Design, Synthesis, and Pharmacological Evaluation of Novel Antipsychotic Drugs Based on Olanzapine That Display Reduced Weight Gain and Metabolic Side Effects

Somayeh Jafari

University of Wollongong

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Design, Synthesis, and Pharmacological Evaluation of Novel Antipsychotic Drugs Based on Olanzapine That Display Reduced Weight Gain and Metabolic Side Effects

A thesis submitted in fulfillment of the requirements for the award of the degree of

Doctor of Philosophy

From

The University of Wollongong

By

Somayeh Jafari

Masters of Science (MedChem)

Supervisors: Prof. Xu-Feng Huang
Dr Francesca Fernandez-Enright

School of Health Sciences

March, 2012
CERTIFICATION

I, Somayeh Jafari, declare that this thesis, submitted in full fulfillment of the requirements for the award of Doctor of Philosophy, in the School of Health Sciences, Faculty of Health and Behavioral Sciences, University of Wollongong, is wholly my own work unless referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Somayeh Jafari

March, 2012
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<tr>
<td>5-HT&lt;sub&gt;2&lt;/sub&gt;</td>
<td>5-hydroxytryptamine</td>
</tr>
<tr>
<td>A</td>
<td>Atypical</td>
</tr>
<tr>
<td>A</td>
<td>Adrenergic receptor</td>
</tr>
<tr>
<td>AMPK</td>
<td>Adenosine monophosphate-activated protein kinase</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>BAT</td>
<td>Brown adipose tissue</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood-brain barrier</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-Butoxycarbonyl</td>
</tr>
<tr>
<td>Calcd</td>
<td>Calculated</td>
</tr>
<tr>
<td>CATIE</td>
<td>Clinical antipsychotic trial on intervention effectiveness</td>
</tr>
<tr>
<td>Cbz</td>
<td>Benzyloxy carbonyl</td>
</tr>
<tr>
<td>CI</td>
<td>Chemical ionization</td>
</tr>
<tr>
<td>Ci</td>
<td>Curie</td>
</tr>
<tr>
<td>CPM</td>
<td>Count per minute</td>
</tr>
<tr>
<td>d</td>
<td>Doublet</td>
</tr>
<tr>
<td>dd</td>
<td>Double of doublets</td>
</tr>
<tr>
<td>D</td>
<td>Dopamine receptor</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DEPT</td>
<td>Distortionless enhancement by polarisation transfer</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DMPK</td>
<td>Drug Metabolism/Pharmacokinetics</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>DOPAC</td>
<td>Dihydroxyphenylacetic acid</td>
</tr>
<tr>
<td>dt</td>
<td>Double of triplet</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethyl(carboxymethyl)amino]acetic acid</td>
</tr>
<tr>
<td>ED&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Median effective dose</td>
</tr>
<tr>
<td>EI</td>
<td>Electron impact</td>
</tr>
<tr>
<td>EPS</td>
<td>Extrapyramidal symptoms</td>
</tr>
<tr>
<td>equiv</td>
<td>Equivalents</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionization</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
<td>-----------</td>
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<tr>
<td>GCOSY</td>
<td>Gradient correlation spectroscopy</td>
</tr>
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<td>GHMBC</td>
<td>Gradient heteronuclear multiple bond correlation</td>
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<tr>
<td>GHSQC</td>
<td>Gradient heteronuclear single quantum correlation</td>
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<tr>
<td>Glu</td>
<td>Glutamate</td>
</tr>
<tr>
<td>h</td>
<td>Hours</td>
</tr>
<tr>
<td>H</td>
<td>Histamine receptor</td>
</tr>
<tr>
<td>H</td>
<td>Hydrogen/proton</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>High resolution mass spectrometry</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Inhibitory concentration (half maximal)</td>
</tr>
<tr>
<td>J</td>
<td>Coupling constant</td>
</tr>
<tr>
<td>Kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>K&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Inhibition constant</td>
</tr>
<tr>
<td>Kcal</td>
<td>Kilo calories</td>
</tr>
<tr>
<td>LRMS</td>
<td>Low resolution mass spectrometry</td>
</tr>
<tr>
<td>m</td>
<td>Multiplet</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>M</td>
<td>Muscarinic receptor</td>
</tr>
<tr>
<td>MHz</td>
<td>Mega hertz</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
</tr>
<tr>
<td>mL</td>
<td>Milliliters</td>
</tr>
<tr>
<td>mmol</td>
<td>Millimole</td>
</tr>
<tr>
<td>m.p.</td>
<td>Melting point</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>MW</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass to charge ratio</td>
</tr>
<tr>
<td>nM</td>
<td>Nanomolar</td>
</tr>
<tr>
<td>NMnDA</td>
<td>N-methyl-D-aspartate acid</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>Olz</td>
<td>Olanzapine</td>
</tr>
<tr>
<td>PCP</td>
<td>Phencyclidine</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>PET</td>
<td>Positron-emission tomography</td>
</tr>
<tr>
<td>pH</td>
<td>Potential of hydrogen</td>
</tr>
<tr>
<td>PPM</td>
<td>Part per million</td>
</tr>
<tr>
<td>PTLC</td>
<td>Preparative thin layer chromatography</td>
</tr>
<tr>
<td>q</td>
<td>Quartet</td>
</tr>
<tr>
<td>quin</td>
<td>Quintet</td>
</tr>
<tr>
<td>rt</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Ref</td>
<td>Reference</td>
</tr>
<tr>
<td>R_f</td>
<td>Retention factor</td>
</tr>
<tr>
<td>s</td>
<td>Singlet</td>
</tr>
<tr>
<td>SAR</td>
<td>Structure activity relationship</td>
</tr>
<tr>
<td>SDA</td>
<td>Serotonin-dopamine antagonist</td>
</tr>
<tr>
<td>SES</td>
<td>2-(trimethylsilyl) ethyl sulfonyle</td>
</tr>
<tr>
<td>SREBP-1</td>
<td>Sterol regulatory element binding protein-1</td>
</tr>
<tr>
<td>t</td>
<td>Triplet</td>
</tr>
<tr>
<td>T</td>
<td>Typical</td>
</tr>
<tr>
<td>TD</td>
<td>Tardive dyskinesia</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>Tetramethylsilane</td>
</tr>
<tr>
<td>TRIS</td>
<td>Tris(hydroxymethyl)aminomethane</td>
</tr>
<tr>
<td>UCP</td>
<td>Uncoupling protein</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>µL</td>
<td>Microliter</td>
</tr>
<tr>
<td>δ</td>
<td>Chemical shift (in parts per million, downfield from TMS)</td>
</tr>
</tbody>
</table>
PUBLICATIONS

The following publications and presentations have arisen directly from a work conducted for this thesis.

Publication in Referred Journals


Jafari S, Bouillon ME, Huang X-F, Pyne SG, and Fernandez-Enright F. Novel olanzapine analogues presenting a reduced H1 receptor affinity and retained SHT2A/D2 binding affinity ratio. BMC Pharmacology 12: 8, 2012 (Chapter 2)

Jafari S, Huang X-F, and Fernandez-Enright F. In vivo pharmacological evaluations of novel olanzapine analogues in rats: potential novel antipsychotic drugs with lower metabolic side effect. (under submission) (Chapters 3 and 4)

Publication in Conference Proceedings


Jafari S, Fernandez-Enright F, Huang, X-F. Development of novel antipsychotic drugs with lower obesogenic side effects. The 32nd Annual Meeting of The Australian Neuroscience Society (2012), Gold Coast, QLD, Australia
I would like to take this opportunity to acknowledge all those people who have aided me in the completion of this PhD project. This dissertation would not have been possible without the guidance and the help of several individuals who in one way or another contributed and extended their valuable assistance in the preparation and completion of this study.

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ABSTRACT

“Design, Synthesis and Pharmacological Evaluation of Novel Antipsychotic Drugs Based on Olanzapine that Display Reduced Weight Gain and Metabolic Side Effects”

Somayeh Jafari
University of Wollongong, 2012

Olanzapine (Olz) is an effective antipsychotic drug for treating severe psychotic disorders, including schizophrenia and bipolar disorders. However, Olz administration is associated with severe weight gain, type II diabetes mellitus, and cardiovascular diseases. Clinical efficiency of Olz has been reported to be linked with its favourable serotoninergic 2A and dopamine 2 (5HT2A/D2) receptor binding affinity ratios, while the blockade of the histamine 1 (H1) receptor is the most likely mechanism for Olz-induced weight gain. Thus, a novel antipsychotic drug with a similar 5HT2A/D2 receptor binding profile as Olz but with reduced affinity for H1 receptor would be an invaluable breakthrough in schizophrenia therapy.

This dissertation focused on the development of novel Olz derivatives with a lower in vitro binding affinity for the H1 receptors, but with a similar 5HT2A/D2 receptor binding affinity ratio to the one observed for Olz. Two new derivatives of Olz, (2-ethyl-4-(4’-methylpiperazin-1’-yl)-10Hbenzo[b]thieno[2,3-e][1,4]diazepine (OlzEt) and (2-ethyl-4-(4’-methyl-1’,4’-diazepan-1’-yl)-10H-benzo[b]thieno[2,3-e][1,4]diazepine (OlzHomo), were synthesized and the affinities of these compounds for the brain 5HT2A, D2, and H1 receptors were evaluated. OlzEt represents a potential
antipsychotic agent characterized by a highly favourable binding profile at 5HT2A ($K_i$: 3.70 ± 0.74 nM) and D2 ($K_i$: 54.51 ± 12.12 nM) receptors, similar to Olz ($K_i$: 4.22 ± 0.77 nM and $K_i$: 67.72± 9.21 nM, respectively), as well as lower affinity for H1 receptors ($K_i$ (Olz): 0.13 ± 0.02 nM and $K_i$ (OlzEt): 1.95 ± 0.33 nM). OlzHomo presented lower binding affinities to all the aforementioned receptors ($K_i$: 81.20 ± 2.43 nM, $K_i$: 791.08 ± 83.19 nM, and $K_i$: 13.63 ± 2.68 nM, respectively). Taking into account that OlzHomo possessed a favourable p$K_i$ ratio 5HT2A/D2 similar to the measured value of Olz (ratio value = 1.17) and that the D2 affinity of thienobenzodiazepine OlzHomo is still comparable with that value of the high potent antipsychotics, it was postulated that both compounds OlzHomo and OlzEt may present therapeutic effectiveness for treating schizophrenia. In addition, these compounds present a lower affinity for H1 receptors, which may have reduced effects on weight gain and metabolic disorders than those reported with Olz.

Further, this study aimed to compare the liability of Olz, OlzEt and OlzHomo to induce weight gain in female rats in detail, incorporating indicate of weight gain, food and water intake, visceral fat deposition and measures of plasma hormones related to body weight maintenance (i.e., insulin, leptin, and adiponectin). The present findings confirmed the obesogenic effect of Olz administration, coupled with down-regulation of the H1 receptors in the hypothalamus. OlzEt and OlzHomo, though, turned up as promising antipsychotic compounds that did not induce enhancing effects on body weight and food intake or detrimental consequences on fat deposition and metabolism. In addition, a PCP-treated rat model was employed to examine the prevention of PCP induced hyperlocomotor activity relevant for the
treatment of schizophrenia. Behavioural assessment in the open-field test predicted the similar effectiveness of OlzEt to Olz for blocking PCP-induced hyperactivities. The study also showed the long lasting down-regulation of D$_2$ and 5HT$_{2A}$ receptors induced by sub-chronic Olz and OlzEt treatment, which may play a part in blocking PCP-induced behaviours. A lower potency of OlzHomo to inhibit PCP-induced behaviours was observed, which could also be explained by its lower affinity for the brain D$_2$ and 5HT$_{2A}$ receptors compared to that of Olz and OlzEt. Therefore, the therapeutic effectiveness of an OlzHomo regime may be delivered at higher dose than that of Olz and OlzEt treatment.

The present findings appear to have reasonable predictive validity for different aspects of Olz-induced weight gain/adiposity and metabolic abnormalities, which mimic the clinical situation. Nevertheless, given the limitations associated with animal models, it is suggested that these results must be taken with caution. Only further behavioural studies and clinical trials will reveal the predictive validity of current preclinical model for therapeutic efficacy and metabolic side effects of OlzEt and OlzHomo.
For decades, a growing number of antipsychotic agents have emerged for treating severe psychotic diseases, including schizophrenia, schizoaffective disorder, bipolar mania, and delusional disorders (Gardner et al. 2005; Mathews and Muzina 2007). Antipsychotic drugs possess various chemical structures that play a crucial role in their interactions with neurotransmitter receptors, resulting in their respective neuropharmacological properties. This chapter will provide an update on the potential contribution of chemical structure to the therapeutic profiles and metabolic side effects of current antipsychotic drugs. The effects of various chemical classes of typical and atypical antipsychotic drugs in treating positive (e.g., hallucination and delusion), negative (e.g., social withdrawal and poverty of speech), and cognitive (e.g., reduction of working memory and attention) symptoms of schizophrenia will be reviewed. The potential mechanism of metabolic side effects associated with the chemical structure of antipsychotics will also be discussed. A better understanding of the structural modifications of atypical antipsychotics that lead to reduced weight gain will play a significant role in the discovery of the next generation of better atypical antipsychotic drugs.
1.1 Contribution of Structure to Therapeutic Actions

Antipsychotics with various chemical structures exert different clinical potencies linked to their affinities for dopaminergic subtype 2 (D₂) receptors. All typical antipsychotic drugs present high affinity for blocking D₂ receptors (Seeman et al. 1976; Dargham and Laruelle 2005). The effects of typical antipsychotics on the dopaminergic system are therapeutically beneficial, but these antipsychotics induce undesirable motor side effects. A positron-emission tomography (PET) study revealed that 60% to 70% of D₂ receptor occupancy is sufficient to provide antipsychotic efficiency, while occupancy higher than 80% produces motor disorders (Farde et al. 1988; Worrel et al. 2000). The molecular imaging studies reported an increased level of presynaptic striatal dopaminergic activities, such as synthesis capacity and release of dopamine, in patients with schizophrenia (Howes and Kapur 2009). Hyperactivity of dopamine receptors in the mesolimbic pathway is responsible for the positive symptoms reported in psychosis (Serretti et al. 2004; Dargham and Laruelle 2005). Blockade of the D₂ receptors in this pathway leads to the alleviation of positive symptoms (Serretti et al. 2004). In contrast, hypoactivity of prefrontal dopamine neurons may contribute to the development of the negative and cognitive signs of schizophrenia. Typical antipsychotic drugs occupy about 70% to 90% of D₂ receptors at the commonly used dosage (Worrel et al. 2000). The blockade of D₂ receptors in the mesocortical dopamine pathway by typical antipsychotic drugs can cause emotional and cognitive disturbances (neuroleptic-induced deficit syndrome). However, the blockade of dopamine receptors by typical antipsychotic treatment in the nigrostriatal pathway is associated, even at moderate doses, with extrapyramidal symptoms (EPS), including tardive dyskinesia (TD),
dystonia, akathisia, and Parkinsonism (Conley and Kelly 2005). While the blockade of the D2 receptors by typical antipsychotics in the mesolimbic dopamine pathway leads to the reduction of positive symptoms, their lack of selectivity for blocking the D2 receptors in the nigrostriatal and mesocortical pathways results in various undesirable motor disorders and worsening of the primary negative and cognitive symptoms of schizophrenia (Oakley et al. 1991; Tandon and Jibson 2002; Zhang et al. 2007).

From a pharmacological perspective, atypical antipsychotics are not only capable of controlling the positive symptoms of schizophrenia, but are also believed to improve the negative signs and cognitive abnormalities (Conley and Kelly 2005). Clinical trials have reported fewer incidents of motor dysfunctions with atypical antipsychotic treatments compared to typical antipsychotic therapy. This may be attributed to the limbic-specific property of some atypical antipsychotics drugs, including clozapine (Serretti et al. 2004). While typical antipsychotics and atypical antipsychotics both have D2 receptor antagonist properties, the atypical antipsychotics are more selective for the D2 receptors in the mesolimbic pathway than those in the mesocortical and nigrostriatal pathways. Additionally, atypical antipsychotics are serotonin and dopamine receptor antagonists (SDAs) (Meltzer et al. 1989b), resulting in their unique therapeutic properties for schizophrenia therapy. Blockade of postsynaptic 5HT2A receptor by atypical antipsychotics in substantia nigra neurons may increase dopamine release in axonal terminals because it removes the inhibitory effect of 5HT2A receptors on dopaminergic neurons. Atypical antipsychotic drugs are therefore able to compensate for the hypoactivity of dopamine in the nigrostriatal
dopamine pathway, which leads to a lower incidence of EPS side effects, such as TD. Similarly, in the mesocortical dopamine pathway, the blockade of serotonin 5HT\textsubscript{2A} receptors by atypical antipsychotics is believed to balance the dopamine activity deficiency and lead to an improvement of the negative and cognitive signs of schizophrenia (Meltzer et al. 1989a; Stahl 1998; Meltzer 1999; Bubser et al. 2001). However, the above discussion should be taken with extreme caution, as not all literature supports these findings. For instance, amisulpride and remoxipride are effective atypical antipsychotics with no affinity for the 5HT\textsubscript{2A} receptor at clinically therapeutic doses, therefore, 5HT\textsubscript{2A} antagonism is not necessary for atypical antipsychotic action (Kapur et al. 2000; Kapur and Seeman 2001; Seeman 2002; Seeman 2006; Seeman 2010). It has also been reported that the high occupancy of 5HT\textsubscript{2A} receptors by Olz and risperidone does not affect the D\textsubscript{2} occupancy required for the antipsychotic effect or the D\textsubscript{2} occupancy at which EPS signs occur (Kapur et al. 1999).

The neuropharmacological properties associated with the different chemical structures of antipsychotics will be compared in the following section. This approach may improve our understanding of the structural contributions to therapeutic efficacy. Receptor binding affinities are also given in Table 1.1.
### Table 1.1: Chemical structures and receptor binding affinity profiles of antipsychotics

<table>
<thead>
<tr>
<th>Structure</th>
<th>Antipsychotic Chemical Class</th>
<th>Phenothiazines</th>
<th>T</th>
<th>D₂</th>
<th>5HT₂A</th>
<th>H₁</th>
<th>5HT₂C</th>
<th>M₁</th>
<th>α₁</th>
<th>Ref*</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Chlorpromazine Structure" /></td>
<td>Chlorpromazine</td>
<td>Phenothiazines</td>
<td>T</td>
<td>1.2</td>
<td>8</td>
<td>6</td>
<td>25</td>
<td>NA</td>
<td>0.28</td>
<td>a, b</td>
</tr>
<tr>
<td><img src="image2" alt="Thiothixene Structure" /></td>
<td>Thiothixene</td>
<td>Thioxanthenes</td>
<td>T</td>
<td>0.15</td>
<td>50</td>
<td>4</td>
<td>1400</td>
<td>NA</td>
<td>11</td>
<td>a, b</td>
</tr>
<tr>
<td><img src="image3" alt="Haloperidol Structure" /></td>
<td>Haloperidol</td>
<td>Butyrophenones</td>
<td>T</td>
<td>0.7</td>
<td>45</td>
<td>440</td>
<td>&gt;10000</td>
<td>&gt;1500</td>
<td>6</td>
<td>c</td>
</tr>
<tr>
<td>0.74</td>
<td>53</td>
<td>180</td>
<td>10000</td>
<td>NA</td>
<td>12</td>
<td>a, b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1 (Continued)

<table>
<thead>
<tr>
<th>Antipsychotic</th>
<th>Chemical Class</th>
<th>Pharmacological Class</th>
<th>( K_i ) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D(_2)</td>
<td>5HT(_{2A})</td>
</tr>
<tr>
<td>Clozapine</td>
<td>Dibenzodiazepines</td>
<td>A</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75</td>
<td>5.4</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>Thienobenzodiazepines</td>
<td>A</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.4</td>
<td>2</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>Dibenzothiazepines</td>
<td>A</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td></td>
<td>140</td>
<td>101</td>
</tr>
<tr>
<td>Loxapine</td>
<td>Dibenzoxazepines</td>
<td>T</td>
<td>9.8</td>
</tr>
</tbody>
</table>

*Ref* indicates the reference(s) for the data.
Table 1 (Continued)

<table>
<thead>
<tr>
<th>Structure</th>
<th>Antipsychotic Chemical Class</th>
<th>Pharmacological 1 Class</th>
<th>$K_i$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D$_2$</td>
<td>5HT$_{2A}$</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>Phenylpiperazines</td>
<td>A</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.8</td>
</tr>
<tr>
<td>Pimozide</td>
<td>Diphenylbutylpiperidines</td>
<td>T</td>
<td>1.4</td>
</tr>
</tbody>
</table>
Table 1 (Continued)

<table>
<thead>
<tr>
<th>Structure</th>
<th>Antipsychotic Chemical Class</th>
<th>Pharmacological Class</th>
<th>( K_i ) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ziprasidone</td>
<td>Indoles</td>
<td>D&lt;sub&gt;2&lt;/sub&gt;</td>
<td>5HT&lt;sub&gt;2A&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Risperidone</td>
<td>Benzisoxazoles</td>
<td>A</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.09</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( K_i \): receptor binding affinity, D<sub>2</sub>: dopamine (2) receptors, 5HT<sub>2A</sub>: serotonin (2A) receptors, M<sub>1</sub>: muscarinic (1) receptors, \( \alpha_1 \): alpha (1) receptors, H<sub>1</sub>: histamine (1) receptors. NA: not available, >> very high value, A: atypical, T: typical. * Data are from "Kroeze et al. 2003", "Seeman 2010", "Bishara and Taylor 2008", "Worreletal.2000", and "Sussman 2003". "a, b" Data presented from human cloned receptors, "a, d, e" Data presented from rat brain receptors.
1.1.1 Phenothiazines and Thioxanthenes

Phenothiazines (e.g., chlorpromazine and promethazine) and thioxanthenes (e.g., flupentixol) are classified as typical antipsychotics due to their high potency for D₂ receptor blockade in both the mesocortical and mesolimbic pathways. These drugs can be effective in treating the positive symptoms but not the negative signs of schizophrenia; the one exception is flupentixol, which demonstrates an alleviating effect on the negative signs of schizophrenia that is comparable to risperidone treatment (Reimold et al. 2007; Ruhrmann et al. 2007). Phenothiazines and thioxanthenes are accompanied with severe EPS, including akathisia, TD, and potential neuroleptic malignant syndromes (Mitterschiffthaler et al. 1989; Chodorowski et al. 2003; Porsolt et al. 2010).

