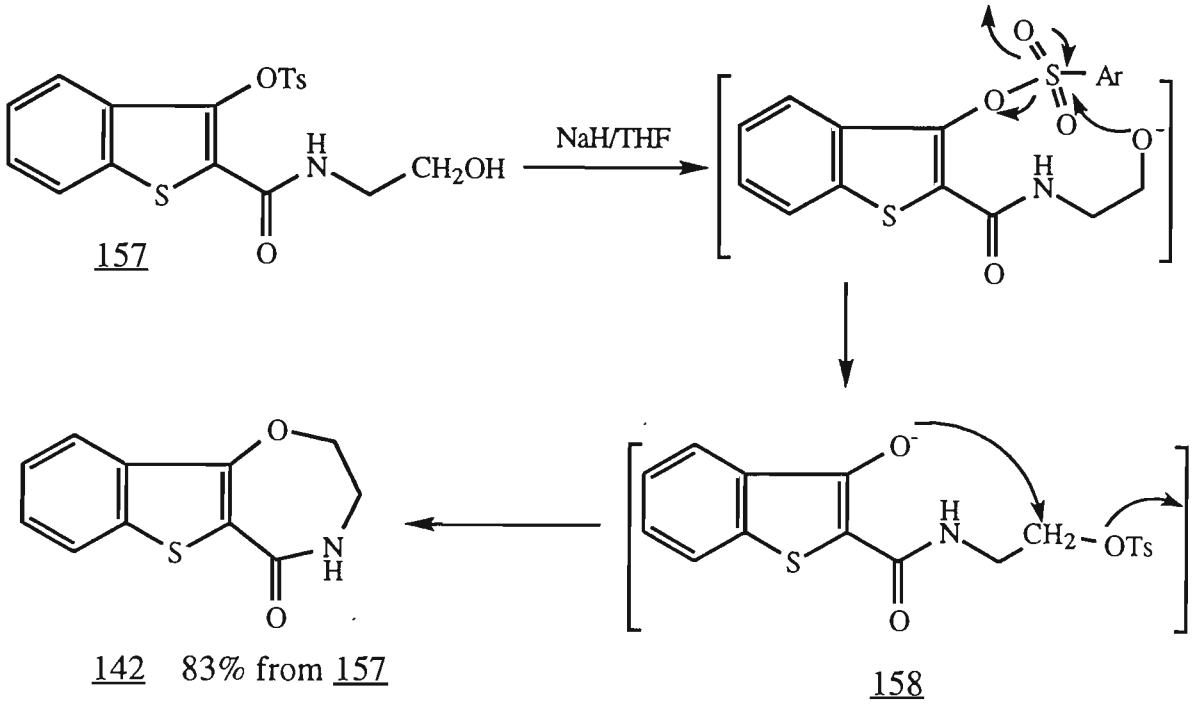
(b)



(c)

Figure 4.2 ^{1}H NMR spectra of compounds 156, 157 and 151

Treatment of the 3-tosylate 157 with sodium hydride in THF at room temperature gave the desired ring closure compound 142 in good yield. The cyclization probably occurred through an intramolecular trans-sulfonylation to give the intermediate 158, which then underwent a 7-exo-tet aliphatic nucleophilic displacement to afford 142 (Scheme 4.7)



157

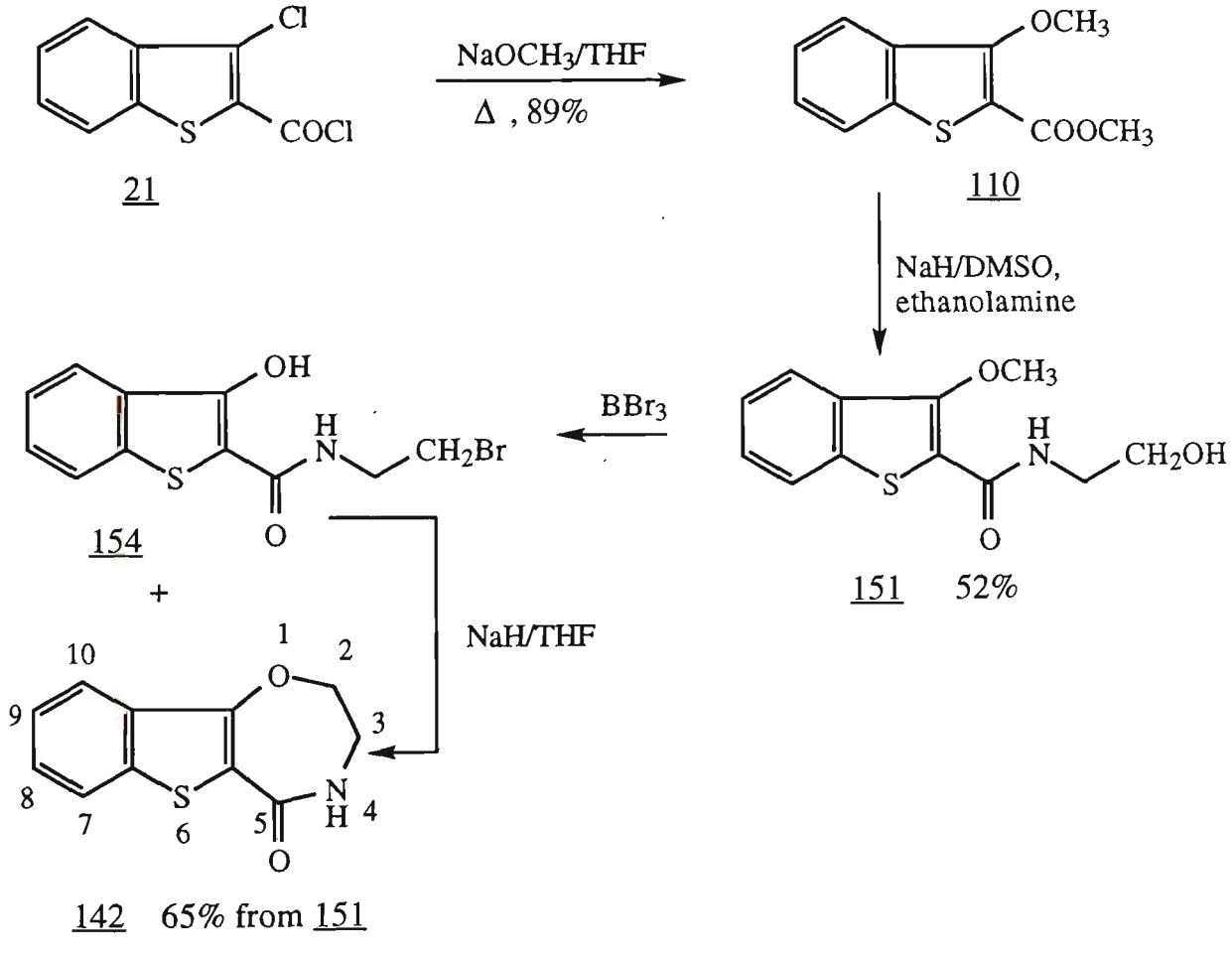
142 83% from 157

158

Scheme 4.7

Another more convenient and efficient approach to the target compound was developed by modifying the foregoing procedure. Previously, the preparation of the 3-methoxy alcohol 151 from the 3-methoxy ester 110 took three steps. Singh[134] has reported, however, that esters can be directly converted to amides through sodium salts of primary amines. Treatment of ethanolamine with sodium hydride in dimethyl sulfoxide, followed by addition of the methyl ester 110, gave the 3-methoxy amide alcohol 151 in 52% yield. Exposure of the alcohol to boron tribromide in dichloromethane at -78°C not only led to demethylation of the 3-methyl ether but also brought

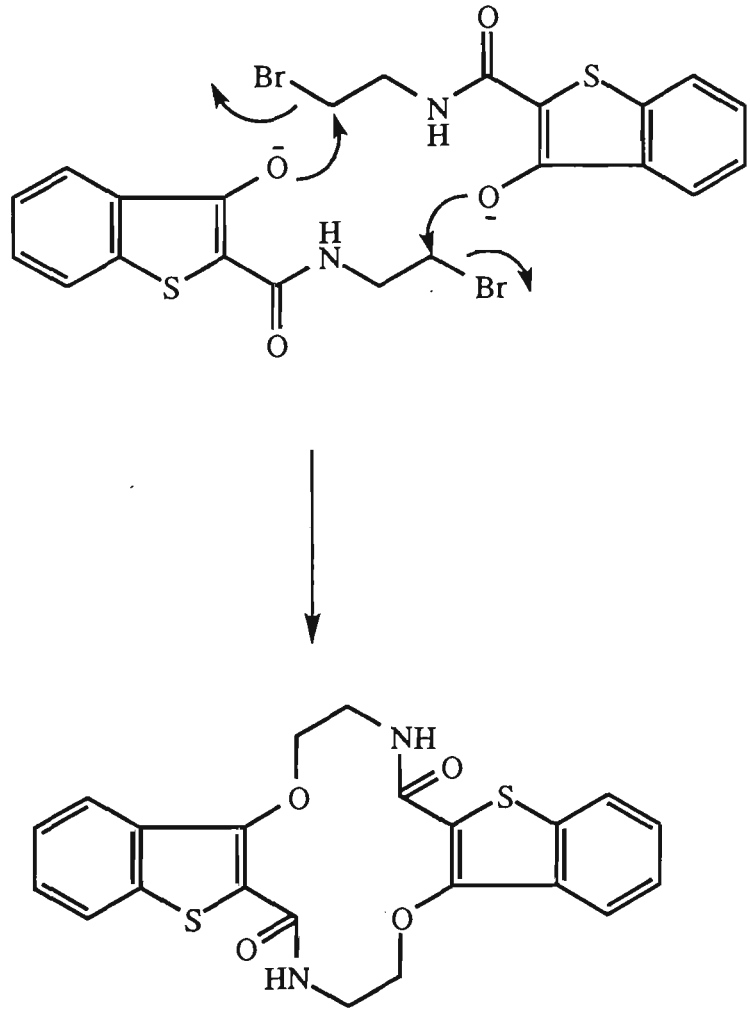
about bromination of the primary hydroxyl group. More interestingly, some cyclized compound 142 accompanied the formation of the 3-hydroxy bromide 154 and in effect it was found that the bromide in $CDCl_3$ would slowly cyclize to give the ring compound 142. Treatment of the crude bromide in THF with sodium hydride for two hours afforded 142 in 68% yield. The overall yield from the acid chloride 21 to the cyclized compound 142 was 30% (Scheme 4.8). This compound showed moderate 5-HT potentiation in the pharmacological testing (*cf* Chapter 5).



Scheme 4.8

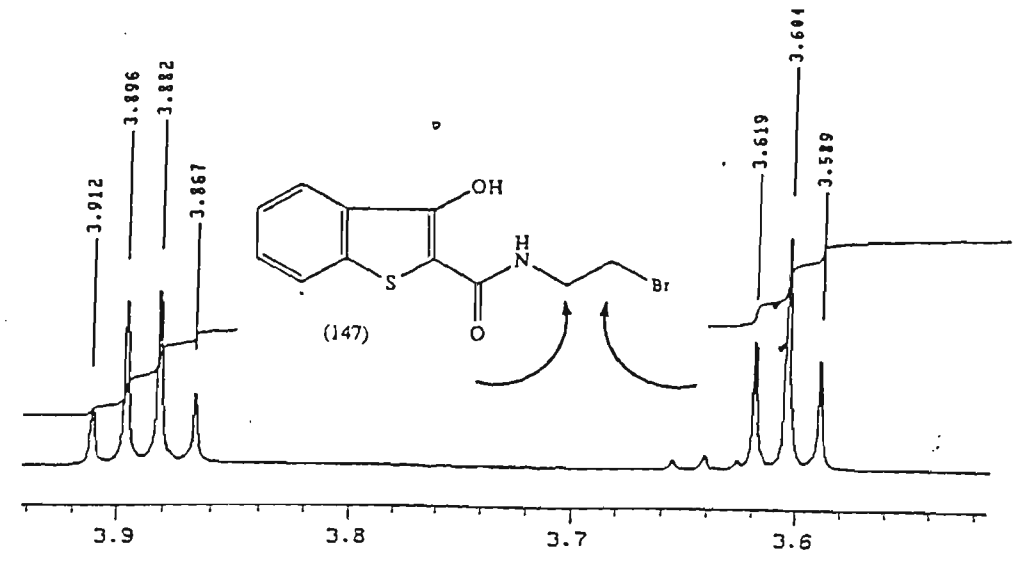
The ES+ mass spectrum of 142 showed a very strong MH^+ peak at *m/z* 220 (Figure 4.3). There were no peaks beyond *m/z* 439, excluding the formation of the dimer 159 (Scheme 4.9). In the ^1H NMR spectrum of 142, no coupling

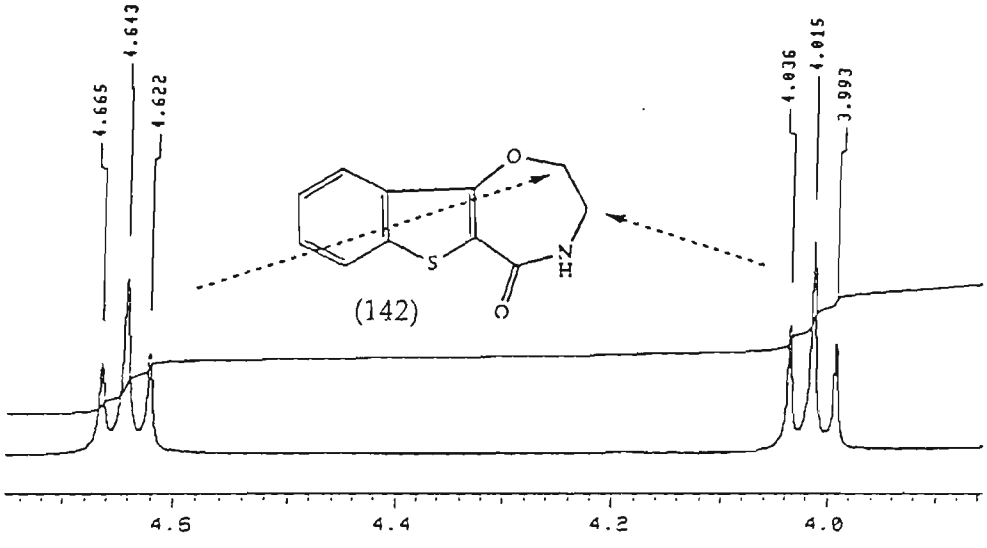
was observed between the amide proton and the adjacent protons, thus two groups of triplets at 4.65 ppm and 3.91 ppm corresponding to the methylene protons on C2 and C3 were found. In the IR absorption spectrum, an amide carbonyl band was present at 1641 cm^{-1}, slightly different from that (1616 cm^{-1}) of its precursor, the acyclic amide 154. The formation of the ring apparently reduces the extent to which the amide function is conjugated with the aromatic ring.



159 M^+ (*m/z*) 438

Scheme 4.9





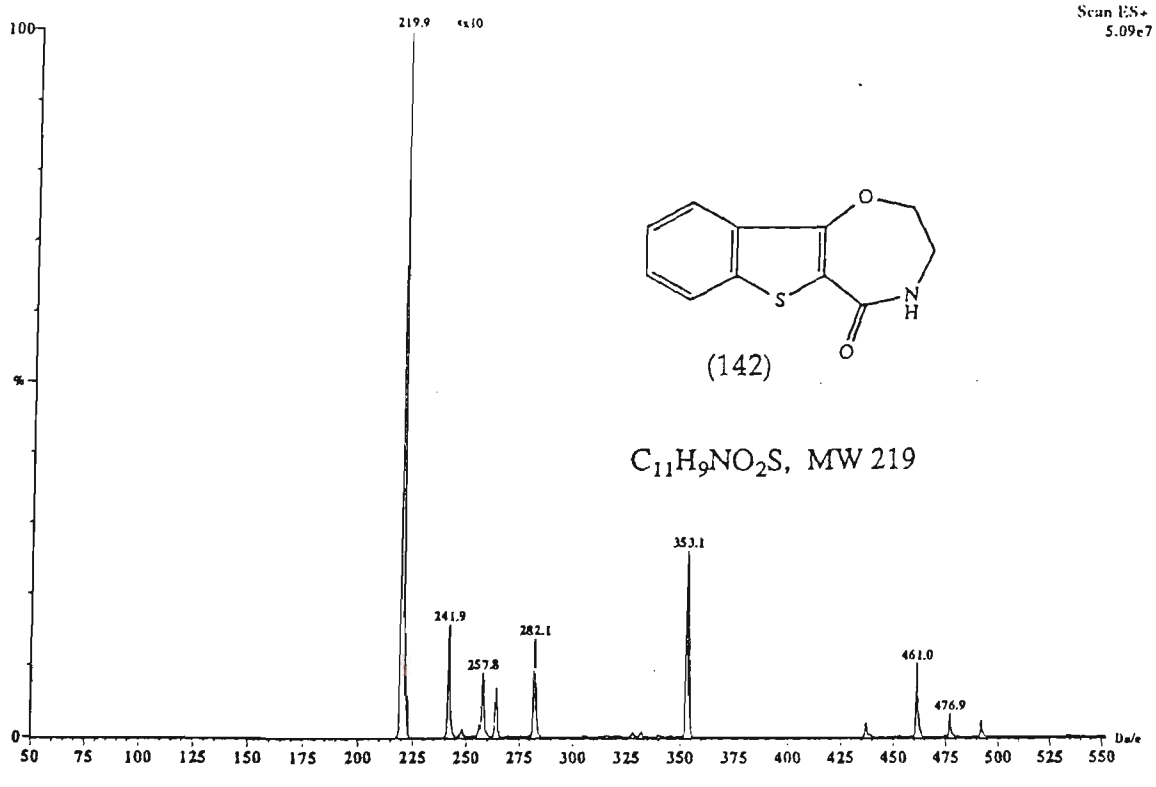
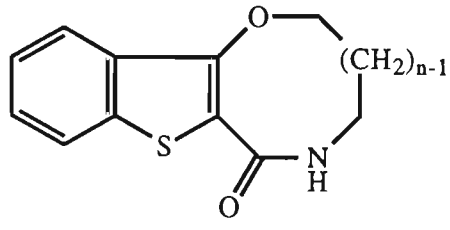


Figure 4.3 ^{1}H NMR spectra of 147 and 142 and ES+ mass spectrum of 142

4.3. Preparation of other medium ring-containing [1]benzothiophene derivatives

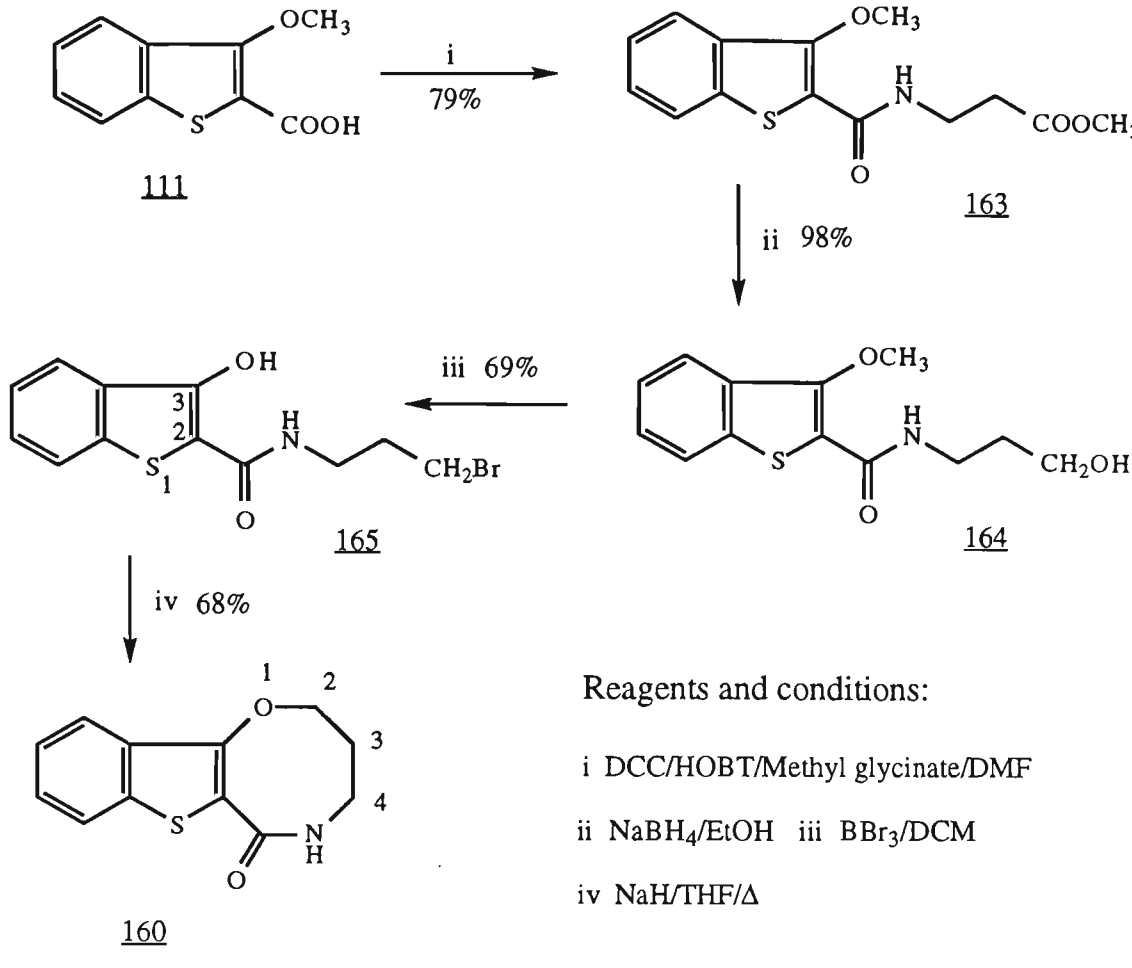
With the success of synthesis of the new 7-membered ring compound 142, extension of this methodology to other new medium ring derivatives of the type shown below was investigated. The compounds with n=3 and 4 also represent new heterocyclic systems.



n = 2, 3, 4.

n= 2, 160
n= 3, 161
n= 4, 162

To prepare the 8-membered ring compound 160, the precursor of the cyclization was obtained by a three-step reaction from the 3-methoxy acid 111 (Scheme 4.10). The 3-methoxy ester 163 was obtained by coupling the acid 111 with methyl β-alaninate in the presence of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole in DMF. The ester was then reduced by sodium borohydride in ethanol to the corresponding alcohol 164, which was converted to the 3-hydroxy bromide 165 by demethylation and concurrent bromination with boron tribromide in dichloromethane at -78°C. The bromide 165, in contrast to the analogue 154, was stable to treatment with sodium hydride in THF at room temperature, but under reflexing conditions it cyclized to afford the desired 8-membered ring compound 160 in fair yield (Scheme 4.10).

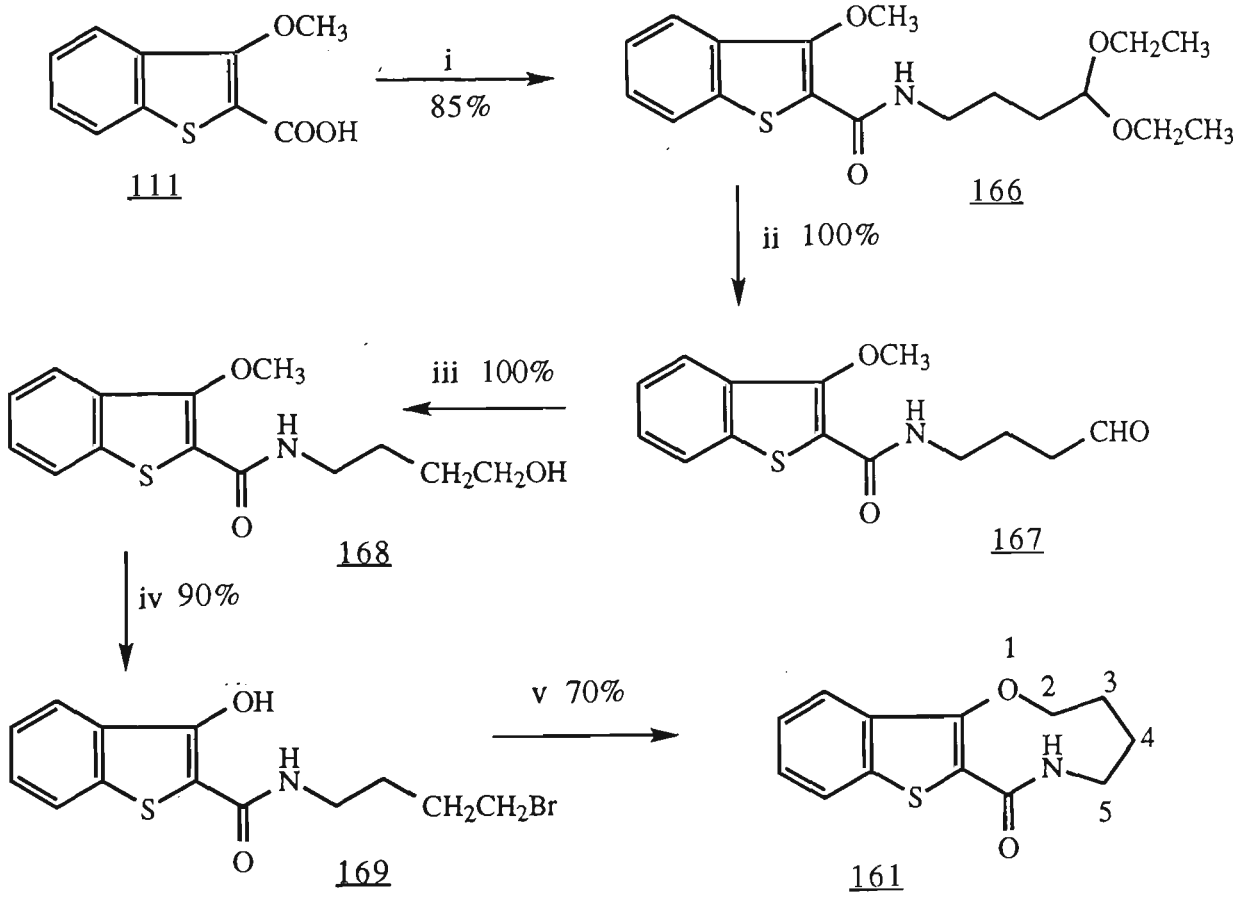


Scheme 4.10

The electrospray (ES+) mass spectrum of 160 displayed an MH^+ base peak at *m/z* 234, consistent with the structure. The ^1H NMR spectrum of 160 showed no coupling between the amide proton and its adjacent protons and as a result two triplets appeared at 4.47 ppm and 3.58 ppm corresponding to the methylene protons on C2 and C4 respectively. Its ^{13}C NMR spectrum had signals at 66 ppm and 38 ppm ascribed to C2 and C4 respectively. In the IR absorption spectrum, the band for the amide carbonyl decreased from 1640 cm^{-1} for the 7-membered ring compound to 1626 cm^{-1}, similar to the conjugated acyclic amide carbonyl of its precursor 165.

The synthesis of the 9-membered ring derivative 161 was performed using a similar route, with slight modifications (Scheme 4.11). The 3-methoxy

acid 111 was coupled with 4-aminobutyraldehyde in THF containing dicyclohexylcarbodiimide and 1-hydroxybenzotriazole to give the amide acetal 166. The acetal in acetone was quantitatively hydrolysed with hydrochloric acid to the corresponding aldehyde 167. Reduction of the aldehyde with sodium borohydride in ethanol, followed by treatment of the resultant alcohol 168 with boron tribromide in dichloromethane at -78°C, afforded the 3-hydroxy bromide 169. Upon treatment of the bromide with sodium hydride in THF at room temperature, it cyclized smoothly to the ring compound 161 in moderate yield (Scheme 4.11).



Reagents and conditions:

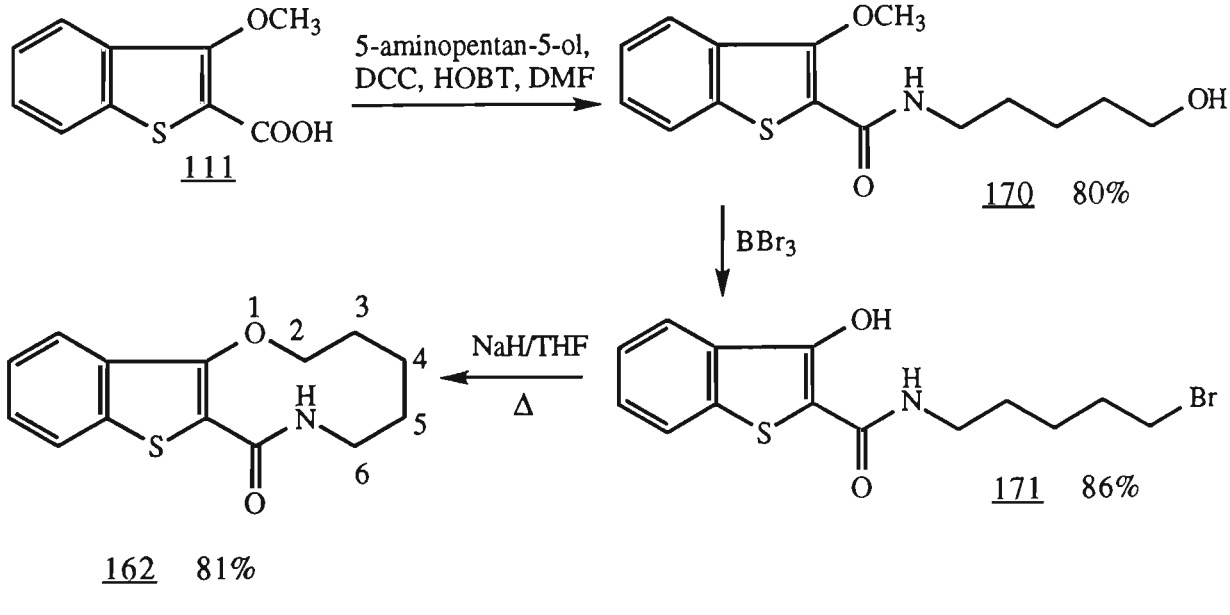
i DCC/HOBt/4-Aminobutyraldehyde/THF ii HCl/Acetone iii $NaBH_4$/EtOH

iv BBr_3/DCM v NaH/THF

Scheme 4.11

An MH^+ base peak was shown in the ES+ mass spectrum of the 9-membered ring compound 161 at *m/z* 248. In the ^1H NMR spectrum of 161, a broad singlet appeared at 3.8 ppm corresponding to the methylene protons on C2 and C5. Another broad singlet appeared at 2.1 ppm corresponding to the methylene protons on C3 and C4. The methylene protons adjacent to oxygen, however, moved upfield by 0.7 ppm compared to the corresponding signal in the 7-membered ring compound 142. In the IR absorption spectrum, an amide carbonyl absorption at 1565 cm^{-1} was observed.

The 10-membered ring compound 162 was obtained *via* a three-step sequence from the 3-methoxy acid 111. The amide alcohol 170, generated from a coupling reaction between the 3-methoxy acid and 5-aminopentan-1-ol in DMF in the presence of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole, was exposed to boron tribromide in dichloromethane at -78°C to give the 3-hydroxy bromide 171. Treatment of the bromide with sodium hydride in THF, after reflux, gave the 10-membered ring derivative 162 in good yield (Scheme 4.12).

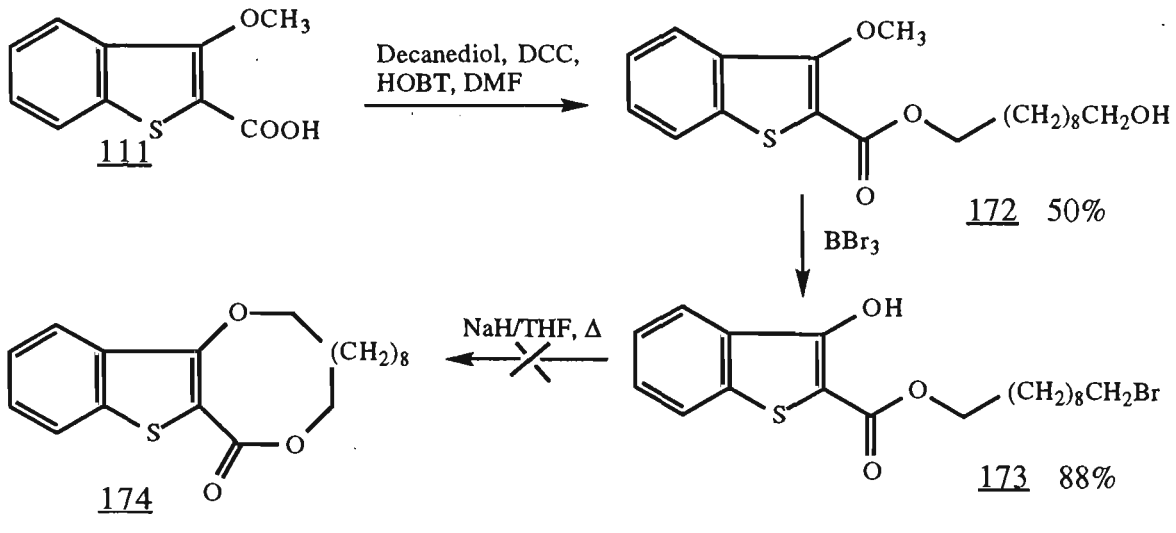


Scheme 4.12

The EI mass spectrum of 162 displayed a strong molecular ion peak at *m/z* 261. Like the 9-membered ring compound 161, the ^{1}H NMR spectrum of 162 showed only two groups of non aromatic peaks in which the methylene protons on C2 adjacent to oxygen and on C6 next to the amide nitrogen overlapped as a multiplet from 3.85 to 3.83 ppm. The other three methylene protons on C3, C4, and C5 were also unresolved appearing from 1.72 to 1.70 ppm. In the ^{13}C NMR spectrum, C2 and C6 overlapped at 46 ppm, while C3 and C5 overlapped at 26 ppm, and C4 had a signal at 24.5 ppm. The IR absorption band of the amide carbonyl group appeared at 1573 cm^{-1}, which was similar to the position in the 9-membered ring compound.

Compared to the formation of the 7- and 9-membered ring compounds, harsher conditions had to be used to form the 8 and 10-membered ring derivatives. It is believed that smooth formation of the 7- and 9-membered rings was due to favourable entropic and enthalpic effects[135].

It was hoped that this ring closure methodology could also be applied to prepare fused macrocyclic compounds. As an example, the preparation of a 15-membered ring compound 174 was attempted. The 3-methoxy acid 111 was converted to the ester alcohol 172 by the application of the coupling reaction with 1,10-decanediol. Demethylation of the alcohol by boron tribromide in dichloromethane at -78°C afforded the 3-hydroxy bromide 173. Unfortunately, no cyclization was observed upon treatment of the bromide with sodium hydride in refluxing THF for 48 hours, even with high dilution. This was presumably due to the unfavourable entropic and enthalpic factors involved (Scheme 4.13).



Scheme 4.13

4.4 Conclusion

Attempts to synthesise the seven-membered ring compound 142 *via* aromatic nucleophilic substitution using the precursors 34, 37 and 144 respectively failed. An efficient route involving cyclization of the 3-hydroxy bromide 154 *via* aliphatic nucleophilic substitution, was developed to prepare the seven-membered ring compound 142. Based on this methodology, other 8-, 9- and 10-membered ring compounds were easily prepared from the corresponding 3-hydroxy bromides. The 7-, 9- and 10-oxaza fused [1]benzothiophenes are novel ring systems. Attempts to synthesise the macrocyclic compound 174 were unsuccessful. The seven-membered ring compound 142 showed moderate activity in the 5-HT potentiation screening. This was consistent with the prediction of molecular modelling studies (*cf* Chapter 5). The methodology developed in this work should be extendable to the synthesis of a range of new aromatic and heteroaromatic ring-fused oxazepine, oxazocine, oxazonine and oxazecine systems.

Chapter 5

Structure-Activity Evaluation

5.1 Introduction

The structural modifications of the [1]benzothiophene derivatives have been described in previous chapters and some pharmacological test results of the derivatives with respect to the 5-HT system have also been noted. As a result, an important pharmacophoric element proposed for the activity according to the molecular modelling studies appeared to involve a specific intramolecular hydrogen bond, though substituents in remote positions also had an influence. In this Chapter, all the pharmacological test results are summarised, and in combination with the molecular modelling studies a pharmacophoric model is proposed with respect to 5-HT potentiation in the [1]benzothiophene derivatives.

5.2 Summary of pharmacological results

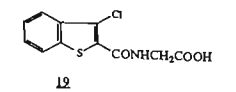
Preliminary pharmacological screening of compounds for biological activity has been used extensively in developing new pharmaceuticals, since this initial screening allows one to exclude those compounds that are inactive or unselective and select those with potential activity for more sophisticated testing. In this study *in vivo* tests were carried out by Ms Winnie Lau in the Department of Pharmacology, Monash University in collaboration with Associate Professor J. O'Neil, to evaluate the 5-HT potentiation activity of the selected [1]benzothiophene derivatives.

The activation of 5-HT receptors on the wall of the right cardioid ventrical results in a reflex stimulation of the vagus nerve eliciting a reflex bradycardia or Bezold-Jarisch effect. Bolus injection of 5-HT results in a Bezold-Jarisch-like reflex bradycardia in the anaesthetised rat and the abrupt, transient fall in heart rate is mediated by reflex stimulation of the vagus nerve following activation of 5-HT receptors on afferent fibres[136, 137]. This effect of 5-HT was used to evaluate a series of novel compounds for 5-HT uptake blocking activities in the anaesthetised rat. Peak falls in heart rate evoked by 5-HT before and after intravenous administration of test compounds can be displayed in a log-dose-response curve to 5-HT. The potency ratios with their 95% confidence limits can be calculated. The bigger potency ratio reflects stronger potentiation activity. Table 5.1 presents a complete list of the potentiation of 5-HT-induced bradycardic responses in anaesthetised rats of all tested [1]benzothiophene derivatives in the order of the potency ratio. Compounds with potency ratios greater than 2.0 were considered as potentiators with significant potentiation of 5-HT uptake inhibition, whereas those with potency ratios close to 1.0 were regarded as non-potentiators. Therefore, compounds 19, 74, 115, 31, 73, 142, 26, and 122 were classified as the 5-HT uptake potentiators, while the others were considered inactive. The 3-aminoquinuclidine derivative 29 was the only [1]benzothiophene derivative found to be a 5-HT_3 antagonist with the potency ratio smaller than 1.0.

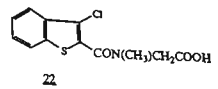
Table 5.1 Cardiovascular Pharmacological Test Results of The [1]Benzothiophene derivatives in the Rat

Compound	Potency ratio[a]	Compound	Activity
19	3.78 (1.97, 8.68)	22	inactive[c]
74	2.64 (1.09, 9.07)	53	"
24	2.39 (1.97, 8.68)	66	"
115	2.38 (1.29, 5.32)	67	"
31	2.36 (1.25, 5.15)	35	"
73	2.18 (1.08, 3.19)	45	"
142	2.06 (1.48, 3.37)	37	"
26	2.04 (1.39, 3.16)	113	"
122	2.03 (1.23, 3.58)	48	"
57	1.82 (1.03, 3.74)	119	"
52	1.82 (0.97, 3.69)	79	"
114	1.54 (1.03, 2.38)	25	"
82	1.45 (1.05, 6.65)	50	
86	1.23 (0.70, 2.26)	29	0.44 (0.18, 0.87)
56	1.21 (0.88, 1.68)	29	0.19 (0.06, 0.44)[d]
48	1.46 (0.8, 3.54)[b]		

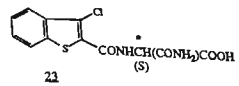
a. Data in brackets indicate 95% confidence limits at the dose of the compounds (3 mg/kg). b. No potentiation at a low dose (3 mg/kg), potency ratio obtained at a high dose (10 mg/kg). c. The potency ratio was within the range 1.20-1.00. d. Dose was 1 mg/kg .



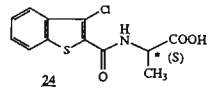
19



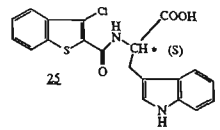
22



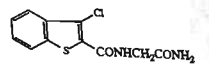
23



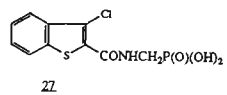
24



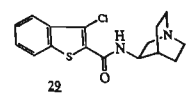
25

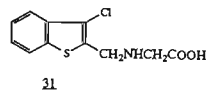


26

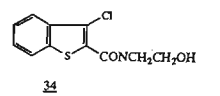


27

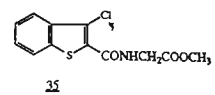

29



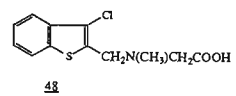
31



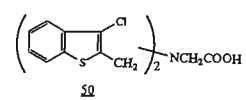
34



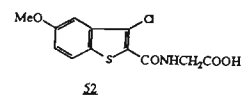
35



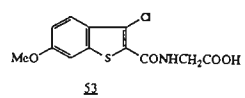
48



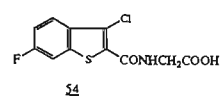
50



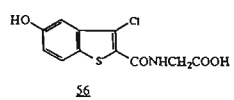
52



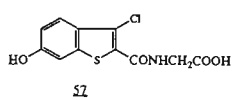
53



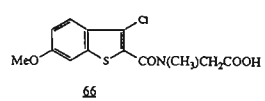
54



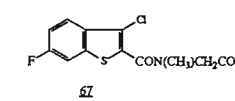
56



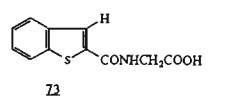
57



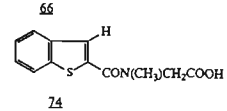
66



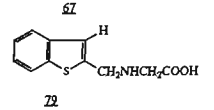
67



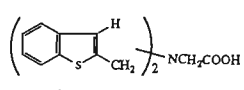
73



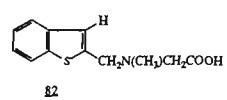
74



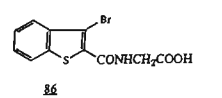
79



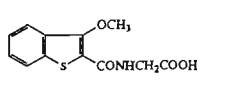
80



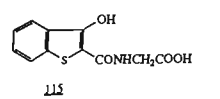
82



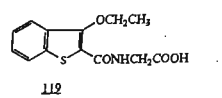
86



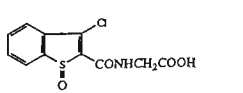
114



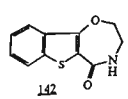
115



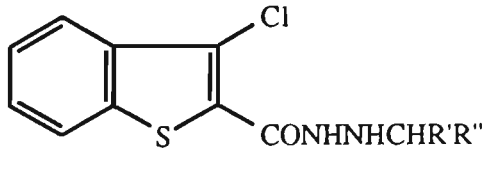
119



122


142

The well known 5-HT uptake inhibitor, fluoxetine 9 (1 mg/kg iv) enhanced the bradycardic effects evoked by 5-HT in the anaesthetised rat[138] and the potency ratio and 95% confidence limits were calculated to be 2.39 (1.21, 6.01). With the [1]benzothiophene derivatives, for example, the effect of 73 on the bradycardic responses to 5-HT is illustrated in Figure 5.1 (a). The compound 73 seemed to show an enhancing effect as the fall in heart rate steadily increased throughout the administration of 5-HT (3 μg-30 μg) in the presence of 73 (2.18, 1.08/3.19) (3 mg / kg). Although it is moderate in comparison with fluoxetine (2.39, 1.21/6.01) (1 mg /kg), it could suggest that the compound may be acting as a 5-HT uptake inhibitor in the anaesthetised rat. In contrast, the administration of 22 resulted in no bradycardic responses as shown in Figure 5.1 (b), indicating that 22 is inactive. Figure 5.1 (c) shows the compound 29 attenuated 5-HT-induced bradycardic responses in a dose-related manner, giving rise to the rightward shift in contrast to the leftward shift caused by 73 as a potentiator, implying it as an antagonist at the 5-HT_3 receptor. The active potentiators shown in Table 5.1 did not show any 5-HT_3 receptor agonist or antagonistic activity, and did not potentiate the activity of the 5-HT agonist, phenylbiguanide. Also the known MAO-type A inhibitors[139] of type 175 did not potentiate the action of 5-HT in this cardiovascular screen. As a result, the active [1]benzothiophene 5-HT potentiators are most likely blocking uptake of serotonin.



R'=H or alkyl group
R"=aromatic group

175

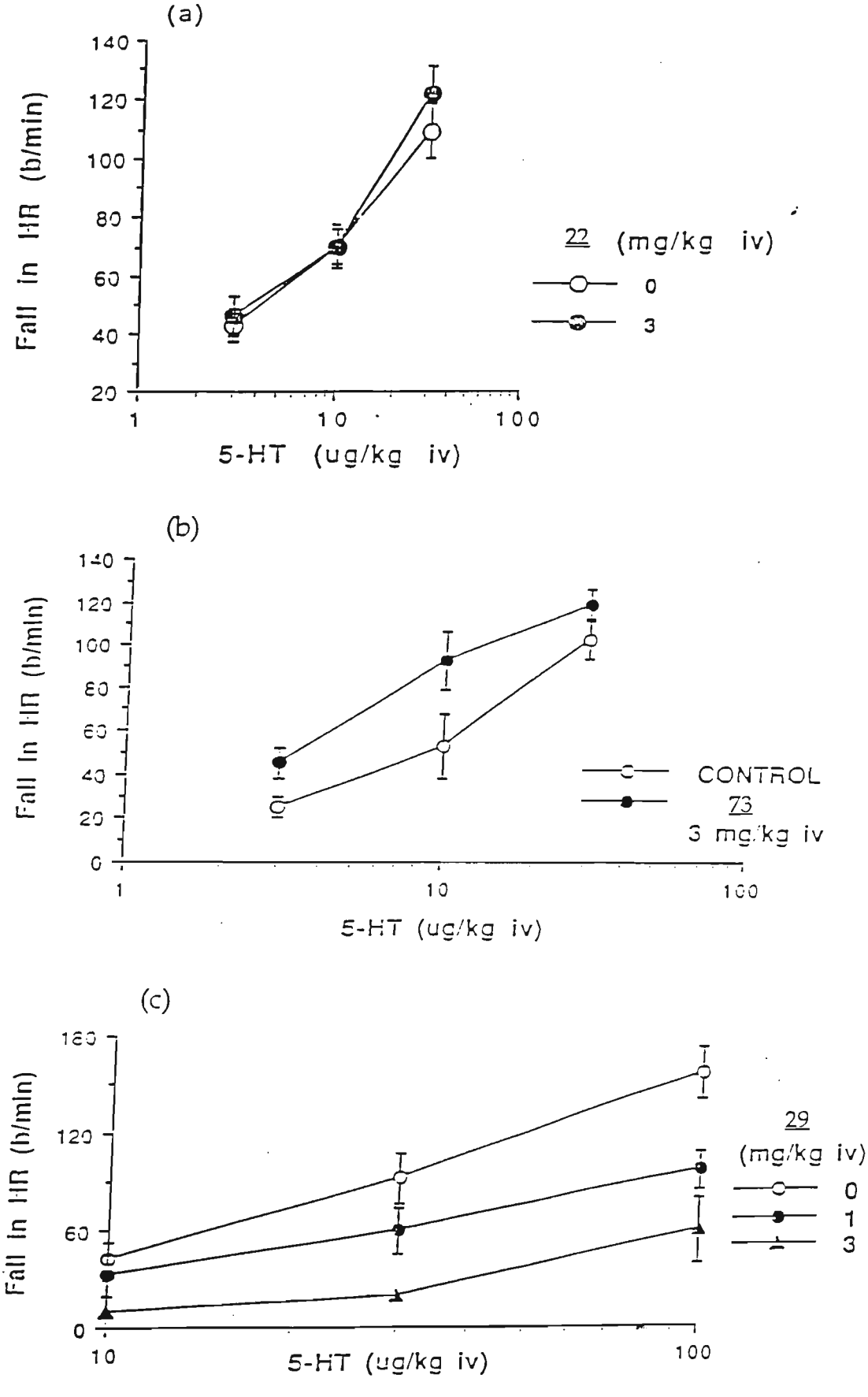
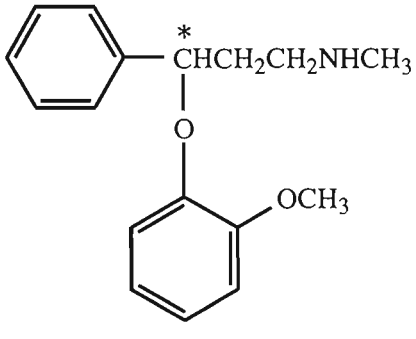
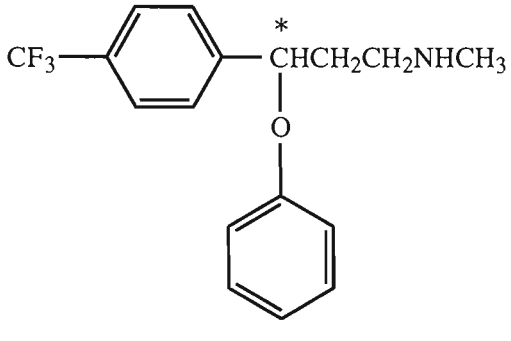


Figure 5.1 5-HT-induced Bezold-Jarisch effect in anaesthetised rats with compounds 22, 73, and 29

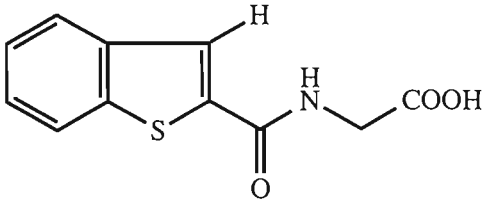
In order to determine whether the [1]benzothiophene compounds had any influence on uptake of other neurotransmitters, a brief study of compounds 73 and 31 on noradrenaline (NA) system was undertaken in comparison with the selective NA uptake inhibitor nisoxetine[140, 141] and the selective 5-HT uptake inhibitor fluoxetine in the rat bisected *vas deferens* preparation.



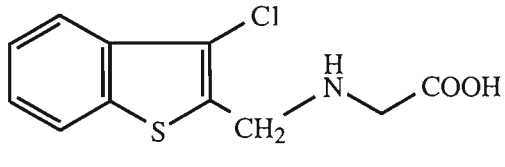
Nisoxetine



Fluoxetine



73



31

Neuronal uptake is the major mechanism of loss of noradrenaline released by field stimulation or to applied noradrenaline to the rat *vas deferens*. Prejunctional α_2-adrenoceptors mediate inhibition of electrically-evoked contractions in the prostatic segments of the *vas deferens*; while postjunctional α_1-adrenoceptors mediate the contractions of the epididymal segments. Thus, the bisected *vas deferens* of the rat offers a very useful preparation for screening the effects of novel compounds acting at α-adrenoceptors and affecting neuronal uptake.

Figure 5.2 (a) and 5.3 (a) show that concentration-response curves to NA were shifted leftwards by the presence of nisoxetine, indicating that nisoxetine potentiated NA responses in the rat isolated *vas deferens* possibly by inhibiting NA uptake. Figure 5.2 (b) and 5.3 (b) show that concentration-response curves to NA were not significantly affected by 73 (0.01 - 1 μM). Responses to NA in the prostatic segments were slightly enhanced in the presence of 10 μM 73. Figure 5.2 (c) and 5.3 (c) show that concentration-response curves to NA were not significantly modified by 31 (0.01 and 0.1 μM). However, 31 (1 μM) shifted the NA curve to the left (Figure 5.2 (c)), indicating that it may potentiate NA responses in the prostatic segments at higher concentration. Finally, Figure 5.2 (d) and 5.3 (d) show that the selective 5-HT uptake inhibitor fluoxetine was without effect on responses to NA in the rat bisected *vas deferens* preparation. So it is believed that both the compounds 73 and 31 neither block neuronal uptake of NA nor activate α_1 or α_2-adrenoceptors in the rat isolated *vas deferens*. It is worth noting that fluoxetine also lacks these effects. In contrast the selective NA uptake inhibitor nisoxetine markedly enhances responses to NA.

Figure 5.2 Epidymal segments: NA-induced contractions and effects of nisoxetine, 31, 73 and fluoxetine

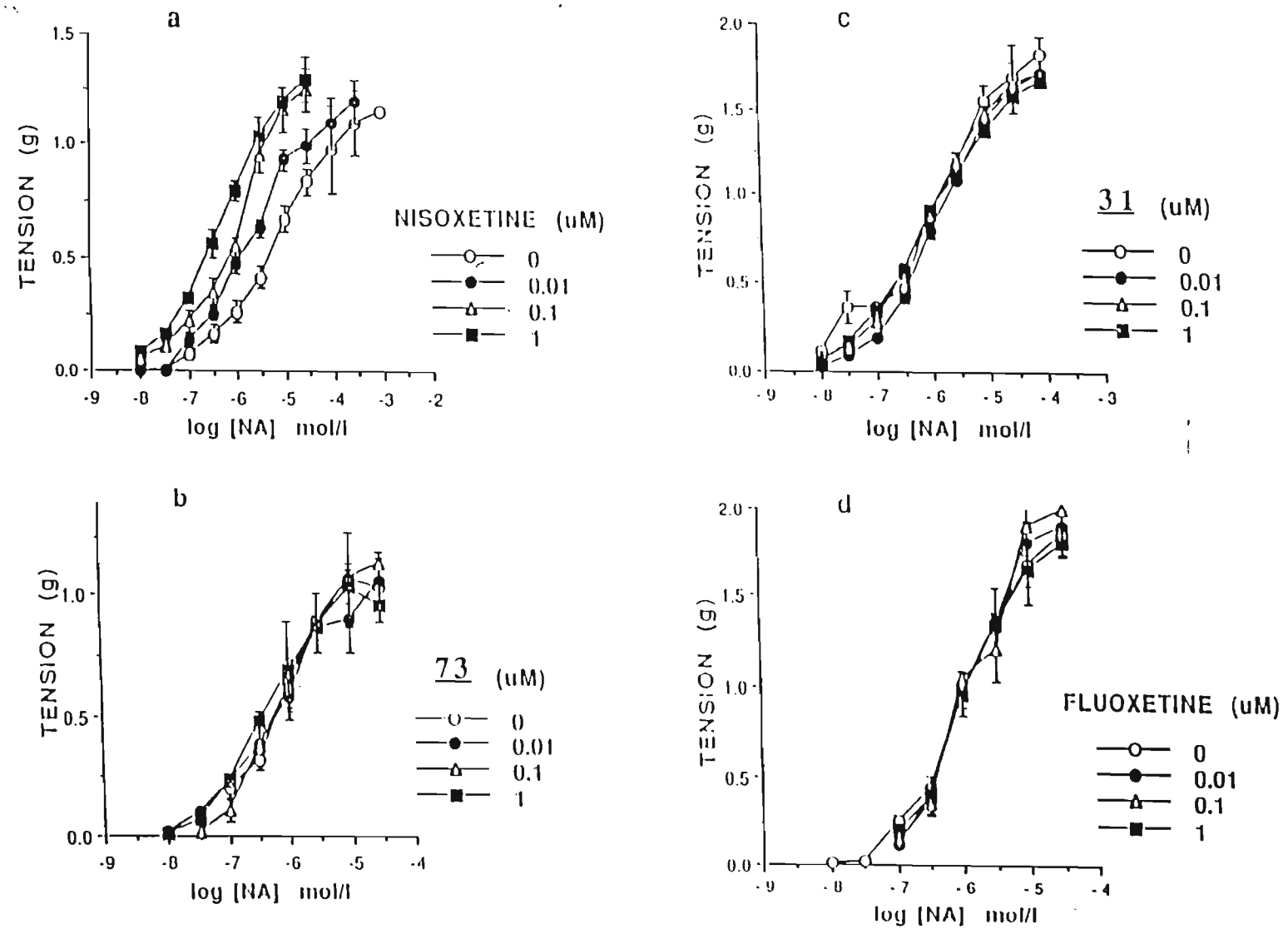
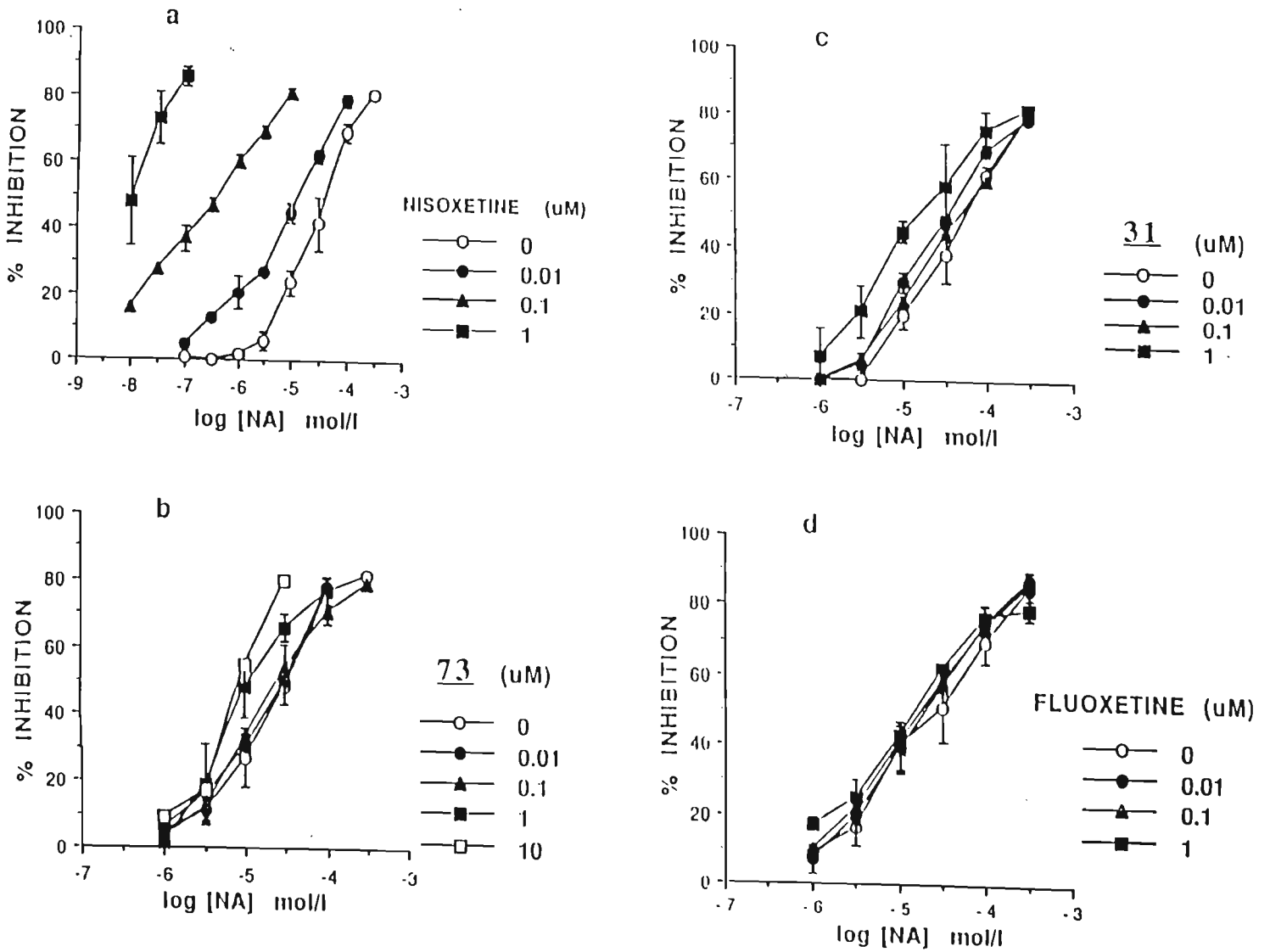


Figure 5.3 Prostatic segments: Inhibition by NA of electrically-evoked contraction



5.3 Computer-aided molecular modelling (CAMM)

5.3.1 Introduction

The cost of bringing a new drug to the market can nowadays amount to many millions of dollars, so any technique that can rationalise the drug design process, and thus reduce the number of compounds to be synthesised therefore is in high demand. Computer-Aided Molecular Modelling (CAMM) is a powerful tool used in this process to help explain and/or predict a variety of molecular properties, and hence to reduce the period of development for new pharmaceutical products by rationalising the drug design approach.

Molecular modelling studies can be approached from two perspectives: i) manipulations involving only the ligands, known as the ligand-ligand approach; ii) modelling interactions between a ligand and receptor macromolecules known as the ligand-receptor approach (Figure 5.4). The former is currently the most frequently employed. This approach attempts to infer information about the macromolecular binding site, and/or modes of binding interactions from comparing the structural features and experimentally determined biological activities of a series of small molecules. The structural features considered include steric attributes (van der Waals surface, solvent accessible surface, hydrophobicity/hydropilicity), flexibility, electronic properties such as potential fields (electrostatic potentials, probe atom affinities) properties from quantum mechanical calculation (semiempirical, *ab inito*) point atomic charges, shapes and energies of molecular orbitals. This method does not directly consider the structure of the binding site. The ligand-receptor approach is used much less frequently because it requires a working knowledge of the receptor structure, which has so

far been very limited. Although this approach has been applied successfully to model the interactions between small molecules and soluble proteins for which detailed structures are available from X-ray crystallographic studies, very few examples of the ligand-receptor approach for neurotransmitter receptor systems have been reported[44]. As far as the serotonin research is concerned, studies have been conducted using the ligand-ligand approach for modelling the 5-HT_{1A}, 5-HT_2 and 5-HT_3 receptors. No studies, however, have been done for the serotonin system using the ligand-receptor approach. In this project the ligand-ligand method was used

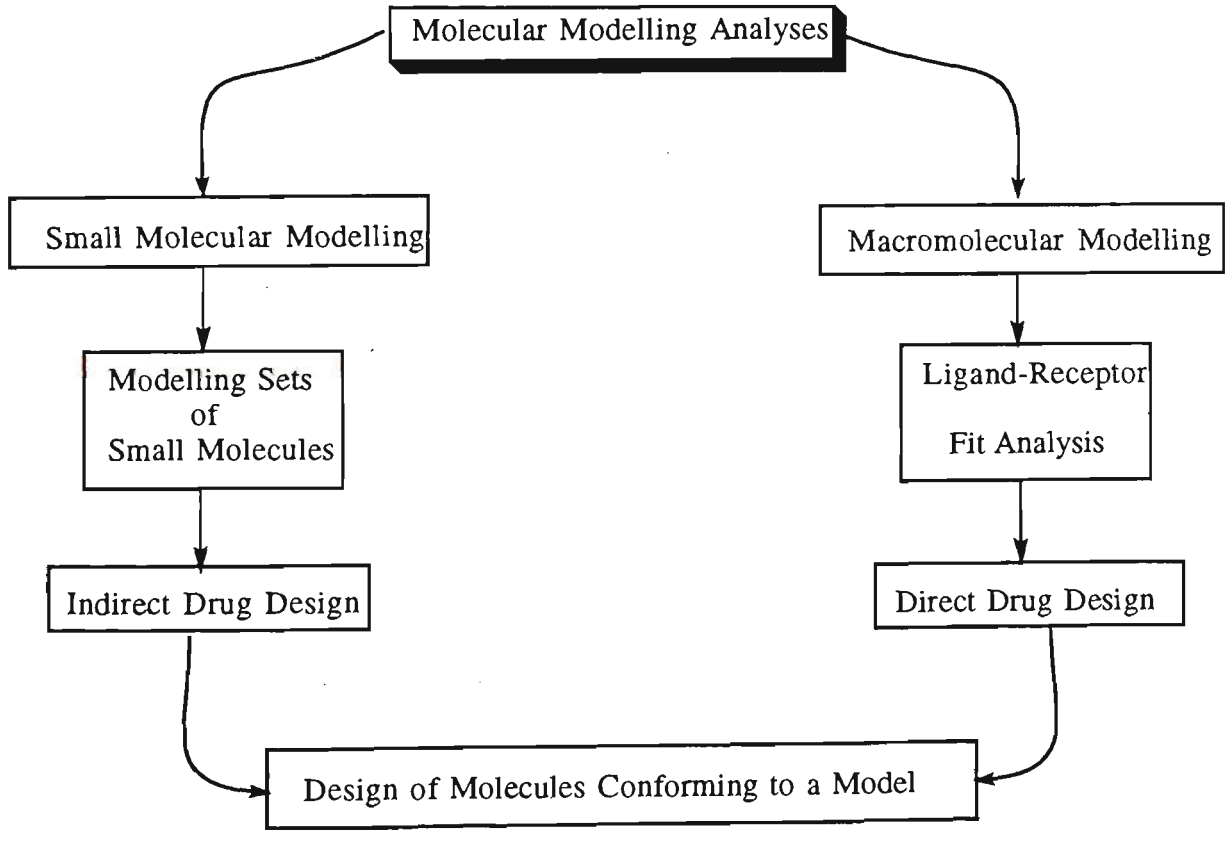
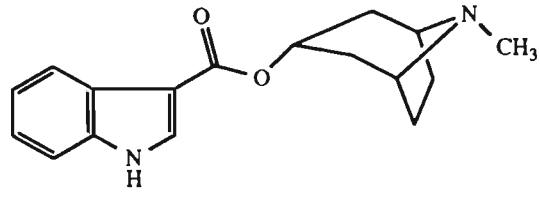


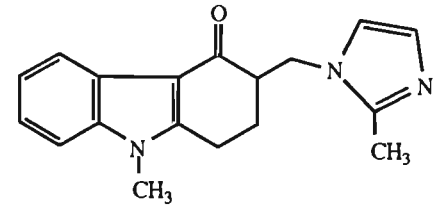
Figure 5.4 Molecular modelling approaches in drug design

5.3.2 The application of molecular modelling in the development of 5-HT_3 antagonists.

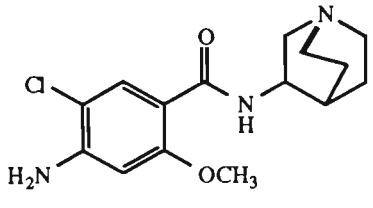
Research on 5-HT_3 receptor ligands is one of the major areas of the serotonin related research, and as a result a large number of 5-HT_3 antagonists have been discovered. Molecular modelling has been instrumental in the recent development of a certain number of 5-HT_3 antagonists. Although the application of molecular modelling to help develop 5-HT_1 and 5-HT_2 ligands has also been reported, most of work has focused on 5-HT_3 receptor antagonist[44]. Rizzi and coworkers[42] modelled three reported 5-HT_3 receptor antagonists ICS-205-930, Ondansetron and Zacopride, and 3-[2-(guanidinylmethyl)-4-thiazoly] indole 176 using QUANTA and CHARMM. These gave a hypothetical model for a specific 5-HT_3 receptor binding site involving a three-component pharmacophore containing a hydrogen-bond-acceptor, a hydrogen-bond-donator and the occupancy of a specific plane by a lipophilic aromatic ring. Hibert and associates[45] examined the structural similarities of known active 5-HT_3 antagonists by overlaying their global minimum conformations. The results suggested a pharmacophore that contained three reference structural features: an aromatic ring, a carbonyl group and a basic atom or centre (Figure 5.5). Furthermore, from this they designed the compound (1H-indol-3-yl) (4-piperidinyl) methanone 177, which satisfied all three requirements of the proposed pharmacophore. This compound displayed a relatively high potency in the Langendroff assay and was only slightly less potent than previously described antagonists in spite of its much simpler structure. In addition, Hibert's hypothetical pharmacophore has received support from the results of Evans and coworkers[46]. They included 15 known 5-HT_3 antagonists to conduct the modelling studies. Their findings suggested that Hibert's model is relatively accurate and may represent a versatile working hypothesis that could easily lead to the design of new original putative 5-HT_3 receptor antagonists.



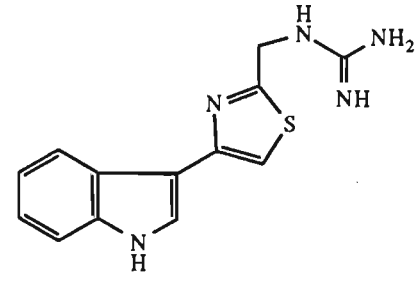
ICS-205-930



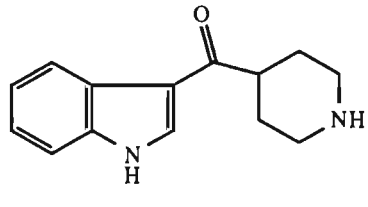
Ondansetron



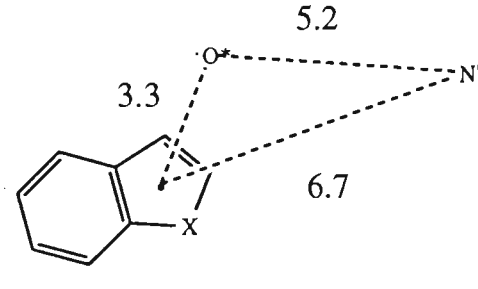
Zacopride



176



177



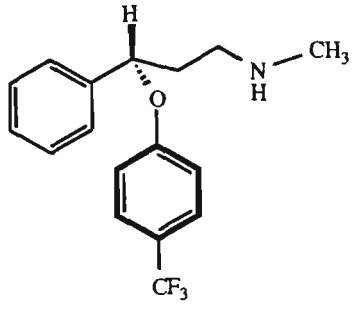
* carbonyl functionality;
\# amine functionality

Figure 5.5 Selected 5-HT3 antagonists and proposed pharmacophoric model

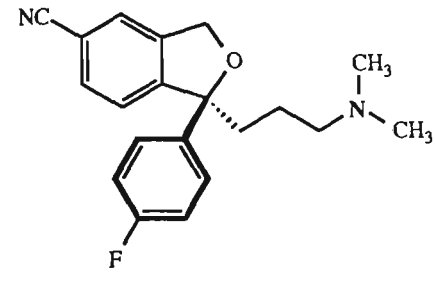
5.3.3 The application of molecular modelling to 5-HT uptake inhibitors

Although there has been a relatively large number of publications concerning molecular modelling-aided design and development of 5-HT antagonists, an extremely limited number of examples dealing with molecular modelling in the development of 5-HT uptake inhibitors are known. Recently, Gundertofte and associates[47] have proposed a common pharmacophoric model containing an aromatic ring and an amine on the basis of conformational analyses of the known active 5-HT uptake inhibitors such as citalopram, fluoxetine, sertraline and paroxetine, as shown below. In addition, the

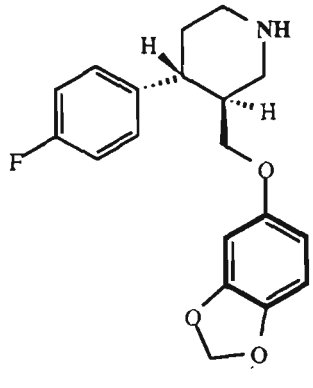
superimposition of citalopram, fluoxetine, sertraline and paroxetine sertraline has shown that the cyano, trifluoromethyl, chloro and dioxolo substituents in the four compounds, respectively, occupy the same region in space.



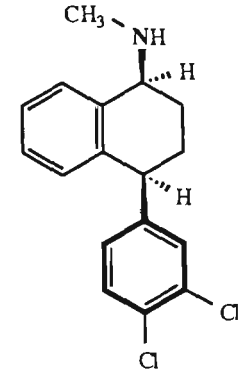
Fluoxetine



Citalopram



Paroxetine



Sertraline

5.3.4 Methods

As mentioned earlier, in the ligand-ligand approach, molecular modelling generally involves structure building, visualisation and manipulation of three-dimensional (3-D) molecular models, molecular mechanics and/or dynamics, conformational analysis and superimposition of a series of molecules. Throughout this study all molecular modelling work was performed on a Silicon Graphics IRIS Indigo workstation using a Biosym software package, i.e. Insight II viewer module (Version 2.2) and Discover module (Version 2.9.5).

Both conformational analysis and molecular superimposition are based on the assumption that molecules must adopt certain shapes before 'docking' into their sites of action. This shape or conformation must be energetically favourable. In general, it is believed that such a conformation has a very close resemblance to the conformation with lowest energy known as global minimum conformation. Therefore, an efficient and reliable method to search for the global minimum energy conformations was examined first. Both Systematic Searching and Molecular Mechanics were chosen to carry out the conformation searching.

5.3.4.1 Determination of the global minimum conformations by systematic searching

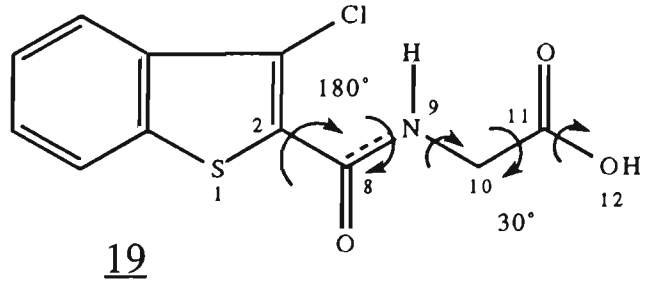
Reliable predictions of molecular structures, energies and properties by molecular modelling are often hindered by the fact that the potential energy surfaces of molecules are complex and have more than one energy minimum. Therefore, in order to be able to map potential energy surfaces and locate the global minimum, all possible conformations must be sampled. This search for the global minimum is commonly referred to as complete conformation analysis. But the global minimum in one forcefield will not necessarily be the global minimum in another forcefield. To prevent neglecting any important conformations, a technique referred to as systematic searching has been introduced[141]. Systematic Searching allows all rotatable bonds to rotate to include all possible conformations of low energy and finally provides a limited number of conformations including one with global minimum energy. This conceptually straightforward method unfortunately only applies to small flexible molecules with less than 7 rotatable bonds, due to it being too impractical timewise for a molecule with 7 or more rotatable bonds. This is

illustrated in Table 5.2[142]. An example using this method to search for the global minimum conformation is illustrated below. The majority of the conformational searches in this project, however, was conducted in *vacuo* using molecular mechanics, since it was found that they yielded the same global minimum as systematic searching.

Table 5.2 Conformer Numbers Generated Using Systematic Searching in Insight II

Rotatable Bonds	Number of Conformations*	Time to generate
1	12	0.5 sec
2	144	7.2 sec
3	1730	86 sec
4	20740	17.3 mins
5	249000	3.5 rhs
6	2986000	41.5 rhs
7	35832000	20.7 days
8	430000000	249 days
9	5160000000	8 years
10	61900000000	98 years

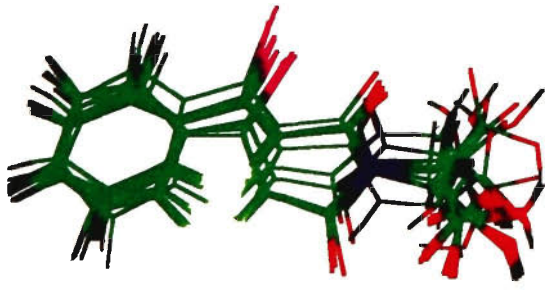
* Based on 30° increments of bond rotation.