1.1.2 Butyrophenones, Diphenylbutylpiperidines and Benzamides

Haloperidol, which has a butyrophenone structure, was the prototype drug for more efficient dopamine antagonist effects (particularly at the D₂ receptors). This drug is commonly prescribed in emergency cases for fast-acting treatment of positive psychosis symptoms (Battaglia 2005); however, it is often accompanied by severe adverse side effects. The co-administration of promethazine and haloperidol is beneficial for enduring tranquillization effects and alleviating the side effects of haloperidol therapy (Huf et al. 2009).

Interestingly, studies before the discovery of atypical antipsychotics suggested that typical neuroleptic diphenylbutylpiperidines improve the negative signs of schizophrenia at low doses (Petit and Dollfus 1991). This effect can be explained by
the considerable calcium channel blocking action of diphenylbutylpiperidines (Meltzer et al. 1986). The benzamides, a class of atypical antipsychotic drugs, has also shown some success in treating the negative signs. Amisulpride, for instance, has been reported as a useful therapeutic agent for the treatment of negative signs, although it has no affinity for 5HT$_{2A}$ receptors (Bressan et al. 2003; Bishara and Taylor 2008; Nuss and Tessier 2010). In further support of this finding, concomitant treatments of the benzamide sulpride and clozapine have been reported to deliver greater clinical efficacy than clozapine along with fewer side effects (Wang et al. 2010).

1.1.3 Benzisoxazolepiperidines and Indoles

The proper correlation between the ratio of the clinical dosage and the D$_2$ receptor affinity to treat the positive symptoms of schizophrenia has not been completely established. Atypical antipsychotics, including indoles (e.g., ziprasidone and sertindole) and benzisoxazoles (e.g., risperidone, iloperidone, and paliperidone), are highly potent D$_2$ antagonists. The greater affinity of risperidone, paliperidone, and sertindole for the 5HT$_{2A}$ receptor relative to the dopamine D$_2$ receptor (with the ratio of 5HT$_{2A}$/D$_2$ receptor $>$ 10) is correlated with a lower risk of EPS and the effective treatment of negative signs (Schotte et al. 1996). These pharmacological properties of risperidone have been shown in separate studies that compared their properties with haloperidol (Cohen 1994; He and Richardson 1995). Iloperidone can also be an efficient antipsychotic agent in schizophrenia treatment (Marino and Caballero 2010). In fact, iloperidone was more effective than other benzisoxazole antipsychotics in antagonizing climbing behaviour in mice (Jain 2000).
1.1.4 Dibenzodiazepines, Thienobenzodiazepines and Dibenzothiazepines

The tetracycle dibenzodiazepine and thienobenzodiazepine antipsychotics (Table 1.1, page 5) have a higher affinity for the mesolimbic dopamine receptors than the receptors present in the nigrostriatal and mesocortical pathways (Minchin and Csernansky 1996; Tehan et al. 2001). Clozapine shows high mesolimbic selectivity in various pharmacological and behavioural studies, and has been reported to block the dopaminergic effects of apomorphine and amphetamine agents (Ashby and Wang 1996). In addition to its limbic selectivity, the unique therapeutic features of clozapine, including the low risk of EPS, arises from its broad pleomorphic receptor pharmacology, with activity for the D_2, dopaminergic subtype 4 (D_4), 5HT_2A, and serotonergic subtype 2C (5-HT_2C) receptors as well as dopaminergic subtype 1 (D_1), adrenergic subtype 1(α_1), adrenergic subtype 2 (α_2), muscarinic subtype 1 (M_1), and histaminergic subtype 1 (H_1) receptors (Marder 1992; Ashby and Wang 1996; Tehan et al. 2001). Occupancy of clozapine at D_2 receptors is between 38% and 63% at normal doses, which is below the threshold for inducing EPS. Long-term clozapine therapy showed greater efficacy in the treatment of the positive symptoms and negative signs of schizophrenia compared to typical antipsychotic drugs (Ravanic et al. 2009). Olz, a clozapine-like antipsychotic, is classified as a thienobenzodiazepine, which is also effective for treating negative signs and positive schizophrenic symptoms but induces low incidence of EPS. Studies with PET technology reported that at an effective dose, Olz occupied approximately 43% to 80% of D_2 receptors (Buckley 2005). In vitro and in vivo studies with Olz showed antagonist activity at 5HT_6, 5HT_2A, 5HT_2B, 5HT_2C, α_1, H_1, D_1, D_2, and M_1 – M_5 receptors (Roth et al. 1994). Quetiapine, which is classified as a dibenzothiazepine, shows high affinity for
blocking 5HT receptors and is able to antagonize H₁ receptors and α₁ and α₂ adrenergic receptors (Mathews and Muzina 2007). However, this drug shows only 30% and 41% D₂ receptor occupancy in the putamen at doses of 450 and 750 mg/day, respectively (Serretti et al. 2004). Due to its fast-off D₂ receptor action, quetiapine does not induce any EPS, even at high doses (Seeman and Tallerico 1999; Baldwin and Scott 2009).

1.1.5 Phenylpiperazines and Azapirones

Phenylpiperazine (e.g., aripiprazole and bifeprunox) and azapirone (e.g., perospirone and lurasidone) compounds are classified as the third generation of antipsychotics due to their unique partial agonist activity at the D₂ and 5HT₁A receptors (Newman-Tancredi 2010). This partial agonist propensity is therapeutically beneficial for the treatment of the positive symptoms and negative signs of schizophrenia. Partial agonists act as antagonists in the presence of the full agonist but play an agonist role in the absence of the full agonist. As previously described, schizophrenic subjects show hyperactivity in the mesolimbic dopamine pathway but present with hypoactivity in the mesocortical and nigrostriatal dopamine pathways. In the mesolimbic dopamine pathway, aripiprazole act as a dopamine antagonist. This effect is similar to the typical antipsychotics and other atypical antipsychotics with respect to the therapeutic mechanism. However, in the mesocortical dopaminergic pathway, aripiprazole effectively acts as an agonist. The same effect applies to the nigrostriatal dopamine pathways, and aripiprazole thus shows a lower tendency for inducing EPS (Bishara and Taylor 2008). With unique pharmacological benefits (low potential for inducing extrapyramidal and metabolic side effects), aripiprazole may
be a tolerable treatment for schizophrenia (Normala and Hamidin 2009; Stip and Tourjman 2010).

In brief, it appears that the structural diversity of antipsychotic drugs is responsible for their various neuropharmacological properties. While all of the aforementioned chemical classes are useful agents in treating the positive symptoms of schizophrenia, atypical antipsychotics, particularly thienobenzodiazepines and dibenzodiazepines, are more effective in inhibiting the negative signs of schizophrenia. The potential effects of flupentixol and diphenylbutylpiperidines (typical antipsychotics) and amisulpride (atypical antipsychotic) on improving negative signs while possessing no affinity for the 5HT$_{2A}$ receptors (Bressan et al. 2003) suggest that the 5HT$_{2A}$/D$_2$ receptor binding affinity ratio hypothesis is not the only mechanism involved in the therapeutic effects of antipsychotics on the negative signs of schizophrenia. Although it has often been reported that the blockade of the 5HT$_{2A}$ receptors may contribute to the antipsychotic effectiveness and reduce EPS, the exact role of 5HT$_{2A}$ blockade by antipsychotics is still unclear.

### 1.1.6 Effectiveness of Antipsychotic Drugs in CATIE Study

Similar effectiveness between typical and atypical antipsychotics has been found in treating nonrefractory patients in the CATIE (clinical antipsychotic trial on intervention effectiveness) study (Lieberman 2006). In CATIE study, the effectiveness of atypical antipsychotics (OIr, ziprasodone, quetiapine, and risperidone but not clozapine and aripiprazole) and typical antipsychotic (perphenazine with phenothiazine structure) on patients with chronic schizophrenia
has been investigated up to 18 months (Lieberman et al. 2005). The main side effect of atypical antipsychotics such as Olz was associated with significant weight gain and changes in glucose and lipid metabolism. The results of CATIE study, that atypical antipsychotics are not the real breakthrough in effectiveness in treating schizophrenia, indicate that the needs to develop novel antipsychotic drugs for schizophrenia remains.

1.2 The Metabolic Side Effects of Antipsychotics

The majority of atypical antipsychotic drugs are associated with severe weight gain, type II diabetes mellitus, hyperglycemia, dyslipidemia, insulin resistance, cardiovascular disease, hyperprolactemia, and hypertension (Allison et al. 1999; Jones et al. 2001; Wetterling 2001; Gardner et al. 2005; Holt 2006; Mathews and Muzina 2007; Han et al. 2008; Breden et al. 2009). The risk of weight gain is high with clozapine and Olz, moderate with quetiapine and typical antipsychotics, low with risperidone and minimal with aripiprazole, molindone and ziprasidone (Sussman 2003; Gardner et al. 2005; Llorente and Urrutia 2006; Mathews and Muzina 2007). A study assessing the short-term (10 weeks) treatment of antipsychotics at standard doses revealed average body weight increases of 4.45 kg with clozapine, 4.15 kg with Olz, 3.19 kg with thioridazine (typical antipsychotic with phenothiazine structure), 2.92 kg with sertindole, 2.10 kg with risperidone, and 1.1 kg with haloperidol (Allison et al. 1999). With the exception of clozapine, this study showed similar weight gain effects of antipsychotic drugs across the different standard doses. Phase I of the CATIE study also reported the following ratios of patients gaining significant weight after long term antipsychotic treatment: 30% with
Olz, 16% with quetiapine, 14% with risperidone, and 7% with ziprasidone (Lieberman et al. 2005). Weight gain associated with antipsychotics is largest within the first three months of therapy and continues over 52 weeks of treatment (Breden et al. 2009). Furthermore, post-synaptic changes can also occur with different doses and durations of antipsychotic treatment. For example, the study showed that histamine H_1 receptor binding density in the ventromedial hypothalamic nucleus was significantly reduced after 1 week of Olz treatment, but not after 12 weeks of treatment (Han et al. 2008). Therefore, the symptoms and pathology can vary depending on the type, dose and duration of antipsychotic drugs used.

Although both typical and atypical antipsychotic drugs are associated with weight gain, atypical antipsychotic drugs induce more weight gain than typical antipsychotics (Gardner et al. 2005; Holt 2006; Mathews and Muzina 2007; Bobo et al. 2010; Muench and Hamer 2010). However, treatment with melperone, an atypical antipsychotic of the butyrophenone chemical class (derivative of haloperidol), showed similar low weight gain comparable to the weight gain induced by typical antipsychotics (Bobo et al. 2010). Among atypical antipsychotics, dibenzodiazepines and thienobenzodiazepines (e.g., Olz and clozapine) have the greatest tendency to induce weight gain, while a lower risk for weight gain has been reported with the use of phenylpiperazines, indoles, benzamides, and benzoxazoles. Among typical antipsychotic drugs, phenothiazines (e.g., thioridazine) showed a higher propensity to cause weight gain (Allison et al. 1999). Weight gain is also associated with some antidepressant agents, including monoamine oxidase inhibitors (e.g., phenelzine, isocarboxazid, and tranylcypromine) and tricyclic antidepressants (e.g., amitriptyline,
imipramine, and doxepin). Tricyclic antidepressants, with a structure similar to typical antipsychotic phenothiazines, induced weight gain in both short-term (< 6 months) and long-term (1 ≥ year) treatments (Deshmukh and Franco 2003).

The prevalence of diabetes in schizophrenic patients is almost 1.5 to 2 times greater than the prevalence reported in the general population (Mathews and Muzina 2007). Major risk factors for diabetes in schizophrenia are particularly high after atypical antipsychotic treatment (Holt 2006). The incidence of diabetes is nearly 10% greater in schizophrenic patients treated with atypical antipsychotic drugs than it is for those treated with typical antipsychotic drugs (Sernyak et al. 2002). Among developed atypical antipsychotic drugs, the dibenzodiazepine and thienobenzodiazepine classes (e.g., clozapine and Olz) have been repeatedly reported to induce the highest incidence of type II diabetes (Sussman 2003; Muench and Hamer 2010; Nielsen et al. 2010; Reynolds and Kirk 2010), whereas treatment with quetiapine, a member of the dibenzothiazepines, has a low incidence of diabetes mellitus type II and hyperglycemia (<1%) in schizophrenic patients (Bloomgarden 2005). In a study conducted among antipsychotic-naïve schizophrenia patients in Denmark, a lower risk of diabetes was reported in patients treated with ziprasidone and aripiprazole compared to schizophrenic sufferers given Olz or clozapine treatment (Nielsen et al. 2010). Olz and clozapine appear to have the highest tendency to disturb glucose metabolism compared to the other antipsychotic drugs available on the market (Llorente and Urrutia 2006).
In addition to a higher prevalence of diabetes induced by antipsychotic treatment, disturbances in lipid metabolism have also been observed in schizophrenia sufferers (McIntyre et al. 2001). It has been suggested that atypical antipsychotics, such as dibenzodiazepines and thienobenzodiazepines, are the most potent antipsychotic treatments that lead to the development of hyperlipidemia (Gardner et al. 2005; Holt 2006; Llorente and Urrutia 2006). Olz medication in particular showed the greatest increase of cholesterol, glycosylated hemoglobin and triglycerides levels in patients with less than 18 months of treatment (Lieberman et al. 2005). Antipsychotic agents, such as risperidone, ziprasidone, and aripiprazole, however, were the least likely to induce dyslipidemia and adverse effects on lipid levels (Llorente and Urrutia 2006). The effect of quetiapine on worsening the lipid profile was intermediate (Nasrallah 2008). Among the lipid fractions, triglyceride concentrations are the most affected by antipsychotic treatment (Holt 2006). The CATIE trial showed an elevating effect of Olz (+42.9 mg/d) and quetiapine (+19.2 mg/dl) but a reducing effect of risperidone (-2.6 mg/dl) and ziprasidone (-9.2 mg/dl) on the triglycerides levels measured in the blood of schizophrenic patients (Lieberman et al. 2005).

In addition to hyperglycemia and lipid metabolism disturbances, many clinical studies have reported an exacerbation of cardiovascular risk factors during antipsychotic treatment (Holt 2006; Mathews and Muzina 2007). Prolongation of the QT interval (measurement of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle) is strongly correlated with cardiac adverse effects (Gardner et al. 2005). QTc intervals exceeding 500 ms can result in sudden cardiac death, ventricular tachycardia and torsades development (Abdelmawla and
Mitchell 2006). Among typical antipsychotic drugs, thioridazine of the phenothiazine class has been observed to have the greatest elevating effect on the QTc interval (35.6 ms) (Glassman and Bigger 2001). Long-term treatment with phenothiazines causes severe effects on dilatation cardiomyopathy (Volkov 2009). Sertindole, ziprasidone, haloperidol, and clozapine administration have been reported to increase the QTc interval in a dose-dependent manner in patients treated for schizophrenia (Breden et al. 2009; Miceli et al. 2010). Quetiapine increased the QTc interval (6.8 ms) to a greater degree than Olz (14.5 ms). Aripiprazole has not been reported to cause QTc prolongation (Mardera et al. 2003). Cardiovascular effects are mostly associated with phenothiazine agents, whereas fewer effects are observed with indoles, dibenzodiazepines, thienobenzodiazepines, phenylpiperazines, indoles, benzamides, and benzoazoles. Overall, the cardiovascular effects of antipsychotics may be secondary to weight gain, hyperglycemia, and lipid metabolism disturbances. They may be caused by the direct effects of these drugs on QTc prolongation. This updated report on the chemical structures of these compounds shows that thienobenzodiazepine and dibenzodiazepine antipsychotics have a greater propensity for inducing obesity, hyperglycemia, and lipid dysfunction, while the cardiovascular effects are mostly associated with phenothiazine agents. Other side effects of antipsychotic drugs should also be taken into consideration. For instance, clozapine can induce serious blood dyscrasias, such as agranulocytosis. The co-administration of clozapine and some mood-stabilizing antiepileptic drugs, including carbamazepine and sulfonamide antibiotics, may contribute to the risk of agranulocytosis (Demler and Trigoboff 2011). Also, though less frequent, effects of
Olz on impaired agranulocytosis have also been reported (Tolosa-Vilella et al. 2002; Maloney and Linmarie 2010).

Potential mechanisms of weight gain and adverse metabolic effects will be briefly discussed below to provide a deeper understanding of the implications raised by the structural diversity of antipsychotic drugs. However, the current data are not sufficient to draw any conclusions concerning the relationship between the chemical structure and the metabolic side effects of antipsychotic drugs. A systematic structure activity relationship (SAR) study on thienobenzodiazepine and dibenzodiazepine agents would be a helpful step toward the discovery of novel antipsychotics with fewer obesogenic effects.

1.3 Potential Mechanisms of Antipsychotic-Induced Weight Gain and Discussion of SAR

Many reports have discussed the potential mechanisms of atypical antipsychotic drug-induced weight gain and the subsequent metabolic dysregulations (Mathews and Muzina 2007; Roerig et al. 2009; Coccurello and Moles 2010; Reynolds and Kirk 2010). Changes in hormonal peptide levels correlated with food intake (such as insulin and leptin) have been suggested to play a part in weight gain induced by atypical antipsychotic drug treatment (Roerig et al. 2009). Clozapine and Olz medications (Melkersson 2004; Bai et al. 2009) in particular significantly influence the regulation of plasmatic insulin and leptin, although the mechanism of action remains unclear. A large number of studies have reported a relevant role for the affinities of atypical antipsychotics for the $5\text{HT}_{2A}$, $5\text{HT}_{2C}$, $5\text{HT}_{6}$, $\alpha_{1A}$, and particularly
H₁ receptors in their obesogenic effects (Reynolds et al. 2002; Kroeze et al. 2003; Mathews and Muzina 2007; Reynolds and Kirk 2010). The blockade of the H₁ receptors has repeatedly been described as the most likely mechanism for atypical antipsychotic drug-induced weight gain (Masaki et al. 2004; Masaki and Yoshimatsu 2006). The blockade of the H₁ receptors is directly involved in the activation of hypothalamic adenosine monophosphate-activated protein kinase signalling (AMPK), which stimulates food intake and positive energy balance and reverses the anorexigenic effect of leptin (Kim et al. 2007). As reviewed briefly in this manuscript, Olz and clozapine showed the highest incidence of weight gain and metabolic disorders, while ziprasidone and aripiprazole (expressing a low affinity for the H₁ receptors) showed a lower risk of weight gain (Llorente and Urrutia 2006). Similarly, the blockade of the H₁ and 5HT₂C receptors and alterations to the regulation of body adiposity by modulating neurotransmitter systems at the hypothalamic level have been reported as the potential mechanisms for antidepressant induced weight gain. Due to their higher affinity for the blockade of the H₁ receptors, tertiary tricyclic antidepressant drugs, including amitriptyline, imipramine, and doxepin, are more likely to cause weight gain than secondary tricyclics (e.g., desipramine and nortriptyline) (Deshmukh and Franco 2003).

Thus, the affinity of the drug for H₁ receptors appears to play a key role in drug-induced weight gain. In fact, a large body of evidence has supported a strong link between the H₁ receptor binding affinity of tetracyclic dibenzodiazepines and thienobenzodiazepines and their weight gain effects in schizophrenic sufferers (Mercer 1997). Clozapine ($K_i$: 6 nM) and Olz ($K_i$: 7 nM), which have a higher
affinity for H₁ receptors, showed a greater propensity to induce weight gain and subsequent hyperlipidemia, hypertension, and hyperglycemia (Mercer et al. 1996; Kroeze et al. 2003). However, dibenzoxazepine compounds with lower H₁ antagonist affinity, such as antipsychotic loxapine and antidepressant amoxapine caused neither weight gain nor weight loss (Pijl and Meinders 1996; Recasens 2001; Apiquian et al. 2003). Quetiapine (Kᵢ: 11 nM) has been shown to have an intermediate effect on weight gain (Lieberman et al. 2005). The chemical structures and receptor binding profiles of clozapine, Olz, quetiapine, and loxapine are given in Table 1.1 (page 5).

In addition to their affinities for binding to different receptors, small changes in the chemical structures of tetracyclic atypical antipsychotic drugs can have an important impact on their ability to induce weight gain. Modifications in the chemical structures of these drugs might lead to a change in their receptor binding affinities for H₁ or 5HT₂C receptors, which could explain the reduction of weight gain and metabolic disturbances. Dibenzoxazepines (e.g., loxapine and amoxapine) and dibenzothiazepines (e.g., quetiapine), which contain central heterocyclic seven-membered rings substituted with oxygen and sulfur, are associated with little or no weight gain. However, dibenzodiazepines (e.g., clozapine) and thienobenzodiazepines (e.g., Olz), which contain a central nitrogen substituted ring, have the highest incident of weight gain. Thus, this report concludes that the heteroatom of the central seven-membered ring and the substituents on the aromatic fused rings may contribute to their receptor binding affinities for H₁ or 5HT₂C receptors. These changes may affect the emergence of weight gain and metabolic disturbances following the administration of these drugs.
However, this theory should be considered with caution because the obesogenic mechanism of the antipsychotics remains unclear. In the last decade, research has been focused on developing novel tetracyclic atypical antipsychotics with high efficacy and low metabolic side effects. Table 1.2 illustrates the major potential tetracyclic antipsychotics in their developmental stages, including a brief summary of their preclinical studies during the last decade. These tetracyclic compounds possess a basic piperazine ring containing a distal nitrogen, which is essential for the receptor interactions and the efficacy of antipsychotic drugs (Chakrabarti et al. 1982). 6-Fluoro-10-[3-(2-methoxyethyl)-4-methyl-piperazin-1-yl]-2-methyl 4H-3-thia-4,9-diaza-benzo[f]azulene (FMPD), 660864-42-6, 660865-03-2, and 221059-44-5 showed high potency for dopaminergic receptors. The methyl, isopropyl, and fluoro substituents at the thiophene and phenyl rings of these compounds, respectively, provide the beneficial effects from their interactions with dopamine receptors (Chakrabarti et al. 1980). In addition, the presence of the methoxyethyl (CH$_2$OCH$_3$) group appears to be favourable for increasing the dopaminergic activity of FMPD and its analogues. This chemical group may also reduce the interaction of these compounds with H$_1$ receptors, leading to a reduction in drug-induced weight gain.

Tetracyclic ST2472, which contains a pyrrolobenzothiazepine structure, showed reduced weight gain and fewer metabolic disturbances than Olz in a mouse study (Coccurello et al. 2008). Moreover, an N-methyl analogue of ST2472 (395073-74-2) showed promising dopaminergic activity in both in vitro and in vivo tests (Campiani et al. 2004). This study supports the hypothesis that these pyrrolobenzothiazepines may represent valuable antipsychotic candidates. Nevertheless, more studies on these
pyrrolobenzothiazepine derivatives are required to elucidate the potential role of their chemical structure in their neuroleptic activity and their metabolic side effects.

In summary, although the SAR of tetracyclic atypical antipsychotics and their neuroleptic effects has been discussed in the literature, the role of their chemical structures in inducing weight gain and metabolic side effects remains unclear. A more comprehensive SAR study on antipsychotic drugs is thus crucial for the discovery of an ideal antipsychotic agent. Novel dibenzodiazepine or thienobenzodiazepine analogues with reduced affinity for H₁ and 5HT₂c receptors and low metabolic disturbances would certainly be a valuable molecular target for new antipsychotic therapy.
Table 1.2: Preclinical studies on potential future antipsychotics

<table>
<thead>
<tr>
<th>Reference</th>
<th>Structure</th>
<th>Name/CAS</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Rasmussen et al. 2005)</td>
<td>FMPD</td>
<td>[Image]</td>
<td>FMPD is a potent antagonist of D₂ (Kᵢ = 6.3 nM), 5HT₂A (Kᵢ = 7.3 nM), and 5HT₆ (Kᵢ = 8.0 nM) receptors but a weak antagonist of H₁ (Kᵢ = 30 nM) and 5HT₃C (Kᵢ = 102 nM) receptors. Fos-induction assays predict that FMPD will have treatment-emergent EPS and weight gain similar to Olz, while behavioural studies and in vivo antagonist activity predict that FMPD will cause more treatment-emergent EPS and fewer weight gain effects than Olz. Clinical trials are required.</td>
</tr>
<tr>
<td>(Aicher et al. 2004)</td>
<td>660864-42-6</td>
<td>[Image]</td>
<td>Compared to Olz, Thieno [2,3-b][1,5]benzodiazepine, 7,8difluoro-2-methyl-4-[3-(2-methoxyethyl)-4-methyl-1-piperazinyl] showed similar and weaker binding affinity for D₂ (Kᵢ = 11 nM) and H₁ receptors (Kᵢ = 80 nM), respectively. The ex vivo binding affinity for H₁ receptors (ED₅₀ &gt; 30 mg/kg) and the DOPAC concentrations (ED₂₅₀ = 6.4 mg/kg) of this compound in the rat brain were comparable to the values obtained from clozapine (ED₅₀ &gt; 10 mg/kg and ED₂₅₀ = 45.6 mg/kg).</td>
</tr>
<tr>
<td>(Aicher et al. 2004)</td>
<td>660865-03-2</td>
<td>[Image]</td>
<td>Thiazolo[5,4-b][1,5]benzodiazepine, -2-(1-methylethyl)-10-[3-(2-methoxyethyl)-4-methyl-1-piperazinyl] showed a binding affinity close to Olz for D₂ receptors (Kᵢ = 9 nM) but lower affinity for H₁ receptors (Kᵢ = 91 nM) in the rat brain. The ex vivo binding affinity for H₁ receptors (ED₅₀ &gt; 30 mg/kg) and the DOPAC concentrations (ED₂₅₀ = 4 mg/kg) of this compound were comparable to the values reported for clozapine (ED₅₀ &gt; 10 mg/kg and ED₂₅₀ = 45.6 mg/kg).</td>
</tr>
</tbody>
</table>
Table 1.2 (Continued)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Structure</th>
<th>Name/CAS</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Coccurello et al. 2008)</td>
<td><img src="image1" alt="Structure" /></td>
<td>ST2472</td>
<td>In contrast to Olz, ST2472 did not induce weight gain, hyperglycemia, hyperinsulinemia, dyslipidemia, ketoacidosis, or visceral fat accumulation in mice treated for 50 days.</td>
</tr>
<tr>
<td>(Campiani et al. 2004)</td>
<td><img src="image2" alt="Structure" /></td>
<td>395073-74-2</td>
<td>Pyrrolo[2,1-b][1,3] benzothiazepine, 9-(4-methyl-1-piperazinyl) has a pharmacological profile greater than Olz and clozapine. In vitro binding affinities for D2 ($K_i = 17.2$ nM), 5HT2A ($K_i = 0.65$ nM), D1 ($K_i = 19.7$ nM), and D3 ($K_i = 8.3$ nM) receptors and a $pK_i 5HT_2A/D_2$ ratio of 1.2 suggests that this compound represents a potential new antipsychotic drug. This compound also antagonized the cognitive impairment induced by phencyclidine. It did not cause catalepsy in the conditioned avoidance response test in rats.</td>
</tr>
<tr>
<td>(Campiani et al. 2005)</td>
<td><img src="image3" alt="Structure" /></td>
<td>798573-34-9</td>
<td>5H-Pyrrolo[1,2-b][2]benzazepine, 11-(4-methyl-piperazinyl) showed an optimal $pK_i 5HT_2A/D_2$ ratio of 1.30 and in vivo atypical antipsychotic pharmacological properties. It was also active in the conditioned avoidance response test at 0.56 mg/kg, showing low cataleptic potential, which was superior to those of Olz and clozapine.</td>
</tr>
<tr>
<td>(Kohara et al. 2002)</td>
<td><img src="image4" alt="Structure" /></td>
<td>221059-44-5</td>
<td>Thieno[2,3-b][1,5]benzoxazepine, 2-methyl-4-(4-methyl-1-piperazinyl) showed high affinity for D2 receptors ($IC_{50} = 36$ nmol/L), and it antagonized the locomotor hyperactivity induced by apomorphine in vivo ($ED_{50} = 0.04$ mg/kg) in mice.</td>
</tr>
</tbody>
</table>
1.4 Conclusions and Future Prospects

As discussed above, the chemical structure of antipsychotic drugs plays an essential role in their receptor binding affinities in the brain and their therapeutic profiles and metabolic side effects in schizophrenia treatment. Various clinical trials have reported that, despite their therapeutic benefits, most of the prescribed atypical antipsychotics can cause excessive weight gain and metabolic disorders. Among the antipsychotic agents, thienobenzodiazepine and dibenzodiazepine compounds, particularly Olz and clozapine, are associated with the greatest weight gain and metabolic disturbances. However, their unique tetracyclic structure results in a low risk of EPS and TD. Most of the current research is focused on SAR studies of tetracyclic antipsychotics and their neuroleptic effects; however, the mechanisms underlying the contribution of thienobenzodiazepine and dibenzodiazepine antipsychotic structures on weight gain remains unclear. This report concludes that the presence of the piperazine ring, a distal nitrogen and a methoxyethyl group on the piperazine ring, a short alkyl group at position 2 of the thiophene ring, and a phenyl with a halogen atom (Cl, F), can enhance the antipsychotic activities of tetracyclic thienobenzodiazepine and dibenzodiazepine medications. Further, SAR studies on these drugs are necessary to identify the potential modification sites and to eventually remove the metabolic side effects while retaining their therapeutic actions. A novel thienobenzodiazepine or dibenzodiazepine analogue with lower affinity for H₁ or 5HT₂C receptors can be a valuable molecular target. Future antipsychotic drugs with reduced side effects will benefit schizophrenic patients and create a new era in the treatment of schizophrenia.
1.5 Aims of This Project

Given the great need for a new therapeutic agent for schizophrenia, this dissertation focused on the development of a novel thienobenzodiazepine antipsychotic that retains the clinical efficacy of Olz but has less treatment-emergent weight gain. As previously discussed, the clinical effectiveness of Olz has been reported to be linked with its favourable $5HT_{2A}/D_2$ receptor binding affinity ratios, whereas the blockade of the $H_1$ receptor is the most likely mechanism for Olz-induced weight gain.

The aims of this project were thus:

- To design and synthesize a series of novel Olz analogues that possess an ideal $5HT_{2A}/D_2$ receptor binding affinity ratio similar to that of Olz but a lower binding affinity to the $H_1$ receptors.

- To assess the SAR between the Olz derivatives synthesized in this project and their binding affinity to the $5HT_{2A}$, $D_2$, and $H_1$ receptors with the ultimate goal of identifying the potential modification sites of the Olz structure.

- To evaluate the *in vivo* effects of the Olz derivatives synthesized in this project on weight gain and adiposity in an animal model.

- To examine the effects of the newly synthesized compounds in this study on prevention of PCP induced hyperlocomotor activity relevant for the treatment of schizophrenia in a PCP treated rat model.
As previously discussed, the antagonism property of multiple receptors particularly H₁, 5HT₂C, α₁A, and mAch are involved in the antipsychotic-induced weight gain and metabolic disorders. A quantitative analysis study revealed that among the mentioned receptors, H₁ receptors predominantly contribute to the incidence of weight gain and morbidity rate of diabetes type 2 mellitus induced by antipsychotics (Matsui-Sakata 2005). Therefore, at this early stage of drug development, this study mainly focused on the modification of olanzapine structure to new analogues with lower affinity to the H₁ receptors only and not to other neurotransmitter receptors such as 5HT₂C.

In Chapter 2, the design and synthesis of novel Olz derivatives as well as their *in vitro* binding affinities for the 5HT₂A, D₂, and H₁ receptors will be discussed. Chapter 3 will cover the *in vivo* validation results of the major compounds synthesised in this project on reducing weight gain and adiposity. Chapter 4 will focus on the therapeutic effectiveness of the synthesized Olz analogues in a PCP treated animal model. Chapter 5 will describe the conclusions, and possible future directions of this project.
CHAPTER 2 SYNTHESIS AND SAR STUDY OF NOVEL POTENTIAL ANTIPSYCHOTICS

2.1 Background

A search for an effective Olz analogue commenced during my Masters of Science project (Jafari 2008). Initially, computer-aided drug design techniques were applied to identify the potential modification sites of the Olz structure aimed at modifying Olz from a H₁ antagonist to a H₁ agonist or to a new derivative with lower binding affinity for the H₁ receptors. A H₁ agonist pharmacophore was designed based on available published data. The generated pharmacophore demonstrated a series of hypothesized models represented as a three-dimensional set of structural elements (e.g., green, blue, and red spheres shown in Figure 2.1). As a result of mapping Olz into the selected H₁ agonist model, two modifications sites were suggested. It was proposed that increasing hydrophobicity and polarity at positions C-2 and C-3, respectively, (Figure 2.1) may give rise to the favourable H₁ receptors interactions.
The syntheses of thienobenzodiazepine compounds 1 (named OlzEt throughout this thesis) and 2, representing the replacement of the methyl group with an ethyl group and the substitution of a hydroxymethyl group at positions C-2 and C-3, respectively, were attempted. OlzEt 1 was successfully synthesized, however, the attempted synthetic routes did not give the desired product 2, but instead formamide compound 3 was serendipitously obtained (Figure 2.2).
The binding affinity of OlzEt 1 and the analogue 3 for the H₁ receptors was evaluated. These studies showed a reduced affinity of OlzEt 1 and compound 3 (IC₅₀: 978 nM and 37.0 nM, respectively) for the H₁ receptors in rat brain slices when compared to that of Olz (IC₅₀ 2.08 nM) (Jafari 2008). Due to the time constraints and lack of sufficient rat brain tissues, these results could not be replicated in my Master’s study.

The synthesis and pharmacological evaluations of an ideal Olz derivative were further developed in this PhD project. This chapter focuses on two main strands of SAR study, including synthesis and in vitro biological testing. Initially, an alternative synthetic approach to the preparation of compound 2 was investigated. Previously (Jafari 2008), it was thought that the formylation reaction of Olz using the Vilsmeier-Haack reaction conditions, followed by the reduction of the formed aldehyde 4, may possibly furnish the desired product 2 (Scheme 2.1). However, instead of producing the desired aldehyde 4, the formamide 3 was obtained. This was partly due to the presence of the N-10 proton, which may interfere with the formylation reaction of Olz. Therefore, it was suggested that Vilsmeier-Haack reaction of Olz required first protection of N-10 (Jafari 2008) (Scheme 2.2). In the present study, a suitable amino (N-10) protecting group for Olz was further explored. Table 2.1 summarises various procedures attempted for protection of the N-10 of Olz. Unfortunately, none of these attempts were successful at affording the desired target and time constraints prevented further investigation.
Scheme 2.1: Vilsmeier-Haack reaction of Olz (Jafari 2008)
Reagents: (ia) POCl$_3$, DMF; (ib) NaOH, H$_2$O; (ii) NaBH$_4$, EtOH

Scheme 2.2: Synthesis of compound 2
Reagents: (i) N-Protection; (ii) Vilsmeier formylation; (iii) Reduction reaction with NaBH$_4$, EtOH; (iv) N-Deprotection
Table 2.1 Various attempted conditions for N-10 protection of Olz

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagent</th>
<th>Base</th>
<th>Solvent</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BnBr</td>
<td>K₂CO₃</td>
<td>THF</td>
<td>85 – 90 °C</td>
</tr>
<tr>
<td>2</td>
<td>BnBr</td>
<td>KOH</td>
<td>THF</td>
<td>85 – 90 °C</td>
</tr>
<tr>
<td>3</td>
<td>BnBr</td>
<td>KOH</td>
<td>1,4-Dioxane</td>
<td>reflux</td>
</tr>
<tr>
<td>4</td>
<td>BnCl</td>
<td>KOH</td>
<td>THF</td>
<td>75 – 80 °C</td>
</tr>
<tr>
<td>5</td>
<td>BnCl</td>
<td>KOH</td>
<td>1,4-Dioxane</td>
<td>reflux</td>
</tr>
<tr>
<td>6</td>
<td>Boc</td>
<td>Na₂CO₃</td>
<td>THF : H₂O (1: 1)</td>
<td>rt</td>
</tr>
<tr>
<td>7</td>
<td>Boc</td>
<td>NaH</td>
<td>THF</td>
<td>0 °C to rt</td>
</tr>
<tr>
<td>8</td>
<td>SES-Cl</td>
<td>Et₃N</td>
<td>DMF</td>
<td>0 °C to rt</td>
</tr>
<tr>
<td>9</td>
<td>SES-Cl</td>
<td>Et₃N</td>
<td>DCM</td>
<td>0 °C to rt</td>
</tr>
<tr>
<td>10</td>
<td>Cbz-Cl</td>
<td>NaHCO₃</td>
<td>DCM : H₂O (1: 1)</td>
<td>0 °C to rt</td>
</tr>
</tbody>
</table>

With the failure of substitution of a hydroxymethyl group at position C-3 of Olz, attention was turned to the new synthetic targets for this project. A SciFinder Scholar database search revealed that Olz analogues modified at position C-4 (Figure 2.3) have not been reported in the primary or patent literature. Notwithstanding, we found the patent in which a large number of 1’,4’ diazapane analogous was introduced at their developmental stage (Seio Koji et al. 1999). These novel compounds possess a general structure of dibenzodiazepine with potential effects on treatments of both positive and negative symptoms of schizophrenia, dementia of Alzheimer type, and periodic psychosis. In particular, a series of compounds presenting the replacement of the N-methylpiperazine with a bicyclic diamine moiety, or/and the compounds which contain a central heterocyclic ring substituted with oxygen caught our attention. The similarity of these tetracyclic dibenzodiazepines with potential antipsychotic property and our proposed Olz analogues in Figure 2.3 prompted us to peruse the development of compounds 8-10. Although these series of Olz derivatives were not included in our rational drug design approach, their potential antipsychotic propensity was of interest from a medicinal chemistry point of view. Therefore, this dissertation focused on an investigation of a novel series of Olz analogues, as shown
in Figure 2.3. These analogues contain the variety of amine groups at position C-4 which introduced patentable novelty into the structures and/or the ethyl group at positions C-2, which appeared to be important for a reduced H1 receptor binding affinity. The new revised synthetic targets led to the successful formation of compounds 8a (named MeHomo), 8b (named OlzHomo), and 9b. In this chapter, the attempted synthetic routes, full characterization, and in vitro binding affinity of OlzEt 1, OlzHomo 8b, MeHomo 8a and compound 3 for the H1, 5HT2A, and D2 receptors are further discussed.

Figure 2.3: Chemical structure of Olz analogues modified at position C-4
2.2 Synthesis of Novel Olz Derivatives

The synthesis of the target compounds (Figure 2.3) followed the procedures previously reported for the preparation of Olz and OlzEt 1 (Shastri et al. 2006; Jafari 2008) with some modifications. Scheme 2.3 illustrates the four step synthesis of OlzEt 1 commencing with a Gewald reaction (step i) followed by aromatic ring condensation of thiophenecarbonitrile (13b) with o-fluoronitrobenzene (step ii), the intramolecular reductive cyclization of the thiophene-3-carbonitrile 14b in the presence of stannous chloride (step iii) and the condensation of the primary amidine hydrochloride with an N-methylpiperazine (step iv).