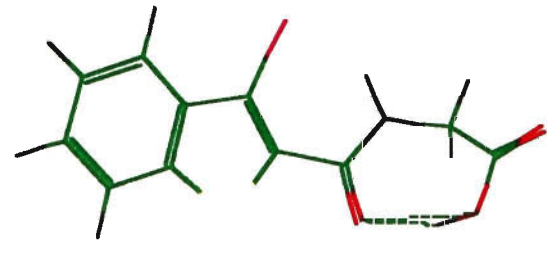
19

A 2-dimensional structure of 19 was sketched and then converted to its 3-dimensional one. Five rotatable bonds were defined, in which bond-8 and bond-9 were confined to rotation by 180° increments due to their geometrical restriction whereas the remaining three bonds (bond-10, 10-11 and 11-12) were

allowed to rotate by 30° increments. The first round of the conformation search gave 6912 conformations. A subsequent search, restricting the number of conformations by applying an energy threshold reduced conformations to 100. The 100 conformations were subjected to a further conformation search, minimising each acceptable conformation to a maximum derivative of 0.01 Cal/mol (duplicates were defined as having the same within energy 0.01 Cal and on superimposition of all atoms a root mean square (RMS) deviation of below 0.01 *A*) to automatically remove any duplicate structures. Forty eight conformations were finally found and the one with the lowest energy was selected and saved separately (Figure 5.6). It should be pointed out that although the lowest energy conformation is not necessarily the binding conformation, it would be expected that the energy of the binding conformation will not be much greater than that of the global minimum, unless the gain in energy obtained from binding outweighs the loss in energy necessary to force the molecule into the required conformation[42].



48 minimised conformations of (19)



The global minimum conformation of (19)

Keys to the figures in colours: green for carbon; black for hydrogen; red for oxygen; pink for halogen; purple for nitrogen and yellow for sulfur

Figure 5.6 Forty eight minimised conformations and the global minimum conformation of (19) obtained using systematic searching

5.3.4.2 Determination of the global minimum conformations using molecular mechanics

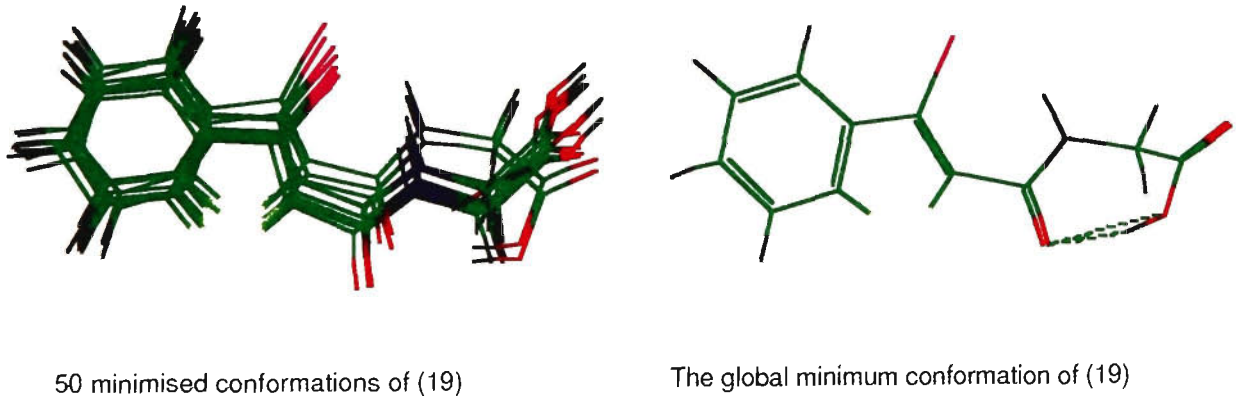
The conformational search of the majority of compounds in this project was achieved using molecular mechanics consisting of minimisation, dynamics and annealing. A protocol edited within InsightII for the conformational search is shown in Appendix I. The compound 19 is exemplified for this search using the protocol.

The 3-D structure of 19 was optimised simply by using Optimise under Discover. The dielectric constant was set to 1.0, but could be changed if required. The next series of commands instructed Discover to carry out an initial minimisation on the molecule to relieve any strain before the dynamics was started. This minimisation invoked 100 steps of steepest descents iteration followed by 100 steps of conjugate gradients iteration until the maximum derivative was less than 0.001 kcal/mol. The dynamics was initialised at 900 K for 1 ps (1000 steps of 1 fs) and coordinates were written 10 times during this initialisation process. A loop was started and the first step in the loop was to resume the dynamics at 900 K for 1 ps, then save the coordinates to an archive file, resume the dynamics for another step, and so on for a total of 50 cycles.

Initialise and *Resume* were two distinct phases during a dynamics run. During the initialise phase the system was raised to and partially equilibrated at the target temperature. During the resume phase the temperature and the energy remained relatively constant. The initial dynamics was carried out at 900 K to include major conformational changes. Finally the 50 structures collected after each 1 ps of dynamics at 900 K were annealed to prevent structures getting caught in high energy minimum if they were minimised

directly. A molecule at a high temperature was gradually cooled down to a lower temperature in this process. The cooling in all experiments throughout the project was approximated by a single temperature drop from 900 K to 300 K, at which the molecules were allowed to equilibrate for 5 picoseconds. As a whole the conformational searches were completed within a few hours, but a background job was usually set up overnight to save time.

All fifty low energy conformations could be displayed under Discover/Conformer display. The most important conformation, the global minimum conformation with the lowest energy, however, was separately saved (Figure 5.7).



50 minimised conformations of (19)

The global minimum conformation of (19)

Figure 5.7 Fifty minimised conformations and the global minimum conformation of (19) obtained using molecular mechanics

5.3.5 Analysis of the global minimum conformations

Conformational analysis of organic molecules which show biological activity is often used in drug design since it may establish a common feature that could be responsible for the activity. The global minimum conformations of the selected [1]benzothiophene derivatives are shown in Appendix II. It is

clear that all glycine derivatives shared a distinctive conformation, in which an intramolecular hydrogen bond was formed between the amide oxygen and the hydroxyl group of the carboxylic acid, so specifying the region occupied by the most polar part of the molecule. Another striking feature among the glycine derivatives was that the aromatic ring and the amide functional group lay in the same plane (Figure 5.8). The *N*-methylated 3-chloro glycine analogue 22, however, did not have this coplanarity, because the incorporation of the methyl on the amide nitrogen reduced the extent to which the amide was conjugated with the aromatic ring. Such non-coplanarity was also seen in the case of the *N*-methyl 3-hydrogen glycine derivative 74. Figure 5.9a and 5.9b show the side views of 22 and 19 respectively. It is obvious that the oxygen of the amide carbonyl of 22 was out of the plane, whereas in 19 it stayed within the plane. A longer C-N bond of 22 and 74 (1.38 Å) was therefore observed in comparison with that in fully conjugated amide 19 (1.34 Å).

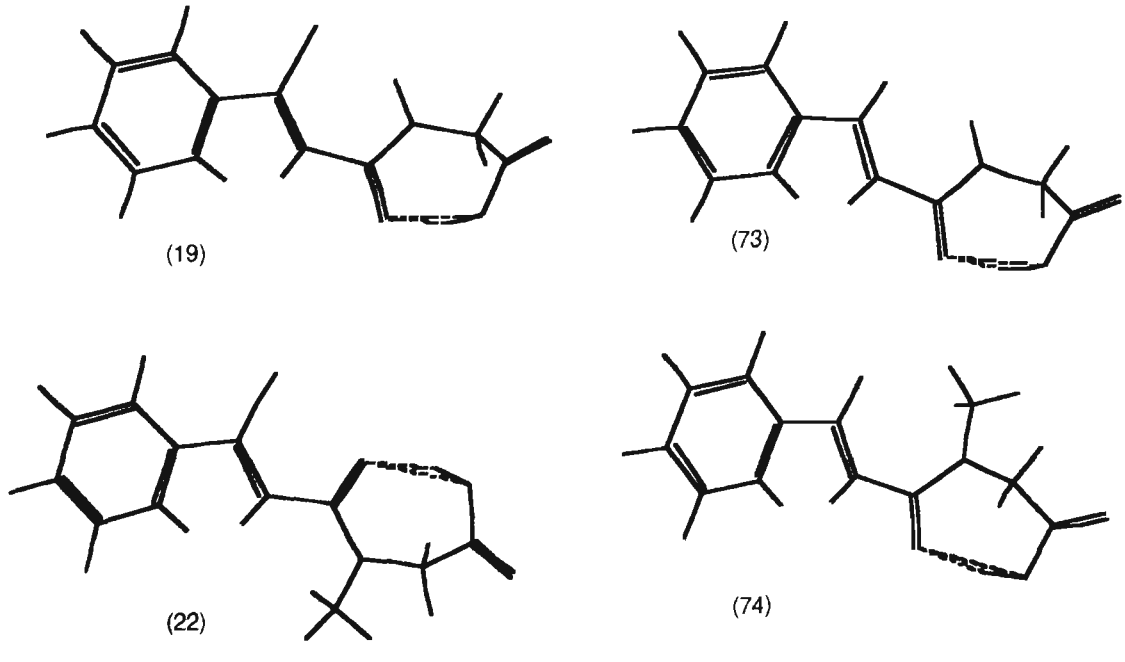


Figure 5.8 The global minimum conformations of 19, 22, 73 and 74

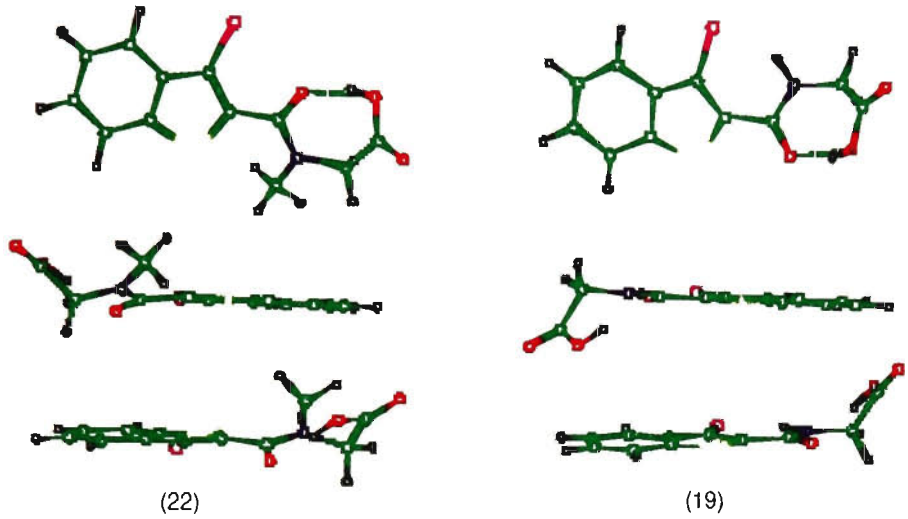


Figure 5.9 Side views of the global minimum conformations of 22 and 19

It is interesting that although both the *N*-methyl derivatives 22 (3-Cl) and 74 (3-H) differed only with respect to the atom attached to the 3-position, their global minimum conformations contrasted considerably. The latter (3-H) had the downwards intramolecular hydrogen bond as in the glycine derivatives, implying that 74 could be active in inhibiting of 5-HT uptake if such a hydrogen bonding was essential. The former (3-Cl) possessed an upwards intramolecular hydrogen bond. The pharmacological test results were in accordance with the prediction that possession of the downwards H-bond conformation could result in potentiation, since 19, 73 and 74 showed 5-HT potentiation and 23 did not.

As it was postulated that there might be some interaction between the 3-substituent and the amide nitrogen, conformational analysis of the glycine derivatives with bromo, methoxy, ethoxy and hydroxy respectively at the position-3 was investigated to help evaluate the structure-activity relationship. The bromo compound 86 possessed the same conformation as the chloro compound 19, but it exhibited only a third of activity of 19. It is believed that

the bigger atomic size and lower electronegativity of the bromine atom might be responsible for this fall in activity. From the global minimum conformations, the methoxy and ethoxy compounds 114 and 119, showed hydrogen bonding between the oxygen of the 3-methoxy or 3-ethoxy group and the amide proton (Figure 5.10). The hydrogen bond was expected to help enhance the potentiation. The 3-methoxy derivative, however, showed significantly lower activity than the 3-chloro derivative 19, and the 3-ethoxy derivative showed no potentiation. The 3-hydroxy compound 115 did show activity. The difference in activity of the hydroxy, methoxy and ethoxy compounds is interesting, as they have similar global minimum conformations. It should be pointed out, though, that the 3-hydroxy compound 115 was water soluble, and the 3-methoxy compound 114 was water insoluble. Whether the solubility of the compounds was partly responsible for the enhancement of the potentiaton of 115 remains unclear, but the size of the substituent is also believed to be a major factor.

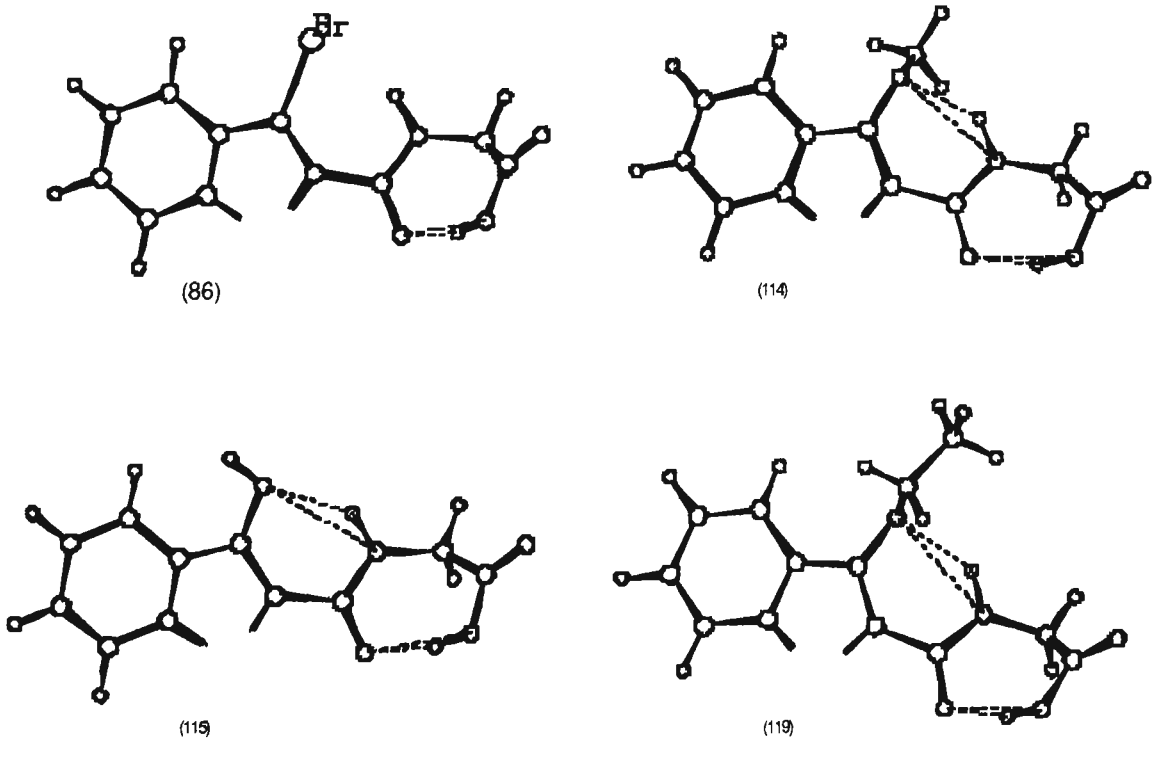
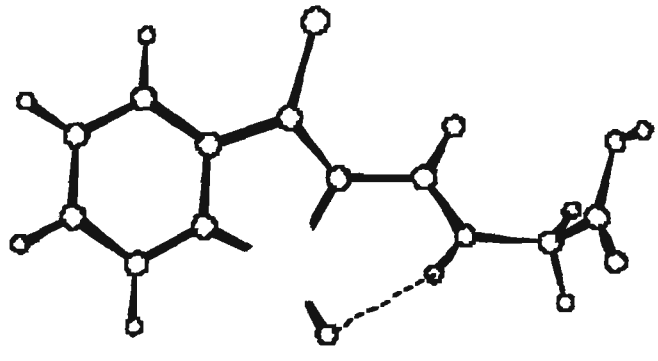
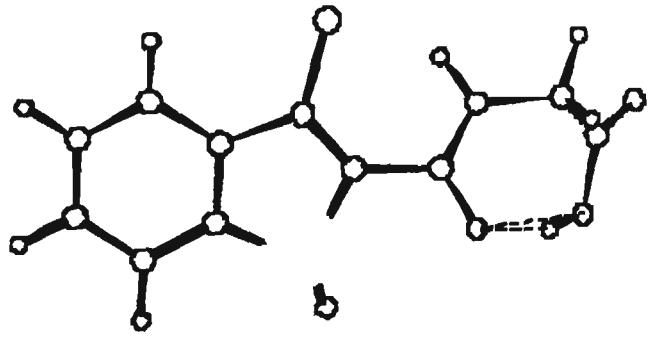


Figure 5.10 The global minimum conformations of 86, 114, 115 and 119.

The global minimum conformation of the sulfoxide 122 did not have the same type of hydrogen bond as the glycine derivative 19. Instead it showed a strong intramolecular hydrogen bond between the sulfoxide oxygen and the amide proton. Another low energy conformation, however, showed a hydrogen bond similar to that of the glycine derivative 19 (Figure 5.11). It is believed the energy compensation on binding to receptors was bigger enough to overcome such a small energy gap (1.9 kcal/mol) and this conformation should be taken into consideration. This may explain the fact that 122 displayed a moderate activity but not as strong as its precursor 19.



Global minimum conformation, 25.9 kcal/mol



Near global minimum conformation, 27.8 kcal/mol

Figure 5.11 The global minimum conformation of the sulfoxide 122 and its near global minimum conformation

The molecular modelling results found that the 5- or 6-substituted compounds still possessed the required global minimum conformations (*cf* Appendix II). The activity of compounds bearing 5, or 6-methoxy and 5-fluoro (63, 61 and 62) dramatically decreased, though both the 5 and 6-hydroxy compounds (68 and 69) exhibited some activity possibly due to the combination of smaller size of the substituents and their good solubility in water. The results may suggest the substituents at 5 and 6-position have acted as blocking groups.

Although the molecular modelling results indicated other amino acid derivatives did not possess the above mentioned conformation (*cf* Appendix II) and as a result they were predicted to be either inactive or slightly active, it was of interest to experimentally demonstrate this prediction. Compounds 23, 25, 27, 50, and 80 showed no significant potentiation, as summarised in Table 5.1, in contrast 24 with the required conformation showed 5-HT potentiation. It is believed that any substituent other than hydrogen on carbon attached to amide nitrogen could block the potentiation property.

As mentioned before, the amide carbonyl was reported to be an essential part of an active 5-HT_3 antagonist due to the coplanarity between the aromatic ring and the amide moiety. It was of interest to see whether this criterion applied in the case of the 5-HT potentiators. Though structurally the methylene amines (31, 48, 79 and 82) lacked such coplanarity, molecular modelling results indicated the intramolecular hydrogen bond between the nitrogen and hydroxy of the carboxylic acid in lower position (Figure 5.12). The positive testing results are believed to have further supported the hypothesis. The low activity of 48 in low concentration (3 μg/kg) may be attributed to the weaker hydrogen bond in a tertiary amino acid.

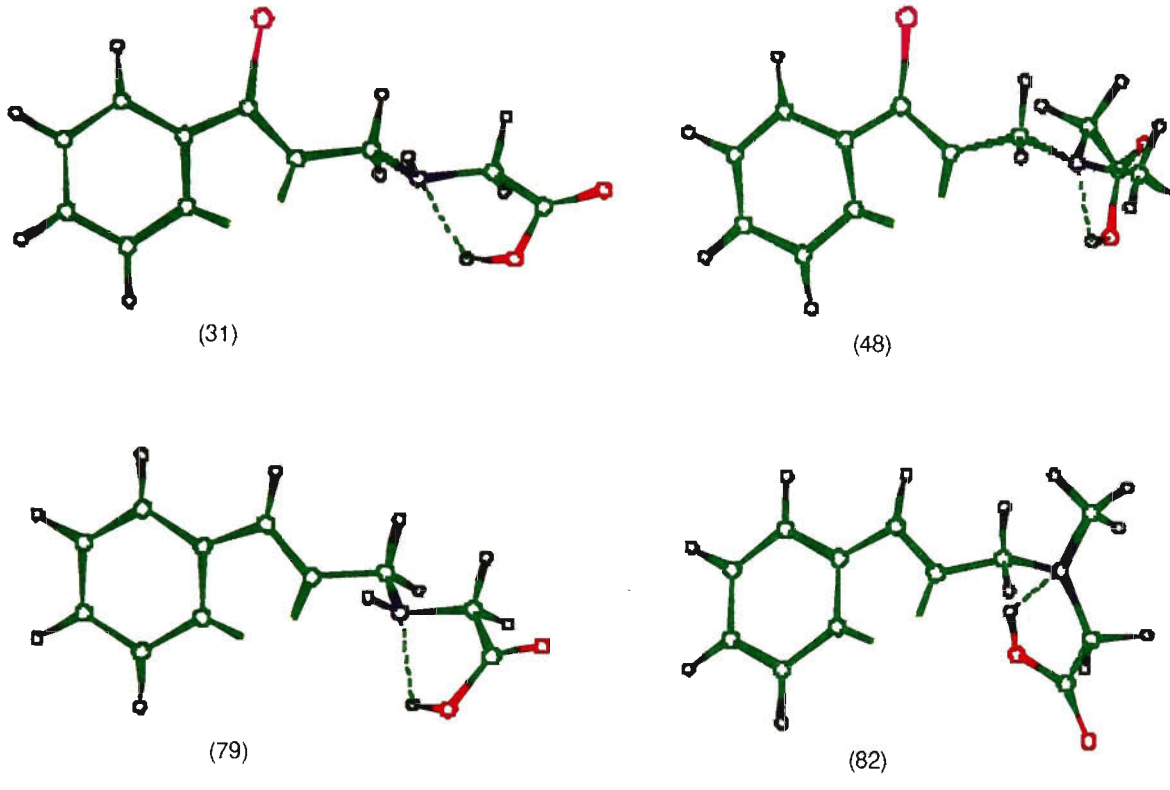


Figure 5.12 The global minimum conformations of the amines 31, 48, 79 and 82

Finally, derivatization of the carboxylic acid functionality led to the ester 35, the alcohol 37, the amide 26, phosphonic acid 27 and the cyclized compound 142. Though the global minimum conformations (*cf* Appendix II) of both the amide and the phosphonic acid were similar to that of 19, the former showed slightly decreased activity whereas the latter was inactive, suggesting that the carboxylic acid probably was the best alternative. In the case of the ester, as expected from its modelling results it was inactive, while the alcohol with a similar conformation to that of the *N*-methyl acid 22 did not show any activity either. All these results seemed to be in favour of a viewpoint that the carboxylic acid was an essential part of an active potentiator (the carbamide was an exception). Without the intramolecular hydrogen bond, though the cyclized compound exhibited a similar level of activity to that of 26. As the seven-membered ring is relatively constrained, it is not surprising that its

superimposition over the glycine derivative 19 showed a close similarity (*cf* Section 5.3.6). Appendix III summarises the conformational analysis results of the selected [1]benzothiophene analogues.

5.3.6 Molecular superimposition

Molecular superimposition is a method that plays an important role in proposing a reasonable pharmacophore for a particular drug by revealing some common structural features that allow qualitative assessment of three dimensional similarity and dissimilarity of structurally different molecules.

It is understood that one would start with a known series of active compounds and try to determine the features they have in common. This leads to the derivation of a pharmacophoric pattern consisting of two or more points or centres and distances between them. The original pharmacophore is refined once experimental results reveal elements that tend to produce significantly decreased activity. One then would first search for or design compounds that satisfy these constraints. On the basis of previous conformational analysis results, molecule superimposition was anticipated to help strengthen the proposed hydrogen bond-containing model, thus resulting in a pharmacophore responsible for the potentiation of the [1]benzothiophene derivatives. Selected 2- and 3-substituted [1]benzothiophene derivatives were superimposed against the best-known 5-HT inhibitor fluoxetine and the most active [1]benzothiophene derivative 19. Superimposition was performed by overlaying the phenyl ring of one molecule over the another one. Though fluoxetine is structurally entirely different from the [1]benthiophene derivatives, the fitting points have their common features as shown in Figure 5.13.

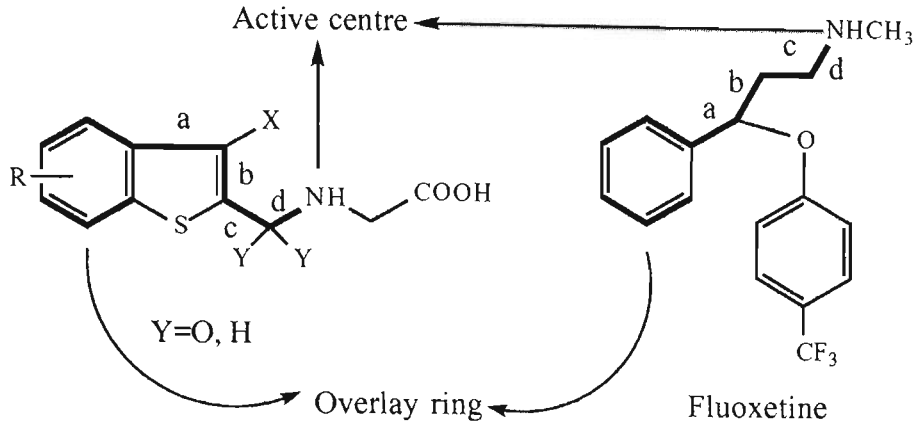


Figure 5.13 Comparison of the [1]benzothiophene-based 5-HT potentiators and fluoxetines

Figure 5.14 shows the superimposed molecules of 19 and fluoxetine with RMS 0.01758 and the distance between the active centres (N15 of fluoxetine and N12 of 19) being 3.21Å. The superimposition of 22 over fluoxetine resulted in a much larger distance between the active centres (N15 of fluoxetine and N12 of 22) due to the different conformation of 22, as also shown in Figure 5.14.

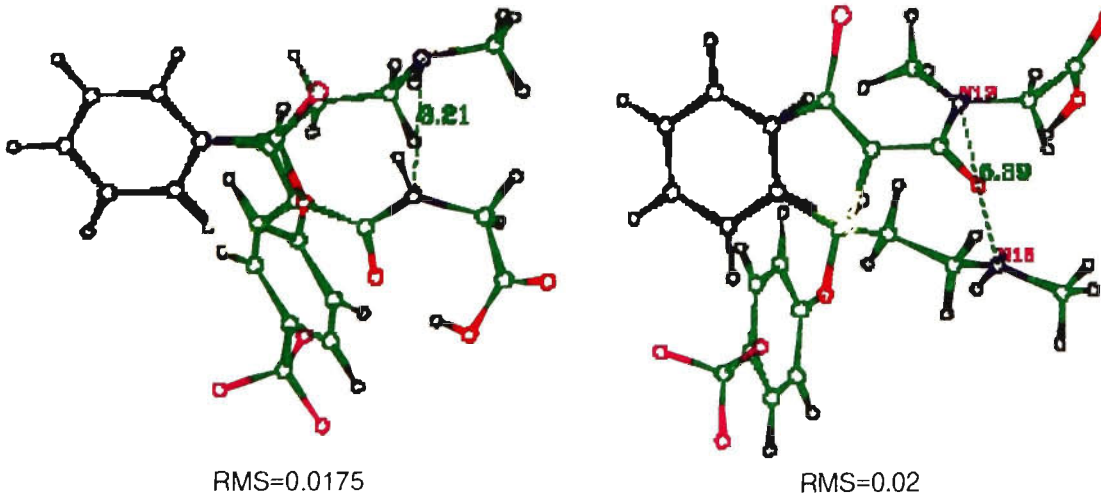
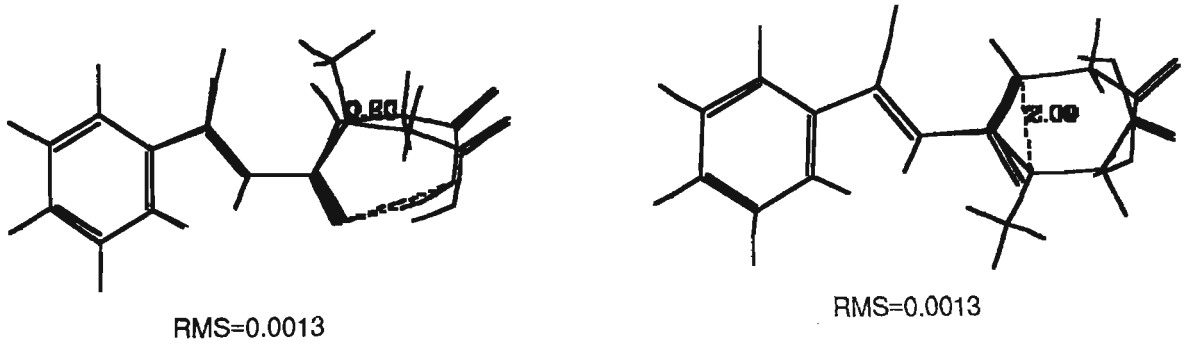


Figure 5.14 Superimpositions of 19 and 22 on fluoxetine

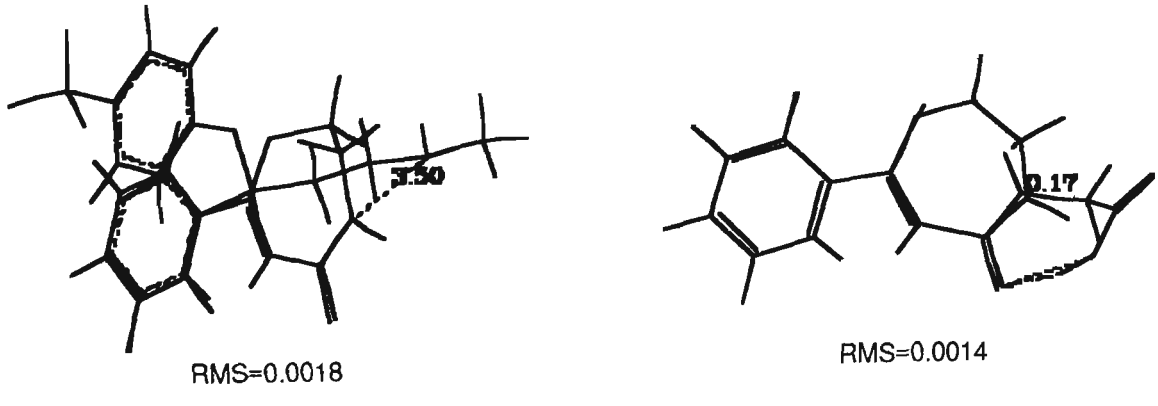
The 3-unsubstituted *N*-methyl compound 74 was superimposed on 19 and the similarity between the two compounds can be viewed from Figure 5.15a. The distance (0.2Å) between the two nitrogens is negligible compared to the distance (2.09Å) in Figure 5.15b, which shows the superimposition of the 3-chloro *N*-methyl compound 22 over 19. The seven-membered ring compound 142 was compared with fluoxetine and 19 respectively and was found to be very similar to 19 (Figure 5.16a and 5.16b).



RMS=0.0013 RMS=0.0013

(a) (b)

Figure 5.15 Superimpositions of 19 on 74 and 22



RMS=0.0018 RMS=0.0014

(a) (b)

Figure 5.16 Superimpositions of 142 on 19 and fluoxtine

The binding of molecules to receptors or at specific sites in proteins is a complicated process which involves many factors. Theoretically, molecules

that have similar structures likely have comparable biological activity[44], however, the binding of different molecules to the specific receptors could be actually quite different. Thus it is necessary that test and modelling results should be taken into consideration in order to propose a pharmacophore, as the previous conformational analysis has demonstrated that the structural similarity is not the only factor governing the activity of the compounds.

By combining the pharmacological test results and molecular modelling results of the [1]benzothiophene derivatives, it is suggested that a likely pharmacophore for the [1]benzothiophene derivatives should consist of the following factors: an unsubstituted phenyl ring; an amide function preferably present; a functional group enable formation of the specific internal hydrogen bonding; a relatively small substituent on the 3-position (Figure 5.17).

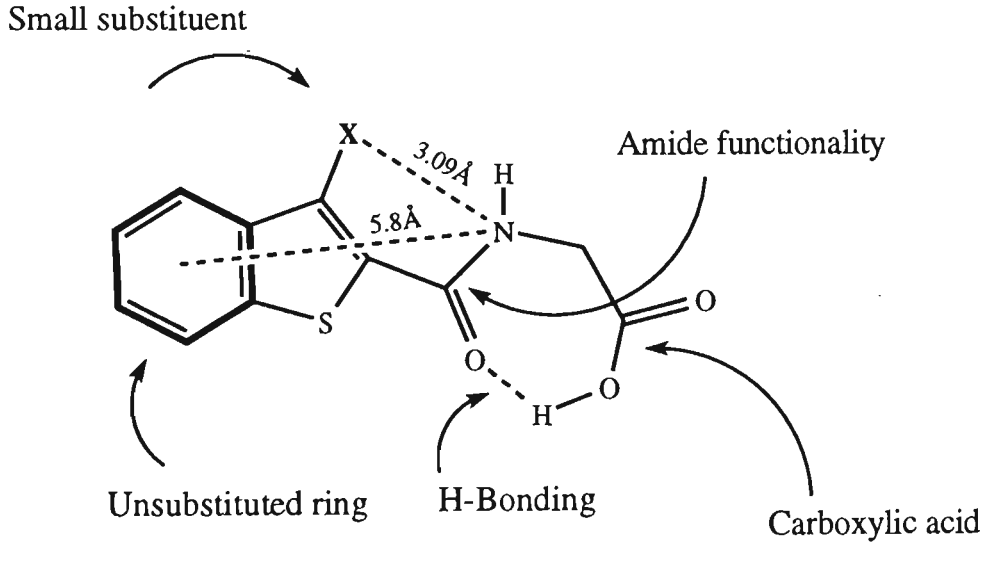


Figure 5.17 Proposed pharmacophore for the [1]benzothiophene derivatives as 5-HT potentiators

The 3-fluoro glycine derivative has the same conformational features as other glycine derivatives (Figure 5.18). But smaller atomic size than chloro and

bromo groups and stronger electronegativity is believed likely to enhance the activity. Its synthesis and pharmacological test remain to be a part of the future work.

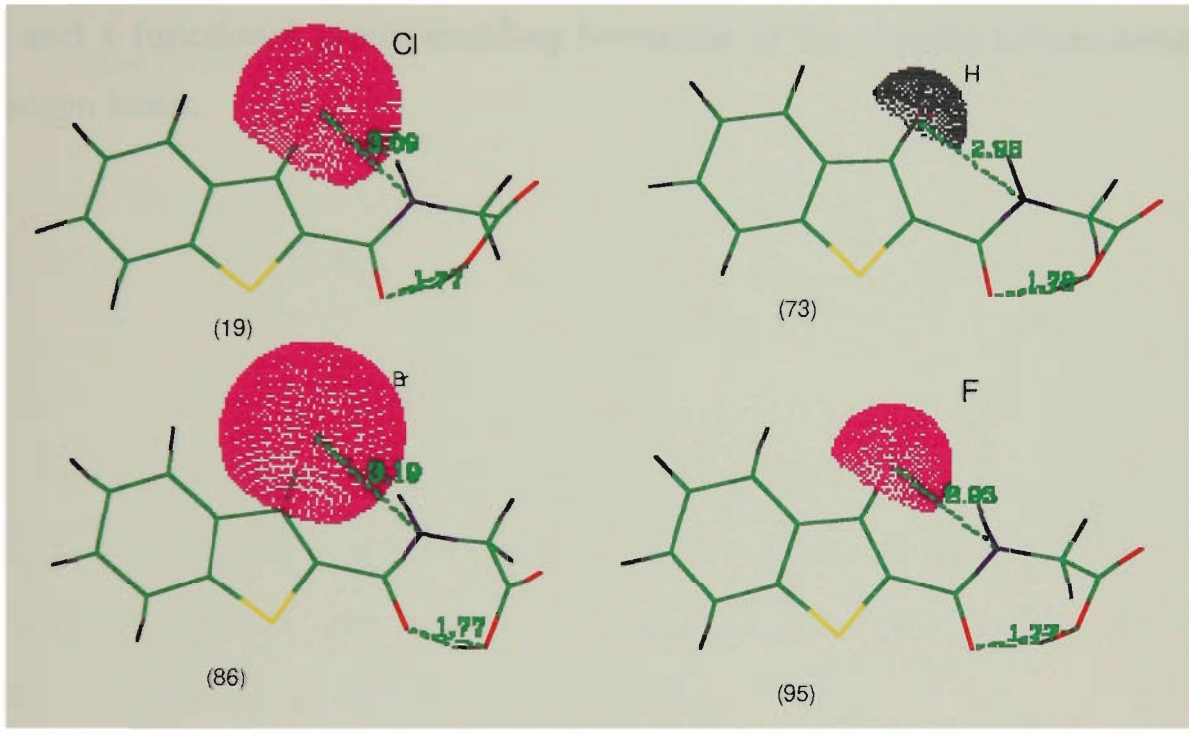


Figure 5.18 Global minimum conformations of 19, 73, 86 and 95 displaying the van der Waals surfaces on the 3-substituents

5.4. Conclusions

About thirty [1]benzothiophene derivatives obtained after structural modifications were screened for their 5-HT potentiation activity at Monash University and several amide derivatives of glycine found to be moderate potentiators of 5-HT action at the 5-HT_3 receptor site. The 3-chloro methylene amino acid 31 was the only methylene amino acid that showed reasonably good activity. Though it adopted the proposed active conformation in part, the reason distinguishing it from other amino acids remains unknown. The structure-activity relationship of the seven-membered ring compound 142 is

required to be further investigated by modification of the structure. In combination with molecular modelling results, a pharmacophoric model containing the [1]benzothiophene ring with no substituent at positions 5 and 6 and with small groups at the 3-position, the amide carbonyl conjugated to the ring and a functional group enabling formation of the specific intramolecular hydrogen bond.