**Scheme 2.3**: Synthesis of compounds OlzEt 1, OlzHomo 8a, and MeHomo 8b

Reagents and conditions: (i) Et₃N, DMF, 18 °C, 20 h, 40% to 55%; (ii) 1-fluoro-2-nitrobenzene, NaH, THF, rt, 20 h, 60%; (iii) SnCl₂, EtOH, 80 °C, 1 h, 80%; (iva) N-methylhomopiperazine (5 equiv), no solvent, microwave heating, 120 °C, 3 h, 55%; (ivb) N-methylhomopiperazine (5 equiv), no solvent, microwave heating, 80 °C, 4 h, 65%; (ivc) N-methylpiperazine (10 equiv), N-methylpiperazine hydrochloride (10 equiv), DMSO, 110-120 °C, 20 h, 56%
First, bulk production of OlzEt 1 was pursued following the optimized reaction conditions described in my Master’s thesis (Scheme 2.3). As previously explained, OlzEt 1 was obtained using the key intermediate amidine hydrochloride 15b and an excess (10 equiv) amount of N-methylpiperazine and N-methylpiperazine hydrochloride (1:1). The reaction mixture was heated at 110 - 120 °C in DMSO for 20 h. After workup and column chromatography OlzEt 1 was obtained in 50% yield as a yellow powder (Jafari 2008). The powder was then crystallized from acetonitrile.

Interestingly, when amidine 15b was reacted with N-methylhomopiperazine and N-methylhomopiperazine hydrochloride under the same reaction conditions, OlzHomo 8b was obtained in low yield (<20%) and purity due to the formation of many by-products, including the dimer of OlzHomo (16) (Figure 2.4). LRMS (Cl+) analysis of the crude product showed a molecular ion [MH+] at m/z of 566.2343 that was consistent with the molecular formula of 16. Optimization of this step was therefore necessary for the more efficient production of OlzHomo 8b.

Figure 2.4: Chemical structure of dimer 16
After many explorations, it was found that the reaction time is the crucial factor of the process. Thus, to accelerate the reaction, microwave irradiation instead of conventional oil bath heating of the reaction mixture was applied. This modification led to a reduction of the reaction time from 20 h to 3 h. Due to the shorter reaction time, the formation of the undesirable by-products was avoided and the yield of OlzHomo 8b consequently increased. Furthermore, it appeared that the addition of N-methylhomopiperazine hydrochloride and a solvent was not necessary for the successful outcome of this reaction. When finally performed under solvent free conditions and microwave heating at 120 ºC for 3 h, OlzHomo 8b was given in an improved yield of 51% (Scheme 2.3). The workup procedure to obtain crude OlzHomo 8b was the same as that developed for OlzEt 1 (Jafari 2008) but with some modifications. As previously described, after cooling to room temperature the reaction mixture was diluted with dichloromethane and washed with hydrochloric acid solution. The hydrochloride phase was basified to pH 7.5 – 8.5, using sodium hydroxide solution. Instead of using hydrochloric acid and sodium hydroxide solutions, this time the reaction mixture was basified under milder conditions using saturated sodium bicarbonate solution that dissolved all the excess N-methylhomopiperazine. Purification of the crude product was quite difficult and time consuming. Several methodologies for the isolation of the desired product, OlzHomo 8b, were applied, including PTLC (methanol/triethylamine/dichrolomethane), crystallization from acetonitrile, ether, acetone, and acidification of the reaction mixture to afford the hydrochloride salt of OlzHomo 8b. However, these attempted methods did not result in the successful isolation of sufficiently pure product for further characterization. The final
purification of OlzHomo 8b was achieved after successive purification by column chromatography (methanol/triethylamine/dichrolo methane).

Structural confirmation of OlzHomo 8b was provided by NMR spectroscopic and mass spectrometric analysis. In the $^1$H-NMR spectrum of OlzHomo 8b an additional methylene signal at 1.97 ppm (2H, quin) when compared to the spectrum of OlzEt 1, indicated the presence of the $N$-methylhomopiperazine moiety in the obtained product. Analysis of the $^{13}$C-NMR and DEPT spectra also supported the structure of OlzHomo 8b where the additional methylene group (CH$_2$) signal appeared at 28.1 ppm. The HRMS (CI$^+$) of OlzHomo 8b showed a [MH$^+$] ion at $m/z$ 341.1800, which was consistent with the desired molecular formula.

The above procedure was applied to the synthesis of MeHomo 8a. A mixture of commercially available amidine 15a and $N$-methylhomopiperazine (5 equiv) was heated at 120 °C under microwave conditions (Scheme 2.3). After 3 h, analytical TLC (20% : 0.1% methanol : triethylamine in dichloromethane) analysis showed that the starting material (R$_f$ : 0) was mainly consumed and several product spots had formed. In the next attempt, milder reaction conditions were applied. This time the reaction mixture was heated at 85 °C under microwave irradiation for 4 h. These new conditions led to the formation of only one major product by TLC analysis. The previously modified workup procedure was used on the reaction mixture. The crude product MeHomo 8a was then subjected to purification by column chromatography. Isolation of MeHomo 8a in 65% yeild was achieved using a gradient elution of 0% - 10% : 2% acetonitrile: triethylamine in ethyl acetate as eluent.
The product was fully characterized by $^1$H-NMR and $^{13}$C-NMR spectroscopic analysis. A comparison of the $^1$H-NMR spectrum of MeHomo 8a and OlzHomo 8b revealed a characteristic signal for the H$_2$C-6' at 1.97 ppm (quin, 2H) which was ascribed to the N-methylhomopiperazine group. Additionally, the presence of a single signal corresponding to the methyl group, at 2.30 (3H) ppm, supported the structure of MeHomo 8a. A resonance at 28.3 ppm and 15.8 ppm in the $^{13}$CNMR spectrum also confirmed the presence of the extra methylene of N-methylhomopiperazine and the methyl group, respectively, in the product. LRMS (CI$^+$) analysis showed a molecular ion [MH$^+$] at m/z of 327.1643, which was consistent with the molecular formula of MeHomo 8a.

Next, the synthesis of compound 9b was attempted following the procedure developed for the preparation of OlzHomo 8b (Scheme 2.4). The mixture of amidine 15b and N-methyloctahydropyrrrolo[3,4-c]pyrrole (5 equiv) was heated to 120 ºC for 3 h in a microwave reactor. After workup and column chromatography (methanol, triethylamine, dichloromethane), the desired 9b was obtained in 65% yield with 80% purity. The LRMS (CI$^+$) of this product gave an [MH$^+$] signal at m/z 352, which was consistent with the molecular weight of 9b. The $^1$H-NMR spectrum of 9b indicated the presence of four characteristic 2H signals at 2.40, 2.75, 2.88, and 3.66 ppm and two multiple 1H signals at 3.42 and 3.44 ppm, which were ascribed to the bicyclic diamine moiety. Unfortunately, the amount of this product obtained was not sufficient to allow further purification and complete spectroscopic characterization.
Due to time constraints, work on the synthetic plan to the remaining target compounds (compounds 10 and 11 - Figure 2.3, page 34) was not pursued further. In general, three novel derivatives of Olz (OlzHomo 8a, MeHomo 8b, and 9b) and the previously reported analogue OlzEt 1 (Chakrabarti et al. 1980; Jafari 2008) were successfully synthesized and characterized. These analogues presented two alterations to the structure of Olz, (i) the substitution of an ethyl group at the C-2 position of the thiophene ring (OlzEt 1, OlzHomo 8b and 9b) and (ii) the replacement of the N-methylpiperazine with an N-methylhomopiperazine ring (OlzHomo 8b and MeHomo 8a) or an N-methyloctahydropyrrolo[3,4-c]pyrrole (9b). Next, Olz, OlzEt 1, OlzHomo 8b, MeHomo 8a, and compound 3 were subjected to a standard in vitro radioligand assay in order to examine their binding affinities for serotonergic, dopaminergic, and histaminergic receptors, which is relevant to their therapeutic and metabolic side effects.
2. 3 General Procedures

2. 3.1 Experimental Procedures, Reagents and Solvents

All reactions were carried out utilizing standard laboratory equipment and standard laboratory glassware. All solvents and reagents used were purchased from Sigma-Aldrich Chemical Co. Inc. and were used as received. Anhydrous THF were distilled over sodium wire, in the presence of benzophenone as an indicator. Anhydrous DMF and DMSO were purchased from Sigma-Aldrich Chemical Co. Inc., in a Sure-seal® bottle and stored under an inert atmosphere. Organic solvent extracts were dried using anhydrous magnesium sulfate. Solvents were removed under reduced pressure at 45 °C with a Büchi rotary evaporator. All solvents were either AR or HPLC grade and were used as received.

2. 3.2 Microwave Reactions

Microwave reactions were conducted in a CEM focused Microwave TM synthesis system running on Synergy software. The reaction temperature was monitored via an internal reaction temperature control. Sealed Teflon reaction vessels were used and the reaction mixtures were magnetically stirred.

2. 3.3 Chromatography

Thin-layer chromatography (TLC) was used to monitor the progress of reaction using aluminum-backed sheets of Merck Silica Gel 60 F254 containing a fluorescent indicator and a UV lamp (254 nm), or via staining of the plate with a ninhydrine or anisaldehyde stain. Preparative TLC was carried out in a manner similar to the
analytical methodology utilizing 20 × 20 cm sheets of the same silica gel. Column chromatography was performed using Merck Kiesel Gel 60 F254 (230-400 mesh) silica gel under medium pressure. Solvent proportions are quoted as volume ratios.

2.4 Characterization and Instrumentation

2.4.1 $^1$H- and $^{13}$C-Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy was performed on a Varian Inova 500 MHz spectrometer, where proton NMR ($^1$H-NMR) spectra and carbon NMR ($^{13}$C-NMR) spectra were obtained at 500 and 125 MHz, respectively. All spectra were recorded in deuterochloroform (CDCl$_3$) or deuteromethanol (CD$_3$OD) with 0.5% tetramethylsilane (TMS), obtained from Cambridge Isotope Laboratories Inc. TMS (0.00 ppm) was utilized as the internal standard. Chemical shifts (δ) were measured in parts per million (ppm) and referenced against (TMS), and coupling constants (J) were measured in Hertz (Hz). NMR assignments were made using standard gradient correlation spectroscopy (gCOSY), gradient heteronuclear single quantum correlation (gHSQC), gradient heteronuclear multiple bond correlation (gHMBC) and distortionless enhancement by polarisation transfer (DEPT) spectroscopy. Multiplicities are denoted as singlet (s), doublet (d), triplet (t), double of doublets (dd), double of triplet (dt), quartet (q), and quintet (quin). The arrangement of $^1$H-NMR spectral data is listed as chemical shift, followed in brackets by multiplicity, integration, coupling constant(s) and attributed assignment.
2. 4. 2 Infrared Spectrometry (IR)

Infrared spectra were obtained as neat samples on Smart Omni-Sampler Avator ESP Nicolet-Brand.

2. 4. 3 Mass Spectrometry (MS)

Chemical Ionisation (CI⁺) low-resolution mass spectrometry was carried out on a Shimadzu QP-5000 MAT-44 quadrupole spectrometer using the direct insertion technique. The mass to charge \((m/z)\) values of the principal ion peaks are stated with their relative intensities in parentheses. High-resolution mass spectrometry (HRMS \(\text{EI}^+\) and \(\text{CI}^+\)) was performed on a Fisons/VG Autospec-TOF mass spectrometer. The accuracy of the measured HRMS relative to the required molecular weight is given in ppm.

2. 4. 4 Melting Points

Melting points were determined on a Reichert melting point apparatus. Temperatures are expressed in degrees Celsius (°C) and are uncorrected.
2.5 Synthesis of (2-ethyl-4-(4'-methylpiperazin-1'-yl)-10Hbenzo[b]thieno[2,3-e][1,4]diazepine (OlzEt 1)

A solution of amidine hydrochloride 15b (1.62 mmol, 455 mg), methyl piperazine (10 equiv, 16.2 mmol, 3300 mg) and methyl piperazine hydrochloride (16.2 mmol, 3400 mg) in DMSO (15 mL) was heated at 110 °C for 20 h. After cooling to room temperature, the reaction mixture was diluted with dichloromethane and washed twice with 1 M aqueous HCl solution. The dark brown coloured hydrochloride was separated and basified to pH 7.5 – 8.5, using 1 M aqueous NaOH solution. The attained solution was extracted with dichloromethane and washed twice with brine, then dried over MgSO₄ and concentrated under reduced pressure to provide a brown oil. The crude product was purified by column chromatography using 6% : 0.1% methanol : triethylamine in dichloromethane and then crystallized from acetonitrile to afford the desired product (265 mg) as bright yellow crystals in 50% yield; m.p. 190-193 °C.

¹H NMR (500 MHz, CDCl₃) δ [ppm]: 1.21 (t, 3H, J = 7.5 Hz, H₂C-2’), 1.97 (quin, 2H, J = 5.7 Hz, H₂C-6’), 2.39 (s, 3H, CH₃ at N-4’), 2.64 (m, 4H, H₂C-1’& H₂C-5’), 2.71 (t, 2H, J = 4.5 Hz, H₂C-3’), 3.68 (t, 2H, J = 5.7 Hz, H₂C-7’), 3.74 (t, 2H, J = 4.5 Hz, H₂C-2’), 5.02 (s, 1H, HN-10), 6.35 (s, 1H, HC-3), 6.60 (dd, 1H, J₁ = 1.3 Hz, J₂ =
7.8 Hz, HC-9), 6.82 (dt, 1H, $J_1 = 1.8$ Hz, $J_2 = 7.8$ Hz, HC-8), 6.94 (dt, 1H, $J_1 = 1.5$ Hz, $J_2 = 7.5$ Hz, HC-7), 7.00 (dd, 1H, $J_1 = 1.8$ Hz, $J_2 = 7.8$ Hz, HC-6).

$^{13}$C NMR (125 MHz, CDCl$_3$) δ [ppm]: 15.6 (CH$_3$, C-2''), 23.6 (CH$_2$, C-1''), 28.1 (CH$_2$, C-6'), 46.8 (CH$_3$, N-4'), 48.4 (CH$_2$, C-7'), 48.5 (CH$_2$, C-2'), 58.0 (CH$_2$, C-5'), 58.6 (CH$_2$, C-3'), 118.9 (CH, C-9), 120.0 (C$_q$, C-3a), 121.2 (CH, C-3), 122.8 (CH, C-8), 124.6 (CH, C-7), 127.9 (CH, C-6), 136.8 (C$_q$, C-2), 141.7 (C$_q$, C-5a), 142.3 (C$_q$, C-9a), 150.9 (C$_q$, C-10a), 157.1 (C$_q$, C-4).

IR (neat) $\nu_{\text{max}}$ [cm$^{-1}$]: 3211(w), 2961(w), 2848(w), 2805(w), 1601(m), 1590(vs), 1561(w), 1510(w), 1469(m), 1449(m), 1399(s), 1369(w), 1350(w), 1309(w), 1297(w), 1284(w), 1272(w), 1262(w), 1247(w), 1223(m), 1202(w), 1177(w), 1157(w), 1143(m), 1133(w), 1105(w), 1081(w), 1050(w), 1037(w), 1005(m), 966(m), 931(w), 890(w), 858(w), 810(w), 785(w), 751(s).

HRMS (ESI) calcd for C$_{18}$H$_{23}$N$_4$S [MH$^+$] 327.1643, found 327.1642.

$R_f$: 0.20 (10% : 0.1% / MeOH : NEt$_3$ in CH$_2$Cl$_2$)
2.6 Synthesis of (2-ethyl-4-(4′-methyl-1′,4′-diazepan-1′-yl)-10H-benzo[b]thieno[2,3-e][1,4]diazepine (OlzHomo 8b)

A mixture of amidine hydrochloride 15b (0.34 mmol, 96 mg) and N-methylhomopiperazine (5 equiv, 1.71 mmol, 195 mg) was stirred at 120 °C and 100 Watt for 3 h under microwave heating in a sealed reactor tube. After cooling to room temperature, the reaction mixture was diluted with dichloromethane (3 mL) and washed twice with saturated sodium bicarbonate solution. The organic phase was washed with brine, then dried over MgSO₄ and concentrated under reduced pressure to provide a brown oil. The crude product was purified by column chromatography utilizing a gradient elution of 1% - 2% : 0.1% methanol : triethylamine in dichloromethane to give the desired product as a brown powder (59 mg) in 51% yield.

¹H NMR (500 MHz, CDCl₃) δ [ppm]: 1.21 (t, 3H, J = 7.5 Hz, H₂C-2′), 1.97 (quin, 2H, J = 5.7 Hz, H₂C-6′), 2.39 (s, 3H, CH₃ at N-4′), 2.64 (m, 4H, H₂C-1′& H₂C-5′), 2.71 (t, 2H, J = 4.5 Hz, H₂C-3′), 3.68 (t, 2H, J = 5.7 Hz, H₂C-7′), 3.74 (t, 2H, J = 4.5 Hz, H₂C-2′), 5.02 (s, 1H, HN-10), 6.35 (s, 1H, HC-3), 6.60 (dd, 1H, J₁ = 1.3 Hz, J₂ = 7.8 Hz, HC-9), 6.82 (dt, 1H, J₁ = 1.8 Hz, J₂ = 7.8 Hz, HC-8), 6.94 (dt, 1H, J₁ = 1.5 Hz, J₂ = 7.5 Hz, HC-7), 7.00 (dd, 1H, J₁ = 1.8 Hz, J₂ = 7.8 Hz, HC-6).
\textbf{Chapter 2}

\textit{Synthesis and SAR Study}

\textbf{\(^{13}\)C NMR} (125 MHz, CDCl\(_3\)) \(\delta\ [ppm]\): 15.6 (CH\(_3\), C-2\('')\), 23.6 (CH\(_2\), C-1\('')\), 8.1 (CH\(_2\), C-6\('')\), 46.8 (CH\(_3\), N-4\('')\), 48.4 (CH\(_2\), C-7\('')\), 48.5 (CH\(_2\), C-2\('')\), 58.0 (CH\(_2\), C-5\('')\), 58.6 (CH\(_2\), C-3\('')\), 118.9 (CH, C-9), 120.0 (C\(_q\), C-3a), 121.2 (CH, C-3), 122.8 (CH, C-8), 124.6 (CH, C-7), 127.9 (CH, C-6), 136.8 (C\(_q\), C-2), 141.7 (C\(_q\), C-5a), 142.3 (C\(_q\), C-9a), 150.9 (C\(_q\), C-10a), 157.1 (C\(_q\), C-4).

\textbf{IR} (neat) \(\nu_{\text{max}}\ [cm^{-1}]\): 2967(w), 2930(w), 2916(w), 2850(w), 1584(vs), 1558(s), 1514(w), 1464(s), 1410(s), 1362(m), 1317(w), 1287(w), 1240(m), 1212(m), 1168(m), 1128(m), 1086(w), 1038(m), 1017(w), 963(w), 912(m), 864(w), 856(w), 837(w), 822(w), 810(w), 804(w), 755(m), 737(m), 722(m), 711(m).

\textbf{HRMS (ESI)} calcd for C\(_{19}\)H\(_{25}\)N\(_4\)S [M\(^+\)] 341.1800, found 341.1802.

\(R_f\): 0.22 (20\% : 0.1\% / MeOH : NEt\(_3\) in CH\(_2\)Cl\(_2\))
2.7 Synthesis of (2-methyl-4-(4′-methyl-1′,4′-diazepan-1′-yl) 10Hbenzo[b]thieno [2,3-e] [1,4]diazepine (MeHomo 8a)

A mixture of amidine hydrochloride 15a (1 mmol, 260 mg) and N-methylhomopiperazine (5 equiv, 5 mmol, and 534 mg) was stirred at 80 °C and 100 Watt for 4 h under microwave heating in a sealed reactor tube. After cooling to room temperature the reaction mixture was diluted with dichloromethane (5 mL) and washed twice with saturated sodium bicarbonate solution. The organic phase was washed with brine, then dried over MgSO₄ and concentrated under reduced pressure to provide a brown oil. The crude product was purified by column chromatography utilizing a gradient elution of 0% - 10% : 2% acetonitrile : triethylamine in ethyl acetate to give the desired product as a dark red powder (212 mg) in 65% yield.

1H NMR (500 MHz, CDCl₃) δ [ppm]: 1.97 (quin, 2H, J = 5.5 Hz, H₂C-6′), 2.30 (s, 3H, H₃C-1″), 2.39 (s, 3H, CH₃ at N-4′), 2.65 (t, 2H, J = 5.5 Hz, H₂C-5′), 2.71 (t, 2H, J = 4.5 Hz, H₂C-3′), 3.68 (t, 2H, J = 5.5 Hz, H₂C-7′), 3.74 (s, 2H, H₂C-2′) 4.96 (s, 1H, HN-10), 6.33 (s, 1H, HC-3), 6.61 (d, 1H, J = 7.5 Hz, HC-9), 6.82 (t, 1H, J = 7.5 Hz, HC-8), 6.95 (t, 1H, J = 7.5 Hz, HC-7), 7.00 (d, 1H, J = 7.5 Hz, HC-6).
$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ [ppm]: 15.8 (CH$_3$, C-1”), 28.3 (CH$_2$, C-6’), 47.0 (CH$_3$, N-4”), 48.6 (CH$_2$, C-7”), 48.7 (CH$_2$, C-2”), 58.2 (CH$_2$, C-5’), 58.9 (CH$_2$, C-3’), 119.0 (CH, C-9), 120.5 (C$_q$, C-3a), 123.0 (CH, C-8), 123.2 (CH, C-3), 124.9 (CH, C-7), 128.1 (CH, C-6), 129.5 (C$_q$, C-2), 141.9 (C$_q$, C-5a), 142.4 (C$_q$, C-9a), 151.2 (C$_q$, C-10a), 157.1 (C$_q$, C-4).

IR (neat) $\nu_{max}$ [cm$^{-1}$]: 2940(w), 2850(w), 2803(w), 1583(vs), 1557(s), 1513(w), 1464(s), 1410(s), 1357(m), 1317(w), 1288(m), 1247(m), 1196(m), 1169(m), 1123(m), 1085(w), 1038(w), 1017(w), 959(w), 910(m), 860(w), 848(w), 834(w), 830(w), 814(w), 753(vs), 740(s), 730(m), 717(w), 711(w), 702(w).

HRMS (ESI) calcd for C$_{18}$H$_{23}$N$_4$S [MH$^+$] 327.1643, found 327.1642.

$R_f$: 0.38 (2% NEt$_3$ in EtOAc)
2.8 Characterization of 2-methyl-4-(4-methylpiperazin-1-yl)-10H-benzo[b]thieno[2,3-e][1,4]diazepine-10carbaldehyde (3)

![Chemical Structure](image)

**^1H NMR** (500 MHz, CDCl$_3$) $\delta$ [ppm]: 2.33 (s, 3H, CH$_3$ at N-4'), 2.43 (s, 3H, H$_3$C-1''), 2.51 (br, 4H, H$_2$C-3' & H$_2$C-5'), 3.60 (br, 4H, H$_2$C-2' & H$_2$C-6'), 6.50 (s, 1H, HC-3), 7.00 (d, 1H, $J = 8.0$ Hz, HC-6 or HC-9), 7.06 (t, 1H, $J = 8.0$ Hz, HC-7 or HC-8), 7.19 (d, 1H, $J = 8.0$ Hz, HC-9 or HC-6), 7.26 (t, 1H, $J = 8.0$ Hz, HC-8 or HC-7), 8.44 (s, 1H, H-CO).

**^13C NMR** (125 MHz, CDCl$_3$) $\delta$ [ppm]: 15.9 (CH$_3$, C-1''), 46.2 (CH$_3$, N-4'), 46.8 (2 x CH$_2$, C-2' & C-6'), 55.0 (2 x CH$_2$, C-3' & C-5'), 121.8 (CH, C-3), 124.0 (CH, C-7 or C-8), 124.8 (CH, C-6 or C-9), 125.5 (C$_q$, C-3a), 128.2 (CH, C-9 or C-6), 128.8 (CH, C-8 or C-7), 133.6 (C$_q$, C-5a or C-9a), 138.9 (C$_q$, C-2), 140.5 (C$_q$, C-10a), 144.4 (C$_q$, C-9a or C-5a), 155.2 (C$_q$, C-4), 162.3 (CHO).

**IR (neat)** $\nu_{\text{max}}$ [cm$^{-1}$]: 2846(w), 2804(w), 1683(vs), 1572(s), 1552(s), 1503(w), 1480(m), 1463(m), 1437(w), 1397(w), 1360(s), 1285(m), 1260(m), 1234(m), 1181(m), 1159(m), 1134(m), 1126(m), 1095(w), 1072(w), 1049(w), 1004(m), 971(m), 937(w), 926(w), 865(w), 837(m), 822(w), 778(w), 766(s), 746(w), 735(m).

2.9 In Vitro Receptor Binding Profiles and SAR Study of Potential Antipsychotics

2.9.1 Method

Male Sprague Dawley rats (200-250g) were obtained from the Animal Resource Centre (Perth, WA, Australia). All animals were sacrificed using carbon dioxide asphyxiation. Brains were immediately removed after death and dissected into prefrontal cortex (for 5HT₂A receptor), striatum (for D₂ receptor), and hypothalamus (for H₁ receptor). Brain structures were stored at -80 ºC until assay. Radioligand binding assays were run under the conditions described previously with some modifications (Durand et al. 2000; Campiani et al. 2004). Tissues were homogenized in about 6 volumes of ice-cold buffer Tris.HCl, 50mM, pH 7.7 (for D₂ and 5HT₂A receptors) or Na⁺/K⁺ phosphate 50mM, pH 7.5 (for H₁ receptors), and the resultant homogenate was then centrifuged (27000g for 15 min at 4 ºC). Each pellet was re-suspended in the same volume of fresh buffer and centrifuged again. The final tissue pellets were resuspended just before the binding assay in the incubation buffer (50 mM Tris.HCl, 5 mM MgSO₄, 0.5 mM EDTA and 0.02% ascorbic acid, pH 7.7 for D₂ receptors; 50 mM Tris.HCl, pH 7.7 for 5HT₂A receptors and Na⁺/K⁺ phosphate 50mM, pH 7.5 for H₁ receptors). [³H]-Spiperone (specific activity, 15 Ci/mmol, 1mCi/ml) binding to D₂ receptor was assayed in the final incubation volume of 500 µL, containing 0.470 µL protein suspension (1.2 mg/mL), 20 µL of radioligand
solution (2 nM) and 10 µL of displacing agent or buffer. [³H]-Ketanserine (specific activity, 67 Ci/mmol, 1mCi/ml) binding to 5HT₂A receptor was assayed in the final incubation volume of 500 µL, containing 0.470 µL protein suspension (0.25 mg/mL), 20 µL of radioligand solution (10 nM) and 10 µL of displacing agent or buffer. [³H]-Pyrilamine (specific activity, 37 Ci/mmol, 1mCi/ml) binding to H₁ receptor was assayed in the final incubation volume of 500 µL, containing 0.470 µL protein suspension (0.3 mg/mL), 20 µL of radioligand solution (2 nM) and 10 µL of displacing agent or buffer. Incubation (40 min at 37 °C for D₂ receptors, 15 min at 37 °C for 5HT₂A receptors, and 30 min at 25 °C for H₁ receptors) were stopped by the addition of 3 mL ice-cold buffer (Tris.HCl, 50mM, pH 7.7 for D₂ and 5HT₂A receptors and Na⁺/K⁺ phosphate 50mM for H₁ receptors) to the test tubes followed by rapid filtration on filter (GF/B Whatman). Filters were then washed with 6 mL of buffer (3 × 2 times) and transferred into scintillation vials. The radioactivity on the filters was measured in 4.5 mL of Ultima gold scintillating liquid (Perkin Elmer) in beta liquid scintillation analyser (Perkin Elmer, Tri-Crab 2800 TR) with a counting efficiency of 47%. IC₅₀ values for Olz (Bosche Scientific) and compounds 3, OlzEt 1, OlzHomo 8b, and MeHomo 8a were determined from competition curves and converted to Kᵢ values using the Cheng-Prusoff equation (Cheng and Prusoff 1973). Protein concentrations were determined by the Bradford method (Bradford 1976).
2.9.2 Results

The receptor binding affinities of compound 3, OlzEt 1, OlzHomo 8b and MeHomo 8a for D$_2$, 5HT$_{2A}$, and H$_1$ receptors were examined and replicated in 4-6 independent experiments. The affinities of compound OlzEt 1 for the D$_2$ and 5HT$_{2A}$ receptors were similar to the values found for Olz ($P = 0.561$ and $P = 0.959$, respectively) (Table 2.2). However, compound OlzHomo 8b showed less binding affinities than Olz for D$_2$ and 5HT$_{2A}$ receptors ($P = 0.002$ and $P = 0.030$, respectively). The pK$_i$ ratios 5HT$_{2A}$/D$_2$ of OlzEt 1 and OlzHomo 8b were similar to the value of Olz ($P = 0.137$ and $P = 0.457$, respectively). This value ratio is consistent with the previously reported one for Olz in the literature (Campiani et al. 2004; Campiani et al. 2005). MeHomo 8a displayed a significant reduction in binding to the D$_2$ and 5HT$_{2A}$ receptors ($P = 0.001$ for both). Similarly, the results of compound 3 indicated a loss of affinity for the D$_2$ receptor and a reduced 5HT$_{2A}$ receptor binding affinity ($P < 0.001$ and $P < 0.05$, respectively) compared to the Olz values. The binding affinity of all tested compounds for H$_1$ receptors was significantly reduced with the following order: Olz > OlzEt 1 > compound 3 > OlzHomo 8b > MeHomo 8a (Table 2.2).
### Table 2.2: Binding affinities of Olz, compounds 3, OlzEt 1, OlzHomo 8b, and MeHomo 8a for 5HT$_{2A}$, D$_2$, and H$_1$ receptors in rats

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_i$ ± SD $^a$ (nM)</th>
<th>pK$_i$ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D$_2$</td>
<td>5HT$_{2A}$</td>
</tr>
<tr>
<td>Olz</td>
<td>67.72 ± 9.21</td>
<td>4.22 ± 0.77</td>
</tr>
<tr>
<td>OlzEt 1</td>
<td>54.51 ± 12.12</td>
<td>3.70 ± 0.74</td>
</tr>
<tr>
<td>OlzHomo 8b</td>
<td>791.08 ± 83.19***</td>
<td>81.20 ± 2.43***</td>
</tr>
<tr>
<td>MeHomo 8a</td>
<td>3939.90 ± 437.35***</td>
<td>787.00 ± 29.81***</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 10000</td>
<td>476.00 ± 65.05***</td>
</tr>
</tbody>
</table>

$^a$ Values are the mean ± SD of four to six independent experiments and represent the concentration giving half maximal inhibition of [$^3$H]-Spiperone (D$_2$), [$^3$H]-Ketanserine (5HT$_{2A}$), and [$^3$H]-Pyrilamine (H$_1$) binding to rat tissue homogenate. *$P < 0.05$, ***$P < 0.001$

### 2.9.3 Discussion

Compound OlzEt 1 with an ethyl substituent at position 2 of the thiophene ring conferred a lower affinity for the H$_1$ receptors but similar affinities for the D$_2$ and 5HT$_{2A}$ receptors when compared to Olz. This may suggest the potential contribution of a C-2 ethyl substituent to the potency of binding to the histaminergic receptors but not to the serotonergic and dopaminergic receptors. On the other hand, the replacement of the N-methylpiperazine ring of OlzEt 1 with an N-methylhomopiperazine ring (OlzHomo 8b) led to a reduction of the affinities for dopamine and serotonin receptors. This decrease of receptor affinities is probably caused by an unfavorable steric interaction of the N-methylhomopiperazine ring with amino acid residues in the binding pocket of the D$_2$ and 5HT$_{2A}$ receptors. Lower affinity of OlzHomo 8b for the H$_1$ receptors compared to Olz could be due to both the ethyl group and the N-methylhomopiperazine ring substitution. Although
OlzHomo 8b has lower affinity than Olz or OlzEt 1 for the D2 and 5HT2A receptors, it possessed a favorable pK\textsubscript{i} ratio 5HT\textsubscript{2A}/D\textsubscript{2} similar to the measured value of Olz (ratio value = 1.17). Moreover, the obtained binding affinity of OlzHomo 8b (Table 2.2, page 54) for the D\textsubscript{2} receptor is still comparable to that of the value of remoxipride (K\textsubscript{i}: 800 nM) and quetiapine (K\textsubscript{i}: 680 nM) (Seeman 2010), atypical antipsychotics with high therapeutic efficacy in the clinic. Therefore, it is assumed that the therapeutic effectiveness of OlzHomo 8b may be delivered at higher doses than that at which Olz is administered.

Similarly, the presence of the N-methylhomopiperazine ring in thienobenzodiazepine MeHomo 8a caused a significant reduction in binding to the D\textsubscript{2} and 5HT\textsubscript{2A} as well as the H\textsubscript{1} receptors. The low binding affinity of MeHomo 8a for the D\textsubscript{2} and 5HT\textsubscript{2A} receptors may be due to a lower bioactive conformational state for the tricyclic thienobenzodiazepine skeleton. Steric effect of the N-methylhomopiperazine ring may also affect the rotation of this ring around the tricyclic thienobenzodiazepine, resulting in a lower interaction with the D\textsubscript{2} receptors in MeHomo 8a and OlzHomo 8b. However, the extension at the C-2 position of the thiophene ring with an ethyl group in OlzHomo 8b seems to improve the affinities for the D\textsubscript{2} and 5HT\textsubscript{2A} receptors when compared to those values of MeHomo 8a.

Taking these results into account, it appears that the N-methylhomopiperazine ring is not a favorable group for the interaction of the molecule with serotonin and dopamine receptors. The distances between the centroid of the fused thiophene and phenyl rings (A) and the basic distal nitrogen (B and C) (Figure 2.5, page 58) as well
as the conformational state of the tricyclic thienobenzodiazepine, are essential parameters for the affinity of the molecule for the D₂ and 5HT₂A receptors (Campiani et al. 2005). Presumably, alteration in these distances in OlzHomo 8b and MeHomo 8a structures is involved in the lower affinity of these compounds for the D₂ and 5HT₂A receptors compared to Olz. Furthermore, substitution of a formamide at position N-10 in Olz structure (compound 3) resulted in a marked reduction of serotonergic, histaminergic and, particularly, dopaminergic binding affinity. These results may shed light on the fact that the N-10 proton is a crucial interaction site of the molecule with these aforementioned receptors.

2.9.4 Conclusion

In this study, two new derivatives of Olz (MeHomo 8a and OlzHomo 8b) were synthesized, and the affinities of these compounds and previously developed OlzEt 1 and compound 3 for the brain 5HT₂A, D₂, and H₁ receptors were evaluated. OlzEt 1 represents a potential antipsychotic agent characterized by a highly favorable binding profile at 5HT₂A and D₂ receptors, similar to Olz, as well as lower affinity for H₁ receptors. These data may suggest the potential role of an ethyl group in reducing binding affinity to the H₁ receptor. A reduction of binding affinity of compound 3 for 5HT₂A, D₂, and H₁ receptors also highlighted the importance of the N-10 proton for the binding pocket interaction with the drug molecule. Compounds MeHomo 8a and OlzHomo 8b, bearing a bulky seven-membered ring, presented lower binding affinities to all the aforementioned receptors. Taking into account that OlzHomo 8b possessed a favorable pKᵢ ratio 5HT₂A/D₂ similar to the measured value of Olz (ratio value = 1.17) and that the D₂ affinity of thienobenzodiazepine OlzHomo 8b is still
comparable with that of the highly potent antipsychotics, it was postulated that both compounds OlzHomo 8b and OlzEt 1 may present therapeutic effectiveness for treating schizophrenia. In addition, these compounds present a lower affinity for H1 receptors, which may have reduced effects on weight gain and metabolic disorders than those reported with Olz. Further in vivo pharmacological validations of these two compounds are discussed in the next chapters.
Figure 2.5: Structural parameters for bioactive conformations of tetracyclic thienobenzodiazepines: A) The distance between the centroid of the fused thiophene and phenyl rings; B) The distance between the centroid of the fused thiophene and the basic distal nitrogen; C) The distance between the centroid of the fused phenyl rings the basic distal nitrogen (Campiani et al. 2005)
CHAPTER 3  THE EFFECT OF OLZ, OLZET, AND OLZHOMO ON WEIGHT GAIN AND METABOLIC SIDE EFFECTS

3.1 Background

As previously discussed in Chapter 1, Olz is associated with the great incidence of weight gain, dyslipidemia, and diabetes as well as increased appetite and food intake in treated patients (Allison et al. 1999; Eder et al. 2001; Zhang et al. 2004; Zipursky et al. 2005). This contributes to a serious noncompliance in taking medication, which drastically increases the relapse rate and leads to a huge cost for society and the families of patients (Bernstein 1987; Fenton et al. 1997; Han et al. 2008). Although weight gain and metabolic abnormalities continue to be a medical and public health concern, the mechanism underlying these remains unclear. To shed light on the elusive dilemma of obesity, a vast number of studies have attempted to establish an animal model that mimics human antipsychotic-induced weight gain (Pouzet et al. 2003; Minet-Ringuet et al. 2005; Minet-Ringuet et al. 2006; Choi et al. 2007; Han et al. 2008; Albaugh et al. 2006). The clinical picture of antipsychotic-induced obesity was confirmed in various models of male and female rodents, although some discrepancies were reported between animal research and the clinical scenario.
Sex sensitivity was one of the major concerns in the animal model of Olz-induced weight gain and metabolic dysfunction. Even though some aspects of metabolic dysfunction, including fat deposition, insulin tolerance and impaired glucose, have been reported in male rats chronically treated with Olz, the modelling of Olz-induced weight gain in these animals was not successful (Minet-Ringuet et al. 2005; Minet-Ringuet et al. 2006; Choi et al. 2007; Albaugh et al. 2011). On the other hand, Olz-induced body weight gain has been successfully modelled in female rats (Arjona et al. 2004; Cooper et al. 2005; Huang et al. 2006). These findings were consistent with a large number of clinical studies reporting sex-related differences, where female patients are more sensitive than males in terms of Olz-induced weight gain and metabolic side effects (Weston-Green et al. 2010; Weston-Green et al. 2011).

In general, it appears that the female rat model may mimic the clinical scenario in relation to the weight gain and metabolic dysfunction associated with Olz treatment, and it can be used as a valid basis for developing a novel potential therapeutic. Thus, in the present study, a female rat model was employed to examine the obesogenic side effects of novel Olz derivatives synthesized in Chapter 2.

The goal of this chapter is to investigate the potential differences in the liability of Olz, OlzEt, and OlzHomo to induce weight gain and metabolic dysfunctions. As discussed in Chapter 2, both OlzEt and OlzHomo presented a lower binding affinity
for the H₁ receptors compared to that of Olz, suggesting that these compounds may have reduced weight gain and metabolic side effects. The present study assessed the extent to which the chronic use of OlzEt and OlzHomo, as compared with Olz, could produce significant weight gain and antipsychotic-associated metabolic dysfunctions. Thus, the impact of a prolonged oral administration (up to 35 days) of Olz, OlzEt, and OlzHomo on body weight, food intake, and visceral fat accumulation at potential therapeutic doses was pursued. Additionally, the association between these compounds and disturbances in insulin, leptin, and adiponectin was explored. Following other reports (Albaugh et al. 2006), to reduce stressogenic procedures the present study employed an oral self-administration method for drug delivery, with doses three times daily to ensure a consistently high concentration of Olz, OlzEt, or OlzHomo in the rat. Oral self-administration also better mirrors the human scenario in the clinic than do other administration methods, including osmotic minipump or daily subcutaneous or intraperitoneal injection (Balcombe et al. 2004).