Chapter 6

Experimental

6.1 General Procedures

All melting points (m.p.) were determined on a Reichert hot stage apparatus. Temperatures are expressed in degree Celsius (C) and are uncorrected.

The infrared spectra (IR) were recorded on a Bio Rad Fourier Transform Infrared Spectrometer FTS-7 as mulls in nujol unless otherwise stated. The peak positions were recorded in wave numbers (cm^{-1}) and are described as strong (s), medium (m) or weak (w).

The ^{1}H nuclear magnetic resonance spectra (n.m.r.) were determined at 400 MHz with a Varian Unity-400 spectrometer. All the spectra were measured in deutertated chloroform ($CDCl_3$) unless otherwise stated. Chemical shifts and the coupling constants (J) are expressed in ppm (δ) and in cycles per second (Hz) respectively; the peaks are described as singlets (s), doublets (d), triplets (t), quartets (q) and multiplets (m). All spectra were referenced against tetramethylsilane, δ=0.00 ppm, unless otherwise indicated. ^{13}C n.m.r. spectra were recorded in $CDCl_3$ unless otherwise stated, relative to $CDCl_3$ (δ=77.0 ppm) using the same instrument.

Mass spectra were obtained using a Vacuum General 12-12, Vacuum General-Quattro and MAT-44 spectrometers using the direct insertion techniquem, with an electron beam energy of 70 eV and a source temperature

of 200°C. The peak intensities, in parentheses, are expressed as the percentage abundance. In CI mass spectroscopy, methane was used as the ionising gas. High resolution mass spectra were run either by Dr X. Song of the School of Chemistry, the University of Sydney or by Dr N. Davies of the Central Science Laboratory, the University of Tasmania.

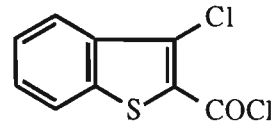
Elemental microanalyses of samples were performed either by the Australian National University Service Unit or Department of Chemistry, the University of Queensland.

Analytical thin layer chromatography (t.l.c.) was performed on Merck Kieselgel $60PF_{254}$ silica on aluminium sheets. Column chromatography was performed under medium pressure using Merck silica gel unless otherwise indicated. All chromatographic solvent proportions are volume for volume.

All extracts were dried over anhydrous sodium sulphate prior to being evaporated under reduced pressure. THF was always freshly distilled out from sodium wire in the presence of benzophenone under nitrogen. Other commercial chemicals and reagents were used as received unless noted otherwise.

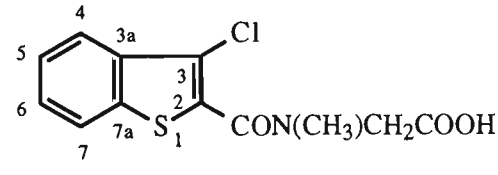
6.2 Experimental for Chapter 2

Preparation of 3-Chloro-[1]benzothiophene-2-carbonyl Chloride 21



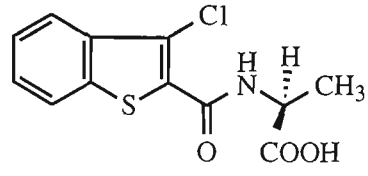
Thionyl chloride (11.1 ml, 90 mmol) was added to a solution of *trans*-cinnamic acid (4.5 g, 30 mmol) in toluene (50 ml) containing pyridine (0.5 ml, 3 mmol), and after addition the mixture was refluxed for 60 hours. The reaction mixture was filtered and the filtrate was concentrated under vacuum. The residue was chromatographed on a short column (2% ethyl acetate/hexane) to give a crude product, which was recrystallized from dichloromethane to afford the acid chloride 21 (4.5 g, 64%) as yellow needles, m.p. 114-116°C, literature m.p. 114-116°C[62].

Preparation of *N*-[3-Chloro-[1]benzothien-2-oyl]sarcosine 22



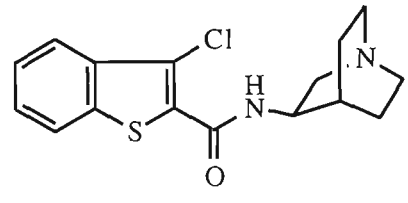
Sarcosine (112 mg, 1.25 mmol) was dissolved in sodium hydroxide solution (0.5 M, 4 ml) , then treated with the acid chloride 21 (231 mg, 1 mmol) for 6 hours. Hydrochloric acid (6 M) solution was added to precipitate the product. The precipitate was collected and washed with cold diethyl ether to afford the acid 22 (240 mg, 81%), m.p. 138-140°C. The sample for microanalysis was recrystalized from methanol. (Found: 48.75, H 3.44, N 4.76. $C_{12}H_{10}ClNO_3S$ requires C 50.80, H 3.55, N 4.94). Mass spectrum: *m/z* (CI) 284 (MH^+, 100). ^1H n.m.r.: δ 7.89-7.82, m, H4, H7; 7.53-7.47, m, H5, H6; 4.38, s, CH_2; 3.19, s, CH_3. υ_{max} 1740 (m), 1600 (s) cm^{-1}.

Preparation of *N*-[3-Chloro-[1]benzothien-2-oyl]L-alanine 24



To a solution of L-alanine (67 mg, 0.75 mmol) in sodium hydroxide (0.5 M, 2.5 ml) was added the acid chloride 21 (116 mg, 0.5 mmol). After stirring overnight, water was added (1 ml) and the mixture extracted with ethyl acetate (3x15 ml). The extracts were washed with brine (10 ml) and dried. Removal of the solvent afforded a residue, which was recrystallized from methanol to give the acid 24 (110 mg, 79%) as colourless crystals. Mass spectrum: *m/z* (EI) 283 (M^+, 10), 239 (12), 212 (100), 195 (90), 167 (33). ^1H n.m.r. (acetone-d6): δ 7.89-7.82, 8.09-7.93, m, H4, H7; 7.66-7.59, m, H5, H6; 4.54, m, NHCH(CH_3)COOH; 2.84, s, NH; 1.58, d, J 6.8 Hz, CH_3. υ_{max} 3368 (w), 1769 (m), 1642 (m) cm^{-1}.

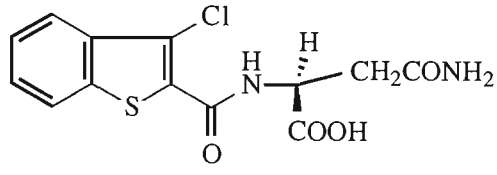
Preparation of *N*-3'-Quinuclidino-3-chloro-[1]benzothiophene-2-carboxamide 29



A mixture of 3-aminoquinuclidine dihydrochloride (300 mg, 1.5 mmol) and triethylamine (0.4 ml) in dichloromethane (10 ml) was stirred for half an hour and the acid chloride 21 (340 mg, 1.5 mmol) was added. After stirring overnight, the reaction mixture was diluted with dichloromethane (30 ml). The mixture was washed with water (2x10 ml), brine (10 ml) and dried. Evaporation of the solvent followed by chromatography of the residue (12% methanol/dichloromethane) afforded the amide 29 (370 mg, 81%) as pale yellow crystals, m.p. 150-152°C. Mass spectrum: *m/z* (EI) 320(M^+, 30), 195 (100),

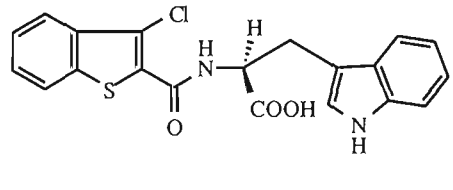
181 (30), 167 (50) (accurate mass: $M^{+\bullet}$, 320.0754. $C_{16}H_{17}Cl_2NOS$ requires 320.0750). ^1H n.m.r. (MeOH-d4): δ 7.93-7.90, m, H4, H7; 7.58-7.52, m, H5, H6; 4.28-4.24, m, NHCH; 3.12-2.91, m, 3xCH_2; 2.18, m, CH; 2.07-1.71, m, 2xCH_2. υ_{max} 3300 (w, broad), 1648 (m), 1565 (m) cm^{-1}.

Preparation of *N*-[3-Chloro-[1]benzothien-2-oylL-asparagine 23



To a solution of L-asparagine (280 mg, 2.2 mmol) in sodium hydroxide (0.5 M, 4.4 ml) was added the acid chloride 21 (432 mg, 2 mmol). The mixture was stirred overnight, then hydrochloric acid (1 M) was added to precipitate a compound. The precipitate was collected and chromatographed (5:60:100, acetic acid/ethyl acetate/hexane) to afford a crude product (390 mg, 64%). The crude product was recrystallized from hot methanol to afford the acid 23 (350 mg, 57%) as colourless crystals, m.p. 150-152°C. Mass spectrum: *m/z* (ES-) 326 (M^-, 100). ^1H n.m.r. (pyridine-d5): δ 7.82-7.73, m, H4, H7; 7.41-7.39, m, H5, H6; 5.26, t, J 4.8 Hz, CH; 2.39, dt, J_1 15.2 Hz, J_2 4.0 Hz, one of CH_2CONH_2; 3.31, dt, J_1 15.2 Hz, J_2 5.6 Hz, one of CH_2CONH_2. υ_{max} 3341 (m, broad), 3182 (m, broad), 1679 (m), 1618 (s), 1519 (s) cm^{-1}.

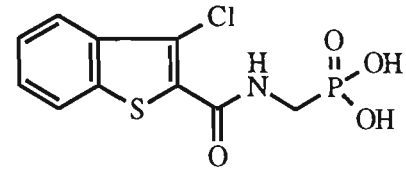
Preparation of *N*-[3-Chloro-[1]benzothien-2-oyl]L-tryptophan 25



The acid chloride 21 (231 mg, 1 mmol) was added to a solution L-tryptophan (204 mg, 1 mmol) in sodium hydroxide (0.5 M, 2 ml) and the mixture stirred overnight. A precipitate was formed, collected and purified by

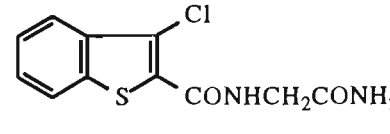
chromatography (0.5:50:100, acetic acid/methanol/dichloromethane) to afford the acid 25 (320 mg, 80%) as colourless crystals, m.p. 226-228°C. Mass spectrum: *m/z* (Fab) 400 (M^+, 10), 354 (12), 238 (20), 196 (100). ^1H n.m.r. (DMSO-d6): δ 8.30, d, J 7.2 Hz, H4; 8.10-7.54, m, H4', 5', 6' and 7'; 7.32, d, J 8.4 Hz, H7; 7.22, d, J 2.4 Hz, H2'; 7.05, t, J 7.6 Hz, H5; 6.95, t, J 8.0 Hz, H6; 4.70, m, CH; 4.01, d, J 6.8 Hz, CH_2. υ_{max} 3379 (w, broad), 1724 (m), 1626 (m), 1535 (s) cm^{-1}.

Preparation of *N*-[3-Chloro-[1]benzothien-2-oyl]methylphosphonic Acid 27



Aminomethylphosphonic acid (55 mg, 0.5 mmol) was dissolved in sodium hydroxide (0.5 M, 2 ml, 1 mmol) and stirred with the acid chloride 21 (115 mg, 0.5 mmol) overnight. The aqueous reaction mixture was subjected to a high vacuum to give a white powder. This powder was washed with diethyl ether to remove organic impurities to afford the acid 27 contaminated with small amount of sodium chloride (160 mg, *ca* 87%) as colourless crystals, m.p. 300°C (decomposition). Mass spectrum: *m/z* (ES+) 306 (M^+, 40), 288 (M^+-OH, 100). ^1H n.m.r. (D_2O): δ 7.85-7.80, m, H4, H7; 7.50-7.46, m, H5, H6; 3.61, d, J 3.6 Hz, CH_2. υ_{max} 3508 (w), 3303 (m, broad), 1634 (s), 1542 (m) cm^{-1}.

Preparation of *N*-[3-Chloro-[1]benzothien-2-oyl]glycinamide 26

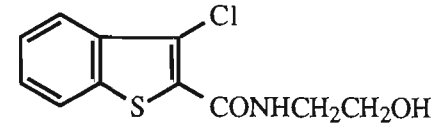


Glycinamide hydrochloride (120 mg, 1.1 mmol) was dissolved in sodium hydroxide solution (0.5 M, 2.2 ml) and stirred with the acid chloride 21 (231 mg, 1 mmol) overnight. A precipitate was formed, collected and washed successively with water (2x5 ml) and diethyl ether (2x10 ml) to yield the amide

26 (200 mg, 74%) as colourless crystals, m.p. 195-196°C. Mass spectrum: *m/z* (ES-) 267 (M^+-1, 83), 167 (93) (accurate mass: $M^{+\bullet}$, 268.0081. $C_{11}H_9ClN_2O_2S$ requires 268.0073). ^1H n.m.r. (DMSO-d6): δ 8.41-7.56, m, H4, 5, 6 and 7); 3.90, d, J 6.0 Hz, CH_2. υ_{max} 3348 (w), 3303 (m, broad), 1680 (s), 1634 (s), 1542 (m) cm^{-1}.

Reduction of the Amide Functionality of the *N*-[3-Chloro-[1]benzothien-2-oyl]glycine 19 and Its Derivatives

(1). Reduction of *N*-[3-Chloro-[1]benzothien-2-oyl]glycine 19 with Borane: formation of *N*-[2'-hydroxyethyl]-3-chloro-[1]benzothiophene-2-carboxamide 34



To a solution of 19 (135 mg, 0.5 mmol) in THF (5 ml) at room temperature, under nitrogen, was added dropwise a solution of borane (1 M in THF, 3 ml). After stirring overnight, the reaction mixture was quenched with saturated ammonium chloride solution, and the aqueous layer was separated and extracted with ethyl acetate (3x10 ml). The combined organic layers were washed with brine (10 ml), dried and the solvent evaporated. Chromatography (40% ethyl acetate/hexane) of the residue gave the alcohol 34 (75 mg, 59%) as colourless crystals, m.p. 114-115°C. (Found: C 51.85, H 4.00, N 5.57. $C_{11}H_{10}ClNO_2S$ requires C 51.67, H 3.94, N 5.48). Mass spectrum: *m/z* (CI) 256 (MH^+, 5), 237 (M^+-H_2O, 18), 211 (10), 195 (100). ^1H n.m.r.: δ 7.89–7.83, m, H4, H7; 7.61, s, NH; 7.52-7.47, m, H5, H6; 3.90, t, J 5.0 Hz, $\underline{CH_2}OH$; 3.73, dt, J_1 5.0 Hz, J_2 4.4 Hz, $NH\underline{CH_2}$. ^{13}C n.m.r.: δ 161.5, $\underline{CO}$; 138.2, C2, 137.4, C3, 133.5, C7a; 127.2, C7; 125.0, C4; 123.0, C5; 122.7, C6; 118.9, C3a; 61.8, CH_2OH; 42.8, $NHCH_2$. υ_{max} 3460 (m, sharp), 3385 (m, sharp), 1612 (s), 1537 (s) cm^{-1}.

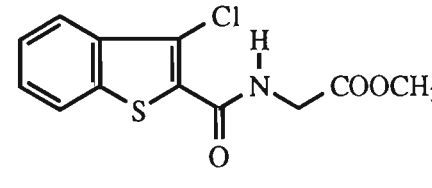
(2). Reduction of the Amide 19 with Sodium Borohydride-Phosphorus Oxychloride Complex

Phosphorus oxychloride (0.05 ml, 0.5 mmol) was added to a solution of 19 (135 mg, 0.5 mmol) in toluene (3 ml). After stirring for 1.5 hours, a brown mixture was formed. This mixture was treated with a suspension of sodium borohydride (100 mg, 2.5 mmol) in ethanol (2 ml) at 0°C. After stirring the reaction mixture overnight, it was found that a mixture of products had been formed on the basis of t.l.c. analysis.

(3). Reduction of the Amide 19 with Sodium Borohydride-Acetic Acid Complex

To a suspension of sodium borohydride (380 mg, 10 mmol) in dioxane (20 ml) was added dropwise acetic acid (0.6 ml, 10 mmol). The mixture was then stirred for 10 minutes. The amide 19 (270 mg, 1 mmol) was then added and the reaction mixture was refluxed for 20 hours. No reaction was observed from t.l.c. analysis, the starting material was recovered after work-up.

Preparation of *N*-[3-Chloro-[1]benzothien-2-oyl]glycine Methyl Ester 35



1. A solution of 19 (1.35 g, 5 mmol) in methanol was refluxed for 5 hours while hydrogen chloride was bubbled into the reaction mixture. The solvent was then evaporated to give a residue, which was chromatographed (30% ethyl acetate/hexane) to yield the ester 35 (1.2 g, 86%) as colourless crystals, m.p. 122-123°C. (Found: C 50.84, H 3.58, N 5.00. $C_{12}H_{10}ClNO_3S$ requires C

50.80, H 3.55, N 4.94). ^{1}H n.m.r.: d 7.90-7.88, m, H4; 7.85-7.82, m, H7; 7.78, s (broad), ArCONH; 7.52-7.48, m, H5, H6; 4.32, d, J 4.8 Hz, CH_2COOCH_3; 3.81, s, OCH_3. ^{13}C n.m.r. d 170.1, $COOCH_3$; 160.8, ArCONH; 138.4, C2; 137.0, C3; 132.4, C7a, 127.5, C4; 125.4, C7; 123.3, C5; 122.8, C6; 119.6, C3a; 52.6, OCH_3; 42.2, CH_2COOCH_3. υ_{max} 3350 (w, sharp), 1733 (s), 1643 (s), 1556 (s) cm^{-1}.

2. Triethylamine (0.25 ml) was added to a suspension of glycine methyl ester hydrochloride (300 mg, 2.2 mmol) in dichloromethane (30 ml) and the mixture was stirred for 2 hours. To this was added the acid chloride 21 (460 mg, 2 mmol). After stirring overnight, the reaction was quenched with hydrochloric acid (1 M) and the mixture extracted with dichloromethane (3x30 ml). The extracts were washed with brine (20 ml), dried and evaporated. The residue was purified by chromatography (30% ethyl acetate/hexane) to give the ester 35 (200 mg, 35%).

4). Reduction of *N*-[3-Chloro-[1]benzothien--2-oyl]glycine Methyl Ester 35 with Borane

Borane (1 M in THF, 3 ml) was added to a solution of 35 (142 mg, 0.5 mmol) in THF (5 ml) at room temperature under nitrogen and the mixture stirred overnight. A solution of saturated ammonium chloride was added to quench the reaction. The aqueous mixture was extracted with ethyl acetate (3x10 ml) and the combined organic layers were washed with brine (15 ml), dried and concentrated to give a residue, which was chromatographed (70% diethyl ether/hexane) to afford the alcohol 34 (60 mg, 43%).

(5). Reduction of the Amide ester 35 with Borane-Triethylamine Complex

A mixture of the methyl ester 35 (144 mg, 0.5 mmol) and borane-triethylamine complex (1 M, 2ml)) in THF (5 ml) at room temperature under nitrogen was stirred overnight. After refluxing for 3 hours no reaction was detected.

(6). Reduction of the Amide Ester 35 with Sodium Borohydride

To a solution of the methyl ester 35 (283 mg, 1 mmol) in ethanol (10 ml) was added sodium borohydride (50 mg, 1.5 mmol) and the reaction mixture was stirred overnight. The reaction was quenched with a saturated ammonium chloride solution. After evaporating to dryness, the residue was dissolved in water and extracted with ethyl acetate (3x15 ml). The extracts were washed with brine (15 ml), dried and concentrated to give a crude product. Chromatography (60% ethyl acetate/hexane) of the crude product yielded the alcohol 34 (250 mg, 98%).

(7). Reduction of the Amide Ester 35 with Sodium Borohydride-Triethyloxonium Tetrafluoroborate Complex

Triethyloxonium tetrafluoroborate (1 M in dichloromethane, 1.5 ml) was added to a solution of 35 (140 mg, 0.5 mmol) in dichloromethane (3 ml) and the reaction mixture stirred overnight. The solvent was removed and the residue was dissolved in ethanol (3 ml). Sodium borohydride (20 mg) was added to the solution at 0°C and the mixture was then stirred overnight. T.l.c. indicated no visible reaction occurred, the starting material was recovered after work-up.

(8). Reduction of the Amide Ester 35 with Sodium Borohydride-Acetic Acid Complex

To a stirred suspension of sodium borohydride (19 mg, 0.5 mmol) in dioxane (3 ml) was added dropwise acetic acid (0.03 ml, 0.5 mmol). After stirring for 15 mins, the methyl ester 35 (144 mg, 0.5 mmol) was added and the mixture was stirred overnight. T.l.c. analysis showed no reaction took place. Additional sodium acetoxyborohydride (2 eq) was then added. After stirring overnight, the alcohol 34 was formed (103 mg, 81%).

(9). Reduction of the Amide Ester 35 with Sodium Borohydride-Phosphorus Oxychloride-Pyridine Complex

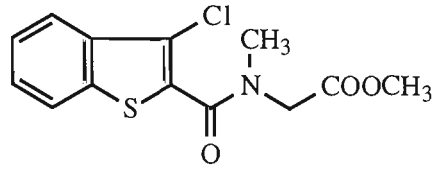
The methyl ester 35 (144 mg, 0.5 mmol) was stirred with phosphorus oxychloride (0.5 ml) in the presence of pyridine (0.04 ml, 0.5 mmol) at room temperature overnight. The excess of phosphorus oxychloride was removed under reduced pressure and the residue was dissolved in diglyme (2-methoxy ethyl ether, 2 ml) and then treated with sodium borohydride (38 mg, 1 mmol) at 0°C. After stirring overnight at room temperature and subsequent refluxing for 4 hours, no reaction was observed. The starting material was recovered after work-up.

(10). Reduction of the Amide Ester 35 with Lithium Diisopropylaminoborohydride

To a solution of borane (1 M in THF, 2 ml) and diisopropylamine (0.29 ml, 2 mmol) in THF (5 ml) at 0°C, under nitrogen, was added dropwise *n*-butyl lithium (2.5 M in hexane, 0.8 ml, 2.0 mmol). After stirring for 30 minutes at

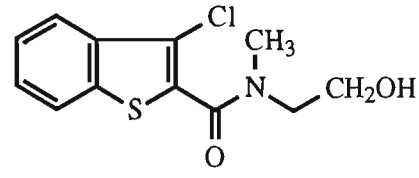
0°C, a solution of 35 (283.5 mg, 1 mmol) in THF (5 ml) was added dropwise. The mixture was stirred at 0°C for 1 hour. The reaction was quenched with saturated ammonium chloride solution and the mixture extracted with ethyl acetate (3x15 ml). The extracts were washed with brine (10 ml), dried and evaporated to afford the alcohol 34 (250 mg, 93%).

Preparation of Methyl *N*-[3-chloro-[1]benzothien-2-oyl]sarcosinate 36



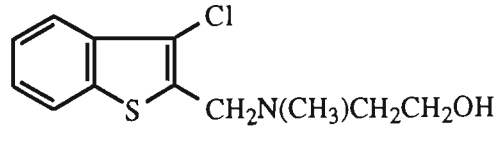
Triethylamine (0.25 ml) was added to a suspension of sarcosine methyl ester hydrochloride (330 mg, 2.2 mmol) in dichloromethane (30 ml). The reaction mixture was stirred for 2 hours and the acid chloride 21 (460 mg, 2 mmol) was added. After stirring overnight, the reaction was quenched with hydrochloric acid (1 M). The reaction mixture was extracted with dichloromethane (3x30 ml) and the extracts were washed with brine (20 ml), dried and evaporated. Chromatography of the residue (40% ethyl acetate/hexane) afforded the ester 36 (100 mg, 25%) as a colourless oil. (Found: C 52.41, H 4.25, N 4.98. $C_{13}H_{12}ClNO_3S$ requires C 52.44, H 4.06, N 4.70). Mass spectrum: *m/z* (EI) 297 (M^+, 8), 238 (6), 195 (65). ^1H n.m.r.: δ 7.90-7.88, m, H4, H7; 7.52-7.43, m, H5, H6; 4.33, s, $\underline{C}H_2COOCH_3$; 3.81, s, OCH_3; 3.16, s, $ArCON(CH_3)$. ^{13}C n.m.r.: δ 168.5, $\underline{C}OOCH_3$; 163.6, CO; 138.5, C2; 136.8, C3; 129.3, C7a; 126.7, C4; 125.5, C7; 122.7, C5; 122.5, C6; 119.7, C3a; 52.5, OCH_3; 49.1, $\underline{C}H_2COOCH_3$; 35.6, $ArCON(\underline{C}H_3)$. υ_{max} (film) 3092 (w), 2985 (s), 1735 (s, $\underline{CO}OCH_3$), 1648 (s, $Ar\underline{CO}N$), 1565 (m) cm^{-1}.

Reduction of *N*-[3-Chloro-[1]benzothien--2-oyl]sarcosine Methyl Ester 36 with Sodium Borohydride: formation of *N*-(2'-hydroxyethyl)-*N*-methyl-3-chloro-[1]benzothiophene-2-carboxamide 37



To a solution of the methyl ester 90 (200 mg, o.67 mmol) in ethanol (5 ml) was added sodium borohydride (45 mg, 1.18 mmol) and the reaction mixture was stirred overnight. A saturated ammonium chloride solution was added to quench the reaction and the mixture was extracted with ethyl acetate (3x15 ml). The extracts were washed with brine (10 ml) and dried. Removal of the solvent gave a residue, which was chromatographed (30% and 80% ethyl acetate/hexane) to afforded the alcohol 142 (150 mg, 83%) as colourless crystals, m.p. 85-86°C. (Found: C 53.51, H 4.39, N 5.18. $C_{12}H_{12}ClNO_2S$ requires C 53.43, H 4.48, N 5.19). Mass spectrum: *m/z* (EI) 269 (M^+, 5), 251 (<1), 238 (3), 195 (100), 167 (22). ^1H n.m.r.: δ 8.03-7.96, m, H4, H7; 7.67-7.60, m, H5, H6; 4.10, s (broad), $\underline{CH_2}CH_2OH$; 3.90, s (broad), $\underline{CH_2}OH$; 3.32, s, ArCON($\underline{CH_3}$). ^{13}C n.m.r.: δ 162.5, CO; 137.4, C2; 135.8, C3; 133.2, C7a; 126.6, C4; 125.4, C7; 124.2, C3a; 122.7, C5; 122.5, C6; 61.5, $\underline{C}H_2CH_2OH$; 52.8, CH_2OH; 37.4, ArCON($\underline{C}H_3$). υ_{max} (thin film) 3404 (s, broad), 3053 (w), 2931 (s), 2876 (m), 1638 (s), 1535 (m) cm^{-1}.

Reduction of *N*-[3-Chloro-[1]benzothien-2-oyl]sarcosine Methyl Ester with Lithium Aluminium Hydride: formation of 2-[(*N*-3'-chloro-[1]benzothien-2'-ylmethyl])-*N*-methyl]aminoethan-1-ol 39

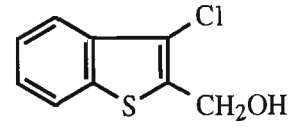


A solution of 36 (390 mg, 1.3 mmol) in THF (3 ml) was added to a suspension of lithium aluminium hydride (48 mg, 1.25 mmol) in THF (7 ml) under nitrogen. After stirring for two hours, a saturated ammonium chloride

solution was added to quench the reaction. The mixture was extracted with ethyl acetate (3x15 ml). The extracts were then washed with brine (10 ml), dried and concentrated to give a crude product. The crude product was chromatographed (30% ethyl acetate/hexane) to afford the alcohol 39 as an oil (200 mg, 60%). (Found: C 56.24, H 5.76, N 5.56. $C_{12}H_{14}ClNOS$ requires C 56.35, H 5.52, N 5.48). Mass spectrum: *m/z* (EI) 255 (M^+, <1), 224 (20), 181 (100). ^1H n.m.r.: δ 7.79-7.75, m, H4, H7; 7.44-7.34, m, H5, H6; 3.93, s, $ArCH_2N$; 3.65, t, J 5.6 Hz, CH_2OH; 2.68, t, J 5.6 Hz, CH_2CH_2OH; 2.37, s, CH_3. υ_{max} (thin film) 3400 (s, broad), 3080 (w) cm^{-1}.

Attempted Conversion of 3-Chloro-[1]benzothiophene-2-carboxylic Acid Chloride 21 to the Corresponding Aldehyde 32

(1). Reduction of 3-Chloro-[1]benzothiophene-2-carbonyl Chloride 21 with Sodium Borohydride-pyridine-dimethylforamide: formation of 2-hydroxymethyl-3-chloro-[1]benzothiophene 40

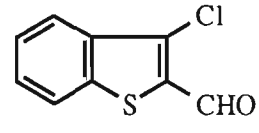


A solution of the acid chloride 21 (231 mg, 1 mmol) in DMF (5 ml) was added in one portion to an ice cold suspension of sodium borohydride (38 mg, 1 mmol) in a mixture of DMF (5 ml), THF (5 ml) and pyridine (0.2 ml, excess). The mixture was stirred for 30 mins at 0°C, then quenched with saturated ammonium chloride solution. The mixture was extracted with diethyl ether (3x15 ml) and the extracts were then washed with brine (10 ml), dried and evaporated to afford the alcohol 40 (190 mg, 97%) as colourless crystals. Mass spectrum: *m/z* (CI) 198 (MH^+, 17), 181 (100). υ_{max} 3440 (s) cm^{-1}.

(2). Reduction of the Acid Chloride with Sodium Borohydride-Cadmium Chloride-*N*, *N*-Dimethylformamide Complex

Cadmium chloride ($CdCl_2$ 1/2 H_2O) (49 mg, 0.25 mmol) was added into a suspension of sodium borohydride (9.5 mg, 0.25 mmol) in DMF (2 ml). The mixture was stirred for 15 mins. The reaction mixture was cooled down with an ice-water bath and a solution of the acid chloride 21 (58 mg, 0.25 mmol) in acetonitrile (1 ml) was then added in one portion. After stirring for 5 mins, t.l.c. indicated most of the starting material had been converted to the alcohol 40.

3. Preparation of 3-Chloro-[1]benzothiophene-2-carbaldehyde 32

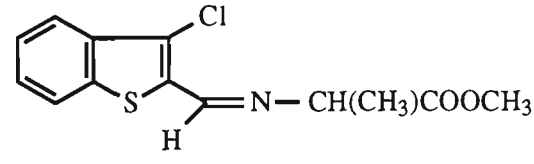


Pyridine (1 ml) was added into a suspension of chromium trioxide (150 mg, 1.5 mmol) in dichloromethane (10 ml) at room temperature and the mixture stirred for 20 minutes. A solution of the alcohol 40 (50 mg, 0.25 mmol) in dichloromethane (2 ml) was added and after 30 mins the reaction was quenched with a mixture of diethyl ether and light petroleum (2:3, 30 ml). The precipitate formed was collected and washed with diethyl ether. The filtrate was concentrated to give a residue that was chromatographed (10% ethyl acetate/hexane) to afford the aldehyde 32 (47 mg, 99%). Mass spectrum: *m/z* (CI) 197 (MH^+, 100). ^1H n.m.r.: δ 10.4, s, CHO; 8.02, d, J 6.8 Hz, H4; 7.87, d, J 7.2 Hz, H7; 7.61-7.51, m, H5, H6. υ_{max} 1680 (m) cm^{-1}.

Attempted Reductive-Amination of 3-Chloro-[1]benzothiophene-2-carbaldehyde with Alanine

A solution of dl-alanine (45 mg, 0.5 mmol) in sodium hydroxide (2 M, 0.25 ml) was treated with a solution of 32 (40 mg, 0.21 mmol) in THF (0.4 ml) at 0°C for half an hour, then with sodium borohydride (6 mg, 0.16 mmol). After 30 minutes, a second portion of 32 (40 mg, 0.21 mmol) was added, as was sodium borohydride (6 mg, 0.16 mmol). After two hours, the reaction mixture was washed with diethyl ether to remove non-polar organic compounds. The aqueous layer was neutralized with hydrochloric acid (1 M) and extracted with ethyl acetate (3x20 ml). Evaporation of the solvents afforded a mixture of the aldehyde 32 and the alcohol 40.

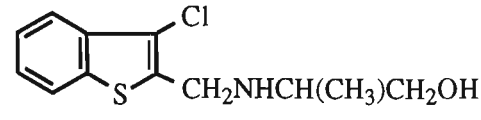
Preparation of *N*-[3-Chloro-[1]benzothien-2-ylmethylidene]-(D,L)-alanine Methyl Ester 41



A mixture of the aldehyde 32 (400 mg, 2 mmol) and dl-alanine methyl ester hydrochloride (280 mg, 2 mmol) in benzene (10 ml) containing triethylamine (0.36 ml, 2.6 mmol) was refluxed for 20 hours. Water was added and the mixture was extracted with ethyl acetate (3x15 ml). The combined extracts were washed with brine (15 ml) and dried. Chromatography (15% ethyl acetate/hexane) gave the imine 41 (450 mg, 80%) as yellow crystals, m.p. 67-69°C. Mass spectrum: *m/z* (EI) 281 (M^+, 5), 266 (5), 222 (100), 195 (60). ^{1}H n.m.r.: δ 8.71, s, ArCH=N; 7.91-7.84, m, H4; 7.82-7.76, m, H7; 7.48-7.42, m, H5, H6; 4.24, q, J 7.2 Hz, ArCH=NCH; 3.77, s, OCH_3; 1.58, d, J 7.2 Hz, CH_3. ^{13}C n.m.r.: δ 172.6, CO; 155.0, ArCH=N; 138.5, C2; 136.7, C2, 394.5, C7a; 127.4, C7; 125.2, C4; 124.8, C3a;

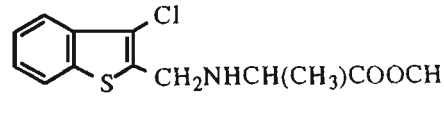
122.9, C5; 122.4, C6; 67.8, ArCH=N<u>C</u>H; 52.4, OCH_3; 19.6, CH_3. υ_{max} (film) 3068 (w), 1743 (s), 1626 (s), 1433 (m) cm^{-1}.

Reduction of the Imine <u>41</u> with Sodium Borohydride: formation of 2-(*N*-3'-chloro-[1]benzothien-2'-ylmethyl)aminoprpan-1-ol <u>42</u>



To a solution of the imine <u>41</u> (100 mg, 0.34 mmol) in ethanol (2.5 ml) at 5 °C was added sodium borohydride (40 mg, 1 mmol). The reaction mixture was stirred at room temperature for six hours and then quenched with saturated ammonium chloride solution and extracted with ethyl acetate (3x15 ml). The combined extracts were washed with brine (15 ml), dried and concentrated. The residue was chromatographed (40% ethyl acetate/hexane containing 1% NH_4OH) to afford the alcohol <u>42</u> (40 mg, 44%) as colourless crystals, m.p. 105-107. (Found: C 56.54, H 5.66, N 5.58. $C_{12}H_{14}ClNOS$ requires C 56.35, H 5.52, N 5.48). Mass spectrum: *m/z* (EI) 255 (M^+, <1), 226 (7), 224 (22), 183 (33), 181 (100). ^1H n.m.r.: δ 7.78, d, J 1.2 Hz, H4; 7.76, d, J 1.2 Hz, H7; 7.45-7.34, m, H5, H6; 4.22, d, J 14.8 Hz, 1H of Ar<u>C</u>H_2NH; 4.10, d, J 14.8 Hz, 1H of Ar<u>C</u>H_2NH; 3.63, dd, J_1 10.8 Hz, J_2 3.6 Hz, 1H of <u>C</u>H_2OH; 3.30, dd, J_1 10.8 Hz, J_2 7.2 Hz, 1H of <u>C</u>H_2OH; 2.92-2.87, m, CH; 1.12, d, J 6.8 Hz, CH_3. ^{13}C n.m.r.: δ 137.6, C2; 136.9, C3, 136.8, C7a; 125.2, C7; 124.8, C4; 122.6, C5; 121.5, C6; 117.8, C3a; 65.6, Ar<u>C</u>H_2NH; 53.8, CH_2OH; 43.7, NH<u>C</u>H; 17.0, CH_3. υ_{max} 3265 (w), 3060 (w) cm^{-1}.

Preparation of *N*-[3-Chloro-[1]benzothien-2-ylmethyl]-(D,L)-alanine Methyl Ester <u>43</u>



(1). To a suspension of sodium cyanoborohydride (10 mg) in methanol (2 ml) (pH adjusted to 5 using 1M hydrochloric acid) was added dropwise a

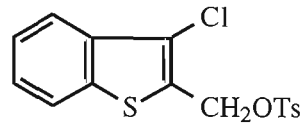
solution of the imine 41 (10 mg) in methanol (0.5 ml). After stirring for 15 minutes, it was found by t.l.c. that the aldehyde 32 and the alcohol 40 had been formed but no reduction product of the imine was observed. After stirring overnight, all starting material was converted to the alcohol 40.

(2). The foregoing reaction was repeated in methanol using acetic acid to adjust the pH to 5. The starting material was reduced quantitatively to the alcohol 40.

(3). A mixture of the imine 41 (10 mg) and Pd-C (10%, 10 mg) in absolute ethanol was exposed to hydrogen stream. After 2 hours a complex mixture was indicated by t.l.c. analysis.

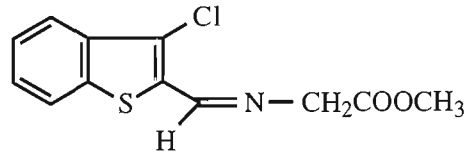
(4). A solution of the imine 41 (281 mg, 1 mmol) in methanol (10 ml) was treated with an excess of sodium cyanoborohydride (80 mg, 1.5 mmol) overnight and the reaction was then quenched with saturated ammonium solution. Methanol was removed under vacuum and the residue was dissolved in water (10 ml) and extracted with ethyl acetate (3x15 ml). The combined extracts were washed with brine (10 ml), dried and then evaporated to give a residue which was chromatographed (8% ethyl acetate/hexane) to give the methyl ester 43 (230 mg, 81%) as colourless crystals, m.p. 102-103°C. Mass spectrum: *m/z* (EI) 283 (M^+, 5), 224 (15), 196 (22), 181 (100). ^{1}H n.m.r.: δ 7.78-7.73, m, H4, H7; 7.44-7.34, m, H5, H6; 4.17, d, J 10.8 Hz, 1H of Ar$\underline{CH_2}$NH; 4.02, d, J 10.8 Hz, 1H of Ar$\underline{CH_2}$NH; 3.73, OCH_3; 3.45, q, J 7.2 Hz, CH; 2.08, s (broad), NH; 1.37, d, J 7.2 Hz, CH_3. υ_{max} 3220 (w), 1720 (s) cm^{-1}.

Preparation of 3-Chloro-[1]benzothien-2-ylmethyl Tosylate 33



To a solution 40 (1.0 g, 5 mmol) in dichloromethane (30 ml) containing triethylamine (0.5 ml, 5.5 mmol) was added *p*-toluenesulfonyl chloride (1.1 g, 5.5 mmol). The reaction mixture was stirred overnight. A solution of sodium bicarbonate (10 ml) was added to quench the reaction and to remove the excess of *p*-toluenesulfonyl chloride. The organic layer was washed with brine (10 ml), dried and evaporated to afford a crude product which was chromatographed on a silica gel column (5% ethyl acetate/hexane) to afford the tosylate 33 (1.5 g, 94%) as colourless crystals, m.p. 95-97°C. Mass spectrum: *m/z* (EI) 216 (M^+-OTs, 23), 181 (100). ^1H n.m.r. (in $CDCl_3$ or acetone-d6): δ 7.83-7.78, m, 4H, H7; 7.49-7.41, m, 4H; 4.91, s, $ArCH_2$; 2.35, s, CH_3. υ_{max} 1542 (m), 1447 (s) cm^{-1}.

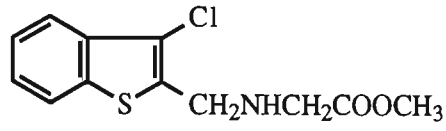
Preparation of *N*-[3-Chloro-[1]benzothien-2-ylmethylidene]glycine Methyl Ester 44



A mixture of the aldehyde 32 (380 mg, 2 mmol), glycine methyl ester hydrochloride (320 mg, 2.4 mmol) and triethylamine (0.4 ml) in benzene (15 ml) was refluxed for 6 hours, then quenched with water. The reaction mixture was extracted with ethyl acetate (3x15 ml) and the extracts were washed with brine (10 ml), dried and evaporated to furnish a crude product. The crude product was chromatographed (10% ethyl acetate/hexane) to give the imine 44 (400 mg, 77%) as yellow crystals, m.p. 83-84°C. Mass spectrum: *m/z* (EI) 267

(M^+, 100). ^1H n.m.r.: δ 8.68, s, ArCH=N; 7.85-7.83, m, H7; 7.81-7.79, m, H4; 7.49-7.43, m, H5, H6; 4.48, s, CH_2; 3.81, s, CH_3. υ_{max} 3120 (w), 1725 (s), 1587(s) cm^{-1}.

Preparation of *N*-[3-Chloro-[1]benzothien-2-ylmethyl]glycine Methyl Ester 45



a. *Via* reduction of the imine 44

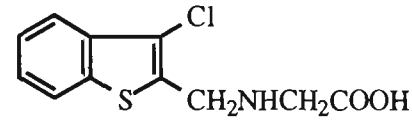
The imine 44 (80 mg, 0.3 mmol) in methanol (5 ml) was stirred with sodium cyanoborohydride (50 mg, 1mmol) for 36 hours, then quenched with saturated ammonium chloride solution. The mixture was extracted with ethyl acetate (3x10 ml) and the extracts were washed with brine (10 ml) and dried. Removal of the solvent, followed by chromatography (15% ethyl acetate/hexane) afforded the methyl ester 45 (55 mg, 69%) as an oil. Mass spectrum: *m/z* (EI) 269 (M^+, 44). (Found: C 53.41, H 4.49, N 5.06. $C_{12}H_{12}ClNO_2S$ requires C 53.43, H 4.48, N 5.19). Mass spectrum: *m/z* (EI) 269 (M^+, 5), 234 (M^+-Cl, 3), 210 (8), 196 (65), 181 (100). ^1H n.m.r.: δ 7.80-7.78, m, H4, H7; 7.44-7.37, H5, H6; 4.18, s, $ArCH_2NH$; 3.75, s, OCH_3; 3.48, CH_2COOCH_3. ^{13}C n.m.r. δ 172.5, CO; 137.0, C2; 136.9, C3; 136.8, C7a; 125.2, C4; 124.8, C7; 122.6, C5; 121.5, C6; 51.8, $ArCH_2NH$; 49.6, OCH_3; 46.0, $ArCH_2NHCH_2$. υ_{max} 3303 (m, sharp), 1725 (s), 1542 (m) cm^{-1}.

b. *Via* nucleophilic substitution

A mixture of the tosylate 33 (352 mg, 1 mmol) and glycine methyl ester hydrochloride (188 mg, 1.5 mmol) in dichloromethane (10 ml) containing triethylamine (0.3 ml, 2 mmol) was refluxed overnight. The solvent was

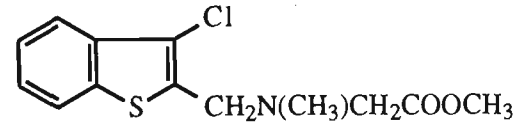
evaporated to give a residue, which was chromatographed (20% ethyl acetate/hexane) to afford the ester 45 (255 mg, 95%).

Preparation of *N*-[3-Chloro-[1]benzothien-2-ylmethyl]glycine 31



A solution of the ester 45 (100 mg, 0.37 mmol) in methanol (3 ml) was treated with sodium hydroxide (2 M, 1 ml) for three hours. Hydrochloric acid (1 M) was added to neutralize the reaction mixture . A precipitate was formed, collected and washed with ether (84 mg, 94%), m.p. 212-214°C. Mass spectrum: *m/z* (EI) 255 (M^+, 7), 196 (20), 181 (32) (accurate mass: $M^{+\bullet}$, 255.0128. $C_{11}H_{10}ClNO_2S$ requires 255.0121). ^1H n.m.r. (DMSO-d6): δ 12.4, s (broad), COOH; 8.00-7.97, m, H4; 7.74-7.72, m, H7; 7.51-7.41, m, H5, H6; 4.40, s, Ar$\underline{CH_2}$NH; 3.70, s, $\underline{CH_2}$COOH. υ_{max} 3111(w), 1622 (s), 1526 (w) cm^{-1}.

Preparation of *N*-[3-Chloro-[1]benzothien-2-ylmethyl]sarcosine Methyl Ester 47



a. *Via* reductive-amination reaction

(1) Formation of the iminium 46

A mixture of the aldehyde 32 (150 mg, 0.76 mmol), sarcosine methyl ester hydrochloride (150 mg, 1.2 mmol) and triethylamine (*ca* 0.2 ml) in toluene (10 ml) was refluxed overnight and then quenched with water. The

mixture was extracted with ethyl acetate (3x15 ml) and the extracts were washed with brine, dried and concentrated to give the crude iminium 46.

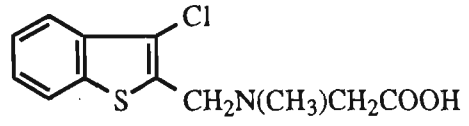
(2) Reduction of the iminium with sodium cyanoborohydride

The above iminium mixture (100 mg, 0.35 mmol) in methanol (3 ml) was stirred with sodium cyanoborohydride (50 mg, 1 mmol) for four hours, then quenched with saturated ammonium solution. The aqueous layer was extracted with ethyl acetate (3x10 ml) and the combined extracts were washed with brine and evaporated to provide a crude product. The crude product was chromatographed (0.2% methanol/dichloromethane) to give the methyl ester 47 (40 mg, 44%). Mass spectrum: *m/z* (EI) 283 (M^+, 10), 224 (20), 210 (15), 181 (100). ^{1}H n.m.r.: δ 7.80-7.77, m, H4, H7; 7.46-7.37, m, H5, H6; 4.10, s, $ArCH_2N$; 3.74, s, OCH_3; 3.41, s, CH_2; 2.50, s, CH_3. ^{13}C n.m.r.: δ 171.2, CO; 137.5, C2; 136.6, C3; 136.2, C7a; 125.2, C7; 124.7, C4; 122.6, C5; 121.6, C6; 118.8, C3a; 57.6, $ArCH_2N$; 53.4, OCH_3; 51.6, CH_2COOCH_3; 42.4, CH_3. υ_{max} (thin film) 3092 (w), 1725 (s) cm^{-1}.

b. *Via* nucleophilic substitution

A mixture of the tosylate 33 (352 mg, 1 mmol) and methyl sarcosinate hydrochloride (210 mg, 1.5 mmol) in dichloromethane (10 ml) containing triethylamine (0.3 ml, 3 mmol) was refluxed for 48 hours. The solvent was removed to give a residue, which was chromatographed (10% ethyl acetate/hexane) to provide the ester 47 (210 mg, 74%).

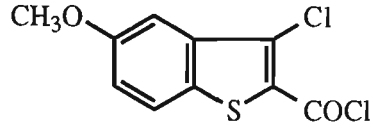
Preparation of *N*-[3-Chloro-[1]benzothien-2-ylmethyl]sarcosine 48



A solution of the ester 47 (120 mg, 0.42 mmol) in methanol (3 ml) was treated with sodium hydroxide (2 M, 1 ml) for three hours. Hydrochloric acid (1 M) was added to neutralize the reaction mixture. A precipitate was formed and collected and washed with ether/hexane to afford the acid 48 (114 mg, 100%) as colourless crystals, m.p. 245°C (decomposition). (Found: C 53.34, H 4.35, N 4.95. $C_{11}H_{12}ClNO_2S$ requires C 53.43, H 4.48, N 5.19). Mass spectrum: *m/z* (EI) 269 (M^+, 7), 224(18), 210 (8), 181 (100). ^1H n.m.r. (DMSO-d6): δ 8.00-7.98, m, H4; 7.74-7.71, m, H7; 7.50-7.42, m, H5, H6; 4.41, s, $ArCH_2N$; 3.36, s, $\underline{CH_2}COOH$; 2.18, s, CH_3. υ_{max} 3340 (m), 1715 (s), 1546(m) cm^{-1}.

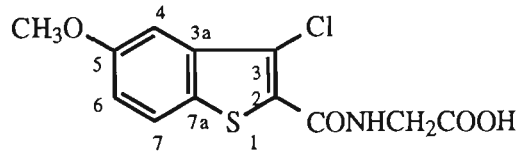
6.3 Experimental for Chapter 3

Preparation of 3-Chloro-5-methoxy-[1]benzothiophene-2-carbonyl Chloride 60



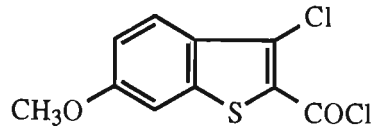
Thionyl chloride (0.50 ml, 7 mmol) was added to a solution of 3-methoxy-*trans*-cinnamic acid (180 mg, 1 mmol) in toluene (20 ml) containing pyridine (0.01 ml, 0.1 mmol). The reaction mixture was refluxed for 48 hours. Toluene was evaporated and the residue was chromatographed (5% ethyl acetate/hexane) to afford the acid chloride 60 (180 mg, 67%) as a yellow cotton-like solid, m.p. 142-143°C, literature[62] m.p. 143-144°C.

Preparation of *N*-[3-Chloro-5-methoxy-[1]benzothien-2-oyl]glycine 52



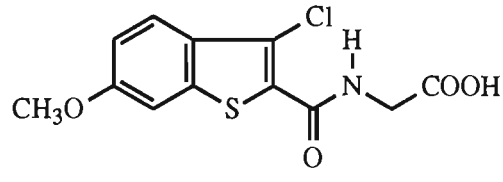
The acid chloride 60 (80 mg, 0.31 mmol) was added to a mixture of glycine (37 mg, 0.5 mmol) and sodium hydroxide (0.5 M, 1 ml), then stirred for 3 hours. Hydrochloric acid (1 M) was added to adjust the pH to 7. The resultant precipitate was collected and washed with cold diethyl ether. Recrystallization of the crude product from methanol gave the acid 52 (70 mg, 77%) as pale crystals, m.p. 208-210°C. Mass spectrum: *m/z* (EI) 299 (M^+, 24), 282 (15), 255 (10), 225 (100) (accurate mass: $M^{+\bullet}$, 299.0100. $C_{12}H_{10}ClNO_4S$ requires 299.0019). ^1H n.m.r. (MeOH-d4): δ 7.75, d, J 8.8 Hz, H7; 7.32, d, J 2.4 Hz, H4; 7.18, dd, J_1 8.8 Hz, J_2 2.4 Hz, H6; 4.10, s, CH_2; 3.92, s, OCH_3. υ_{max} 3200 (w, broad), 1762 (m), 1622 (m), 1567 (m) cm^{-1}.

Preparation of 3-Chloro-6-methoxy-[1]benzothiophene-2-carbonyl Chloride 58



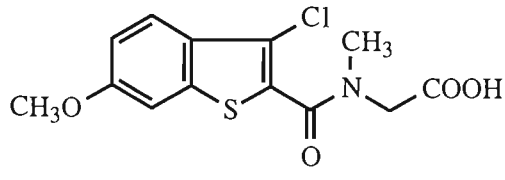
Thionyl chloride (3.63 ml, 5 mmol) was added to a solution of 4-methoxy-*trans*-cinnamic acid (178g, 10 mmol) in toluene (25 ml) containing pyridine (0.08 ml, 1 mmol). The reaction mixture was refluxed for 48 hours. Toluene was evaporated and the residue was chromatographed (5% ethyl acetate/hexane) to afford the acid chloride 58 (1.51 g, 58%) as a yellow cotton-like solid.

Preparation of *N*-[3-Chloro-6-methoxy-[1]benzothien-2-oyl]glycine 53



The acid chloride 58 (131 mg, 0.5 mmol) was added to a mixture of glycine (57 mg, 0.75 mmol) and sodium hydroxide (0.5 M, 2.5 ml). The mixture was stirred overnight. Hydrochloric acid (1 N) was added to precipitate the product. The precipitate was collected and washed with cold diethyl ether. Recrystallization of the crude product from a mixture of ethanol and water gave the acid 53 (135 mg, 91%) as pale yellow crystals, m.p. 218-220°C. (Found: C 47.53, H 3.48, N 4.72. $C_{12}H_{10}ClNO_4S$ requires C 48.09, H 3.36, N 4.67). Mass spectrum: *m/z* (EI) 299 (M^+, 36), 255 (20). ^1H n.m.r. (MeOH-d4): δ 7.78, d, J 8.8 Hz, H4; 7.45, d, J 2.0 Hz, H7; 7.14, dd, J_1 8.8 Hz, J_2 2.0 Hz, H5; 4.20, s, CH_2; 3.92, s, OCH_3. υ_{max} 3400 (m, broad), 1720 (m), 1600 (m), 1567 (m) cm^{-1}.

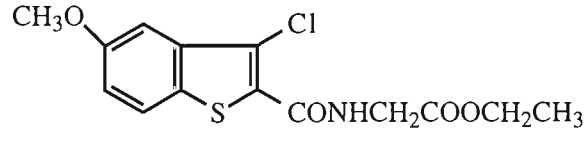
Preparation of *N*-[3-Chloro-6-methoxy-[1]benzothien-2-oyl]sarcosine 66



The acid chloride 58 (131 mg, 0.5 mmol) was added to a mixture of sarcosine (67 mg, 0.75 mmol) and sodium hydroxide (0.5 M, 2.5 ml). After stirring overnight, hydrochloric acid (1 N) was added. The precipitate formed was collected and washed with cold diethyl ether. Recrystallization of the crude product from a mixture of ethyl acetate and hexane afforded the acid 66 (150 mg, 91%) as pale yellow crystals, m.p. 158-160°C. Mass spectrum: *m/z* (CI) 314 (MH^+, 100), 225 (20). ^1H n.m.r. (MeOH-d4): δ 7.78, d, J 8.8 Hz, H4; 7.45, d, J 2.0 Hz, H7; 7.14, dd, J_1 8.8 Hz, J_2 2.0 Hz, H5; 4.30, s, CH_2; 3.92, s, OCH_3; 3.20, s, CH_3. υ_{max} 1730 (m), 1600 (m), 1567 (m) cm^{-1}.

Attempted Demethylation of *N*-[3-Chloro-5-methoxy-[1]benzothien-2-oyl]glycine 52

1. With iodotrimethylsilane in chloroform: formation of ethyl *N*-[3-chloro-5-methoxy-[1]benzothien-2-oyl]glycinate 68



A solution of 52 (15 mg, 0.05 mmol) in chloroform (5 ml) at -78°C under nitrogen was treated with an excess of iodotrimethylsilane (0.1 ml). The reaction mixture was allowed to warm up to room temperature and stirred for 2 hours before being quenched with a mixture of methanol and water (1:3, 10 ml). The aqueous mixture was extracted with chloroform (3x10 ml). The combined organic extracts were washed with a saturated solution of sodium thiosulphate to remove iodine produced during the reaction, then with brine

(10 ml) and dried. Removal of the solvent afforded the ester 68 (17 mg, 100%). Mass spectrum: *m/z* (EI) 327 (M^+, 20), 298 (2, M^+-Et), 282 (3, M^+-OEt), 225 (100), 197 (17). ^1H n.m.r.: δ 7.73, s, NH; 7.70, d, J 9.2 Hz, H7; 7.25, d, J 2.8 Hz, H4; 7.14, dd, J_1 9.2 Hz, J_2 2.8 Hz, H6; 4.30, d, J 4.4 Hz, $NHCH_2$; 4.29, dt, J_1 7.2 Hz, J_2 6.8 Hz, OCH_2CH_3; 3.92, s, OCH_3; 1.34, t, J 7.2 Hz, CH_2CH_3. υ_{max} (thin film in dichloromethane) 3256 (w, NH), 1720 (m, CO), 1600 (m, CONH), 1567 (m) cm^{-1}.

2. With iodotrimethylsilane in quinoline:

A mixture of 52 and iodotrimethylsilane (excess) in quinoline under nitrogen was heated at ~180°C for 70 min. T.l.c. indicated no reaction, except small amount of starting material was decomposed. Reflux overnight completely decomposed the starting material.

3. With sodium cyanide in dimethyl sulfoxide:

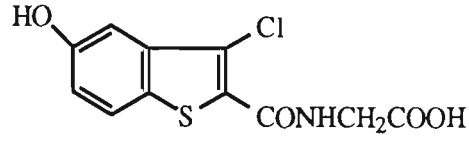
A mixture of 52 (50 mg) and sodium cyanide (*ca* 20 mg) in dimethyl sulfoxide (5 ml) was heated under nitrogen for 2.5 hours. After cooling to room temperature, the mixture was quenched with hydrochloric acid (6 M, 1ml) and the aqueous mixture was extracted with chloroform (2x10 ml). Evaporation of the solvent gave decomposed mixtures.

4. With lithium diphenylphosphide:

To a suspension of lithium (18 mg, 2.6 mmol) in THF (5 ml) at room temperature under nitrogen was added dropwise diphenylphosphine chloride (0.2 ml, 1.3 mmol). After 1.5 hours of stirring the lithium had fully dissolved and a brown solution was generated. The solution was treated with 52 (78 mg,

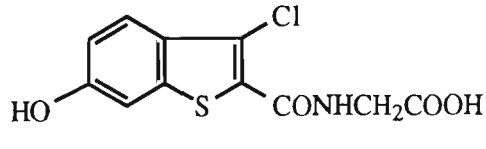
0.26 mmol). After stirring overnight, the starting material was completely decomposed.

Preparation of *N*-[3-Chloro-5-hydroxy-[1]benzothien-2-oyl]glycine 56



A solution of 52 (40 mg, 0.13 mmol) in dichloromethane (5 ml) at -78°C under N_2 was treated with an excess of boron tribromide (0.1 ml) overnight. The reaction mixture was diluted with dichloromethane (15 ml) before being filtered through a short column of silica gel under vacuum, eluting with a mixture of ethyl acetate, hexane and acetic acid (60:40:1). Removal of the solvent afforded the acid 56 (37 mg, 97%) as colurless crystals, m.p. 220°C (decomposition). Mass spectrum: *m/z* (EI) 285 (M^+, 7), 267 (2), 241 (6), 211 (40), 183 (15) (accurate mass: $M^{+\bullet}$, 284.9875. $C_{11}H_8ClNO_4S$ requires 284.9863). ^1H n.m.r. (D_2O): δ 7.62, d, J 8.8 Hz, H7; 7.08, d, J 2.4 Hz, H4; 6.95, dd, J_1 8.8 Hz, J_2 2.4 Hz, H5; 3.81, s, CH_2. υ_{max} 3223 (s, broad), 1746 (m), 1602 (m), 1560 (m) cm^{-1}.

Preparation of *N*-[3-Chloro-6-hydroxy-[1]benzothien-2-oyl]glycine 57

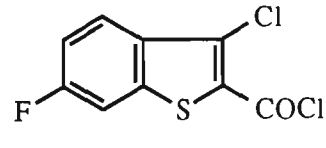


1. A mixture of 53 (30 mg, 0.1 mmol) and sodium sulfide (20 mg) in *N*-methylpyrolidone (NMP, 5 ml) under nitrogen was heated at 140-160°C for 3 hours. Although t.l.c. displayed a clear reaction, it was too difficult to remove high boiling point solvent (NMP).

2. A mixture of 53 (50 mg, 0.17 mmol) and sodium iodide (30 mg, 1.2 eq) in hydrogen bromide aqueous solution (w/w 47%) was heated under a sealed tube at 90-94°C for 3 hours. The reaction mixture was left to cool to room temperature and water (20 ml) was added. The starting material was removed by extraction with dichloromethane and the aqueous layer was concentrated to give the acid 57 (40 mg, 84%)

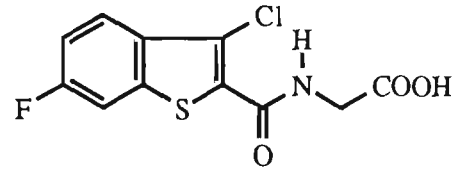
3. To a solution of 53 (90 mg, 0.3 mmol) in dichloromethane (15 ml) at -78°C under nitrogen was added dropwise boron tribromide (*ca* 0.2 ml) and the mixture was stirred overnight. The reaction mixture filtered through a column of silica gel followed by washing with a large amount of ethyl acetate to give the acid 57 (75 mg, 87%) as colourless crystals, m.p. 180°C. Mass spectrum: *m/z* (EI) 285 (M^+, 8), 241 (5), 227 (6), 211 (40), 183 (15). ^1H n.m.r. (acetone-d6): δ 7.75, dd, J_1 8.8 Hz, J_2 0.4 Hz, H4; 7.40, dd, J_1 2.0 Hz, J_2 0.4 Hz, H7; 7.15, dd, J_1 8.8 Hz, J_2 2.0 Hz, H5; 4.10 s, CH_2. υ_{max} 3223 (s, broad), 1744 (m, CO), 1602 (s, ArCONH) cm^{-1}.

Preparation of 3-Chloro-6-fluoro-[1]benzothiophene-2-carbonyl Chloride 59



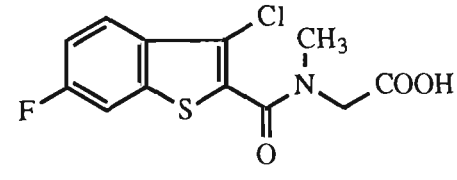
To a solution of 4-fluoro-*trans*-cinnamic acid (5 g, 30 mmol) in toluene (50 ml) containing pyridine (0.5 ml, 3 mmol) was added thionyl chloride (11.1 ml, 150 mmol). After 66 hours of refluxing, toluene was evaporated to give a crude product, which was recrystallized from dichloromethane to give the acid chloride 59 (4.4 g, 59%) as pale yellow needles.

Preparation of *N*-[3-Chloro-6-fluoro-[1]benzothien-2-oyl]glycine 55



The acid chloride 59 (250 mg, 1 mmol) was added to a mixture of glycine (114 mg, 1.5 mmol) and sodium hydroxide (0.5 M, 5 ml). The mixture was then stirred overnight. Hydrochloric acid (1 M) was added to precipitate the product, which was collected and washed with cold diethyl ether to give the acid 55 (260 mg, 91%) as colourless crystals, m.p. 220-221°C. Mass spectrum: *m/z* (CI) 288 (MH^+, 100), 243 (20), 213 (75). ^1H n.m.r. (MeOH-d4): δ 7.95, dd, J_1 9.2 Hz, J_2 4.8 Hz, H4; 7.75, dd, J_1 2.0 Hz, J_2 8.8 Hz, H7; 7.35, ddd, J_1 9.2 Hz, J_2 8.8 Hz, J_3 2.0 Hz, H5; 4.20, s, CH_2. υ_{max} 3350 (w, broad), 1760 (m, $CH_2\underline{CO}OH$), 1600 (m, Ar$\underline{CO}$NH), 1567 (m) cm^{-1}.

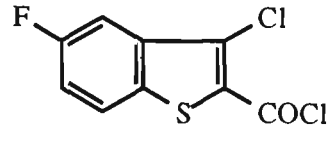
Preparation of *N*-[3-Chloro-6-fluoro-[1]benzothien-2-oyl]sarcosine 67



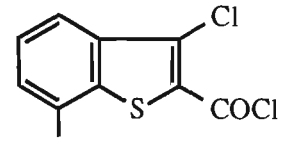
The acid chloride 59 (250 mg, 1 mmol) was added to a mixture of sarcosine (134 mg, 1.5 mmol) and sodium hydroxide (0.5 M, 5 ml). After stirring overnight, the reaction was quenched with hydrochloric acid (1 M). The precipitate formed was collected and washed with a mixture of diethyl ether and hexane (1:1) to afford the acid 67 (290 mg, 97%) as colourless crystals, m.p. 164-166°C. (Found: C 47.92, H 2.96, N 4.59. $C_{12}H_9ClFNO_3S$ requires C 47.77, H 3.01, N 4.64). Mass spectrum: *m/z* (CI) 302 (MH^+, 55), 221 (100). ^1H n.m.r. (MeOH-d4): δ 7.95, dd, J_1 9.2 Hz, J_2 4.8 Hz, H4; 7.75, dd, J_1 2.0 Hz, J_2 8.8 Hz,

H7; 7.35, ddd, J_1 9.2 Hz, J_2 8.8 Hz, J_3 2.0 Hz, H5; 4.30, s, CH_2; 3.20, s, CH_3. υ_{max} 3100 (m, broad), 1750 (m, CO), 1600 (m, ArCON) cm^{-1}.

Preparation of 3-Chloro-5-fluoro-[1]benzothiophene-2-carbonyl Chloride 62



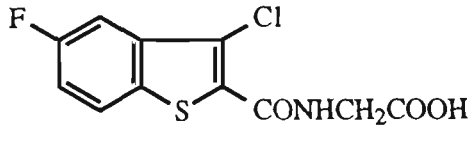
62



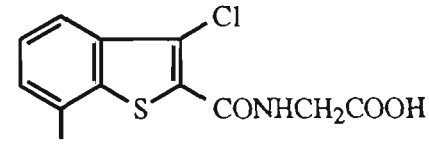
63

To a solution of 3-fluoro-*trans*-cinnamic acid (2.49 g, 14 mmol) in toluene (50 ml) containing pyridine (0.2 ml, 1.5 mmol) was added thionyl chloride (5 ml, 70 mmol). After refluxing for 48 hours, toluene was evaporated to give a crude product, which was chromatographed (5% ethyl acetate/hexane) to give a mixture of the acid chloride 62 and the acid chloride 63 (2:1, 2.1 g, 57%).

Preparation of *N*-[3-Chloro-5-fluoro-[1]benzothien-2-oyl]glycine 54



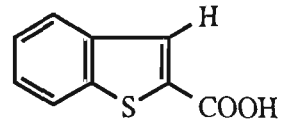
54



65

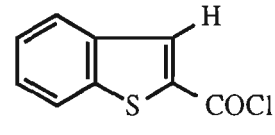
A mixture of acid chloride 62 and acid chloride 63 (500 mg, 2 mmol) was treated with a solution of glycine (228 mg, 3 mmol) in sodium hydroxide (0.5 M, 5 ml). The reaction mixture was stirred overnight. Hydrochloric acid (1 M) was added to precipitate the product. The precipitate was chromatographed to give the product (400 mg, 71%) as a mixture of the acid 54 and the acid 65.

Preparation of [1]Benzothiophene-2-carboxylic Acid 71



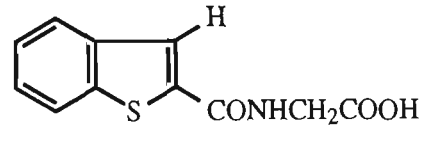
Methyl [1]benzothiophene-2-carboxylate 70 (3.95 g, 20 mmol) in methanol (20 ml) was treated with sodium hydroxide (1 N, 25 ml, 25 mmol), and the reaction mixture was then refluxed for two hours. After cooling down, the mixture was acidified with hydrochloric acid (1 M) to precipitate the product. The crystals were collected, washed with water, then ether to afford the acid 71 (3.41 g, 93%) as colourless crystals, m.p. 236-238°C.

Preparation of [1]Benzothiophene-2-carboxylic Acid Chloride 72



A mixture of acid 71 (0.89 g, 5 mmol) and thionyl chloride (0.73 ml, 10 mmol) was refluxed for 3 hours. The excess of thionyl chloride was removed under reduced pressure to give the acid chloride 72 (0.94 g, 95%) as pale yellow crystals .

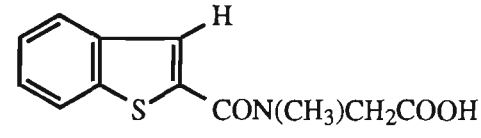
Preparation of *N*-([1]Benzothien-2-oyl)glycine 73



To a solution of glycine (90 mg, 1.2 mmol) in sodium hydroxide (0.5 M, 2.4 ml, 1.2 mmol) was added [1]benzothiophene-2-carbonyl chloride 72 (197 mg, 1 mmol). The reaction mixture was stirred for three hours, hydrochloric acid (1 M, 2.4 ml) was added. The resultant precipitate was collected to afford the acid 73 (199 mg, 85%) as colourless crystals, m.p. 200-202°C. (Found: C 55.98, H 4.01, N 6.06. $C_{11}H_9NO_3S$ requires C 56.16, H 3.86, N 5.95). Mass spectrum: *m/z* (EI)

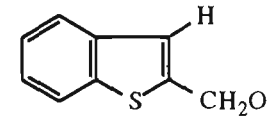
235 (M^+, 20), 217 (1), 191 (12), 161 (100), 133 (19). ^{1}H n.m.r. (DMSO-d6): δ 9.14, t, J 4.8 Hz, NH; 8.10, s, H3; 8.03-8.01, m, H4; 7.96-7.94, m, H7; 7.48-7.43, m, H5, H6; 3.87,d, J 4.8 Hz, $\underline{CH_2}COOH$. υ_{max} 3410 (w, sharp), 3359 (w, sharp), 1738 (s), 1526 (s) cm^{-1}.

Preparation of *N*-([1]Benzothien-2-oyl)sarcosine 74



A solution of sodium hydroxide (0.5 M, 2.6 ml, 1.3 mmol) was added to sarcosine (116 mg, 1.3 mmol) and the mixture stirred for 15 mins. The acid chloride 72 (196.5 mg, 1 mmol) was added. After 3 hours, the reaction was quenched with hydrochloric acid (1 M). The precipitate formed was collected and washed with diethyl ether to provide the acid 74 (154 mg, 62%) as colourless crystals, m.p. 142-144°C. (Found: C 57.66, H 4.43, N 5.66. $C_{12}H_{11}NO_3S$ requires C 57.82, H 4.45, N 5.62). Mass spectrum: *m/z* (EI) 249 (M^+, 12), 205 (10), 161 (100), 133 (23). ^{1}H n.m.r. (DMSO-d6): δ 8.01-7.89, m, H4; 7.96-7.92, m, H7; 7.88, s, H3; 7.46-7.39, m, H5, H6; 4.20, s, $\underline{CH_2}COOH$; 3.03, s, CH_3. υ_{max} 3200 (m, broad), 1740 (m), 1593 (s), 1563 (m) cm^{-1}.

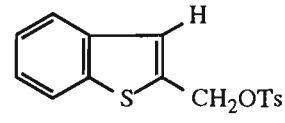
Preparation of 2-Hydroxymethyl-[1]benzothiophene 75



A solution of 70 (1 g, 5.2 mmol) in ethanol (10 ml) was treated with sodium borohydride (300 mg, 8 mmol) and the mixture was stirred overnight before being quenched with saturated ammonium solution. The mixture was extracted with ethyl acetate (3x30 ml). The extracts were washed with brine (20

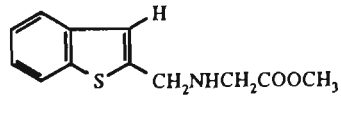
ml), dried and evaporated, to give a crude product. Chromatography of the crude product (50% ethyl acetate/hexane) afforded the alcohol 75 (0.8 g, 95%) as colourless crystals, m.p. 95-97°C. (Found: C 65.77, H 4.96. C_9H_8OS requires C 65.82, H 4.91). Mass spectrum: *m/z* (EI) 164 (M^+, 40), 147 (80). ^1H n.m.r.: δ 7.82-7.80, m, H4; 7.76-7.70, m, H7; 7.48-7.39, m, H5, H6; 7.22, s, H3; 4.94, s, $\underline{CH_2}OH$; 1.60, s, broad, $CH_2\underline{OH}$. ^{13}C n.m.r.: 144.8, C2; 139.9, C7a; 139.5, C3a, 124.5, C4; 124.4, C7, 123.5, C5; 122.5, C6; 121.5, C3, 61.2, CH_2OH. υ_{max} 3243 (m, broad), 3061 (w) cm^{-1}.

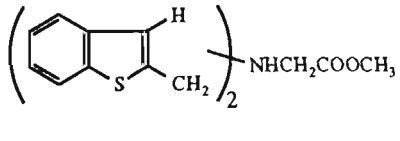
Preparation of [1]Benzothien-2-ylmethyl Tosylate 76



A solution of *p*-toluenesulfonyl chloride (0.83 g, 4 mmol) in dichloromethane (5 ml) was added dropwise to a stirred solution of 75 (0.6 g, 3.7 mmol) in dichloromethane (30 ml) containing triethylamine (0.4 ml, 4 mmol) and the mixture stirred overnight. The solvent was evaporated to afford a residue, which was chromatographed (1% ethyl acetate/hexane) to yield the tosylate 76 (320 mg, 82%, on the basis of the recovery of the starting material) as pale yellow crystals, m.p. 148-150°C. Mass spectrum: *m/z* (EI) 147 (M^+-OTs, 100). ^1H n.m.r.: δ 7.82-7.66, m, 4H; 7.60-7.48, m, 4H; 7.32, s, H3; 4.75, s, CH_2; 2.34, s, CH_3. υ_{max} 3065 (w) 1547 (m) cm^{-1}.

Preparation of *N*-([1]Benzothien-2-ylmethyl)glycine Methyl Ester 77 and *N*, *N*-[Di-[1]benzothien-2-ylmethyl]glycine Methyl Ester 78





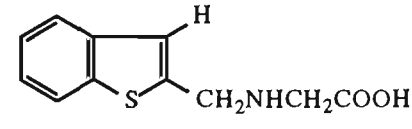
Triethylamine (0.2 ml, 1 mmol) was added to a suspension of glycine methyl ester hydrochloride (200 mg, 0.75 mmol) in dichloromethane (10 ml).

After stirring for 30 mins, tosylate 76 was added and the mixture refluxed for 12 hours. The solvent was removed to leave a residue, which was chromatographed (10%, then 50% ethyl acetate/hexane) to give the ester 77 (93 mg, 79%) as colourless crystals, m.p. 87-89°C and the ester 78 (40 mg, 17%) as colourless crystals, m.p. 79-81°C.

For the ester 77: Mass spectrum: *m/z* (EI) 235 (M^+, 55), 176 (30), 162 (100), 147 (100). ^1H n.m.r.: δ 7.82-7.807.79, dd, J_1 0.8 Hz, J_2 8.0 Hz, H4; 7.70, dd, J_1 0.8 Hz, J_2 8.0 Hz, H7; 7.35-7.26, m, H5, H6; 7,17, s, H3; 4.12, s, $\underline{CH_2}COOCH_3$; 3.74, s, OCH_3; 3.52, s, $Ar\underline{CH_2}NH$. υ_{max} (thin film in dichloromethane) 3346 (m, NH), 3059 (m), 1740 (s, CO) cm^{-1}.