3.2 Materials and Methods

3.2.1 Animals and drug treatment regimes

Female Sprague Dawley rats (7 weeks old) were used to investigate weight gain and adiposity effects of a chronic treatment with Olz, OlzEt, and OlzHomo. Animals were obtained from the Animal Resources Centre (Perth, WA, Australia) and housed individually at 22 °C, on a 12 h light-dark cycle with an ad libitum access to water and standard laboratory chow diet (3.9 kcal/g, 74% carbohydrate, 16% protein, and 10% fat). Animals were then randomly assigned to one of the following treatment groups: 3.0 or 6.0 mg/kg/day of Olz (Bosche Scientific, NJ, USA), OlzEt (Lichem,
Hebei Boyuan Co., China), OlzHomo (Lichem, Hebei Boyuan Co., China), or vehicle (n = 8), three times daily at eight-hourly intervals. Following 1 week habituation with the new environment, the animals underwent a training period to self-administer a sweet cookie dough pellet for 1 week. Cookie dough (62% carbohydrate, 22% protein, 10% vitamins, 6% fiber, and minerals) administration method was performed as previously reported for 5 weeks (Han et al. 2008) (Figure 3.1, page 63). Over the course of this experiment, animals were weighed twice per week. Food and water consumption were also monitored every 48 hours for each animal and results were corrected for spillage. All experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, and conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

3.2.2 Hormonal and post mortem measurement

At the end of the study, female rats were fasted for 10 h and then sacrificed using carbon dioxide asphyxiation. Upon sedation, blood was removed and collected in the Lavender Vacutainer tubes containing EDTA for hormonal testing. Samples were immediately centrifuged (1000g for 10 min at 4 °C), after which plasma was aliquoted and stored at −20 °C until use. Fasting plasma insulin, leptin, and adiponectin levels were measured using commercially available Milliplex kits (Millipore Corp, USA) and Luminex 100. White fat pads and sub-scapula brown fat pads were dissected for each animal and individually weighed. Brains were immediately removed, dissected into hypothalamus, frozen in liquid nitrogen, and then stored at -80 °C.
Chapter 3  In Vivo Study on Metabolic Side Effects

Figure 3.1: Oral administration of Olz to a female Sprague Dawley rat using the sweet cookie-dough pellet method
3.2.3 Radioligand binding assay

Receptor binding was performed as described in Chapter 2. In brief, the prefrontal cortex, striatum, and hypothalamus were homogenized in about 6 volumes of ice-cold buffer Tris.HCl, 50 mM, pH 7.7 (for D₂ and 5HT₂A receptors) or Na⁺/K⁺ phosphate 50 mM, pH 7.5 (for H₁ receptors), and the resultant homogenate was then centrifuged (27000g for 15 min at 4 °C). Each pellet was resuspended in the same volume of fresh buffer and centrifuged again under the same conditions as above. The final tissue pellets were resuspended just before the binding assay in the incubation buffer (50 mM Tris.HCl, 5 mM MgSO₄, 0.5 mM EDTA and 0.02% ascorbic acid, pH 7.7 for D₂ receptors; 50 mM Tris.HCl, pH 7.7 for 5HT₂A receptors and Na⁺/K⁺ phosphate 50 mM, pH 7.5 for H₁ receptors). [³H]-Spiperone (specific activity, 15 Ci/mmol, 1mCi/ml; Perkin Elmer, Australia) binding to D₂ receptor was assayed for 40 min at 37 °C in the presence of 2 nM radioligand solution, 1.2 mg/mL protein suspension in the absence or presence of 2 µM (+)-butaclamol (Sigma, Australia). The binding affinity at 5HT₂A receptors was measured in the presence of 10 nM [³H]-Ketanserine (specific activity, 67 Ci/mmol, 1mCi/ml; Perkin Elmer, Australia), protein suspension (0.25 mg/mL), in the absence or presence of 10µM methysergide (Sigma, Australia). The reaction was carried out for 15 min at 37 °C. [³H]-Pyrilamine (specific activity, 37 Ci/mmol, 1mCi/ml; Perkin Elmer, Australia) binding to H₁ receptor was assayed for 30 min at 25 °C in the presence of radioligand solution (2 nM) in the absence or presence of 2µM doxepin (Sigma, Australia). Incubation reactions were stopped by the addition of ice-cold buffer (Tris.HCl, 50 mM, pH 7.7 for D₂ and 5HT₂A receptors and Na⁺/K⁺ phosphate 50 mM for H₁ receptors) followed by rapid filtration on filter (GF/B Whatman). The filters were
then washed twice with the buffer. The radioactivity on the filters was measured by beta liquid scintillation analyzer (Perkin Elmer, Tri-Crab 2800 TR) with a counting efficiency of 47%.

3.2.4 Data analysis

Data were statistically analyzed using SPSS 17.0 program (SPSS, Chicago, IL, USA). Total weight gain, total food intake, energy efficiency, insulin, leptin, adiponectin, fat mass, and binding density were analyzed by one-way ANOVA for each doses of Olz, OlzEt, and OlzHomo. Repeated ANOVA (COMPOUNDS×DAYS as repeated measures) was employed for cumulative weight gain, food and water intake. Multiple comparisons were performed using post hoc Tukey or Games-Howell tests. Where Kolmogorov–Smirnov tests showed nonparametric data distribution, Kruskall–Wallis tests were applied followed by Mann–Whitney U post hoc analysis. Correlations were identified using Pearson’s correlation tests or Spearman’s correlation tests for non-parametric data. Linear regression was performed in groups with significant correlations. Significance was set at $P < 0.05$.

3.3 Results

3.3.1 Body weight gain, food and water intake

Body weight gain was found significant following both 3 mg/kg ($F_{3,27} = 7.11, P = 0.001$) and 6 mg/kg ($F_{3,27} = 23.27, P < 0.001$) Olz administration compared to the control group. However, the effect of both OlzEt and OlzHomo on total body weight
gain was not significant compared to the control groups. OlzEt and OlzHomo showed notably less weight gain compared to Olz group with 3 mg/kg (-26%, \( P < 0.05 \) for both compounds) and 6 mg/kg doses (-32% and -48%, \( P < 0.001 \), respectively) (Figure 3.2, page 70). A repeated ANOVA (treatment × days) revealed a significant effect of time on the progressive enhancement of body weight for both doses of Olz (3mg/kg and 6mg/kg) \( (F_{3,37,90.90} = 246.06, P < 0.001 \) and \( F_{3,32,92.89} = 289.38, P < 0.001 \), respectively) and the interaction between these two factors \( (F_{10.10,90.90} = 2.34, P < 0.05 \) and \( F_{9.95,92.89} = 5.53, P < 0.001 \), respectively). As illustrated in Figure 3.3, page 71, administration of Olz (at both tested doses) gradually increased weight gain from day 4 to the end of treatment period. In contrast, the effect of OlzEt and OlzHomo on cumulative body weight was not significant compared to controls (Figure 3.3, page 71).

Higher doses of Olz treatment (6 mg/kg) induced a significant food intake increase \( (F_{3,27} = 10.73, P = 0.001) \) that started after 6 days of treatment. In contrast, OlzEt and OlzHomo administration did not affect food intake for both tested doses. Post hoc analysis showed that total food intake after 5 weeks of treatment with OlzEt and OlzHomo (6 mg/kg) was significantly lower than Olz administration (14% and \( P = 0.001 \), 29% and \( P = 0.006 \), respectively) (Figure 3.2, page 70). Energy efficiency (grams of weight gained/grams of food consumed) was significantly increased following Olz (3mg/kg and 6mg/kg) \( (F_{3,26} = 6.50, P = 0.002 \) and \( F_{3,27} = 3.06, P = 0.001 \), respectively), but not with OlzEt and OlzHomo treatment, compared to controls (Figure 3.2, page 70). A significant positive correlation between total body weight gain and total food intake was found after 5 weeks treatment \( (r = 0.48, P < \)
0.001) (Table 3.3, page 75). A repeated measurement of cumulative water intake showed as well a significant effect of time ($P < 0.001$) on water consumptions (Figure 3.3, page 71). However, water intake in drug treated animals was not different with controls.

### 3.3.2 Fat deposition

Visceral fat deposition was significantly increased in 6 mg/kg Olz treated rats compared to the control group ($F_{3,28} = 8.98$, $P = 0.002$). However, OlzEt and OlzHomo treated animals (3 mg/kg and 6 mg/kg, $P > 0.05$) did not show any significant difference in relation to visceral fat deposition compared to controls (Table 3.1, page 73). Significant positive correlations were found between body weight gain and food intake with fat mass ($r = 0.42$, $P = 0.002$ and $r = 0.59$, $P < 0.001$, respectively) (Table 3.3, page 75). There was no effect of treatments on subscapula brown adipose tissue mass (BAT) compared to the control group (Table 3.1, page 73).

### 3.3.3. Plasma hormonal level

Olz treatment with 6 mg/kg caused a significant reduction effect on the fasting plasma insulin levels in tested animals compared to the control rats ($F_{3,23} = 10.46$, $P < 0.01$), while OlzEt and OlzHomo administration did not affect the insulin levels in the tested rats compared to the controls ($P > 0.05$). Additionally, both compounds showed higher insulin levels than those measured in Olz treated animals with 6 mg/kg doses (48%, $P = 0.001$ and 54%, $P = 0.013$, respectively) (Figure 3.4, page 72). The insulin levels were similar in the treated groups (Olz, OlzEt, and OlzHomo).
for 3 mg/kg doses compared to the control animals ($P > 0.05$). Surprisingly, the post hoc analysis did not show any difference in leptin levels of animals treated with Olz at both 6 mg/kg ($F_{3,22} = 6.92, P = 0.95$) and 3 mg/kg ($F_{3,21} = 5.33, P = 1.00$) doses compared to the control groups. Plasma leptin levels were significantly decreased following 3 mg/kg and 6 mg/kg OlzHomo ($P = 0.011$ and $P < 0.01$, respectively) compared to controls. Fasting plasma adiponectin levels were significantly higher after chronic administration of Olz (at the 3 mg/kg and 6 mg/kg doses, $F_{3,23} = 22.45$, $P < 0.01$ and $F_{3,24} = 18.28$, $P = 0.000$, respectively) but lower following OlzHomo treatment (at the 3 mg/kg doses, $P = 0.039$) compared to controls (Figure 3.4, page 72). Leptin and adiponectin levels for the animals treated with OlzEt, though, were not significantly different at both tested doses compared to the controls (Figure 3.4, page 72). Significant correlation was found between insulin level and food intake ($r = -0.46$, $P = 0.002$), as well as between leptin level and fat deposition ($r = 0.34$, $P = 0.026$) (Table 3.3, page 75).

### 3.3.4 Alterations in H$_1$, D$_2$, and 5HT$_{2A}$ receptor densities

Olz (3 mg/kg and 6 mg/kg) significantly reduced the H$_1$ receptor density in the hypothalamus of the female animals treated for 5 weeks ($F_{3,12} = 4.01$, $P < 0.05$ and $F_{3,12} = 23.06$, $P < 0.001$, respectively) compared to controls, while the H$_1$ receptor density remained unchanged in animals treated with either OlzEt or OlzHomo for both tested doses (Table 3.2, page 74). There was a significant negative correlation between total body weight gain and visceral fat mass with H$_1$ receptor density in the hypothalamus ($r = -0.59$, $P = 0.001$ and $r = -0.61$, $P = 0.001$, respectively) (Table 3.3, page 75).
The D₂ receptor binding density in the striatum was significantly reduced following the chronic treatment of Olz (3 mg/kg and 6 mg/kg) ($F_{3,15} = 23.83$, $P = 0.002$ and $F_{3,15} = 5.911$, $P = 0.005$, respectively) and OlzEt (3 mg/kg and 6 mg/kg) ($F_{3,15} = 23.83$, $P < 0.05$ and $F_{3,15} = 5.911$, $P = 0.05$, respectively) but not HomoEt (both doses, $P > 0.05$) in rats. The same result was observed in the prefrontal cortex of the rats treated with Olz (3 mg/kg and 6 mg/kg) ($F_{3,15} = 34.27$, $P < 0.001$ and $F_{3,15} = 39.039$, $P < 0.001$, respectively) and OlzEt (3 mg/kg and 6 mg/kg) ($F_{3,15} = 34.27$, $P < 0.01$ and $F_{3,15} = 39.09$, $P < 0.01$, respectively). However, this study showed that the binding density of 5HT₂A receptor did not differ between HomoEt treatment groups and controls (both doses, $P > 0.05$) (Table 3.2, page 74).
Figure 3.2: A. total body weight gain (g), B. Total food intake (g), C. Energy efficiency (g weight gain/g food intake) in female Sprague Dawley rat treated with Olz, OlzEt, OlzHomo (3 mg/kg or 6 mg/kg), or vehicle (Control) for 5 weeks. The data point are the mean ± SEM, **$P < 0.01$ and ***$P < 0.001$ vs. Control
Figure 3.3: A. cumulative body weight gain (g), B. cumulative food intake (g) and C. cumulative water intake (ml) in female Sprague Dawley rat treated with Olz, OlzEt, OlzHomo (3 mg/kg or 6 mg/kg), or vehicle (Control) for 5 weeks. The data points are the mean ± SEM. A. *P < 0.05, **P < 0.01: Olz 3 mg/kg vs. control, ***P < 0.01, ****P < 0.001: Olz 6 mg/kg vs. Control. B. **P < 0.01, ***P < 0.001: Olz 6 mg/kg vs. control. (*: Control, Δ: Olz 3 mg/kg, ▲: 6 mg/kg, ◊: OlzEt 3 mg/kg, ●: OlzEt 6 mg/kg, ○: OlzHomo 3 mg/kg and ●: OlzHomo 6 mg/kg)
Figure 3.4: A. plasma insulin (PM), B. Leptin (PM), C. Adiponectin (pg/ml) in female Sprague Dawley rat treated with Olz, OlzEt, OlzHomo (3 mg/kg or 6 mg/kg), or vehicle (Control) for 5 weeks. The data point are the mean ± SEM, *P < 0.05 and **P < 0.01 vs. Control.
Table 3.1: Mean body weight and fat pad mass in female Sprague Dawley rats following 5 weeks treatment with Olz, OlzEt, OlzHomo (3 mg/kg or 6 mg/kg), or vehicle (Control)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Olz</th>
<th>OlzEt</th>
<th>OlzHomo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 mg</td>
<td>6 mg</td>
<td>3 mg</td>
<td>6 mg</td>
</tr>
<tr>
<td><strong>Body Weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IBW</td>
<td>226.43 ± 10.74</td>
<td>225.38 ± 10.04</td>
<td>225.18 ± 8.79</td>
<td>228.09 ± 8.46</td>
</tr>
<tr>
<td>FBW</td>
<td>258.16 ± 11.10</td>
<td>274.20 ± 12.14</td>
<td><strong>290.38 ± 7.16</strong>*</td>
<td>266.75 ± 9.8</td>
</tr>
<tr>
<td><strong>Fat pad mass (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total White fat</td>
<td>6.31 ± 0.93</td>
<td>8.01 ± 1.01</td>
<td><strong>12.18 ±1.14</strong>*</td>
<td>6.32 ± 0.73</td>
</tr>
<tr>
<td>Subscapula (BAT)</td>
<td>0.32 ± 0.04</td>
<td>0.30 ± 0.04</td>
<td>0.27 ± 0.3</td>
<td>0.27 ± 0.2</td>
</tr>
<tr>
<td>Total white fat/FBW (%)</td>
<td>2.36 ± 0.29</td>
<td>2.85 ± 0.28</td>
<td><strong>4.28 ± 0.31</strong>*</td>
<td>2.32 ± 0.19</td>
</tr>
</tbody>
</table>

Data is expressed as Mean ± SEM, *P < 0.05, and ***P < 0.001 vs. Control.
Table 3.2: H₁, D₂, and 5HT₂A receptor specific densities in the hypothalamus, striatum, and prefrontal cortex of female Sprague Dawley rats following 5 weeks treatment with Olz, OlzEt, OlzHomo (3 mg/kg or 6 mg/kg), or vehicle (Control)

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Control</th>
<th>Olz</th>
<th>OlzEt</th>
<th>OlzHomo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3 mg</td>
<td>6 mg</td>
<td>3 mg</td>
</tr>
<tr>
<td>H₁</td>
<td>365.2 ± 15.9</td>
<td>289.7 ± 4.6*</td>
<td>209.2 ± 14.9***</td>
<td>344.2 ± 26.9</td>
</tr>
<tr>
<td>D₂</td>
<td>2012 ± 111</td>
<td>1343 ± 76.2**</td>
<td>1577 ± 71.1**</td>
<td>1747.5 ± 94*</td>
</tr>
<tr>
<td>5HT₂A</td>
<td>4817.9 ± 359.6</td>
<td>1590.5 ± 43.6***</td>
<td>1420.2 ± 60.7***</td>
<td>3000.5 ± 40.9**</td>
</tr>
</tbody>
</table>

Data is expressed as CPM; Mean ± SEM, *P < 0.05, **P < 0.01, and ***P < 0.001 vs. Control
Table 3.3: Results of correlation and regression analysis

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>P</th>
<th>r²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total body weight gain</td>
<td>0.48</td>
<td>0.000</td>
<td>0.197</td>
<td>0.001</td>
</tr>
<tr>
<td>Total body weight gain</td>
<td>0.42</td>
<td>0.002</td>
<td>0.170</td>
<td>0.002</td>
</tr>
<tr>
<td>Total body weight gain</td>
<td>-0.59</td>
<td>0.001</td>
<td>0.343</td>
<td>0.001</td>
</tr>
<tr>
<td>Total body weight gain</td>
<td>-0.310</td>
<td>0.036</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total body weight gain</td>
<td>0.63</td>
<td>0.000</td>
<td>0.394</td>
<td>0.001</td>
</tr>
<tr>
<td>Total food intake</td>
<td>-0.46</td>
<td>0.002</td>
<td>0.297</td>
<td>0.001</td>
</tr>
<tr>
<td>Total food intake</td>
<td>0.36</td>
<td>0.020</td>
<td>0.223</td>
<td>0.002</td>
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<tr>
<td>Total food intake</td>
<td>0.46</td>
<td>0.001</td>
<td>0.161</td>
<td>0.005</td>
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<tr>
<td>Total white fat</td>
<td>0.29</td>
<td>0.041</td>
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<tr>
<td>Total white fat</td>
<td>-0.61</td>
<td>0.001</td>
<td>0.375</td>
<td>0.001</td>
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<tr>
<td>Total white fat</td>
<td>0.34</td>
<td>0.019</td>
<td>0.116</td>
<td>0.019</td>
</tr>
<tr>
<td>Total white fat</td>
<td>0.59</td>
<td>0.000</td>
<td>0.329</td>
<td>0.001</td>
</tr>
<tr>
<td>Total white fat</td>
<td>0.344</td>
<td>0.026</td>
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</table>
3.4 Discussion

3.4.1 Prevention of weight gain and adiposity following OlzEt and OlzHomo regimes

In agreement with other animals (Goudie et al. 2002; Pouzet et al. 2003; Fell et al. 2004; Cooper et al. 2005; Choi et al. 2007; Fell et al. 2007; Han et al. 2008; Weston-Green et al. 2011; Albaugh et al. 2006) and clinical reports (Nemeroff 1997; Nasrallah 2003), this study showed that chronic treatment of Olz (at both 3 and 6 mg/kg doses) induced an increased body weight gain, with higher doses causing a greater effect. In contrast, OlzEt and, particularly, OlzHomo prevented weight gain over the period of treatments. This study also confirmed the previous findings with relation to hyperphagia and enhancing energy efficiency induced by Olz treatment (Gothelf et al. 2002; Huang et al. 2006; Weston-Green et al. 2011; Albaugh et al. 2006). Notably, food intake increased in animals treated with only 6 mg/kg of Olz, indicating an important contribution of energy consumption to body weight gain in this group of animals. On the other hand, food intake was not significantly increased in the 3 mg/kg Olz-treated rats, despite a significant increase in body weight in these animals. These results may suggest the potential involvement of decreased energy expenditure in Olz-induced weight gain with the 3 mg/kg dose (Han et al. 2008). Coinciding with previous findings (Cooper et al. 2005; Han et al. 2008; Albaugh et al. 2006), this finding reported the positive correlation between body weight gain and visceral fat deposition, despite Olz, OlzEt, and OlzHomo inducing low visceral fat deposition, which is important for reduction of metabolic disorders. Various studies have suggested that both peripheral and central factors may be involved in Olz-induced weight gain and adiposity, nevertheless, the exact mechanisms by which
this drug causes metabolic adverse side effects still remain elusive (Coccurello and Moles 2010; Reynolds and Kirk 2010). The fat deposition may be associated with the increase of body weight or be due to the direct effect of Olz administration on adipose tissue (Cooper et al. 2007; Coccurello et al. 2008). Olz-mediated peripheral adipogenesis in 3T3-L1 cell model showed an over-expression of a transcript factor gene involved in the regulation of lipid homeostasis (sterol regulatory element binding protein-1, SREBP-1), which is accompanied by over-expression of fatty acid synthase and adiponectin (Yang et al. 2007).