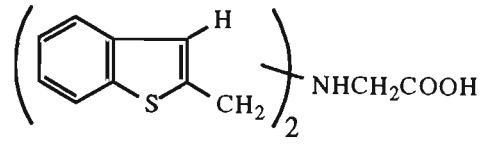
For the ester 78: Mass spectrum: *m/z* (EI) 381 (M^+, 11), 322 (4), 234 (20), 174 (5), 147 (100). ^1H n.m.r.: δ 7.83-7.81, m, 2xH4; 7.71-7.69, m, 2xH7; 7.34-7.26, m, 2xH5, H6; 7.16, s, 2xH3; 4.24, s, $\underline{CH_2}COOCH_3$; 3.73, s, OCH_3; 3.54, s, $2xCH_2$. υ_{max} 1733 (m) cm^{-1}.

Preparation of *N*-([1]Benzothien-2-ylmethyl)glycine 79



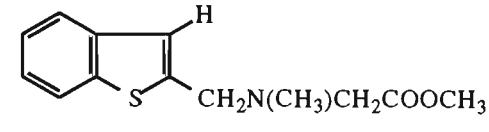
A mixture of 77 (70 mg, 0.3 mmol) and sodium hydroxide (1 M, 0.6 ml, 0.6 mmol) in methanol (5 ml) was stirred overnight. Hydrochloric acid (1 M) was added to precipitate the product. The precipitate was collected by filtration and washed with water, then a mixture of diethyl ether and hexane (1:1) to afford the acid 79 (65 mg, 97%) as colourless crystals, m.p. 192-194°C. Mass spectrum: *m/z* (EI) 221 (M^+, 5), 175 (18), 162 (40), 147 (100) (accurate mass: $M^{+\bullet}$, 221.0511. $C_{11}H_{11}NO_2S$ requires 221.0511). ^1H n.m.r. (MeOH-d4): δ 7.90-7.78, m, H4; 7.85-7.82, m, H7; 7.52, s, H3; 7.41-7.38, m, H5, H6; 4.51, s, $\underline{CH_2}COOH$; 3.51, $\underline{CH_2}NH$ υ_{max} 1682 (m) cm^{-1}.

Preparation of *N*, *N*-[Bis-[1]benzothien-2-ylmethyl]glycine 80



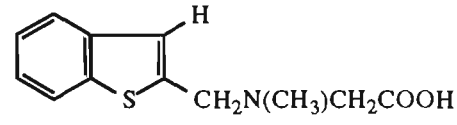
A mixture of 78 (50 mg, 0.13 mmol) and sodium hydroxide (0.5 M, 2 ml) in methanol (5 ml) was refluxed for three hours. Methanol was removed and hydrochloric acid (1 M) added to provide a precipitate. The precipiate was collected and washed with hexane to afford the acid 80 (45 mg, 94%) as colourless crystals, m.p. 250°C decomposition. Mass spectrum: *m/z* (ES+) 368 (MH^+, 100) (accurate mass: $M^{+\bullet}$, 367.0692. $C_{20}H_{17}NO_2S_2$ requires 367.0700). ^1H n.m.r. (DMSO-d6): δ 7.88, dd, J_1 1.2 Hz, J_2 8.0 Hz, 2xH4; 7.72, dd, J_1 1.2 Hz, J_2 8.0 Hz, 2xH7; 7.32-7.26, 2xH5&H6; 7.24, s, Ar-2xH3; 4.25, s, $\underline{CH_2}COOH$; 3.32, s, $2xCH_2$. υ_{max} 1686 (m) cm^{-1}.

Preparation of *N*-([1]Benzothien-2-ylmethyl)sarcosine Methyl Ester 81



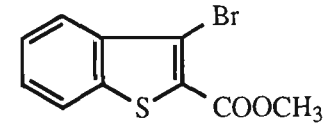
A mixture of tosylate 76 (80 mg, 0.25 mmol), sarcosine methyl ester hydrochloride (150 mg, 1 mmol) and triethylamine (0.15 ml) in dichloromethane was refluxed for 40 hours. The solvent was then evaporated to give a residue, which was chromatographed (10%, then 40% ethyl acetate/hexane) to afford the ester 81 (20 mg, 74% based on the recovery of 45 mg of the starting material). Mass spectrum: *m/z* (EI) 249 (M^+, 5), 190 (20), 176 (15), 147 (100) (accurate mass: $M^{+\bullet}$, 249.0822. $C_{13}H_{15}NO_2S$ requires 249.0824). ^1H n.m.r.: δ 7.81-7.79, m, H4; 7.71-7.69, m, H7; 7.34-7.26, m, H5, H6; 7.16, s, H3; 4.02, s, $\underline{CH_2}COOCH_3$; 3.72, s, OCH_3; 3.37, s, $\underline{CH_2}N$; 2.44, s, CH_3. υ_{max} 1720 (s, CO) cm^{-1}.

Preparation of *N*-([1]Benzothien-2-ylmethyl)sarcosine 82



A mixture of the ester 81 (20 mg, 0.08 mmol) and sodium hydroxide (1 M, 1 ml, 1 mmol) in methanol (5 ml) was refluxed for three hours. Methanol was removed and hydrochloric acid (1 M, 1 ml) added to give a precipitate, which was collected by filtration and washed with a mixture of diethyl ether and hexane (1:3) to provide the acid 82 (18 mg, 95%) as colourless crystals, m.p. 174-176°C. Mass spectrum: *m/z* (CI) 236 (MH^+, 35), 190 (100), 176 (90), 147 (100) (accurate mass: $M^{+\bullet}$, 235.0667. $C_{12}H_{13}NO_2S$ requires 235.0667). ^1H n.m.r. (MeOH-d4): δ 7.92-7.90, m, H4; 7.87-7.85, m, H7; 7.58, s, H3; 7.43-7.39, m, H5, H6; 4.63, s, Ar$\underline{CH_2}$N; 3.64, s, $\underline{CH_2}$COOH; 2.87, s, CH_3. υ_{max} 3420 (broad, w, CO$\underline{OH}$), 3221 (w), 1674 (m), 1622 (m) cm^{-1}.

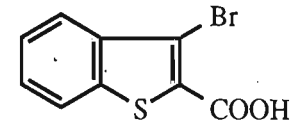
Preparation of Methyl 3-Bromo-[1]benzothiophene-2-carboxylate 83.



Bromine (0.28 ml, 5.04 mmol) was added dropwise to a suspension of methyl [1]benzothiophene-2-carboxylate (0.96 g, 5 mmol) and anhydrous sodium acetate (500 mg, 6 mmol) in acetic acid (15 ml). After stirring 12 hours at room temperature, some of the starting material was unreacted (t.l.c analysis). The mixture was warmed up to 40°C and maintained that temperature for additional 40 hours. Acetic acid was removed under reduced pressure and the resultant residue was dissolved in chloroform, and a solution of sodium thiosulphate was added to remove the rest of bromine. The mixture was extracted with chloroform (3x20 ml). The combined extracts were washed

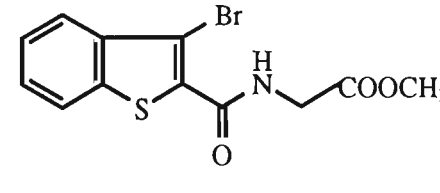
with brine (10 ml), dried and concentrated to give a mixture, which was chromatographed (2% ethyl acetate/hexane) to provide a crude product. After recrystallization from a mixture of ethyl acetate and hexane (2:100), the ester 83 (0.92 g, 70%) was obtained as colourless crystals, m.p. 57-58°C. Mass spectrum: *m/z* (EI) 272 (M^+, 48), 241 (75), 213 (23), 192 (13), 161 (25), 132 (100). ^1H n.m.r.: δ 7.22-8.26, m, H4, 5, 6 and7; 3.98, s, CH_3. υ_{max} 1730 (s, ArCO), 1516 (m) cm^{-1}.

Preparation of 3-Bromo-[1]benzothiophene-2-carboxylic Acid 84.



A solution 83 (271 mg, 1 mmol) in methanol (5 ml) was treated with an excess of sodium hydroxide (5 M, 0.5 ml), and stirred for two hours. The solvent was evaporated to give a residue, which was dissolved in small amount of water and neutralized with hydrochloric acid (1 M) to form a precipitate. The precipitate was collected and washed with water, then diethyl ether, to give the acid 84 as a white powder in quantitative yield. Mass spectrum: *m/z* (EI) 258 (M^+, 66), 241 (35), 211 (15), 178 (38), 161 (32), 132 (65). ^1H n.m.r. (DMSO-d6): δ 8.10, m, H4, H7; 7.62, m, H5, H6. υ_{max} 1680 (m, CO) cm^{-1}.

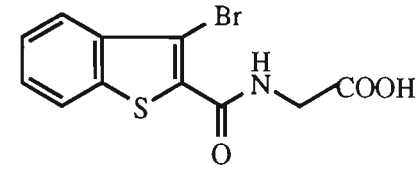
Preparation of Methyl *N*-[3-Bromo-[1]benzothien-2-oyl]glycinate 85.



A mixture of the acid 84 (136 mg, 0.5 mmol), 1,3-dicyclohexylcarbodiimide (104 mg, 0.5 mmol), 1-hydroxybenzotriazole (75 mg, 0.5 mmol) and glycine methyl ester (150 mg, 1.2 mmol) in THF was stirred overnight. Removal of the solvent followed by chromatography (15% ethyl

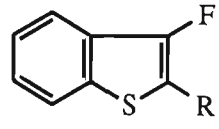
acetate/hexane) afforded the ester 85 (100 mg, 61%) as colourless crystals, m.p. 86-87°C, 61.0%). Mass spectrum: *m/z* (EI) 329 (M^+, 10), 268 (10), 239 (100), 211 (18) (accurate mass: $M^+\bullet$, 326.9563. $C_{12}H_{10}{}^{79}BrNO_3S$ requires 326.9565). ^1H n.m.r.: δ 7.91 d, J 0.8 Hz, H4; 7.78, dd, J_1 8.0 Hz, J_2 0.8 Hz, H7; 7.56, dd, J_1 7.6 Hz, J_2 0.8 Hz, H5; 7.28, t, J 8.0 Hz, H6; 6.85, s, broad, NH; 4.27, d, J 4.8 Hz, $\underline{CH_2}COOCH_3$; 3.82, s, OCH_3. ^{13}C n.m.r.: 170.1 $\underline{C}OOCH_3$; 161.8, CONH; 141.6, C2; 139.0, C3, 138.6, C7a, 128.2, C4, 127.3, C7, 125.3, C5; 121.8, C6; 118.7, C3a, 52.6, OCH_3; 41.7, $\underline{C}H_2COOCH_3$. υ_{max} 3342 (m, NH), 3076 (w), 1755 (s, $\underline{CO}OCH_3$), 1633 (s, Ar$\underline{CO}$NH) cm^{-1}.

Preparation of *N*-[3-Bromo-[1]benzothien-2-oyl]glycine 86.



A solution of 85 (50 mg, 0.15 mmol) in methanol (5 ml) was treated with sodium hydroxide solution (5 M, 1 ml) and then stirred overnight. Hydrochloric acid (1 M) was added to neutralize the solution and the mixture was extracted with ethyl acetate (3x15 ml). The combined extracts were washed with brine (10 ml), then dried and evaporated to give the acid 86 (47 mg, 99%) as colourless crystals, m.p. 185-187°C. Mass spectrum: *m/z* (ES-) 312 (M^-, 100), 213 (15) (accurate mass: $M^+\bullet$, 312.9302. $C_{11}H_8{}^{79}BrNO_3S$ requires 312.9408). ^1H n.m.r.: δ 8.14 d, J 0.8 Hz, H4; 7.92, td, J_1 8.0 Hz, J_2 0.8 Hz, H7; 7.62, dd, J_1 8.0 Hz, J_2 0.8 Hz, H5; 7.35, t, J 8.0 Hz, H6; 6.85, s, broad, NH; 4.12, s, $\underline{CH_2}COOH$. υ_{max} 3486 (m), 3273 (m), 1732 (s), 1626 (s) cm^{-1}.

Attempted Introduction of Fluorine into the 3-Position of [1]Bezothiophene Derivatives

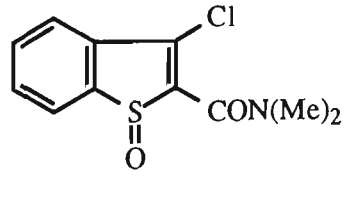


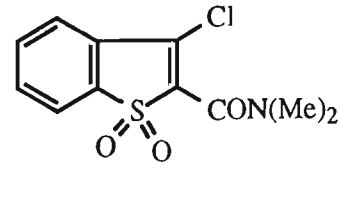
1. A mixture of 35 (150 mg, 0.5 mmol) and potassium fluoride (87 mg, 1.5 mmol) in dimethyl sulfoxide (5 ml) was refluxed for 5 hours. T.l.c. analysis showed no reaction had occurred. Refluxing overnight gave a decomposed mixture.

2. A mixture of acid chloride 21 (20 mg, 0.09 mmol) and potassium fluoride (*ca* 15 mg) dimethyl sulfoxide (5 ml) was refluxed for 2 hours. To this was added methanol (2 ml) and the reaction mixture was stirred for 1 hour. Solvents were removed under high vacuum and the residue was chromatographed (2% ethyl acetate) affording a product as 3-chloro-[1]benzothiophene 88 (10 mg, 67%).

3. A mixture of potassium fluoride (300 mg, excess) and the ester 89 (120 mg, 0.5 mmol) in dimethyl sulfoxide (3 ml) was refluxed for three hours. T.l.c. analysis revealed the starting material had decomposed.

Preparation of *N,N*-Dimethyl-3-chloro-[1]benzothiophene-2-carboxamide Sulfoxide 91 and Sulfone 92.





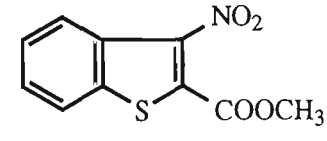
To a solution of 90 (240 mg, 1 mmol) in dichloromethane (5 ml) at 0°C was added *meta*-chloroperoxybenzoic acid (350 mg, 56% content, 1 mmol). After stirring overnight at room temperature overnight, the reaction was

quenched with sodium bicarbonate solution and extracted with dichloromethane (3x15 ml). The solvent was removed to give a crude product which was chromatographed to afford the sulfoxide 91 (121 mg, 45%), m.p. 122-124°C, and the sulfone 92 (77 mg, 30%), m.p. 130-132°C.

For the sulfoxide 91: Mass spectrum: *m/z* (EI) 271 (M^+, 10), 167 (10). ^1H n.m.r. (DMSO-d6): δ 8.08-8.04, m, H4; 7.90-7.84, m, H7; 7.82-7.78, m, H5, H6; 3.08, s, CH_3; 3.01, s, CH_3. υ_{max} 1637 (m), 1571 (w) cm^{-1}. Mass spectrum: *m/z* (EI) 271 (M^+, 10), 167 (10).

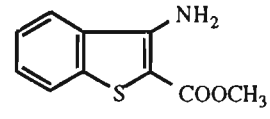
For the sulfone 92: Mass spectrum: *m/z* (CI) 256 (MH^+, 15), 238 (20), 195 (100), 183 (20), 167 (35). ^1H n.m.r. (DMSO-d6): δ 8.12-8.10, m, H4; 7.82-7.78, m, H7; 7.74-7.72, m, H5, H6; 3.12, s, CH_3; 3.04, s, CH_3. υ_{max} 1638 (m), 1571 (w) cm^{-1}.

Preparation of Methyl 3-Nitro-[1]benzothiophene-2-carboxylate 93



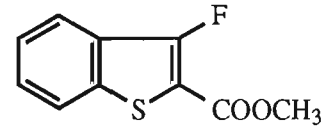
To a suspension of methyl [1]benzothiophene-2-carboxylate 70 (1.92 g, 10 mmol) in acetic acid (15 ml) at 0°C was added dropwise concentrated nitric acid (4.86 ml, 68 mmol). After stirring overnight at room temperature, no reaction was observed. The mixture was gently refluxed until no starting material remained (*ca* 8 hours). The reaction mixture was poured into an ice-water mixture (50 ml), then extracted with ethyl acetate (3x30 ml). The combined extracts were washed with brine (20 ml) and dried. Evaporation of the solvent led to a crude product, which was chromatographed (8% ethyl acetate/hexane) to afford the ester 93 (500 mg, 21%) as yellow crystals, m.p. 101-102°C. Mass spectrum: *m/z* (EI) 272 (M^+, 48), 241 (75), 213 (23), 192 (13), 161 (25), 132 (100). ^1H n.m.r.: δ 8.02-7.98, m, H4; 7.88-7.85, m, H7; 7.59-7.55, m, H5, H6; 3.98, s, $COOCH_3$. υ_{max} 1726 (s, CO) cm^{-1}.

Preparation of Methyl 3-Amino-[1]benzothiophene-2-carboxylate 94



Stannous chloride dihydrate (200 mg, 1 mmol) was added to a solution of 93 (200 mg, 0.84 mmol) in methanol (5 ml) containing concentrated hydrochloric acid (10 drops). After 3 hours of stirring , sodium hydroxide solution (5 M) was added to neutralize the acidic solution. The precipitate was formed and removed by suction. The filtrate was extracted with ethyl acetate (3x20 ml) and the combined extracts were washed with brine (15 ml) and dried. Removal of the solvent yielded the ester 94 (170 mg, 98%) as yellow crystals, m.p. 109-110°C, literature[106] m.p. 110-111°C.

Attempted Preparation of Methyl 3-Fluoro-[1]benzothiophene-2-carboxylate 95



1. Finely crushed 94 (2.07 g, 10 mmol) was added to a stirred hydrochloric acid solution (5 M, 5 ml) at 0°C and the mixture was then stirred for 30 mins. A solution of sodium nitrite (750 mg, 11 mmol) in water (10 ml) was added dropwise to the mixture at 5°C until a drop of reaction mixture turned KI-starch paper purple instantly. After 5 minutes stirring at ~5°C, an ice-chilled solution of sodium tetrafluoroborate was added. The temperature of the reaction mixture was maintained below 10°C for 20 mins, then it was filtered by suction to give a residue, which was successively washed with cold water, methanol and diethyl ether to afford a dry yellow powder. The yellow powder was heated at 120°C until the colour turned to dark brown (~ 20 mins). The brown residue was dissolved in dichloromethane (50 ml) and washed with

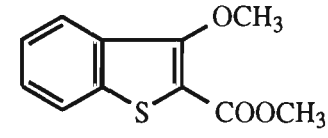
water (2x10 ml) and brine (10 ml). The solvent was evaporated to give a crude product that was chromatographed (10% ethyl acetate in hexane) to provide a mixture (0.5 g) of mainly methyl 3-chloro-[1]benzothiophene-2-carboxylate 89, and a little (*ca.* 5%) of the 3-fluoro compound 95.

2. The reaction was repeated as above except sulphuric acid (6 M) was used instead of hydrochloic acid to afford a trace (< 1 mg) of the 3-fluoro ester 95 in a tiny amount.

3. A mixture of 94 (6.2 g, 30 mmol) and hydrochloric acid (1.25 M, 70 mml) was boiled until the carboxylate was dissolved. After cooling down, a solution of sodium nitrite (3 g, 43 mmol) in water (7 ml)was added dropwise to the mixture at 5°C. The mixture was stirred for 5 mins. Hexafluorophosphoric acid (65% w/w in water, 8 ml) was then added to the reaction mixture to afford a black precipitate immediately. After 30 mins, the reaction mixture was filtered under vacuum. The solid was washed with cold water (50 ml), and a mixture of diethyl ether and methanol (4:1, 20 ml). A black solid was obtained and dried under vacuum. The solid was heated at 170°C for 5 mins. The residue was dissolved in dichloromethane (30 ml) and washed with water (2x15 ml) and brine (10 ml), and dried. The solvent was evaporated to give a crude product, which was chromatographed (5% ethyl acetate in hexane) to afford the ester 70 (1.2 g).

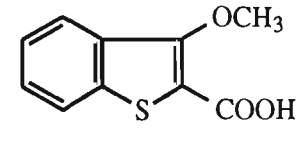
4. A mixture of 70 (91 mg, 0.5 mmol) and 1-fluoropyridinum triflate (123 mg, 1.05 mmol) in dichloromethane (5 ml) was refluxed overnight. No reaction was observed by t.l.c. analysis. The starting material was recovered after work-up.

Preparation of Methyl 3-Methoxy-[1]benzothiophene-2-carboxylate 110



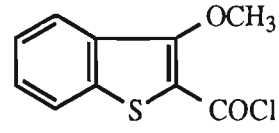
Methanol (0.9 ml, 22.2 mmol) was added dropwise to a suspension of sodium hydride (860 mg, 60% content, 21.5 mmol) in THF (15 ml) at room temperature under nitrogen. After stirring for 1 hour, a solution of the acid chloride 21 (1.7 g, 7.4 mmol) in THF (15 ml) was added dropwise. The reaction mixture was refluxed overnight and then allowed to cool to room temperature before being quenched with a saturated ammonium chloride solution. The mixture was extracted with ethyl acetate (3x30 ml). The combined extracts were washed with brine (20 ml), and dried. Removal of the solvent afforded the ester 110 (1.46 g, 89%) as colourless crystals, m.p. 110-112°C. Mass spectrum: *m/z* (EI) 222 (M^+, 100%), 207 (10), 191 (60), 176 (45). ^{1}H n.m.r.: δ 8.08, m, H4; 7.92, m, H7; 7.60, m, H5, H6; 4.10, s, 3-OCH3; 3.9, s, $COOCH_3$. υ_{max} 1718 (s, C=O), 1593 (m) cm^{-1}.

Preparation of 3-Methoxy-[1]benzothiophene-2-carboxylic Acid 111



A mixture of the ester 110 (1 g, 4.5 mmol) and sodium hydroxide (1 M, 5 ml) in methanol (25 ml) was stirred overnight. The solvent was removed and the residue dissolved in water (5 ml) and neutralized with hydrochloric acid (1 M) to give a precipitate. Filtration under vacuum afforded the acid 111 (0.9 g, 97%) as colourless crystals, m.p. 250°C (decomposition). Mass spectrum: *m/z* (EI) 208 (M^+, 97), 164 (15), 149 (40). ^{1}H n.m.r.: δ 8.08, m, H4; 7.92, m, H7; 7.60, m, H5, H6; 4.10, s, OCH_3. υ_{max} 1686 (m, C=O) cm^{-1}.

Attempted Synthesis of 3-Methoxy-[1]benzothiophene-2-carbonyl Chloride 112



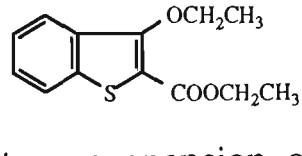
1. A suspension of 111 (750 mg, 3.6 mmol) in thionyl chloride (5 ml) was stirred at room temperature for 2 hours, no reaction was observed. After refluxing for 3 hours, t.l.c. analysis showed the starting material had decomposed.

2. A mixture of 111 (208 mg, 1 mmol) and thionyl chloride (excess) in carbon tetrachloride (5 ml) was refluxed under nitrogen for three hours. No reaction was noticed by t.l.c analysis. The starting material was recovered after work-up.

3. A mixture of 111 (1.06 g, 5.1 mmol) and phosporous pentachloride (1.06 g, 5.1 mmol) was heated at 60°C overnight under notrogen. A complex mixture was indicated by t.l.c. analysis.

4. Phosporous pentachloride (104 mg, 0.5 mmol) was added in portions to a suspension of 111 (104 mg, 0.5 mmol) in diethyl ether (5 ml) under nitrogen, and the mixture was then stirred vigorously overnight. Very little product was formed and after three hours of refluxing no further progress was observed by t.l.c. analysis.

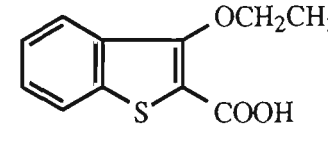
Preparation of Ethyl 3-Ethoxy-[1]benzothiophene-2-carboxylate 116



Ethanol (1.7 ml, 30 mmol) was added dropwise to a suspension of sodium hydride (1.2 g, 60% content, 30 mmol) in THF (80 ml) under nitrogen.

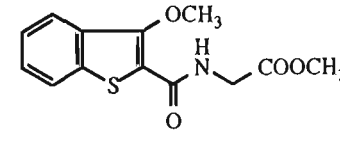
After stirring for 30 mins, a solution of the acid chloride 21 (2.31 g, 10 mmol) in THF (20 ml) was added and the mixture refluxed until no starting material remained. The reaction was quenched with saturated ammonium chloride solution (20 ml), and the aqueous mixture extracted with ethyl acetate (2x30 ml). The combined extracts were washed with brine (20 ml), dried and evaporated to afford the ester 116 (2.2 g, 88%). Mass spectrum: *m/z* (CI) 251 (MH^+, 20), 205 (20), 176 (80). ^1H n.m.r.: δ 7.86-7.83, m, H4; 7.76-7.73, m, H7; 7.47-7.43, m, H5; 7.41-7.39, m, H6; 4.394, q, J 6.8 Hz, $COO\underline{CH_2}CH_3$; 4.388, q, J 7.2 Hz, $O\underline{CH_2}CH_3$; 1.48, t, J 6.8 Hz, $COOCH_2\underline{CH_3}$; 1.41, t, J 7.2 Hz, 3-$OCH_2\underline{CH_3}$. υ_{max}3020 (w), 1711 (s), 1526 (m) cm^{-1}.

Preparation of 3-Ethoxy-[1]benzothiophene-2-carboxylic Acid 117



A mixture of the ester 116 (1 g, 4.0 mmol) and sodium hydroxide (0.5 M, 15 ml) in methanol (30 ml) was stirred overnight. The solvent was removed and the residue dissolved in water (5 ml) and neutralized with hydrochloric acid (1 M) to afford the acid 117 (0.85 g, 96%) as colourless crystal, m.p. 205°C (decomposition). Mass spectrum: *m/z* (EI) 237 (M^+, 50), 220 (23). ^1H n.m.r.: δ 7.80-7.83, m, H4; 7.77-7.71, m, H7; 7.47-7.43, m, H5; 7.41-7.39, m, H6; 4.38, q, J 7.2 Hz, $O\underline{CH_2}CH_3$; 1.41, t, J 7.2 Hz, $OCH_2\underline{CH_3}$. υ_{max}3028 (w), 1711 (s), 1538 (m) cm^{-1}.

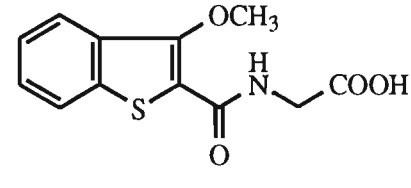
Preparation of Methyl *N*-[3-Methoxy-[1]benzothien-2-oyl]glycinate 113



To a solution of the acid 111 (208 mg, 1 mmol) in dimethylformamide (10 ml) was added dicyclohexylcarbodiimide (206 mg, 1mmol). The mixture

was stirred for 30 mins before 1-hydroxybenzotriazole (150 mg, 1.1 mmol) was added. After an additional half an hour of stirring, a solution of methyl glycinate (90 mg, 1.2 mmol) in chloroform was added. The reaction mixture was allowed to stir overnight and then quenched with water. The mixture was extracted with ethyl acetate (3x15 ml) and the extract was washed with brine (15 ml), then dried. Removal of the solvent gave a crude product, which was purified by chromatography (60% diethyl ether/hexane) to afford the ester 113 (223 mg, 80%) as colourless crystals, m.p. 120-121°C. (Found: C 56.42, H 4.77, N 4.99. $C_{13}H_{13}NO_4S$ requires C 55.90, H 4.69, N 5.01). Mass spectrum: *m/z* (EI) 279 (M^+, 35%), 248 (5), 191 (100), 120 (40). ^1H n.m.r.: δ 8.10, s (broad), NH; 7.80, m, H4, H7; 7.40, m, H5, H6; 4.30, d, J 5.2 Hz, CH_2; 4.20, s, 3-OCH_3; 3.8, s, CH_3. ^{13}C n.m.r.: δ 170.5, $\underline{CO}OCH_3$; 162.0, CONH; 151.0, C3; 139.0, C2; 132.5, C7a; 127.00, C4; 124.6, C7; 123.70, C5, 122.8, C3a; 122.3, C6; 62.3, 3-OCH_3; 52.5, $COO\underline{C}H_3$; 41.4, $NH\underline{C}H_2COOCH_3$. υ_{max} 3398 (m, sharp, NH), 1747 (s, $\underline{CO}OCH_3$), 1644 (s, $Ar\underline{CO}NH$) cm^{-1}.

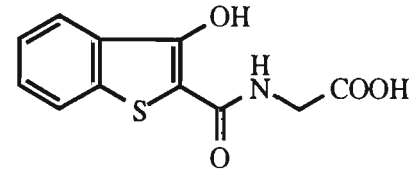
Preparation of *N*-[3-Methoxy-[1]benzothien-2-oyl]glycine 114



A solution of the ester 113 (190 mg, 0.68 mmol) in methanol (3 ml) was treated with sodium hydroxide (0.5 M, 3 ml) and the mixture was stirred until no ester remained. Methanol was evaporated to give a residue. Neutralization with hydrochloric acid (1 M) gave a precipitate, which was collected and washed with a mixture of diethyl ether and hexane (1:3) to provide the acid 114 (180 mg, 100%) as colourless crystals, m.p. 140-142°C. (Found: C 54.55, H 4.26, N 5.31. $C_{12}H_{11}NO_4S$ requires C 54.33, H 4.18, N 5.28). Mass spectrum: *m/z* (CI) 248 (M^+-OH, 20), 220 (5), 191 (100). ^1H n.m.r.: δ 7.92-

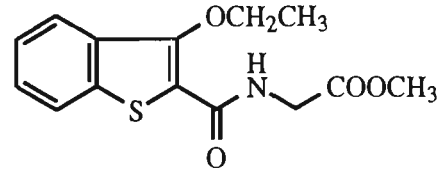
7.90, H4; 7.86-7.83, m, H7; 7.46-7.40, m, H5, H6; 4.12, s, CH_3. υ_{max} 3331 (m, sharp), 1720 (m), 1625 (m), 1587 (m) cm^{-1}.

Preparation of *N*-[3-Hydroxy-[1]benzothien-2-oyl]glycine 115



Boron tribromide (0.5 ml, excess) was added dropwise to a stirred solution of the ester 113 (140 mg, 0.5 mmol) in dichloromethane (10 ml) at -78°C under nitrogen. The reaction mixture was left to warm up to room temperature. After stirring overnight, the reaction was quenched with and the mixture was extracted with chloroform (3x10 ml). The extracts were washed with brine (10 ml), dried and evaporated to furnish the acid 115 (124 mg, 99%) as a colourless powder, m.p. 270°C (decomposition). Mass spectrum: *m/z* (EI) 251 (M^+, 24), 233 (5), 205 (10), 176 (83), 148 (10) (accurate mass: $M^{+\bullet}$, 251.0249. $C_{11}H_9NO_4S$ requires 251.0252). ^1H n.m.r. (D_2O): δ 7.87, d, J 8.0 Hz, H4; 7.82, d, J 8.0 Hz, H7; 7.50, dt, J_1 8.0 Hz, J_2 0.8 Hz, H5; 7.43, dt, J_1 8.0 Hz, J_2 0.8 Hz, H6; 4.03, s, $\underline{CH_2}COOH$. υ_{max} 3395 (m, sharp), 3204 (w), 1724 (s, $\underline{CO}OH$), 1611 (s, $\underline{CO}NH$), 1551 (s) cm^{-1}.

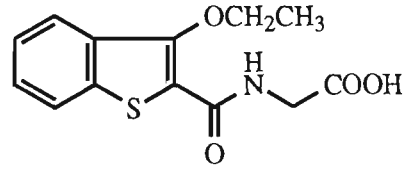
Preparation of *N*-[3-Ethoxy-[1]benzothien-2-oyl]glycine Methyl Ester 118



A mixture of the acid 117 (222 mg, 1 mmol) and 1,3-dicyclohexylcarbodiimide (227 mg, 1.05 mmol) in THF (10 ml) was stirred for 1 hour. To this was added a suspension of glycine methyl ester hydrochloride

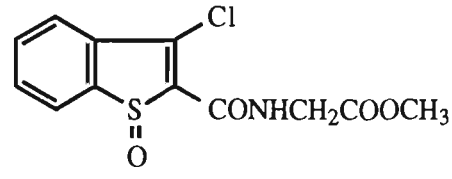
(250 mg, 2 mmol) and triethylamine (0.3 ml, 2.2 mmol) in THF (10 ml). The reaction mixture was then stirred overnight before being quenched with water. The aqueous mixture was extracted with ethyl acetate (3x15 ml). The combined extracts were washed with brine (15 ml) and dried. Evaporation of the solvent afforded a crude product, which was chromatographed to furnish the ester 118 (120 mg, 41%) as colourless crystals, m.p. 129-130°C. Mass spectrum: *m/z* (CI) 294 (MH^+, 45), 219 (15), 205 (100), 177 (70). ^1H n.m.r.: δ 8.17, s (broad), NH; 7.83-7.81, m, H4; 7.80-7.79, m, H7; 7.46-7.38, m, H5, H6; 4.44, q, J 6.8 Hz, 3-O$\underline{CH_2}CH_3$; 4.31, d, J 5.2 Hz, CH_2; 3.82, s, OCH_3; 1.60, t, J 6.8 Hz , 3-$OCH_2\underline{CH_3}$. υ_{max} 3368 (m), 3062 (w), 1747 (m), 1629 (s), 1530 (m) cm^{-1}.

Preparation of *N*-[3-Ethoxy-[1]benzothien-2-oyl]glycine 119



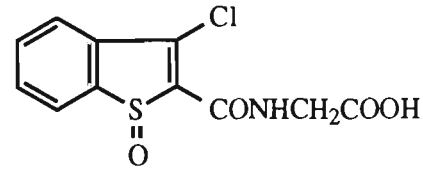
A solution of the ester 118 (100 mg, 0.34 mmol) in methanol (10 ml) was treated with sodium hydroxide (0.5 M, 3 ml) and the mixture was stirred overnight. Methanol was evaporated to give a residue, which was neutralized with hydrochloric acid (1 M) to form a precipitate. The precipitate was collected and washed with a mixture of diethyl ether and hexane (1:3), to afford the acid 119 (90 mg, 95%) as colourless crystals, m.p. 140-142°C. Mass spectrum: *m/z* (EI) 280 (12) (accurate mass: $M^{+\bullet}$, 279.0570. $C_{13}H_{13}NO_4S$ requires 279.0565). ^1H n.m.r. (acetone-d6): δ 8.22, broad s, NH; 7.94-7.92, m, NH4, H7; 7.52-7.44, m, H5, H6; 4.47, q, J 7.2 Hz, 3-O$\underline{CH_2}CH_3$, 4.24, d, J 5.6 Hz, CH_2; 1.55, t, J 7.2 Hz, 3-$OCH_2\underline{CH_3}$. υ_{max} 3331 (m, sharp), 1725 (m), 1622 (m), 1587 (m) cm^{-1}.

Preparation of *N*-[3-Chloro-[1]benzothien-2-oyl]glycine Methyl Ester Sulfoxide 124



To a solution of 35 (236 mg, 0.83 mmol) in dichloromethane was added *meta*-chloroperoxybenzoic acid (256 mg, 56% content, 0.83 mmol) and the mixture was stirred for 20 hours. The solvent was removed to give a residue, which was chromatographed (5% methanol/dichloromethane) to provide the sulfoxide 124 (120 mg, 48%) as colourless crystals, m.p. 105-107°C. Mass spectrum: *m/z* (CI) 300 (MH^+, 30), 268 (10), 240 (18), 211 (40). ^1H n.m.r.: δ 7.76-7.73, m, H4, H7; 7.58-7.54, m, H5, H6; 4.32, dd, J_1 18.4 Hz, J_2 5.2 Hz, 1H of CH_2; 4.24, dd, J_1 18.4 Hz, J_2 5.2 Hz, 1H of CH_2; 3.80, s, CH_3. υ_{max} 1742 (w), 1696 (m) cm^{-1}.

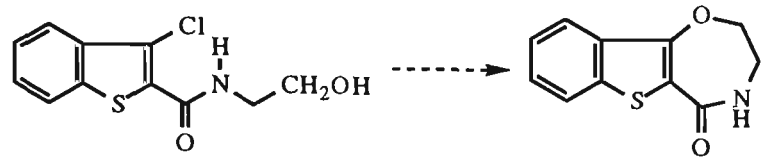
Preparation of *N*-[3-Chloro-[1]benzothien-2-oyl]glycine Sulfoxide 122



A solution of the methyl ester sulfoxide 124 (50 mg, 0.17 mmol) in methanol (2 ml) was treated with sodium hydroxide (1 M, 1 ml) for two hours. Methanol was removed and hydrochloric acid (1 M) was added to neutralize the reaction mixture. Water was evaporated under vacuum to give a residue containing sodium chloride. Sephadex G-10 chromatography eluting with water, furnished the acid 122 (30 mg, 64%) as colourless crystals, m.p. 160-162°C. Mass spectrum: *m/z* (ES+) 284 (M^+-1, 100). ^1H n.m.r. (D_2O): δ 7.83-7.69, m, H4, H7; 7.54-7.41, m, H5, H6; 3.98, d, J 17.6 Hz, 1H of CH_2; 3.92, d, J 17.6 Hz, 1H of CH_2. υ_{max} 3309 (m, broad), 1600 (m), 1563 (m) cm^{-1}.

6.4 Experimental for Chapter 4

Attempted Cyclization of *N*-[2'-Hydroxyethyl]-3-chloro-[1]benzothiophene-2-carboxamide 34

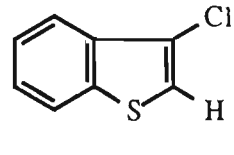


1. Sodium (25 mg, 1 mmol) was reacted with methanol (5 ml) under nitrogen, and the mixture stirred until the sodium had dissolved. The solvent was evaporated to give a white precipitate. The precipitate was dissolved in THF (5 ml) and treated with a solution of the alcohol 34 (43 mg, 0.17 mmol) in THF (2 ml) for 3 hours at room temperature. No visible reaction was oberved by t.l.c. analysis, after refluxing the mixture overnight under nitrogen. The starting material was recovered after work-up.

2. The reaction was repeated using potassium *tert*-butoxide instead of sodium methoxide. After refluxing overnight, t.l.c. analysis showed no reaction had occurred. The starting material was recovered after work-up.

3. A solution of the alcohol 34 (50 mg, 0.2 mmol) in THF (2 ml) was added to a suspension of sodium hydride (24 mg, 1 mmol) in THF (5 ml). The mixture was then refluxed overnight. No evident reaction was observed by t.l.c analysis. The starting material was recovered after work-up.

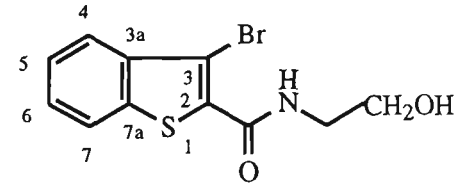
Preparation of 3-Chloro-[1]benzothiophene 150



A solution of the carboxamide 37 (210 mg, 0.8 mmol) in THF (5 ml) was added dropwise to a suspension of sodium hydride (24 mg, 1 mmol) in THF (10

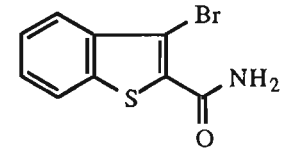
ml) at room temperature under nitrogen. The reaction mixture was refluxed for two hours, then quenched with a saturated ammonium chloride solution. The aqueous mixture was extracted with ethyl acetate (3x15 ml) and the extracts were washed with brine (10 ml), dried and concentrated. Chromatography (20% ethyl acetate/hexane) of the residue yielded the compound 150 (80 mg, 60%) as a colourless oil . Mass spectrum: *m/z* (EI) 168 (M^+, 95), 133 (20). ^1H n.m.r.: δ 7.86-7.81, m, H4, H7; 7.48-7.37, m, H5, H6; 7.32, s, H2. υ_{max} (thin film) 3103 (m), 3059 (w), 1504 (m), 1416 (s) cm^{-1}.

Preparation of *N*-[2'-Hydroxyethyl]-3-bromo-[1]benzothiophene-2-carboxamide 144



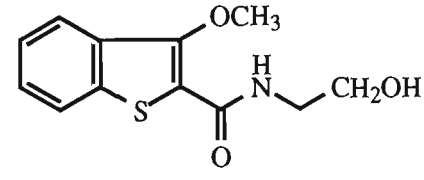
A solution of 85 (100 mg, 0.3 mmol) in ethanol (5 ml) was treated with sodium borohydride (38 mg, 1 mmol), and the reaction mixture stirred overnight. The reaction was quenched with a saturated ammonium chloride solution and then extracted with ethyl acetate (3x10 ml). The extracts were washed with brine and dried to give the alcohol 144 (90 mg, 99%). Mass spectrum: *m/z* (Fab) 302 (M^+, 10), 283 (5), 255 (25), 239 (80), 213 (20), 132 (100) (accurate mass: $M^{+\bullet}$, 298.9620. $C_{11}H_{10}{}^{79}BrNO_2S$ requires 298.9616). ^1H n.m.r.: δ 7.88, s, H4; 7.80, d, J 8.0 Hz, H7; 7.57, d, J 7.6 Hz, H5; 7.30, d, J 7.6 Hz, H6; 6.68, s (broad), ArCO<u>NH</u>; 3.89, t, J 4.8 Hz, $CH_2\underline{CH_2}OH$; 3.67, dt, J_1 4.8 Hz, J_2 5.2 Hz, $\underline{CH_2}CH_2OH$. υ_{max} 3448 (s, broad), 1625 (s), 1459 (s), 1376 (m), 1019 (w) cm^{-1}.

Attempted Cyclization of *N*-[2'-Hydroxyethyl]-3-bromo-[1]benzothiophene-2-carboxamide: formation of 3-bromo-[1]benzothiophene-2-carboxamide 147



The carboxamide 144 (20 mg, 0.067 mmol) in THF (5 ml) was treated with sodium hydride (10 mg, 0.4 mmol), then refluxed for four hours. The reaction was quenched with a saturated ammonium chloride solution and extracted with ethyl acetate (2x10 ml). The extracts were washed with brine (10 ml), dried and evaporated to give a residue, which was chromatographed (40% ethyl acetate/hexane) to afford the amide 147 (13 mg, 77%) as colourless crystals, m.p. 171-172°C. Mass spectrum: *m/z* (EI) 257 (M^+, 45), 241 (50), 213 (10), 132 (62). ^1H n.m.r.: δ 7.89, d, J 0.8 Hz, H4; 7.81, dd, J_1 0.8 Hz, J_2 8.8 Hz, H7; 7.59, dd, J_1 0.8 Hz, J_2 7.6 Hz, H5; 7.30, t, 8.0 Hz, H6. υ_{max} 3449 (w), 3174 (w), 1656 (m), 1611 (m) cm^{-1}.

Preparation of *N*-[2'-Hydroxyethyl]-3-methoxy-[1]benzothiophene-2-carboxamide 151

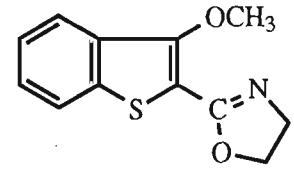


1. Methyl *N*-[3-methoxy-[1]benzothien-2-oyl] glycinate 113 (120 mg, 0.43 mmol) in ethanol (5 ml) was treated with an excess of sodium borohydride (38 mg, 1 mmol) and stirred overnight. The reaction was quenched with a saturated ammonium chloride solution and the aqueous solution was extracted with ethyl acetate (3x10 ml). The combined ethyl acetate extracts were washed with brine (10 ml), dried and concentrated. Chromatography of the residue (50% ethyl acetate/hexane) gave the alcohol 151 (101 mg, 94%) as colourless crystals, m.p. 93-94°C. A satisfactory elemental analysis for this compound could not be obtained. Mass spectrum: *m/z* (ES-) 250 (M^-, 32), 148

(10). ^{1}H n.m.r.: δ 7.88, s (broad), ArCONH; 7.82-7.78, m, H4, H7; 7.44-7.41, m, H5, H6; 4.15, s, OCH_3; 3.80, dt, J_1 5.6 Hz, J_2 4.8 Hz, $NHCH_2CH_2OH$; 3.70, t, 4.8 Hz, $NHCH_2CH_2OH$; 1.27, s (broad), CH_2OH. ^{13}C n.m.r.: δ 164.0, CO; 152.7, C3; 139.0, C2; 133.7, C7a; 128.1, C4; 125.7, C7; 124.5, C5; 123.5, C6; 123.2, C3a; 62.6, OCH_3; 61.8, CH_2OH; 42.9, CH_2CH_2OH. υ_{max} 3379 (m, OH), 1625 (s, C=O), 1534 (m) cm^{-1}.

2. A mixture of methyl 3-methoxy-[1]benzothiophene-2-carboxylate 110 (111 mg, 0.5 mmol), ethanolamine (34 mg, 0.55 mmol) and sodium hydride (44 mg, 60%, 1.1 mmol) in DMSO (5 ml) was stirred overnight. A saturated ammonium chloride solution was added and the mixture was extracted with ethyl acetate (3x15 ml). The combined extracts were washed with brine (10 ml), dried and evaporated to yield a residue, which was chromatographed (acetic acid: ethyl acetate: hexane 1: 60: 100) to give the alcohol 151 (65 mg, 52%).

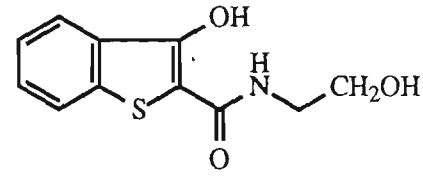
Preparation of 2-[2'-5,6-Dihydro-4H-oxazinyl]-3-methoxy-[1]benzothiophene 153



To a stirred solution of the alcohol 151 (40 mg, 0.16 mmol) in dichloromethane (5 ml) containing triethylamine (0.1 ml) at 0°C was added dropwise a solution of *p*-toluenesulfonyl chloride (100 mg, 0.5 mmol) in dichloromethane. The reaction mixture was then stirred at room temperature, then warmed up to 40°C. The temperature was maintained at 40°C until no starting material remained. A solution of saturated sodium bicarbonate was added to quench the reaction and the reaction mixture was extracted with dichloromethane (3x10 ml). The extracts were washed with brine (10 ml), dried and evaporated. Chromatography of the residue (20% ethyl acetate/hexane) afforded the dihydrooxazole 153 (30 mg, 81%) as colourless crystals, m.p. 105-

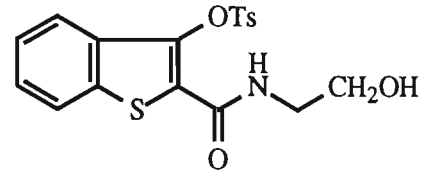
106°C. Mass spectrum: *m/z* (ES+) 234 (MH^+, 100) (accurate mass: $M^{+\bullet}$, 233.0511. $C_{12}H_{11}NO_2S$ requires 233.0511). ^1H n.m.r.: 7.86–7.81, m, H4; 7.78-7.73, m, H7; 7.46-7.40, m, H5, H6; 4.49, t, J 9.6 Hz, $\underline{C}H_2CH_2N$; 4.10, t, J 9.6 Hz, $CH_2\underline{C}H_2N$. ^{13}C n.m.r.: δ 159.8, $\underline{C}$-O-CH_2; 154.6, C3; 137.6, C2; 133.7, C7a; 126.9, C4; 124.4, C7; 1229, C5; 122.1, C6; 113.0, C3a; 68.0, $\underline{C}H_2CH_2N$; 62.4, $CH_2\underline{C}H_2N$. υ_{max} 3053 (w), 1634 (s), 1451(s) cm^{-1}.

Preparation of *N*-[2'-Hydroxyethyl]-3-hydroxy-[1]benzothiophene-2-carboxamide 156



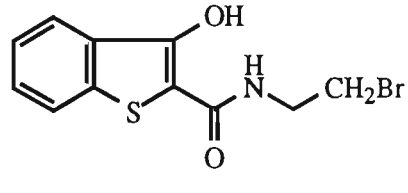
To a solution of the acid 115 (50 mg, 0.2 mmol) in THF (5 ml) at room temperature under nitrogen was added dropwise borane (1 M in THF, 3 ml). After addition, the mixture was stirred overnight. The reaction was quenched with hydrochloric acid (1 M) and the reaction mixture was extracted with ethyl acetate (3x10 ml). The extracts were washed with brine (15 ml), dried and concentrated to give the alcohol 156 (40 mg, 94%) as colourless crystals, m.p. 141-142°C. Mass spectrum: *m/z* (EI) 237 (M^+, 26), 219 (16), 176 (100), 148 (13) (accurate mass: $M^{+\bullet}$, 237.0457. $C_{11}H_{11}NO_3S$ requires 237.0460). ^1H n.m.r.: δ 7.94, dd, J_1 8.0 Hz, J_2 0.8 Hz, H4; 7.72, dd, J_1 8.0 Hz, J_2 0.8 Hz, H7; 7.52, dt, J_1 8.0 Hz, J_2 0.8 Hz, H5; 7.42, J_1 8.0 Hz, J_2 0.8 Hz, H6; 3.86, t, J 4.8 Hz, $CH_2\underline{C}H_2OH$; 3.65, dt, J_1 4.8 Hz, J_2 5.0 Hz, $\underline{C}H_2CH_2OH$. υ_{max} (thin film) 3348 (w, OH), 3280 (m), 3060 (w), 2931 (s), 2855 (m), 1603 (s, C=O), 1521 (m) cm^{-1}.

Preparation of *N*-[2'-Hydroxyethyl]-3-tosyl-[1]benzothiophene-2-carboxamide 157



The alcohol 156 (50 mg, 0.21 mmol) in dichloromethane (10 ml) in the presence of triethylamine (0.1 ml) at 0°C was treated with *p*-toluenesulfonyl chloride (80 mg, 0.42 mmol). The mixture was stirred at room temperature for 4 hours, then quenched with a solution of saturated sodium bicarbonate. The aqueous mixture was extracted with dichloromethane (2x10 ml). The extracts were washed with brine (10 ml), dried and concentrated. The residue was chromatographed (60% ethyl acetate/hexane) to give the tosylate 157 (50 mg, 61%) as colourless crystals, m.p. 132-133°C. Mass spectrum: *m/z* (EI) 391 (M^+, 5), 373 (7), 218 (15), 177 (75) (accurate mass: $M^{+\bullet}$, 391.0553. $C_{18}H_{17}NO_5S_2$ requires 391.0548). ^1H n.m.r.: δ 7.20–7.82, m, aromatic protons (8H); 7.10, s (broad), ArCONH; 3.83, t, J 5.2, $\underline{CH_2}OH$; 3.52, dt, J_1 5.2 Hz, J_2 4.0 Hz, $\underline{CH_2}CH_2OH$; 2.62, s (broad), $CH_2\underline{OH}$; 2.52, s, CH_3. ^{13}C n.m.r.: δ 161.0, $\underline{CO}$; 146.6, C3; 136.9, OTs; 136.7, C2; 132.3, OTs; 131.5, C7a; 130.1, 2xOTs; 129.1, C3a; 128.6, 2xOTs; 127.0, C4; 125.2, C7; 122.8, C5; 122.6, C6; 61.8, C10; 42.6, C9; 21.8, OTs-CH_3. υ_{max} (thin film in dichloromethane) 3409 (m, broad, OH), 3068 (w), 1641 (s), 1535 (s) cm^{-1}.

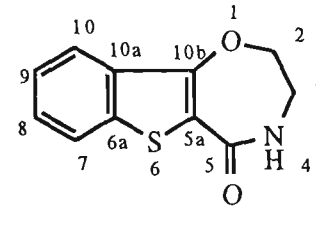
Preparation of *N*-[2'-bromoethyl]-3-hydroxy-[1]benzothiophene-2-carboxamide 154



To a solution of the alcohol 151 (125 mg, 0.5 mmol) in dichloromethane (10 ml) at -78°C under nitrogen was added dropwise boron tribromide (0.15 ml). The dry ice bath was then removed and the reaction mixture stirred

overnight, then quenched with water. The mixture was extracted with dichloromethane (3x10 ml). The combined extracts were washed with brine (10 ml), dried and concentrated to furnish a crude product. Chromatography of the crude product (25% ethyl acetate/hexane) afforded the bromide 154 (142 mg, 95%) as a grey powder, m.p. 192-193°C. Mass spectrum: *m/z* (ES+) 302 (MH^+, 10), 220 (100) (accurate mass: $M^{+\bullet}$, 298.9616. $C_{11}H_{10}{}^{79}BrNO_2S$ requires 298.9616). ^1H n.m.r.: δ 8.00, d, J 8 Hz, H4; 7.80, d, J 8 Hz, H7; 7.57-7.54, m, H5; 7.50-7.48, m, H6; 6.00, broad s, ArCONH; 3.95, dt, J_1, 6.0 Hz, J_2, 6.0 Hz, $\underline{CH_2}CH_2Br$; 3,65, t, J 6.0 Hz, $CH_2\underline{CH_2}Br$. υ_{max} (thin film, in dichloromethane) 3440 (s, OH), 2924 (m), 2855 (m), 1618 (s, C=O), 1534 (m) cm^{-1}.

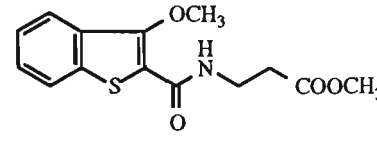
Preparation of 3,4-Dihydro[1]Benzothieno[2,3-f][1,4]oxazepin-5(2*H*)-one 142



To a solution of the bromide 154 (30 mg, 0.1 mmol) in THF (20 ml) under nitrogen at room temperature was added an excess of sodium hydride (10 mg, 0.4 mmol). The mixture was stirred for one hour, then quenched with a saturated ammonium chloride solution. The aqueous mixture was extracted with ethyl acetate (3x10 ml). The combined extracts were washed with brine (15 ml), then dried. The solvent was evaporated and the residue was chromatographed (30%, ethyl acetate/hexane; then 70% ethyl acetate/hexane & 10% methanol) to afford the cyclized compound 142 (15 mg, 68%) as a yellow powder, m.p. 215°C, decomposition. Mass spectrum: *m/z* (EI) 219 (M^+, 40), 175 (39) (accurate mass: M^+-CH_2CH_2O, 175.0073. $C_{11}H_9NO_2S$-CH_2CH_2O requires 175.0092). ^1H n.m.r.: δ 7.95, d, J 8.0 Hz, H10; 7.65, dd, J_1 8.0 Hz, J_2 8 Hz, H7; 7.45, dt, J_1 0.8 Hz, J_2 8.0 Hz, H9; 7.32, dt, J_1 0.8 Hz, J_2 8.0 Hz; H8; 4.65, t, J 8.8 Hz, $NHCH_2\underline{CH_2}O$; 4.02, t, J 8.8 Hz, $NH\underline{CH_2}CH_2O$; 3.70 s (broad), CONH. ^{13}C n.m.r.: δ

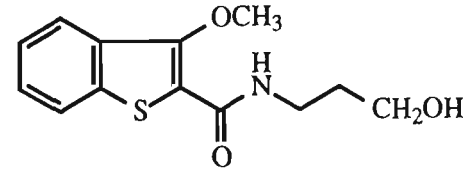
165.4, C5 (CO); 156.6, C10b; 141.6 C6, C5a; 133.1, C6a; 130.8, C10b; 130.0, C10; 124.2, C7, 123.9; C9, 123.0, C8; 68.5, C2; 45.8, C3. υ_{max} 3200 (w), 1641 (s, C=O), 1459 (s) cm^{-1}.

Preparation of *N*-[3-Methoxy-[1]benzothien-2-oyl]-β-alanine Methyl Ester 163



A solution of β-alanine methyl ester (150 mg, 1.4 mmol) was added to a solution of the acid 111 (208 mg, 1 mmol), 1,3-dicyclohexylcarbodiimide (206 mg, 1 mmol) and 1-hydroxybenzotriazole (150 mg, 1.1 mmol) in DMF (10 ml). The reaction mixture was stirred overnight. The solvent was removed under vacuum and the residue was chromatographed (20%, then 40% ethyl acetate/hexane) to give the ester 163 (230 mg, 79%) as colourless crystals, m.p. 61-62°C. Mass spectrum: *m/z* (ES+) 294 (MH^+, 100) (accurate mass: $M^{+\bullet}$, 293.0728. $C_{14}H_{15}NO_4S$ requires 293.0722). ^1H n.m.r.: δ 8.10, s (broad), CONH; 7.82-7.80, m, H4, H7; 7.43-7.39, m, H5, H6; 4.10, s, OCH_3; 3.75, dt, J_1 6.0 Hz, J_2 6.8 Hz, $\underline{C}H_2CH_2COOCH_3$; 3.72, s, $COOCH_3$; 2.68, t, J 6.0 Hz, $\underline{C}H_2COOCH_3$. ^{13}C n.m.r.: δ 172.9, $\underline{C}OOCH_3$; 161.6, CO; 150.5, C3; 137.8, C2; 132.4, C7a; 126.6, C4; 124.3, C7; 123.6, C3a; 123.5, C5; 122.0, C6; 61.9, OCH_3; 51.7, $ArCONH\underline{C}H_2$; 48.8, $COO\underline{C}H_3$; 34.8, $\underline{C}H_2COOCH_3$. υ_{max} (film) 3387 (m, $ArCO\underline{NH}$), 3065 (w), 2946 (m), 2848 (w), 1732(s, $COOCH_3$), 1641 (s, $Ar\underline{CO}NH$), 1535 (s) cm^{-1}.

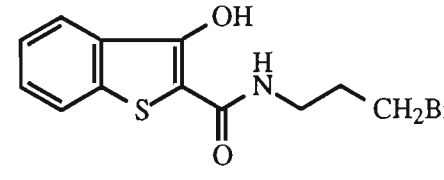
Preparation of *N*-[3'-Hydroxypropyl]-3-methoxy-[1]benzothiophene-2-carboxamide 155



To a solution of the methyl ester 163 (140 mg, 0.48 mmol) in ethanol (5 ml) was added an excess of sodium borohydride (20 mg, 0. 8 mmol) and the

mixture was stirred overnight. A saturated ammonium chloride solution was added and the aqueous mixture was extracted with ethyl acetate (3x10 ml). The extract was washed with brine (10 ml), dried and evaporated to afford a crude product, which was chromatographed (60% ethyl acetate/hexane) to yield the alcohol 164 (125 mg, 98%) as colourless crystals, m.p. 64-66°C. Mass spectrum: *m/z* (ES+) 266 (MH^+, 100) (accurate mass: $M^{+\bullet}$, 265.0776. $C_{13}H_{15}NO_3S$ requires 265.0773). ^1H n.m.r.: δ 7.84–7.80, m, H4, H7; ; 7.46-7.41, m, H5, H6; 4.13, s, OCH_3; 3.74, t, J 5.6 Hz, $\underline{CH_2}OH$; 3.67, dt, J_1 6.4 Hz, J_2 6.0 Hz, $NH\underline{CH_2}$; 1.85-1.82, m, $\underline{CH_2}CH_2OH$. ^{13}C n.m.r.: δ 162.8, CO; 150.7, C3; 137.8, C2; 132.3, C7a; 126.7, C4; 124.4, C7; 123.5, C5; 1229.9, C3a; 122.0, C6; 61.9, 3-O$\underline{C}H_3$; 59.5, CH_2OH; 36.5, $NHCH_2$; 32.1, $\underline{C}H_2CH_2OH$. υ_{max} (thin film) 3379 (s, broad, OH, NH), 3060 (w), 2878 (w). 1625 (s, Ar$\underline{CO}$NH), 1542 (s) cm^{-1}.

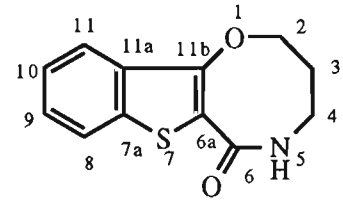
Preparation of *N*-[3'-Bromopropyl]-3-hydroxyl-[1]benzothiophene-2-carboxamide 165



To a solution of the alcohol 164 (100 mg, 0.38 mmol) in dichloromethane (5 ml) at -78°C under nitrogen was added dropwise an excess of boron tribromide (0.25 ml). The dry-ice bath was then removed and the reaction mixture stirred overnight. The reaction was quenched with water and the reaction mixture extracted with dichloromethane (3x15 ml). The combined extracts were washed with brine (10 ml), dried and evaporated . Chromatography of the residue (60% ethyl acetate/hexane) afforded the bromide 165 (80 mg, 69%) as a white powder, m.p. 135-136°C. Mass spectrum: *m/z* (EI) 315 (M^+, 10), 297 (5) (accurate mass: $M^{+\bullet}$, 312.9771. $C_{12}H_{12}{}^{79}BrNO_2S$ requires 312.9772). ^1H n.m.r.: δ 7.94, d, J 8.0 Hz, H4; 7,71, d, J 8.0 Hz, H7; 7.48, dt, J_1 1.2 Hz, J_2 8.0 Hz, H5; 7.42, dt, J_1 1.2 Hz, J_2 8.0 Hz, H6; 3.63, dt, J_1 6.4 Hz J_2 6.4 Hz,

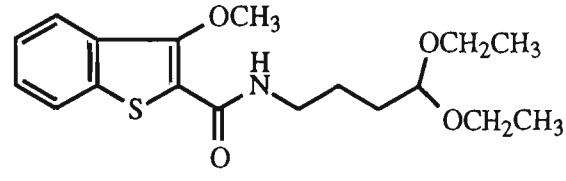
$NHCH_2$; 3.50, t, J 6.4 Hz CH_2Br; 2.21, quintuplet, J 6.4 Hz, $CH_2\underline{CH_2}CH_2Br$. ^{13}C n.m.r.: δ 167.1, C3, 158.9, CO; 136.2, C2; 131.2, C7a; 128.5, C4; 124.7, C7; 123.0, C5; 122.9, C6; 102.1, C3a; 38.2, $NHCH_2$; 32.1, CH_2Br; 30.7, $\underline{C}H_2CH_2Br$. υ_{max} 3348 (w, NH), 1611 (m, C=O), 1559 (s) cm^{-1}.

Preparation of 4,5-Dihydro-2*H*-[1]benzothieno[3,2-b][1,5]oxazocin-6(3*H*)-one



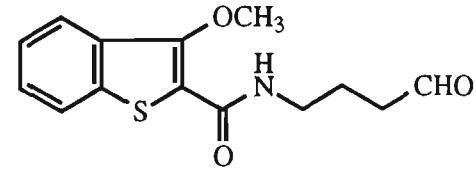
Sodium hydride (10 mg, 0.4 mmol) was added to a solution of the bromide 165 (30 mg, 0.1 mmol) in THF (10 ml) under nitrogen. After stirring for 2 hours, no reaction was observed by t.l.c. analysis (20% ethyl acetate/hexane). The reaction mixture was refluxed for two hours and quenched with water. The mixture was extracted with ethyl acetate (3x15 ml) and the combined extracts were washed with brine (10 ml), and dried. The solvent was evaporated to furnish a crude product, which was chromatographed (methanol: ethyl acetate:hexane 10:80:100) to give the cyclized compound 160 (15 mg, 68%) as a yellow powder, m.p. 196-197°C. Mass spectrum: *m/z* (ES+) 248 (MH^+, 100) (accurate mass: $M^{+\bullet}$, 233.0462. $C_{12}H_{11}NO_2S$ requires 233.0511) ^1H n.m.r.: δ 7.98-7.96, m, H11; 7.74-7.71, m, H8; 7.48, dt, J_1 1.2 Hz, J_2 8.0 Hz, H10; 7.41, dt, J_1 1.2 Hz, J_2 8.0 Hz, H9; 3.83-3.80, m, 2xCH_2 ; 2.01-1.98, m, CH_2. ^{13}C n.m.r.: δ 166.60, C11b; 160.90, C6 (CO); 137.90, C6b; 130.80, C7a; 128.15, C11; 124.35, C8; 122.80, C10; 122.40, C9; 101.80, C11a; 47.02, (C2, C3 and C4 overlapped). υ_{max} 1621 (w, C=O), 1565 (s) cm^{-1}.

Preparation of *N*-[3'-Methoxy-[1]benzothien-2'-oyl]-4-amino-butyraldehyde Diethyl Acetal 166



A mixture of the acid 111 (208 mg, 1 mmol), 1,3-dicyclohexylcarbodiimide (206 mg, 1 mmol), 1-hydroxybenzotriazole (150 mg, 1.1 mmol) and 4-aminobutyraldehyde diethyl acetal (0.3 ml) in THF (15 ml) was stirred overnight. The reaction was quenched with water and the aqueous mixture extracted with ethyl acetate (3x15 ml). The combined extracts were washed with brine and dried. Removal of the solvent gave a residue, which was chromatographed (20% ethyl acetate/hexane) to give the acetal 166 (300 mg, 85%) as colourless crystals, m.p. 110-111°C. Mass spectrum: *m/z* (EI) 322 (M^+-Et, <1), 305 (3), 259 (20), 191(100), 176 (60) (accurate mass: $M^{+\bullet}$, 351.1489. $C_{18}H_{25}NO_4S$ requires 351.1504). ^1H n.m.r.: δ 7.80-7.78, m, H4, H7; 7.54, s (broad), ArCONH; 7.43-7.40, m, H5, H6; 4.53, t, J 4.8 Hz, CH$(OEt)_2$; 4.10, s, OCH_3; 3.65, q, J 7.2 Hz, 2xOCH_2CH_3; 3.55-3.48, m, 4H; 1.74-1.72, m, 2H; 1.21, t, J 7.2 Hz, 2xOCH_2CH_3. υ_{max} 3387 (m, ArCONH), 3065 (w), 2969 (s), 2870 (s), 1648 (s), 1534 (s) cm^{-1}.

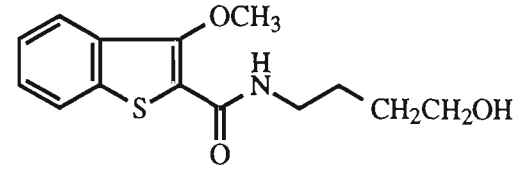
Preparation of *N*-[3'-Methoxy-[1]benzothien-2'-oyl]-4-aminobutyraldehyde 167



A solution of the acetal 166 (70 mg, 0.2 mmol) in acetone (10 ml) was treated with a few drops of hydrochloric acid (1 M) at room temperature and stirred overnight. The solvent was removed and the residue was chromatographed (40% ethyl acetate/hexane) to give the aldehyde 167 (55 mg,

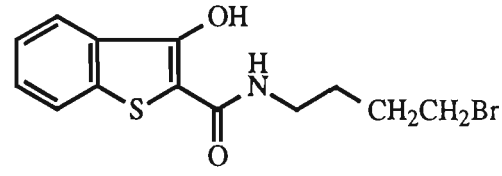
100%) as an oil. Mass spectrum: *m/z* (EI) 277 (M^+, 8), 249 (5), 191 (100) (accurate mass: $M^{+\bullet}$, 277.0769. $C_{14}H_{15}NO_3S$ requires 277.0773). ^1H n.m.r.: δ 9.80, t, J 0.8 Hz, $CH_2\underline{CHO}$; 7,82-7.80, m, H4, H7; 7.60, s (broad), ArCO<u>NH</u>; 7.43-7.40 m, H5, H6; 4.10, s, 3-O<u>C</u>H_3; 3.5, dt, J_1 7.2 Hz, J_2 6.8 Hz, NH<u>C</u>H_2CH_2; 2.60, t, J 6.8 Hz, $CH_2\underline{CH_2}CHO$; 2.02, t, J 6.8 Hz, 1.98, t, J 6.8 Hz, <u>C</u>H_2CH_2CHO. υ_{max} 3387 (s, ArCO<u>NH</u>), 3060 (w), 2939 (s), 2856(m), 1725 (s, <u>CHO</u>), 1641 (s, Ar<u>CO</u>NH), 1527 (s) cm^{-1}.

Preparation of *N*-[4'-Hydroxylbutyl]-3-methoxy-[1]benzothiophene]-2-carboxamide <u>168</u>



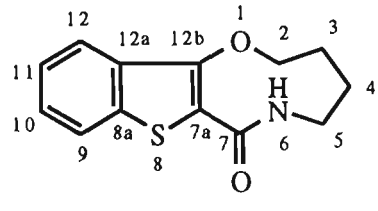
To a solution of the aldehyde <u>167</u> (55 mg, 0.2 mmol) in ethanol (10 ml) was added an excess of sodium borohydride (20 mg, 0.8 mmol) and the mixture was allowed to stir overnight. The reaction was quenched with a saturated ammonium chloride solution and the mixture extracted with ethyl acetate (3x10 ml). The combined extracts were washed with brine (10 ml) and dried. Removal of the solvent gave the alcohol <u>168</u> (55 mg, 100%) as colourless crystals, m.p. 139-140°C. Mass spectrum: *m/z* (ES+) 280 (MH^+, 100), 191 (10) (accurate mass: $M^{+\bullet}$, 279.0929. $C_{14}H_{17}NO_3S$ requires 279.0929). ^1H n.m.r.: δ 7.82, dd, J_1 2 Hz, J_2 6.8 Hz, H4, H7; 7.59, s (broad), ArCO<u>NH</u>; 7.42-7.40, m, H5, H64.10, s, OCH_3; 3,74, t, J 6.0 Hz, <u>C</u>H_2OH; 3.55, dt, J_1 6.8 Hz, J_2 6.4 Hz, CONH<u>C</u>H_2; 1.73-1.70, m, <u>C</u>$H_2\underline{CH_2}CH_2OH$. ^{13}C n.m.r.: δ 162.0 CO; 151.0, C3; 139.1, C2; 132.5, C7a; 126.7, C4; 124.5, C7; 124.0, C3a; 123.7, C5; 122.0, C6; 62.3, OCH_3; 61.9, CH_2OH; 39.0, CONH<u>C</u>H_2; 30.1, <u>C</u>H_2CH_2OH; 26.50, <u>C</u>$H_2CH_2CH_2OH$. υ_{max} (film) 3243 (s, broad, OH), 2939 (s), 2863 (m), 1641 (s, Ar<u>CO</u>NH), 1535 (s) cm^{-1}.

Preparation of *N*-[4'-Bromobutyl]-3-hydroxy-[1]benzothiophene-2-carboxamide 169



To a solution of the alcohol 168 (100 mg, 0.36 mmol) in dichloromethane (10 ml) at -78°C under nitrogen was added dropwise boron tribromide (0.2 ml). The dry ice bath was then removed and the reaction mixture stirred overnight. The reaction was quenched with water and the mixture extracted with dichloromethane (3x10 ml). The combined extracts were washed with brine (10 ml) and dried. Chromatography of the residue (10% ethyl acetate/hexane) afforded the bromide 169 (105 mg, 90%) as a grey powder, m.p. 87-88°C. Mass spectrum: *m/z* (EI) 327 (M^+, 20), 247 (18), 176 (100) (accurate mass: $M^{+\bullet}$, 326.9926. $C_{13}H_{14}{}^{79}BrNO_2S$ requires 326.9929). ^1H n.m.r.: δ 7.94, dd, J_1 0.8 Hz, J_2 8.0 Hz, H4; 7.72, dd, J_1 0.8 Hz, J_2 8.0 Hz, H7; 7.49, dt, J_1 1.2 Hz, J_2 8.0 Hz, H5; 7.42, dt, J_1 1.2 Hz, J_2 8.0 Hz, H6; 5.60, s (broad), OH; 3.51, dt, J_1 7.2 Hz, J_2 6.8 Hz, NH$\underline{CH_2}$; 3.47, t, J 6.4 Hz, $\underline{CH_2}Br$; 1.99-1.95, m, $\underline{CH_2}CH_2CH_2Br$; 1.85-1.81, m, $\underline{CH_2}CH_2CH_2Br$. ^{13}C n.m.r.: δ 166.9, C3; 158.9, CO; 136.1, C2; 131.2, C7a; 128.5, C4; 124.7, C7; 122.9, C5; 122.8, C6; 102.5, C3a; 38.6, $NHCH_2$; 33.1, CH_2Br; 29.8, $\underline{C}H_2CH_2CH_2Br$; 28.3, $\underline{C}H_2CH_2Br$. υ_{max} 3354 (w, ArCO$\underline{NH}$), 1626 (m, Ar$\underline{CO}$NH), 1586 (s) cm^{-1}.

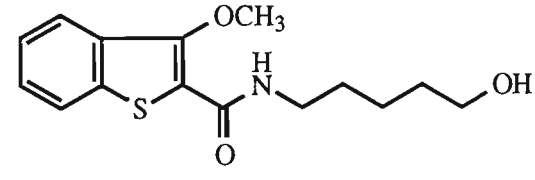
Preparation of 3,4,5,6-Tetrahydro[1]benzothieno[3,2-b][1,5]oxazonin-7(2*H*)-one 161



Sodium hydride (10 mg, 0.4 mmol) was added to a solution of the bromide 169 (40 mg, 0.12 mmol) in THF (20 ml) under nitrogen. After stirring overnight, water was added to stop the reaction. The reaction mixture was

extracted with ethyl acetate (3x15 ml). The extracts were washed with brine (10 ml), and dried. The solvent was evaporated to furnish the cyclized compound 161 (28 mg, 70%) as colourless crystals, m.p. 95-96°C. (Found: C 63.55, H 5.80, N 5.19. $C_{13}H_{13}NO_2S$ requires C 63.14, H 5.30, N 5.66). Mass spectrum: *m/z* (ES+) 248 (MH^+, 100) (accurate mass: $M^{+\bullet}$, 247.0670. $C_{13}H_{13}NO_2S$ requires 247.0667) ^1H n.m.r.: δ 7.99-7.96, m, H12; 7.75-7.73, m, H9; 7.48, dt, J_1 1.2 Hz, J_2 8.0 Hz, H11; 7.41, dt, J_1 1.2 Hz, J_2 8.0 Hz, H10; 3.84-3.80, m, 2xCH_2; 2.01-1.98, m, 2xCH_2. ^{13}C n.m.r.: δ 166.60, C12b; 160.90, CO; 137.90, C7a; 130.80, C8a; 128.15, C12; 124.35, C9; 122.80, C11; 122.40, C10; 101.80, C12a; 47.02, (C2, C3, C4 and C5, overlapped). υ_{max} 1621 (w, ArCONH), 1565 (s) cm^{-1}.