Olz, OlzEt, and OlzHomo did not increase the BAT mass in this study. BAT has been found to contribute to the regulation of energy homeostasis, and to be increased as a compensatory response to hyperphagia in rodents (Stock 1999). Previously, an increase in interscapula BAT mass but not body weight in male rats following 8-weeks treatment with Olz was reported. Although Olz did not alter the mitochondrial thermogenic activity of BAT in those animals, it was suggested that increased BAT mass may have attenuated the weight gain (Minet-Ringuet et al. 2006). However, the study on female rats (Stefanidis et al. 2009) showed that Olz decreased the BAT temperature and the expression of the mitochondrial uncoupling protein (UCP1) in the interscapula BAT while inducing weight gain (Stefanidis et al. 2009). In the present study, where hyperphagia was observed at 6 mg/kg, the lack of increased BAT mass to compensate weight gain with increased metabolism may suggest an Olz-induced dysfunction of thermogenesis. However, further study is required to investigate BAT mitochondrial thermogenic activity in Olz-treated rats in this study.
In agreement with the previous *in vitro* adipogenesis results (Yang *et al.* 2007) and animal and clinical studies (Togo *et al.* 2004; Cooper *et al.* 2005), this finding supported the effect of Olz administration on increasing plasma adiponectin levels. However, this data may appear counterintuitive given that other studies reported the reduction of adiponectin in obesity (Hotta *et al.* 2000; Asayama *et al.* 2003). The correlation between plasma leptin levels and visceral adiposity found in this study suggested that leptin may be a useful indicator of fat mass deposition induced by Olz. Interestingly, plasma leptin levels in animal treated with OlzHomo was significantly reduced, which may confirm the prevention effect of OlzHomo on visceral adiposity. The similar outcome was found in the leptin levels of female rats treated with ziprasidone, which has a low effect on weight gain and fat deposition (Fell *et al.* 2007). A number of studies have shown that Olz-induced weight gain is associated with elevated leptin levels in schizophrenic patients (Kraus *et al.* 1999; Eder *et al.* 2001; Fitzgerald *et al.* 2003; McIntyre *et al.* 2003). Since we have observed a link between enhanced adiposity and leptin levels, the lack of a significant effect of Olz treatment on leptin levels in this study may appear surprising. However, similar outcomes have been reported in both animal (Cooper *et al.* 2005; Fell *et al.* 2007) and clinical studies (Esen-Danaci *et al.* 2008), suggesting, as a possible reconciliation of this discrepancy, that orexigenic effect of Olz may not be related to leptin levels.

This study also showed a marked reduction in insulin secretion in the 6 mg/kg Olz treatment group and no significant change in the 3 mg/kg Olz, OlzEt, and OlzHomo-treated animals. These results support some recent findings that short-term treatment with Olz decreased insulin levels in rats and schizophrenic patients (Chiu *et al.* 2006;
Weston-Green et al. 2011). On the other hand, an extensive amount of literature reported increased insulin levels following chronic treatment with Olz, particularly in patients who gained a significant amount of weight (Oriot et al. 2008; Wu et al. 2008). It is possible that Olz enhances insulin hypersensitivity as a negative response to hypoinsulinemia induced following the acute phase of treatment (Choi et al. 2009; Weston-Green et al. 2011). However, as a result of increasing weight gain, chronic administration of Olz may lead to compensatory hyperinsulinemia and insulin-resistance, which are commonly observed in clinical cases. The direct effect of Olz on pancreatic β-cell has been previously reported, suggesting that the current results may also be related to the direct effects of Olz on decreased insulin secretions in the pancreas (Johnson et al. 2005). In addition, adiponectin plays a crucial part in glucose homeostasis and enhances insulin sensitivity (Goldfine and Kahn 2003; Cooper et al. 2005). It has been previously established that fasting hyperinsulinemia is accompanied by low plasma adiponectin serum concentration in humans and in rodents (Stefan et al. 2002; Cooper et al. 2005). The insulin-sensitizing action of adiponectin could be explained by the negative correlation found between basal insulin receptor tyrosine phosphorylation and plasma adiponectin levels (Stefan et al. 2002). A low concentration of adiponectin promotes fasting hyperinsulinemia, which may elevate basal phosphorylation of the insulin receptors and precede a reduction in insulin sensitivity (Stefan et al. 2002). In contrast to these reports, a previous study in rats found increased adiponectin levels together with hyperinsulinemia following treatment with Olz (Cooper et al. 2005). It was postulated that an increase in adiponectin levels was a compensatory response to normalize the Olz-induced insulin resistance (Cooper et al. 2005). The elevated fasting plasma adiponectin
concentration in the current study, though, was associated with hypoinsulinemia in rats treated with Olz (6 mg/kg). It is possible that adiponectin reduces basal phosphorylation by enhancing insulin signalling downstream of the insulin receptor, leading to increased insulin sensitivity and decreased plasma insulin levels. However, the exact mechanisms explaining these results need to be determined. Nevertheless, it appears that the current data are still consistent with the hypothesis that adiponectin plays a part in insulin sensitivity in humans and rodents.

3.4.2 A study on neurochemical alterations following the treatments with Olz, OlzEt, and OlzHomo

A relevant role of the affinities of atypical antipsychotics for the 5HT<sub>2A</sub>, 5HT<sub>2C</sub>, 5HT<sub>6</sub>, α<sub>1A</sub>, and H<sub>1</sub> receptors in their obesogenic effects has been repeatedly reported in various studies (Reynolds et al. 2002; Kroeze et al. 2003; Mathews and Muzina 2007; Reynolds and Kirk 2010). In such a framework, particular emphasis was placed on the affinity of antipsychotics to block the H<sub>1</sub> receptors (Masaki et al. 2004; Masaki and Yoshimatsu 2006). The central role of hypothalamic H<sub>1</sub> receptors and histamine neurons in the regulation of body mass has been well established. It is reported that hypothalamic H<sub>1</sub> receptor and histamine neurons are directly involved in the leptin signalling pathway. The blockade of the H<sub>1</sub> receptors can activate hypothalamic AMPK signalling, which stimulates food intake and positive energy balance and reverses the anorexigenic effect of leptin (Kim et al. 2007). On the other hand, studies in obese mice showed that administration of histamine in the hypothalamus can alter food intake and energy expenditure, resulting in the attenuation of adiposity and body mass in these animals (Masaki et al. 2001a; Masaki et al. 2001b). By stimulating the sympathetic nervous system, neuronal
histamine can also influence lipolysis in adipose tissues (Tsuda et al. 2002). Additionally, neuronal histamine and H₁ receptors affect UCP expression in BAT, which explains their central regulation of energy homeostasis (Masaki et al. 2001a; Masaki et al. 2001b). Taken together, these results indicate that H₁ receptors and histamine neurons are crucial in the development of obesity.

This study supports the potential role of H₁ receptor affinity in antipsychotics-induced weight gain and fat deposition. As shown in Chapter 2, OlzEt and OlzHomo have a lower affinity for binding to the H₁ receptors ($K_i = 1.95$, $K_i = 13.63$, respectively) compared to that of Olz ($K_i = 0.13$). Therefore, the pronounced antagonisms of OlzEt and OlzHomo at H₁ receptor may be responsible for their significantly attenuated propensity to induce weight gain and metabolic dysfunctions, which are associated with Olz treatment. Furthermore, corresponding with previous reports, the present study demonstrated the significant negative correlation between hypothalamic H₁ receptor density and weight gain and accumulative fat mass in rats. H₁ receptor density in the hypothalamus has been markedly reduced following chronic treatment with Olz (at both doses) but not with OlzEt and OlzHomo, supporting the importance of H₁ receptor in Olz-induced obesogenic side effect. In agreement with these observation, down-regulation of H₁ expression has been previously reported in hypothalamic nuclei of rats treated with Olz (Han et al. 2008). In addition, the nonsignificant alterations of the H₁ receptor density following OlzEt and OlzHomo treatments may explain the lack of orexigenic effect of these two compounds in the treated rats. Thus, the involvement of H₁ receptor in Olz-induced obesity and fat deposition might be closely related.
As previously discussed, the mechanisms of Olz-induced weight gain involve both peripheral and central factors. Peripheral adiposity and nutrient-related signals converge on the hypothalamus, which plays an imperative role in the maintenance of body energy balance and appetite. The complete picture of eating behaviour that is mainly modulated by interrelated neurobiological mechanisms, incorporating energy homeostasis and food reward system and cognitive control (Saper et al. 2002; Hinton et al. 2004; Kelley 2004), has been extensively reviewed by Elman et al. (Elman et al. 2006). In brief, reciprocal innervation of hypothalamic–limbic areas with prefrontal cortex (Berthoud 2004) together with the peripheral adiposity signals and hormonal biomarkers of body fat mass (i.e., insulin and leptin) contribute to the mechanism of food consumption. Previous studies in patients with schizophrenia suggested that the incidence of glucose intolerance and insulin resistance affects the energy homeostasis and reward regulation (Mukherjee et al. 1996; Ryan et al. 2003), leading to an increase of body weight in these patients. Moreover, mesolimbic hyperdopaminergic state and hypofunctional prefrontal cortex, which is associated with reward deficiency and poor cognitive control, may also contribute to the increase of food intake in patients with schizophrenia. And while Olz treatment (and most of the other atypical antipsychotics) may improve the mesolimbic dopamine-mediated reward system and cognitive impairment in the prefrontal cortex in schizophrenia, it is accompanied by an increase of food intake and weight gain, mostly due to its hypothalamic H₁ receptor antagonism activity. Taken together, it appears that further exploration of the involvement of prefrontal cortex cognitive control and limbic reward system in antipsychotic-induced hypoplasia and weight
gain can provide an invaluable direction for the development of new, highly targeted antipsychotic drugs.

This study evaluated the effect of Olz, OlzEt and OlzHomo treatments on the alterations of D_2 and 5HT_{2A} receptor densities in the striatum and prefrontal cortex in rats, respectively. A markedly lower density of D_2 and 5HT_{2A} receptors was observed following the chronic treatment with Olz and OlzEt but not with OlzHomo. These findings were compatible with the in vitro results reported in Chapter 2 (page 54), suggesting that a higher affinity of Olz and OlzEt for binding to D_2 and 5HT_{2A} receptors may be involved in down-regulation of these receptors. Investigation of the potential involvement of the prefrontal cortical 5HT_{2A} and striatal D_2 receptor binding capacities in cognitive control and limbic reward function with regard to the modulation of food intake and weight gain was beyond the scope of this dissertation. Further study is required to provide more insight into this concept. The overview of the potential neuropathology underlying increased food intake and obesity pertinent to schizophrenia is depicted in Figure 3.5 (a-b).
Figure 3.5 (a): Systematic overview of the potential neuropathology underlying increased food intake and obesity pertinent to schizophrenia. Reciprocal innervation of hypothalamic–limbic areas with prefrontal cortex together with the peripheral adiposity signals and hormonal biomarkers of body fat mass (i.e., insulin and leptin) contribute to the mechanism of food consumption. The incidence of glucose intolerance and insulin resistance in patients with schizophrenia affects the energy homeostasis and reward regulation and leads to an increase of body weight in these patients. Additionally, mesolimbic hyperdopaminergic state and hypofunctional prefrontal cortex, which is associated with the reward deficiency and poor cognitive control, may also contribute to the increase of food intake in patients with schizophrenia. Adapted from Elman et al. (2006)
Figure 3.5 (b): Systematic overview of the potential neuropathology underlying increased food intake and obesity following treatment with Olz. Although Olz treatment (and most of the other atypical antipsychotics) may improve the mesolimbic dopamine-mediated reward system and cognitive impairment in the prefrontal cortex in schizophrenia, it is accompanied by an increase of food intake and weight gain, mostly due to its hypothalamic H1 receptor antagonism activity. Adapted from Elman et al. (2006)
3.5 Conclusion

In general, while this study confirmed the effect of Olz administration on metabolic alterations in rats, the current results showed that OlzEt and OlzHomo administrations did not induce either enhancing effects on body weight and food intake or detrimental consequences on fat deposition and metabolism. These findings appear to have reasonable predictive validity for different aspects of Olz-induced weight gain/adiposity and metabolic abnormalities, which mimic the clinical situation.
CHAPTER 4  BEHAVIOURAL STUDY: A COMPARISON BETWEEN OLZ, OLZET, AND OLZHOMO

4.1 Introduction

NMDA receptor antagonists such as PCP and ketamine are psychotomimetic drugs, which produce both positive and negative signs of schizophrenia as well as impairment of cognitive functions in normal humans. They also exacerbate symptoms in patients with schizophrenia (Javitt and Zukin 1991; Moller and Husby 2000; Silver et al. 2003; Choi et al. 2009). In animal experiments, these drugs cause schizophrenia-relevant behaviours, including hyperlocomotion (Facchinetti et al. 1993; Wang et al. 2001) and deficits in working memory tasks (Stefani and Moghaddam 2005; Mouri et al. 2007).

The observation that NMDA receptor antagonists mimic the core symptoms of schizophrenia suggested that this psychiatric disorder may be linked to a state of NMDA receptor hypofunction. This hypothesis was supported by the evidence reporting alterations in NMDA receptor expression and function in the brains of
patients with schizophrenia (Harrison and Owen 2003; Konradi and Heckers 2003; Moya et al. 2004; Large 2007). In addition to NMDA receptor hypofunction state, D2 receptor-mediated hyperdopaminergic state also contributes to the pathophysiology of schizophrenia (Sato et al. 1993; Abekawa et al. 2007). In animal experiments, methamphetamine/amphetamine induced abnormal hyperlocomotion and stereotyped behaviours that are suppressed by dopamine D2 receptor antagonists such as haloperidole and clozapine. However, the behavioural and neurochemical changes induced by PCP are ameliorated only by an atypical antipsychotic, but not by haloperidol (Bakshi et al. 1994; Corbett et al. 1995; Gleason and Shannon 1997; Abekawa et al. 2007). These results were consistent with the evidence for the involvement of both serotonergic and dopaminergic mechanisms in the PCP-induced behaviours (Johnson and Jones 1990; Hori et al. 1996; Gleason and Shannon 1997).

Additionally, it is reported that clozapine blocked PCP-induced hyperlocomotion at doses about ten times lower than those that inhibited amphetamine-induced hyperlocomotion (Maurel-Remy et al. 1995; Gleason and Shannon 1997). Mixed D2/5HT2A antagonists have been also shown to block the hyperlocomotion effects of PCP at doses lower than those that reduce activity when administrated alone (Jackson et al. 1994; Maurel-Remy et al. 1995). Taken together, these findings support the hypothesis that the propensity of antipsychotics in blocking PCP-induced hyperlocomotion is closely linked to their affinity for 5HT2A receptors and that 5HT2A receptor antagonism may contribute to the unique therapeutic profile of atypical antipsychotic agents (Meltzer and Nash 1991; Meltzer 1996).
Having established the very low propensity of OlzEt and OlzHomo to elicit weight gain and metabolic abnormalities, this study further assessed the potential of these compounds to retain the effectiveness of Olz in blocking PCP-induced hyperlocomotion. As shown in Chapter 2, OlzEt represents a potential antipsychotic agent characterized by a highly favourable in vitro binding profile at 5HT$_{2A}$ and D$_2$ receptors similar to Olz, while OlzHomo presents reduced binding affinities to the aforementioned receptors compared to those of Olz. Bearing in mind that OlzHomo possessed a favourable $pK_i$ ratio 5HT$_{2A}$/D$_2$ similar to the measured value of Olz and that the D$_2$ affinity of OlzHomo is still comparable with that value of the high potent antipsychotics, it was postulated that both compounds OlzHomo and OlzEt may present therapeutic effectiveness for treating schizophrenia.

In the present chapter, the effect of pre-treatment with Olz, OlzEt, and OlzHomo on PCP-induced hyperactivities in rats has been evaluated in the open-field test. Furthermore, the effect of these treatments on the alteration of the brain D$_2$ and 5HT$_{2A}$ receptor densities following PCP challenge in the striatum and prefrontal cortex respectively was examined.

4.2 Materials and Methods

4.2.1 Animals and drug treatment regimes

The effects of Olz, OlzEt and OlzHomo subchronic administration on PCP-induced behaviours were tested in male Sprague Dawley rats (180-200 g). Animals were housed in pairs in the same conditions as described above. Following a 1 week habituation period, rats were treated orally with a sweet cookie dough pellet
containing 3.0 mg/kg of Olz, OlzEt, OlzHomo, or vehicle (n = 12), three times daily at eight-hourly intervals for 2 weeks. Animals were injected subcutaneously with either saline vehicle or PCP (10 mg/kg, synthesized in the School of Chemistry, Wollongong University, Wollongong, Australia) 30 min following the final drug/cookie administration. Open-field behavioural testing was performed 15 min after this injection.

**4.2.2 Behavioural analysis and post mortem measurement**

Open field test was used to determine the behavioural effects of Olz, OlzEt and OlzHomo pre-treatment on PCP treated animals. To minimise stress during the experiment, animals underwent a 10 min-habituation period for the open-field test one day prior to the experiment. As previously described (Du Bois et al. 2008), the locomotor activity has been recorded for each tested animal in an open black square box (60cm×60cm×40cm) (Figure 4.1, page 91). Behavioural parameters including total distance travelled (cm), mean velocity (cm/s), central and peripheral duration (s), and frequency of rearing were measured for 30-min and then analysed via Ethovision video-tracking software (Nodulus Information Technology, Wageningen, The Netherlands). Animals were euthanized 120 min following the open-field test as described above; brains were rapidly removed from the skull and dissected into prefrontal cortex and striatum until receptor binding assays.
Figure 4.1: Rat performing in the open field test
4.2.3 Data analysis

Data were statistically analysed using SPSS 17.0 program (SPSS, Chicago, IL, USA). Open-field parameters were also analysed by one-way ANOVA. Student’s $t$-test was used to determine the significance of differences between saline and PCP-treated rats. Multiple comparisons were performed using post hoc Tukey or Games-Howell tests. Where Kolmogorov–Smirnov tests showed nonparametric data distribution, Kruskall–Wallis tests were applied followed by Mann–Whitney U post hoc analysis. Significance was set at $P < 0.05$.

4.2.3 Radioligand binding assay

The striatum and prefrontal cortex were used in radioligand binding assay to measure the receptor binding density $D_2$ and $5HT_{2A}$ receptors, respectively. The assay was performed following the procedure described previously in Chapter 2. In brief, the striatum and prefrontal cortex were homogenized separately and then centrifuged (27000g for 15 min at 4 ºC). The resultant membrane was incubated in the presence of 2 nM [$^3$H]-Spiperone (specific activity, 15 Ci/mmol, 1mCi/ml; Perkin Elmer, Australia), with or without 2µM (+)butaclamol (Sigma, Australia) or 10 nM [$^3$H]-Ketanserine (specific activity, 67 Ci/mmol, 1mCi/ml; Perkin Elmer, Australia) in the absence or presence of 10µM methysergid (Sigma, Australia), for $D_2$ and $5HT_{2A}$ receptor binding assay respectively. Radioactivity was measured by beta liquid scintillation analyser (Perkin Elmer, Tri-Crab 2800 TR).
4.3 Results

4.3.1 Behavioural testing

Behavioural results are illustrated in Table 4.1 (page 95). In saline groups, no significant difference was found following 2 weeks of treatment with Olz, OlzEt, OlzHomo in all the parameters measured in the open-field test. However, in animal acutely administered with PCP, total distance moved, mean velocity, and centre and periphery durations differed significantly according to the tested treatment (Olz, OlzEt, and OlzHomo). Total travelled distance was significantly reduced in Olz and OlzEt ($F_{3,31} = 4.97$, $P = 0.023$ and $P = 0.008$) treated rats compared to controls. However, the effect of OlzHomo treatment on reducing motor activity was not significant compared to the controls ($P = 0.42$). Mean velocity was also reduced in animals treated with Olz ($F_{3,44} = 8.22$ and $P < 0.05$). One–way ANOVA revealed significant changes in the centre and periphery durations following 2 weeks treatment with OlzEt ($F_{3,33} = 4.37$, $P < 0.01$ and $F_{3,41} = 3.21$, $P = 0.017$, respectively). Moreover, t-test results showed a significant effect of acute PCP administration on the travelled distance in animals treated with Olz, OlzEt, OlzHomo, or control compared to the saline groups.

4.3.2 Alteration in D$_2$ and 5HT$_{2A}$ receptor densities

In saline groups, 2 weeks of treatment with Olz and OlzHomo induced a significant reduction effect on D$_2$ receptor density in the striatum of the male rats compare to controls ($F_{3,20} = 34.83$, $P < 0.001$ and $P < 0.001$, respectively). However, in PCP groups, a significant reduction was found following Olz and OlzEt treatments ($F_{3,20}$
= 40.75, \( P = 0.005 \) compared to controls. Similarly, 5HT\(_{2A}\) receptor density in the prefrontal cortex of the animals treated with Olz and OlzEt was significantly decreased in both saline \( (F_{3,20} = 125.03, \ P < 0.001 \) and \( P = 0.002 \), respectively) and PCP \( (F_{3,20} = 92.13, \ P < 0.001 \) groups (Table 4.2, page 96).
Table 4.1 Open field testing in male Sprague Dawley rats following 2 weeks treatment with Olz, OlzEt, OlzHomo, or vehicle (Control)

<table>
<thead>
<tr>
<th>Open field test</th>
<th>Control</th>
<th>Olz</th>
<th>OlzEt</th>
<th>OlzHomo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total distance moved (%)</td>
<td>135.5 ± 36.6</td>
<td>110.2 ± 31.9</td>
<td>143.1 ± 49.1</td>
<td>118.7 ± 33.1</td>
</tr>
<tr>
<td>Mean velocity (cm/s)</td>
<td>20.2 ± 3.7</td>
<td>18.2 ± 3.3</td>
<td>14.0 ± 1.0</td>
<td>16.9 ± 1.8</td>
</tr>
<tr>
<td>Rearing frequency (%)</td>
<td>118.6 ± 19.5</td>
<td>109.4 ± 30.5</td>
<td>122.7 ± 23.1</td>
<td>116.9 ± 22.1</td>
</tr>
<tr>
<td>Centre duration (s)</td>
<td>40.68 ± 10.1</td>
<td>40.34 ± 6.4</td>
<td>51.52 ± 93</td>
<td>86.16 ± 28.9</td>
</tr>
<tr>
<td>Periphery duration</td>
<td>1820.5 ± 17.5</td>
<td>1827.8 ± 28.3</td>
<td>1792.0 ± 16.2</td>
<td>1818.3 ± 14.6</td>
</tr>
<tr>
<td>Centre frequency</td>
<td>22.2 ± 2.7</td>
<td>18.5 ± 2.5</td>
<td>23.6 ± 2.8</td>
<td>23.7 ± 3.6</td>
</tr>
<tr>
<td>Periphery frequency (%)</td>
<td>106.5 ± 28.9</td>
<td>133.0 ± 39.0</td>
<td>132.5 ± 20.8</td>
<td>118.3 ± 63.4</td>
</tr>
<tr>
<td><strong>PCP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total distance moved (%)</td>
<td>357.6 ± 33.5</td>
<td>217.9 ± 39.6</td>
<td>198.1 ± 28.9</td>
<td>280.8 ± 26.6</td>
</tr>
<tr>
<td>Mean velocity (cm/s)</td>
<td>22.0 ± 1.5</td>
<td><strong>11.7 ± 2.1</strong></td>
<td>15.1 ± 2.9</td>
<td>26.4 ± 2.4</td>
</tr>
<tr>
<td>Rearing frequency (%)</td>
<td>209.6 ± 40.0</td>
<td>233.3 ± 71.0</td>
<td>258.6 ± 69.6</td>
<td>176.8 ± 29.8</td>
</tr>
<tr>
<td>Centre duration (s)</td>
<td>101.2 ± 19.5</td>
<td>234.4 ± 62.9</td>
<td><strong>281.0 ± 36.1</strong></td>
<td>127.6 ± 38.4</td>
</tr>
<tr>
<td>Periphery duration</td>
<td>1770.5 ± 24.8</td>
<td>1666.6 ± 93.6</td>
<td><strong>1585.4 ± 48.7</strong></td>
<td>1768.0 ± 36.7</td>
</tr>
<tr>
<td>Centre frequency</td>
<td>47.1 ± 4.9</td>
<td>36.5 ± 6.1</td>
<td>54.3 ± 9.2</td>
<td>45 ± 11.8</td>
</tr>
<tr>
<td>Periphery frequency (%)</td>
<td>245.6 ± 24</td>
<td>285.9 ± 99.6</td>
<td>376.4 ± 54.8</td>
<td>238.7 ± 53.3</td>
</tr>
</tbody>
</table>

Data is expressed as Mean ± SEM, *P < 0.05 and **P < 0.01 vs. Control. *P < 0.05, ***P < 0.01, and ****P < 0.001 vs. Saline

*Data normalized from baseline
**Table 4.2:** D<sub>2</sub> and 5HT<sub>2A</sub> receptor specific densities in the striatum and prefrontal cortex of male Sprague Dawley rats, respectively, following 2 weeks pre-treatment with Olz, OlzEt, OlzHomo, or vehicle (Control) and subcutaneous injection of Saline or PCP

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Olz</th>
<th>OlzEt</th>
<th>OlzHomo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D&lt;sub&gt;2&lt;/sub&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Saline</em></td>
<td>2009.2 ± 27.9</td>
<td><strong>1677.7 ± 23.6</strong>*</td>
<td>1938.2 ±18.6</td>
<td><strong>1826 ± 26.9</strong>*</td>
</tr>
<tr>
<td><em>PCP</em></td>
<td>2031.8 ± 42.9</td>
<td><strong>1403.2 ± 68.6</strong></td>
<td><strong>1651.7 ± 41.2</strong></td>
<td>2088.5 ± 45.9</td>
</tr>
</tbody>
</table>

| **5HT<sub>2A</sub>** |             |             |             |             |
| *Saline*       | 7391 ± 264.8 | **2919.8 ± 76.3*** | **5528 ± 117.9**  | 6604.2 ± 178.2 |
| *PCP*          | 8835.8 ± 187 | **2742.7 ± 125.8*** | **5682.8 ± 76.3**  | 8421.2 ± 537.9  |

Data is expressed as CPM; Mean ± SEM, **P < 0.01 and ***P < 0.001 vs. Control
4.4 Discussion

As previously discussed, the PCP animal model has been widely used in the development of new drugs that may have potential applicability in schizophrenia therapy (Large 2007). They offer reasonable validity with respect to the clinical symptoms of schizophrenia, and predict to some degree the efficacy of drugs in patients. In this study, the PCP animal model was used to determine whether sub-chronic pre-treatment with a single dose of Olz, OlzEt, or OlzHomo could attenuate the characteristic of PCP-induced behaviours in adult male rats. Coinciding with previous reports (Castellani and Adams 1981; Steinpreisa et al. 1999; Sircara and Soliman 2003), the results of this study showed that PCP-treated rats evidenced a remarkable increase in spontaneous locomotor activity (i.e., total distance travelled or travel velocity) and anxiety/exploratory related parameters (duration/frequency in centre or periphery) of the open-field test compared to the saline group. It has been suggested that the behavioural effect of PCP treatment is due to the multiple mechanisms of action that may partly include altered dopamine, serotonin and noradrenaline transmission (Martin et al. 1998; Ballmaier et al. 2001; Large 2007). For instance, a disruption of the firing pattern of dopamine neurons, which increases dopamine release in the frontal cortex and activates the mesolimbic dopamine neuron, may play a part in PCP-induced psychotic behaviour as schizophrenia. Altered activity of glutamatergic neurons in the cortex leading to an elevated glutamate release and a reduced inhibitory feedback onto principal neurons is also involved in this mechanism (Large 2007).
In accord with the clinical picture of schizophrenia, Olz attenuated the psychotic (e.g., hyperlocomotion and anxiety) effect of PCP administration in animals (Moya et al. 2004). In this study, the observed PCP-induced behaviours were largely blocked in the Olz and OlzEt treatments in rats. Pre-treatment with Olz and OlzEt significantly inhibited the hyperlocomotion induced by PCP. Interestingly, OlzEt was more efficient than Olz treatment in suppressing anxiety-like behaviours of PCP such as longer time spent in the outer field and the lower entries to centre field, suggesting the potential antipsychotic propensity of OlzEt. In contrast, OlzHomo did not suppress the PCP induced behaviours measured in the open-field test.

It is suggested that the mixed antagonist activity of atypical antipschotics at multiple receptors, including D₁-D₄, 5HT₂A, 5HT₂C, M₁, α₁, and α₂ receptors may underlie their efficacy in blocking the PCP induced behaviours (Seeman 1992; Large 2007). In particular, atypical antipsychotic action on 5HT₂ receptors is largely believed to be involved in the blockade of NMDA-antagonist-induced stereotyped activities (Gleason and Shannon 1997). For instance, increased synaptic level of serotonin at synapses containing 5HT₂A receptors plays a part in PCP-induced hyperlocomotion. This effect may be prevented by 5HT₂A antagonism activity of Olz at the level of motor pathways in the spinal cord or the brain (Gleason and Shannon 1997). In adult animals, evidence also indicates the involvement of both dopaminergic and serotonergic receptor antagonists in suppressing PCP-induced locomotion (Freed et al. 1980; Kitaichi et al. 1994). Additionally, in agreement with the glutamate (Glu) hypothesis of schizophrenia (Goff and Coyle 2001), mGlu2/3 receptor agonist has
been shown to inhibit the behaviour induced by PCP administration (Moghaddam and Adams 1998; Clark et al. 2002; Swanson and Schoepp 2002).

As shown in Chapter 2, OlzEt showed a similar affinity to Olz for blocking the D$_2$ and 5HT$_{2A}$ receptors in the striatum and prefrontal cortex, respectively, which may partly explain the *in vivo* effect of OlzEt at inhibiting the PCP behaviours. On the other hand, OlzHomo demonstrated a lower affinity at blocking these two receptors, which may eventually contribute to its lack of efficacy for alleviating the PCP-induced hyperactivity. In fact, since the ambulation in OlzHomo/PCP-treated rats was comparable to the control/PCP group, it is postulated that the potential therapeutic effectiveness of an OlzHomo regime may be achieved at a higher dose than at which Olz and OlzEt are administered.

To further validate our hypothesis regarding the effect of PCP on levels of D$_2$ and 5HT$_{2A}$ receptors, the neurochemical changes in the brain following PCP challenge were measured. Previously, Tomic *et al.* (Tomic *et al.* 1997) showed that a single injection of PCP (10 mg/kg) induced a significant increase in the D$_2$ receptor specific binding in the striatum only 30 min following the treatment. PCP-induced striatal D$_2$ receptor up-regulation was completely abolished at the 120 min point. In agreement with this report, in the present study, where PCP-treated animals were euthanized 120 min after injection, D$_2$ receptor density in the striatum in adult male PCP-treated rats did not differ from saline-treated controls. However, PCP-induced hyperlocomotion recorded between 15 and 45 min following the injection could be explained by the possible alterations of striatal dopaminergic neurotransmission in these animals over this time period. Consistent with the literature (Choi *et al.* 2009),
this study also reported no significant alteration in 5HT$_{2A}$ receptor density in prefrontal cortex in PCP-treated rats compared to the saline-treated vehicles. However, subchronic treatment with Olz and OlzEt induced a long-lasting down-regulation in the binding capacities of D$_2$ and 5HT$_{2A}$ receptors in both saline and PCP treated animals. As previously suggested, down-regulation of these two receptors may partly contribute to blockade of PCP induced behavioural changes, including hyperlocomotion (Gleason and Shannon 1997; Gandolfi et al. 2003). However, this hypothesis should be taken with a degree of caution, since the altered dopamine and serotonin receptors densities are not the only mechanisms underlying the behavioural changes induced by PCP administration (Large 2007). The extent to which such changes are involved in the therapeutic effects of Olz and OlzEt remains to be investigated.

4.5 Conclusion

Behavioural assessment in the open-field test predicted the similar effectiveness of OlzEt to Olz for blocking PCP-induced hyperactivities. These findings suggest that the long lasting down-regulation of D$_2$ and 5-HT$_{2A}$ receptors induced by sub-chronic Olz and OlzEt treatment may play a part in blocking PCP-induced behaviours. A lower potency of OlzHomo to inhibit PCP-induced behaviours could also be explained by its lower affinity for the brain D$_2$ and 5HT$_{2A}$ receptors compared to that of Olz and OlzEt. Therefore, the therapeutic effectiveness of the OlzHomo regime may be delivered at a higher dose than that of Olz and OlzEt treatment. Given the limitations associated with animal models, it is suggested that the present results be taken with caution. Only further behavioural studies and clinical trials will reveal the
predictive validity of current preclinical model for the therapeutic efficacy and metabolic side effects of these two compounds.
CHAPTER 5   GENERAL CONCLUSIONS 
AND FUTURE PROSPECTS

5.1 Conclusion

This dissertation focused on the preclinical development of novel antipsychotic drugs without obesogenic side effects. A structure-activity based approach to drug design was utilised, centring on the lead thienobenzodiazepine antipsychotic drug, Olz. Olz with tetracyclic thienobenzodiazepine structure offers superior clinical efficacy in schizophrenia treatment, but is unfortunately notorious for inducing severe metabolic derangement in patients. In the last decade, research has been focused on developing novel tetracyclic atypical antipsychotics with high efficacy and low metabolic side effects (reviewed in Jafari et al. 2012). Nevertheless, the crucial need for developing an ideal antipsychotic agent for schizophrenia still continues.

It is suggested that the simultaneous blockade of the D$_2$ and 5HT$_{2A}$ receptors with Olz in the various dopamine pathways is involved in the molecular mechanisms of therapeutic efficacy and results in the distinct clinical property of this drug (Meltzer et al. 1989b; Meltzer et al. 1989a; Meltzer and Nash 1991; Stahl 1998; Meltzer 1999). Additionally, the implication of the H$_1$ receptor and neural histamine in
regulation of weight gain and body mass has been well established (Masaki et al. 2001a; Masaki et al. 2004; Masaki and Yoshimatsu 2006; Masaki et al. 2001b). Olz-induced weight gain and metabolic abnormalities are strongly associated with its H1 receptor antagonism property (Kroeze et al. 2003). Thus, this study focused on the development of novel Olz derivatives with a lower in vitro binding affinity for the H1 receptors, but with a similar 5HT2A/D2 receptor binding affinity ratios to the one observed for Olz. In this regard, two novel derivatives of Olz (MeHomo and OlzHomo) and the previously reported analogue OlzEt (Chakrabarti et al. 1982; Monte 2003; Jafari 2008) were successfully synthesized. These compounds present two alterations to the structure of Olz, including the substitution of an ethyl group at the C-2 position of the thiophene ring (OlzHomo and OlzEt) and the replacement of the N-methylpiperazine with an N-methylhomopiperazine ring (MeHomo and OlzHomo). Further, the in vitro binding affinities of these compounds for serotoninergic, dopaminergic, and histaminergic receptors in the prefrontal cortex, striatum, and hypothalamus respectively, relevant to their therapeutic and metabolic side effects, were evaluated. OlzEt showed a highly favourable binding profile at 5HT2A and D2 receptors, similar to Olz, but approximately 15-fold lower affinity for H1 receptors, suggesting the potential role of a C-2 ethyl group in attenuating binding affinity to the H1 receptors. MeHomo and OlzHomo with a bulky seven-membered ring, though, presented lower binding affinities to all the aforementioned receptors. Nevertheless, since the D2 binding affinity of OlzHomo was comparable with those values of the current potent antipsychotics (i.e., remoxipride) in the clinic (Seeman 2010), further examination of this compound remains of interest from the perspective of drug development.
Next, this study employed a female rat model to compare in detail the liability of Olz, OlzEt, and OlzHomo to induce weight gain, incorporating indices of weight gain, food and water intake, visceral fat deposition and measures of plasma hormones related to body weight maintenance (i.e., insulin, leptin, and adiponectin). Chronic administration of Olz induced overweight, hyperphagia (at higher dose), and visceral fat accumulation. In contrast, OlzEt and OlzHomo presented a promising atypical antipsychotic compound, which did not induce weight gain, visceral adiposity, and metabolic dysregulation in rats. This study also reported an Olz-induced down-regulation of the H₁ receptor in the hypothalamus, which may be a key factor contributing to Olz-induced obesity/adiposity. However, OlzEt and OlzHomo did not alter H₁ receptor density in the hypothalamus, supporting the previous report that H₁ receptor affinity may predict the body weight gain liability of antipsychotics (Kroeze et al. 2003). Taken together, these findings provide a valid model for Olz-induced weight gain and metabolic alterations in female rats, and also suggested that OlzEt and OlzHomo represent promising antipsychotic agents characterized by a low propensity to induce metabolic side effects.

After having established their low propensity to induce weight gain and adiposity, the present study assessed the potential effectiveness of OlzEt and OlzHomo relevant to the treatment of schizophrenia. In an open-field behavioural test, pre-treatment of Olz and OlzEt showed a similar effectiveness for blocking PCP-induced hyperactivities in rats. However, OlzHomo showed a lower potency to inhibit these stereotype behaviours. This study also revealed that subchronic treatment with Olz and OlzEt induced a down-regulation in the binding capacities of D₂ and 5HT₂A
receptors in both saline and PCP treated animals. Down-regulation of these two receptors may contribute to blockade of PCP induced-hyperlocomotion, as observed with Olz and OlzEt treatment (Gleason and Shannon 1997; Gandolfi et al. 2003). A lower potency of OlzHomo to inhibit PCP-induced behaviours could also be explained by its lower affinity for the brain D₂ and 5HT₂A receptors compared to that of Olz and OlzEt. Hence, it was concluded that the therapeutic effectiveness of the OlzHomo regime may be delivered at higher dose than that of Olz and OlzEt treatment.

On the other hand, without appropriate comparison of DMPK (Drug Metabolism/Pharmacokinetics) properties between Olz, OlzEt, and OlzHomo the above conclusion could be an over-prediction. By providing more insight into the quantity of circulating compounds in the blood and the brain, further studies on the distribution coefficient (D) and the blood-brain barrier (BBB) values of OlzEt and OlzHomo could strengthen the results presented in this dissertation. Indeed, as a part of drug development process, it is crucial to investigate the preclinical toxicity, pharmacokinetics, and pharmacodynamics properties of the candidate drugs before risking and enhancing the possibility of further costly development.

5.2 Future Directions

Based on the findings presented in this thesis, recommendations for further study are listed below.
1) The present study supported the notion that H₁ receptor antagonism is a key factor in the mechanism of Olz-induced weight gain and adiposity. Nevertheless, it is likely that multiple receptors are involved in the emergence of weight gain following treatment with antipsychotics. In particular, there is much compelling evidence of the involvement of 5HT₂C receptors in food intake and body weight (reviewed in Coccurello and Moles 2010). It is reported that 5HT₂C antagonism or inverse agonism may contribute to Olz-induced weight gain (Kirk et al. 2009). Since this study showed that OlzEt and OlzHomo treatment did not produce weight gain and adiposity in rats, it would be of interest to examine the effect of these compounds on 5HT₂C receptors. The result of this study would provide more insight into the role of this receptor in antipsychotic-induced weight gain.

2) In this study the crucial role of reciprocal integration between hypothalamic-limbic areas and prefrontal cortex in regulation of food intake and body weight has been addressed. As briefly discussed, palatable food promotes activation of reward centre by hunger signalling in hypothalamic orexigenic networks and increases appetite, which can be modulated via reciprocal innervation with prefrontal cortex. It is suggested that the prevalence of obesity in patients with schizophrenia is strongly linked to the impairment of hypothalamic nutrient signalling and reward system as well as attenuated cognitive control from the hypofunctional prefrontal cortex (Elman et al. 2006). Additionally, it emerges that the effect of Olz treatment on ameliorating cognitive control and dopamine regulated reward aspect could not sufficiently counteract its obesogenic side effect. Further study would be valuable to determine whether the effect of OlzEt and OlzHomo treatments on the prefrontal cortex and reward system can favour the prevention of weight gain and adiposity.
3) This study mainly focused on the central mechanism involved in Olz-induced weight gain and adiposity, particularly the involvement of the \( H_1 \) receptor in body weight gain. However, the contribution of periphery factors (e.g. adipogenesis) in Olz-induced metabolic dysregulation should not be underestimated. For instance, it would be invaluable to examine the Olz, OlzEt, and Olz-mediated adipogenesis by testing their liability to express the transcription factor involved in the regulation of lipid homeostasis (e.g., SREBP) in liver and in adipocyte cell lines. This study would provide a better understanding of mechanisms underlying Olz-induced dysmetabolism.

4) In the present study, in contrast to Olz and OlzEt treatments which significantly blocked the PCP-induced hyperactivity, OlzHomo treatment showed a lower potency to alleviate these stereotype behaviours in rats. Hence, it was concluded that potential therapeutic effectiveness of an OlzHomo regime may be delivered at a higher dose than that at which Olz and OlzEt are administered. Therefore, further investigation of the effect of OlzHomo on inhibiting PCP-induced behaviour at higher doses (higher than 3 mg/kg) may produce a more conclusive result in future studies. Moreover, further behavioural tests on working memory and cognitive flexibility are required to shed light on the therapeutic effectiveness of both, OlzEt and OLzHomo, pertinent to the clinical picture of schizophrenia therapy.
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