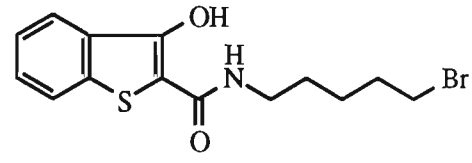
Preparation of *N*-[5'-Hydroxylpentyl]-3-methoxy-[1]benzothiophene-2-carboxamide 170



A mixture of the acid 111 (208 mg, 1 mmol), 1,3-dicyclohexylcarbodiimide (206 mg, 1 mmol), 1-hydroxybenzotriazole (150 mg, 1.1 mmol) and 5-aminopentan-1-ol in DMF (10 ml) was stirred overnight. The solvent was removed under high vacuum, and the residue was chromatographed (30%; 70% Ethyl acetate/hexane) to afford the alcohol 170 (240 mg, 80%) as colourless crystals, m.p. 68-69°C. Mass spectrum: *m/z* (ES-) 292 (M^+-1, 65), 278 (12), 207 (8), 163 (52) (accurate mass: $M^{+\bullet}$, 293.1090. $C_{15}H_{19}NO_3S$ requires 293.1086) ^1H n.m.r.: δ 7.83-7.80, m, H4, H7; 7.53, s (broad), ArCONH; 7.42-7.39, m, H5, H6; 4.1, s, OCH_3; 3.67, t, J 6.4 Hz, CH_2OH; 3.50, dt, J_1 7.2 Hz, J_2 6.8 Hz, $NHCH_2$; 1.76, s (broad), CH_2OH; 1.66-1.68, m, 2xCH_2; 1.52-1.49, m, $CH_2CH_2CH_2OH$. ^{13}C n.m.r.: δ 161.8 CO; 150.0, C3; 137.7; C2, 132.4, C7a; 126.6, C4; 124.4, C7, 123.6, C5; 121.9, C6, 124.2, C3a; 62.5, OCH_3; 61.9, CH_2OH; 39.3, $NHCH_2$; 32.3, CH_2CH_2OH; 29.6,

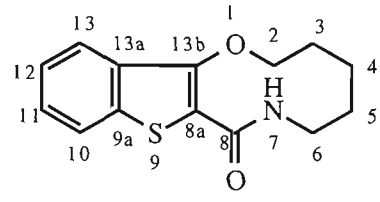
$NHCH_2\underline{C}H_2$; 23.1, $NHCH_2CH_2\underline{C}H_2$. υ_{max} (film) 3463 (m, OH), 3387 (m, ArCO<u>NH</u>), 1634 (s, Ar<u>CO</u>NH), 1535 (s), 1451 (s) cm^{-1}.

Preparation of *N*-[5'-Bromopentyl]-3-methoxy-[1]benzothiophene-2-carboxamide <u>171</u>



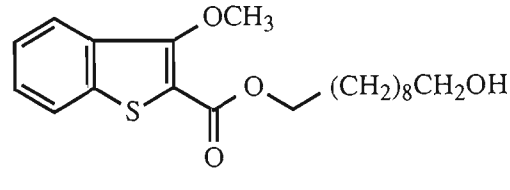
To a solution of the alcohol <u>170</u> (100 mg, 0.34 mmol) in dichloromethane (10 ml) at -78°C under nitrogen was added dropwise an excess of boron tribromide (0.2 ml). After addition, the dry ice bath was removed and the reaction mixture stirred overnight. The reaction was quenched with water and the mixture was extracted with dichloromethane (3x10 ml). The combined extracts were washed with brine (10 ml) and dried. Chromatography of the residue (15% ethyl acetate/hexane) yielded the bromide <u>171</u> (100 mg, 86%) as pink crystals, m.p. 94-95°C. Mass spectrum: *m/z* (ES-) 342 (M^+-1, 95), 81 (89) (accurate mass: $M^{+\bullet}$, 341.0079. $C_{14}H_{16}{}^{79}BrNO_2S$ requires 341.0085). ^1H n.m.r.: δ 7.93, dd, J_1 0.8 Hz, J_2 8.0 Hz, H4; 7.71, dd, J_1 0.8 Hz, J_2 8.0 Hz, H7; 7.48, dt, J_1 1.2 Hz, J_2 8.0 Hz, H5; 7.40, dt, J_1 1.2 Hz, J_2 8.0 Hz, H6; 5.60, s (broad), OH; 3.46, dt, J_1 7.2 Hz, J_2 6.8, $NH\underline{C}H_2$; 3.42, t, J 6.4, $\underline{C}H_2Br$; 1.90, m, $\underline{C}H_2CH_2CH_2CH_2Br$; 1.66, m, $\underline{C}H_2CH_2Br$; 1.54, m, $\underline{C}H_2CH_2CH_2Br$. ^{13}C n.m.r.: δ 166.9, C3, 158.7; CO; 136.1, C2; 131.2, C7a; 128.40, C4; 124.6, C4; 122.9, C5; 122.8, C6; 102.4, C3a; 39.3, $ArCONH\underline{C}H_2$; 33.5, CH_2Br; 32.1, $\underline{C}H_2CH_2CH_2CH_2Br$; 28.9, $\underline{C}H_2CH_2Br$; 25.3, $\underline{C}H_2CH_2CH_2Br$. υ_{max} 3356 (m, ArCO<u>NH</u>), 1610 (s, Ar<u>CO</u>NH), 1550 (m) cm^{-1}.

Preparation of 3,4,5,6-Tetrahydro-2*H*-[1]benzothieno[3,2-b][1,5]oxazecin-8(3*H*)-one 162



An excess of sodium hydride (10 mg, 0.4 mmol) was added to a solution of the bromide 171 (68 mg, 0.2 mmol) in THF (20 ml). After stirring for 1 hour, no reaction occurred. The reaction mixture was refluxed for 1 hour, then quenched with water. The mixture was extracted with ethyl acetate (3x10 ml). The extracts were washed with brine (10 ml) and dried. The solvent was evaporated and the residue was chromatographed (15% ethyl acetate/hexane) to give the cyclized compound 162 (42 mg, 81%) as pink crystals, m.p. 69-70°C. (Found: C 64.15, H 5.97, N 5.36. $C_{14}H_{15}NO_2S$ requires C 64.34, H 5.79, N 5.36). Mass spectrum: *m/z* (EI) 261 (M^+, 65), 176 (94) (accurate mass: $M^{+\bullet}$, 261.0787. $C_{14}H_{15}NO_2S$ requires 261.0824). ^1H n.m.r.: δ 7.98-7.96, m, H13; 7.72-7.70, m, H10; 7.47-7.45, m, H12; 7.42-7.39, m, H11; 3.86-3.83, m, 2xCH_2; 1.72-1.70, m, 3xCH_2. ^{13}C n.m.r.: δ 167.0, C13b; 161.8, C8 (CO); 137.3, C8a; 130.8, C9a; 128.4, C13; 124.4, C10; 122.8, C12; 122.2, C11; 101.2, C13a; 46.1, C2 and C6; 26.3, C3 and C5; 24.5, C4. υ_{max} 1585 (s, ArCONH), 1452 (s) cm^{-1}.

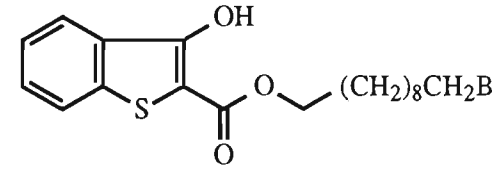
Preparation of 10'-Hydroxydecyl 3-Methoxy-[1]benzothiophene-2-carboxylate 172



A solution of the acid 111 (208 mg, 1 mmol), 1,3-dicyclohexylcarbodiimide (206 mg, 1 mmol), 1-hydroxybenzotriazole (150 mg, 1.1 mmol) and 1,10-decanediol (174 mg, 1 mmol) in dimethylformamide (10 ml) was stirred overnight. The solvent was then removed under vacuum and

the residue chromatographed (30% ethyl acetate/hexane) to give the ester 172(180 mg, 50%) as colourless crystals, m.p. 53-54°C. Mass spectrum: *m/z* (ES+) 365 (MH^+, 70), 332 (8), 316 (32), 288 (100) (accurate mass: $M^{+\bullet}$, 364.1713. $C_{20}H_{28}O_4S$ requires $M^{+\bullet}$, 364.1708). ^1H n.m.r.: δ 7.87, td, J_1 8.8 Hz, J_2 0.8 Hz, H4; 7.74, d, J 8.8 Hz, H7; 7.46, dt, J_1, J_2, H5; 7.39, dt, J_1, J_2, H6; 4.32, t, J 6.4 Hz, $COOCH_2CH_2$; 4.15, s, OCH_3; 3.63, t, J 6.8 Hz, CH_2OH; 1.80-1.30, m, $COOCH_2(CH_2)_8CH_2OH$. ^{13}C n.m.r.: δ 162.3, CO; 146.6, C2; 138.2, C3; 134.0, C7a; 127.6, C4; 124.3, C7; 122.6, C5; 122.6, 116.6, C3a; 65.3, $COOCH_2CH_2$; 62.9, OCH_3; 62.6, CH_2OH; 29.4-25.6, $8xCH_2$. υ_{max} (thin film in dichloromethane) 3387 (m, broad, NH), 3061 (w), 2932 (m), 2856 (s), 1702 (s), 1527 (s) cm^{-1}.

Preparation of 10'-Bromodecyl 3-Hydroxy-[1]benzothiophene-2-carboxylate 173



To a solution of the ester 172 (40 mg, 0.12 mmol) in dichloromethane (10 ml) at -78°C under nitrogen was added dropwise neat boron tribromide(0.1 ml), The dry ice bath was then removed and the reaction mixture was stirred overnight. The reaction was then quenched with water and the reaction mixture extracted with dichloromethane (3x10 ml). The combined extracts were washed with brine (10 ml), dried and evaporated. Chromatography of the residue (5% ethyl acetate/hexane) afforded the bromide 173 (40 mg, 88%) as colourless crystals, m.p. 74-76°C. Mass spectrum: *m/z* (EI) 315 (M^+, 10), 297 (5). (accurate mass: $M^{+\bullet}$, 412.0709. $C_{19}H_{25}{}^{79}BrO_3S$ requires $M^{+\bullet}$, 412.0708). ^1H n.m.r.: δ 7.96-7.93, m, H4; 7,73, dd, J_1 8.0 Hz, J_2 0.8 Hz, H7; 7.50, tt, J_1 1.2 Hz, J_2 8.0 Hz, H5; 7.41, tt, J_1 1.2 Hz, J_2 8.0 Hz, H6; 4.36, t, J 6.8 Hz, $COOCH_2CH_2$; 3.40, t, J 6.8, CH_2CH_2Br; 1.88-1.31, m, $CH_2(CH_2)_8CH_2$. ^{13}C n.m.r.: δ 167.4, CO; 159.4, C3; 138.8, C2; 135.6, C7a; 128.8, C4; 130.0, C3a; 124.4, C7; 123.1, C5; 122.9, C6; 65.4, $COOCH_2$;

41.1, CH_2Br; 29.4-25.8, 8xCH_2. υ_{max} (thin film in dichloromethane) 3318 (w, OH), 3067 (w), 2931 (s), 2862 (s), 1663 (s), 1542 (s) cm^{-1}.

Attempted Cyclization of 10'-Bromodecyl 3-Hydroxy-[1]benzothiophene-2-carboxylate 173

To a suspension of sodium hydride (10 mg, 0.4 mmol) in THF under nitrogen (5 ml) was added a solution of the bromide 173 (100 mg, 0.24 mmol) in THF (15 ml). The mixture was refluxed for 20 hours. No reaction was detected by t.l.c. analysis of the reaction mixture. The starting material 173 was recovered after work-up.

6.5 Experimental for Chapter 5

General Procedure for the Determination of the Potentiation of 5-HT-induced Bradycardic Responses: (Ms. W. Lau, Department of Pharmacology, Monash University).

Male Wistar rats (250-400 g) were anaesthetised with urethane (1.25 g/kg ip) and the trachea intubated to facilitate spontaneous respiration. Blood pressure was recorded from the carotid artery using a pressure transducer. The heart rate was derived from the pulse signal via a tachograph. The femoral vein was cannulated to allow intravenous administration of compounds, the body temperature of rats was maintained at 37°C throughout the experiment. After a 15 minutes equilibration period, each rat was given doses of 3, 10 or 30 mg/kg iv 5-HT every 10 minutes, evoking dose-dependent bradycardic responses.

General Procedure for the Determination of Effects of the Selected Compounds on Noradrenaline Reuptake Mechanisms:

Prostatic and epididymal segments of *vasa deferentia* from Wistar rats (280-330 g) were rapidly removed and suspended under 0.8-1 g tension in 5-ml organ baths containing warm (37°C) oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit solution. Prostatic segments were field-stimulated at 0.1 Hz with pulses of 1ms duration and a supramaximal voltage of 60V; epididymal segments were not stimulated. Prazosin (0.5 μM) was added to the solution bathing prostatic halves of the *vas deferens* to inhibit effects on postjunctional α_1-adrenoceptors. Isometric contractions were recorded with *GRASS FT03C* transducers. The transducer signals were digitally transformed and registered in a *Macintosh* SE/30 computer using *Maclab Chart* software system.

Molecular Modelling

All minimisation of structures was done for molecules in *vacuo* in the Consistent Valence Forcefield (CVFF) within Insight II, using the Biosym software package. The energy tolerance was defined as 0.001 kcal/mol. The constant dielectric was set to its full scale (1.00). Heating was done at 900 K with a time step of 1.00 and the cooling was achieved at 300 K over 5 ps. The Biosym software was run on a Silicon Graphics Iris Indigo computer.

References

1. Feighner JP and Boyer WF, *Selective Serotonin Re-uptake Inhibitors*, Wiley, Chichester, 1991, p11-12.
2. Fuller RW and Wong DT, *Serotonin Uptake and Serotonin Uptake Inhibition*, Annals New York Academy of Sciences, 1990, **Vol 600**, 68.
3. Wong DT, Bymaster FP, Horng JS and Molloy BB, *J. Pharmacol. Exp. Ther.*, 1975, **193**, 804.
4. Le Fur G and Uzan A, *Biochem. Pharmacol.*, 1977, **26**, 497.
5. Glennon RA, *J. Med. Chem.*, 1987, **30**, 1.
6. Bradley PB, *Introduction to Neuropharmacology*, Butterworth and Co., 1989, p351.
7. Feighner JP and Boyer WF, *Selective Serotonin Re-uptake Inhibitors*, Wiley, Chichester, 1991, p17.
8. Singh S and Dryhurst G, *J. Med. Chem.*, 1992, **35**, 2667.
9. Falck B, Hillarp N-A and Thieme G, *J. Histochem. Cytochem.*, 1962, **10**, 348.
10. Williams M, *Ann. Reports in Med. Chem.*, 1986, **21**, 211.
11. Gaddum JH and Picarelli ZP, *Br. J. Pharmacol.*, 1957, **12**, 323.
12. 1994 Receptor & Ion Channel Nomenclature Supplement, 5th ed. *Trends in Pharmacological Sciences*, p25-27.
13. Fozard JR, *The Peripheral Actions of 5-Hydroxytryptamine*, Oxford Medical Publication, Oxford, 1989, p354.
14. Bradley G, Ward TJ, White JC, Coleman J, Taylor A and Rhodes KF, *J. Med. Chem.*, 1992, **35**, 1515 and references cited therein.
15. Nagel AA, Rosen T, Rizzi J, Daffeh J, Guarino K, Nowakowski J, Vincent LA, Heym J, McLean S, Seeger T, Connolly M, Schmidt AW and Siok C, *J. Med. Chem.*, 1990, **33**, 13.
16. Leibundgut U and Lancranjan I, *Lancet*, 1987, No 1, 1198.
17. Andrews PR, Rapeport WG and Sanger GT, *Trends Pharmacol. Sci.*, 1988, **9**, 334.

18. Swain CT, Baker R, Kneen C, Moseley J, Saunders J, Seward EM, Stevenson G, Beer M, Stanton J and Watling K, *J. Med. Chem.*, 1991, **34**, 140.

19. Schmidt AW and Peroutka SJ, *Molecular harmacology*, 1990, **38**, 511.

20. Hayashi H, Miwa Y, Ichikawa S, Yoda N, Miki I, Ishii A, Kono M, Yasuzawa T and suzuki F, *J. Med. Chem.*, 1993, **36**, 617.

21. Fuller RW, *Advances in Drug Research*, 1988, **17**, 349.

22. Marcusson JO, Norinder U, Hogberg J and Ross SB, *Europ. J. Pharmacol.*, 1992, **215**, 191.

23. Ives JL and Heym J, *Ann. Reports in Med. Chem.*, Academic Press, 1989, p21.

24. Fuller RW, *Neurotransmissions*, 1993, **vol IX**, No 2.

25. Blier P, de Montigny C and Chaput Y, *J. Clin. Psychiatry*, **51** (Suppl. 4), 14.

26. Feighner JP and Boyer WF, *Selective Serotonin Re-uptake Inhibitors*, Wiley, Chichester, 1991, p37-40.

27. Mendels J, *J. Clin. Psychiatry*, 1987, **48** (suppl), 26.

28. Montgomery SA, *Br. J. Psychiatry*, 1988, **153** (suppl. 3), 7.

29. Pollack MH and Rosenbaum JF, *J. Clin. Psychiatry*, 1987, **48**, 3.

30. Feighner JP and Boyer WF, *Selective Serotonin Re-uptake Inhibitors*, Wiley, Chichester, 1991, p89-108.

31. Blier P and de Montigny C, *Current Advances and Trends in the Treatment of Depression,TiPS*, 1994, July, **15**, 225.

32. Corey EJ and Reichard GA, *Tetrahedron Lett.*, 1989, 5207.

33. Robertson DW, Krushinski JH, Fuller RW and Leander JD, *J. Med. Chem.*, 1988, **31**, 1412.

34. Wong DT, Bymaster FP, Reid LR, Fuller RW and Perry KW, *Drug Dev. Res.*, 1985, **6**, 397.

35. Gao Y and Sharpless KB, *J. Org. Chem.*, 1988, **53**, 4081.

36. Kumar A, Ner DH and Dike SY, *Tetrahedron Lett.*, 1991, **32**, 1901.

37. Hjorth S, *J. Neurochem.*, 1993, **60**, 776.

38. Feighner JP and Boyer WF, *Selective Serotonin Re-uptake Inhibitors*, Wiley, Chichester, 1991, p142.

39. Nelson JC, Mozure CM, Bowers MB and Jatlow PI, *Arch. Gen. Psychiatry*, 1991, **48**, 303.

40. Feighner JP and Boyer WF, *Selective Serotonin Re-uptake Inhibitors*, Wiley, Chichester, 1991, p140.

41. Cohen NC, Blaney JM, Humblet C, Gund P and Barry DC, *J. Med. Chem.*, 1990, **33**, 883.

42. Rizzi JP, Nagel AA, Rosen T, Mclean S and Seeger T, *J. Med. Chem.*, 1990, **33**, 2721.

43. Schmidt AW and Peroutka SJ, *Molecular Pharmacology*, 1989, **36**, 505.

44. Westkaemper RB and Glennon RA, *Pharmacology Biochemistry & Behaviour*, 1991, **40**, 1019.

45. Hibert MF, Hoffmann R, Miller RC and Carr AA, *J. Med. Chem.*, 1990, **33**, 1594.

46. Evans SM, Galdes A and Gall M, *Pharmacology Biochemistry & Behaviour*, 1991, **40**, 1033.

47. Gundertofte K and Bøgesø KP, *Development of A Pharmacophoric Model of the Serotonin Reuptake Site*, 207th ACS National Meeting, 1994, March, San Diego, CA, USA.

48. Pinder RM, Green DM and Thompson PB, *J. Med. Chem.*, 1971, **14**, 626.

49. Kawakubo H, Takagi S, Yamaura Y, Katoh S, Ishimoto Y, Nagatani T, Mochizuki D, Kamata T and Sasaki Y, *J. Med. Chem.*, 1993, **36**, 3526.

50. Nussbaumer P, Petranyi G and Stutz A, *J. Med. Chem.*, 1991, **34**, 65.

51. Boyle EA, Mangan FR, Markwell RE, Smith SA, Thomson MJ, Ward RW and Wyman PA, *J. Med. Chem.*, 1986, **29**, 894.

52. Carter GW, *J. Pharm. Exp. Ther.*, 1991, **256**, 929.

53. He X-S, Raymon LP, Mattson MV, Eldefrawi ME and de Costa BR, *J. Med. Chem.*, 1993, **36**, 1188.

54. Graham SL, Shepard KL, Anderson PS, Baldwin JJ, Best DB, Christy ME, Freedman MB, Gautheron P, Habecker CN, Hoffman JM, Lyle PA, Michelson SR, Ponticello GS, Robb CM, Schwam H, Smith AM, Smith RL, Sondey JM, Strohmaier KM, Sugrue MF and Varga SL, *J. Med. Chem.*, 1989, **32**, 2548.

55. Aboulwara OM and El-Metwaldi MAE, *Arch. Pharm.* (Eng), 1992, 603.

56. Malleron J-L, Gueremy C, Mignani S, Peyronel J-F, Truchon A, Blanchard J-C, Doble A, Laduron P, Piot O, Zundel J-L, Betschart J, Canard H, Chaillou P, Ferris O, Huon C, Just B, Kerphirique R, Martin B, Mouton P and Renaudon A, *J. Med. Chem.*, 1993, **36**, 1194.

57. Bosin TR and Campaign EE, *Advances in Drug Research*, 1977, **1**, 191.

58. Browne EJ, unpublished results, Department of Chemistry, University of Tasmania.

59. Rao RN, *[1]Benzothiophene Derivatives*, 1990 Honours thesis, University of Tasmania.

60. Frankcombe K, *Synthetic, Modelling and Pharmacological Studies of [1]Benzothiophene-3-carboxamides* , 1992 Honours thesis, University of Tasmania.

61. Wright Jr. WB and Brabander HJ, *J. Heterocyclic Chem.*, 1971, **8**, 711.

62. Higa T and Krubsack AJ, *J. Org. Chem.*, 1975, **40**, 3037.

63. Katritzky AR and Rees CW, *Comprehensive Heterocyclic Chemistry*, Pergamon, Oxford, 1984, **Vol 4**, p731-732.

64. Silverstein RM, Bassler GC and Morrill TC, Spectrometric Identification of Organic Compounds, 5th edition, Wiley, New York, 1991, p122.

65. Baxter GS, Boyland P, Gaster LM and King FD, *Bioorganic & Medicinal Chemistry Letters*, 1993, **3**, 633.

66. Challis JA, *The Chemistry of Amides*, Wiley, New York, 1970, p795-801.

67. March J, *Advanced Org. Chem.*, 4th edition, Wiley, New York, 1992, p417.

68. Muller E, *Methoden Org. Chem.*, (Houben-Weyl), 1957, **XI/1**, 1.

69. Rahman A-U, Basha A and Waheed N, *Tetrahedron Lett.*, 1976, 219.

70. Trost BM and Fleming I, *Comprehensive Organic Syhthesis*, Pergamon, Oxford, 1991, **Vol 8**, p248-251.

71. Kropf H, *Methoden Org. Chem.*, (Houben-Weyl), 1984, **VI/Ib**, 1.

72. Brown HC, *Organic Synthesis via Boranes*, Wiley, New York, 1975, p15.

73. Brown HC and Heim P, *J. Am. Chem. Soc.*, 1964, **86**, 3566.

74. Brown HC and Heim P, *J. Org. Chem.*, 1973, **38**, 912.

75. March J, *Advanced Org. Chem.*, 4th edition, Wiley, New York, 1992, p1208.

76. Barton D and Ollis WD, *Comprehensive Organic Chemistry*, Pergamon, Oxford, 1979, **Vol 3**, p730.

77. Fisher GB, Fuller JC, Harrison J, Goralski CT and Singaram B, *Tetrahedron Lett.*, 1993, **34**, 1091.

78. Guida WC, Entreken EE and Guida AR, *J. Org. Chem.*, 1984, **49**, 3024.

79. Hutchins RO, Learn K, El-Telbany F and Stercho YP, *J. Org. Chem.*, 1984, 49, 2438.

80. Maki Y, Kikuchi K, Sugiyama H and Seto S, *Chem. Ind.*, 1976, 322.

81. Lalancette JM and Brindle JR, *Can. J. Chem.*, 1971, 49, 2990.

82. Umino N, Iwakuma T and Itoh N, *Tetrahedron Lett.*, 1976, 763.

83. Wann SR, Thorsen PT and Kreevoy MM, *J. Org. Chem.*, 1981, **46**, 2579.

84. Satoh T, Suzuki S, Suzuki Y, Miyaji Y ad Imai Z, *Tetrahedron. Lett.*, 1969, 4555.

85. Kuehne ME and Shannon PJ, *J. Org. Chem.*, 1977, **42**, 2082.

86. Borch RF, *Tetrahedron. Lett.*, 1968, 61.

87. March J, *Advanced Org. Chem.*, 4th edition, Wiley, New York, 1992, p1207.

88. March J, *Advanced Org. Chem.*, 4th edition, Wiley, New York, 1992, p898-900.

89. Babler J and Invergo BJ, *Tetrahedron Lett.*, 1981, **22**, 11.

90. Babler J, *Synth. Commun.*, 1982, 839.

91. Entwistle ID, Boehm P, Johnstone RW and Telford RP, *J. Chem. Soc. Perkin I*, 1980, 27.

92. Collins JC, Hess WW and Frank FJ, *Tetrahedron Lett.*, 1968, 3363.

93. Quitt P, *Helv. Chim. Acta*, 1963, 327.

94. O'Donnell MJ, Boniece JM and Earp SE, *Tetrahedron Lett.*, 1978, **34**, 264.

95. Bey P and Vevert JP, *Tetrahedron Lett.*, 1977, 1455.

96. March J, *Advanced Org. Chem.*, 4th edition, Wiley, New York, 1992, p918.

97. Morrison RT and Boyd RN, *Organic Chemistry*, 5th ed, Allyn and Bacon Inc., Boston, 1980, p900.

98. Lane CF, *Synthesis*, 1975, 135.

99. Seyden-Penne J, *Reduction by the Alumino- and Borohydrides in Organic Synthesis*, VCH, New York, 1991, p105.

100. Jung ME and Lyster MA, *J. Org. Chem.*, 1977, **42**, 3761.

101. McCarthy JR, Moore JL and Cregge RJ, *Tetrahedron Lett.*, 1978, **34**, 5183.

102. Ireland RE and Walba DM, *Org. Synth. Coll.*, **VI**, 1988, 567.

103. Minamikawa J and Brossi A, *Tetrahedron Lett.*, 1978, **34**, 3086.

104. Newman MS, Sankaran V and Olson DR, *J. Am. Chem. Soc.*, 1976, **98**, 3237.

105. McOmie JFW and West DE, *Org. Synth. Coll.*, **V**, 1973, 412.

106. Beck JR, *J. Org. Chem.*, 1972, **37**, 3224.

107. Cross PE, Dickinson RP, Parry MJ and Randall MJ, *J. Med. Chem.*, 1986, **29**, 1643.

108. O'Neil IA, Miller ND, Peake J, Barkley JV, Low CR and Kalindjian SB, *Synlett*, 1993, 515.

109. Larock RC, *Comprehensive Organic Transformation*, VCH, New York, 1989, p340.

110. Sauer J and Huisgen R, *Angew. Chem.*, 1960, 72, 294.

111. Dolby-Glover L, *Chemistry & Industry*, 1986, 518.

112. Suschitzky H, *Adv. Fluorine Chem.*, 1965, 1030.

113. Woodard RB, *Org. Synth. Coll.*, **III**, 1955, 453.

114. Flood DT, *Org. Syn. Coll.*, **II**, 1943, 295.

115. Rutherford KG, Redmond W and Rigamonti J, *J. Org. Chem.*, 1961, 5149.

116. Alker D, Barton D, Hesse RH, James JL, Markwell RE, Pechet MM, Rozen S, Takeshita T and Toh HT, *Noueveau J. De Chemie*, 1980, 239.

117. Schlosser M and Heinz G, *Chem. Ber.*, 1969, **102**, 1944.

118. Rozen S and Lerman O, *J. Org. Chem.*, 1980, **45**, 672.

119. Rozen S, Lerman O, Kol M and Hebel D, *J. Org. Chem.*, 1985, **50**, 4753 and references cited therein.

120. Umemoto T, Kawada K and Tomita K, *Tetrahedron Lett.*, 1986, **27**, 4465.

121. Gillet JP, Sauvetre R and Normant JF, *Synthesis*, 1982, 297.

122. Connor DT, Cetenko WA, Mullican MD, Sorenson RJ, Unangst PC, Weikert RJ, Adolphson RL, Kennedy JA, Thueson DO, Wright CD and Conroy MC, *J. Med. Chem.*, 1992, **35**, 958.

123. Conde S, Corral C and Lissavetzky J, *J. Heterocyclic Chem.*, 1980, **17**, 937.

124. Monkovic I, Willner D, Adam MA, Brown M, Crenshaw RR, Fuller CE, Juby PF, Luke GM, Matiskella JA and Montzka TA, *J. Med. Chem.*, 1988, **31**, 1548.

125. Derieg ME and Sternbach LH, *J. Heterocyclic Chem.*, 1966, **3**, 237.

126. Schenker von K and Druey J, *Helv. Chim. Acta*, 1963, 1696.

127. Levai A, Toth G, Halasz J, Timar T, Frank L and Hosztafi S, *Heterocycles*, 1994, **38**, 305.

128. Wong PL and Moeller KD, *J. Am. Chem. Soc.*, 1993, **115**, 11434.

129. Voloveko YM, Litenko VA, Khrapak TVand Babichev FS, *Khim. Geterotsikl. Soedin*, 1983, **(11)**, 1476 (Russ.)

130. Koji O, Masashi T and Otohiko T, *Hukusokan Kagaku Toronkai Koen Yoshishu* (Japan), 1975, **8,** 14.

131. Gilchrist TL, *Heterocyclic Chemistry,* Longman Scientific & Technical, New York, 1992, p68 and references cited therein.

132. Golding BT and Hall DR, *J. Chem. Soc. Perkin I,* 1975, 1302.

133. Kenner GW and Murray MA, *J. Chem. Soc.,* 1949, S178.

134. Singh B, *Tetrahedron Lett.,* 1971, 321.

135. Calli C, Illuminati G, Mardolini L and Tamborra P, *J. Am. Chem. Soc.,* 1977, **99,** 2591.

136. Paintal AS, *Physiol. Rev.,* 1973, **53 (1),** 159.

137. Fozard JR and Host M, *Br. J. Pharmacol.,* 1982, **77,** 520.

138. Stark P, Fuller RW and Wong DT, *J. Clin. Psychiatry,* 1985, **46,** 7.

139. AboulWafa OM and El-Metwalli MAE, *Arch. Pharm,* 1992, **325,** 603.

140. Lednicer D, Mitscher LA and Georg GI, *Organic Chemistry of Drug Synthesis,* Wiley, New York, 1990, p30.

141. Baeckstroem IT and Marcusson JO, *Neuropsychobiology,* 1990, **23 (2),** 68.

142. *An Introduction to Molecular Modelling with Insight and Discover,* p51, Handbook, Biosym Workshop on Molecular Modelling, 1993, April, Melbourne, Australia.

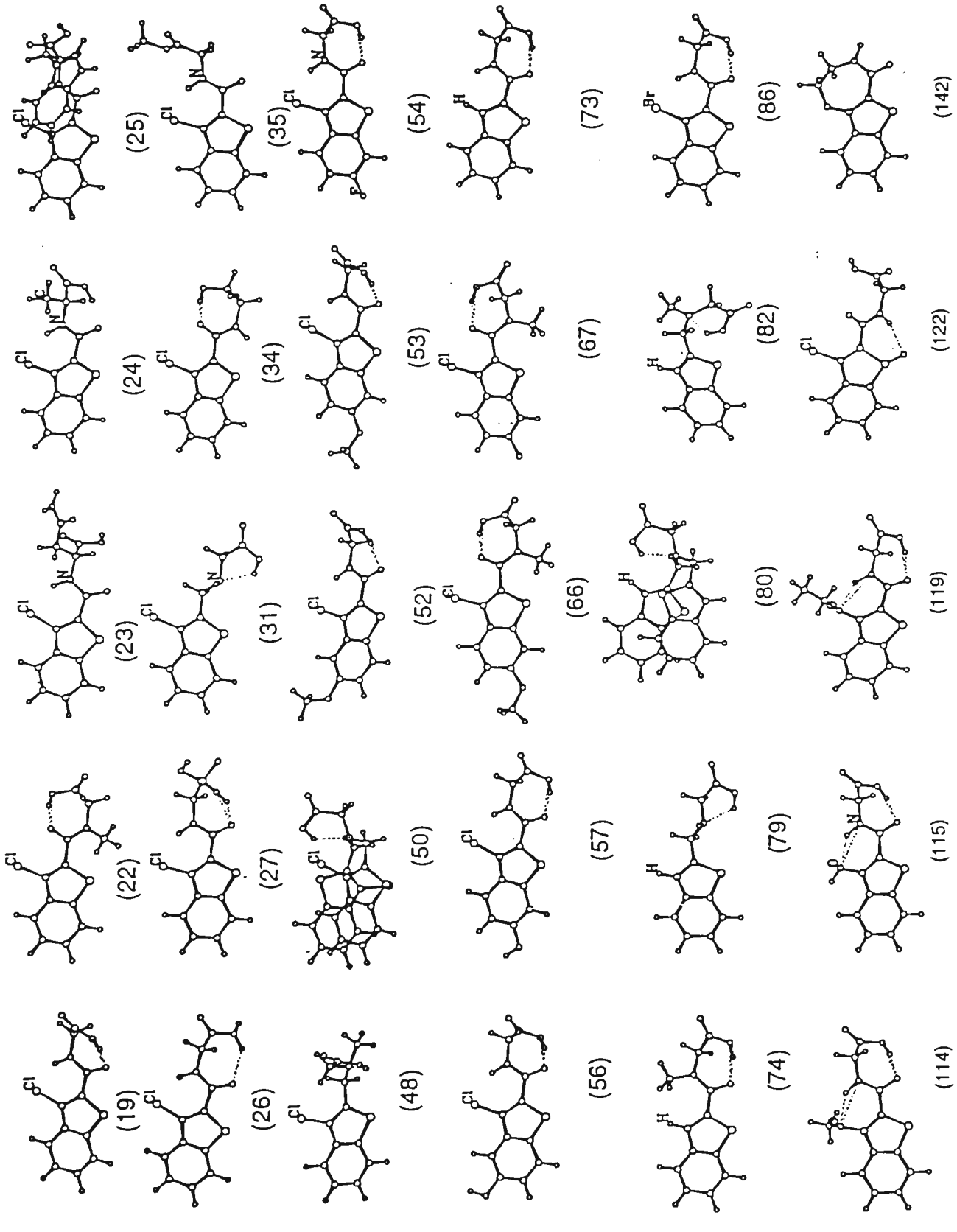
The input file for Discover generated in InsghtII

```
!
      overlap = 0.01

      begin simulation
*     add-automatic bond torsion valence out-of-plane
      reduce
!
      set dielectric  = 1.000000
!
!
!
      Minimize
*     no cross terms
*     no morse
*     for 100 iterations
*     using steepest descents
*     until the maximum derivative is less than 0.001000000 kcal/A
      initialize dynamics
      Minimize
*     no cross terms
*     no morse
*     for 100 iterations
*     using conjugate gradients
*     until the maximum derivative is less than 0.001000000 kcal/A
      initialize dynamics
*     for 1000 iterations
*     at  900.000 K
*     steps of 1.000
*     no cross terms
*     no morse
*     write history  file every 100 steps
      ifile = 1
loop  resume dynamics
*     for 1000 iterations
*     no cross terms
*     no morse
!
      archive as file number ifile
      ifile = ifile + 1
      if ifile.le.50 then loop
!
      loop=1
200   retrieve file number loop
      initialize dynamics
*     for 1000 iterations
*     at  300.000 K
*     steps of    1.000
*     no cross terms
*     no morse
*     write history  file every 100 steps
      resume dynamics at 300 K for 1000 iterations
      mimimize using steepest descents for 50 cycles
*     until the maximum derivative is less than 0.001000000 kcal/A
*     no cross terms
*     no morse
      minimize using conjugate gradients
*     no cross terms
*     no morse
*     until the maximum derivative is less than 0.001000000 kcal/A
      archive as file number loop
      loop= loop+1
      if loop.le.50 then 200
!
      end
```

Appendix II

The global minimum conformations of the [1]benzothiophene derivatives



Appendix III

Conformational analysis results of the selected [1]benzothiophene derivatives

Compounds	Energy of the global minimum conformation	Hydrogen bonding	Distance between the 3-substituent and the nitrogen	Distance between the aromatic ring and the nitrogen
19	14.0328 kcal/mol	yes	3.09Å	5.79Å
22	42.5176	yes	3.45	5.95
23	-36.8508	No	3.08	5.79
24	17.5162	yes	3.09	5.79
25	82.0310	No	3.08	5.76
26	5.6145	yes	3.09	5.79
27	-22.3561	yes	3.09	5.79
31	50.5867	yes	4.37	5.75
34	20.1042	yes	4.65	5.78
35	25.8698	No	3.08	5.79
48	53.7993	yes	4.41	5.78
50	90.0010	yes	4.46	5.69
52	16.9733	yes	3.09	5.79
53	19.9863	yes	3.09	5.79
54	15.3066	yes	3.09	5.84
56	3.2672	yes	3.09	5.79
57	5.5179	yes	3.09	5.79
66	47.9482	yes	4.66	5.95
67	43.4672	yes	4.66	5.95
73	24.2994	yes	2.96	5.81
74	49.6932	yes	3.24	5.95
79	50.6921	yes	4.03	5.74
80	91.1011	yes	3.47	5.66
82	58.0635	yes	3.18	5.76
86	17.4678	yes	3.19	5.81
114	19.6301	yes	2.94	5.77
115	8.6279	yes	2.94	5.86
119	15.6563	yes	2.94	5.77
122	25.8698	yes	4.51	5.73