The development of radiolabelled peripheral benzodiazepine receptor ligands for imaging cancer and neurodegenerative disorders

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The Development of Radiolabelled Peripheral Benzodiazepine
Receptor Ligands for Imaging Cancer and Neurodegenerative
Disorders

Taryn P Homes

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

Doctor of Philosophy
from
University of Wollongong

Department of Chemistry
December 2007
I, Taryn P. Homes, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Department of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institute.

Taryn P. Homes

December 2007
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My fiancé Brad Angel for his love and support
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<tbody>
<tr>
<td>$^{13}$C NMR</td>
<td>Carbon Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>$^1$H NMR</td>
<td>Proton Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>ACN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>AcOH</td>
<td>Acetic Acid</td>
</tr>
<tr>
<td>AIBN</td>
<td>Azobisisobutyronitrile</td>
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<tr>
<td>ANT</td>
<td>Adenine Nucleotide Translocase</td>
</tr>
<tr>
<td>Ar</td>
<td>Aryl</td>
</tr>
<tr>
<td>bs</td>
<td>Broad singlet</td>
</tr>
<tr>
<td>CAT</td>
<td>Chloramine-T</td>
</tr>
<tr>
<td>CBR</td>
<td>Central Benzodiazepine Receptor</td>
</tr>
<tr>
<td>CI</td>
<td>Chemical Ionisation</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>DBI</td>
<td>Diazepam Binding Inhibitor</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>dd</td>
<td>Doublet of doublets</td>
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<tr>
<td>ddd</td>
<td>Doublet of doublets of doublets</td>
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<tr>
<td>DEPT</td>
<td>Distortionless Enhancement by Polarisation Transfer</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
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<tr>
<td>dt</td>
<td>Doublet of triplets</td>
</tr>
<tr>
<td>EAE</td>
<td>Experimental Autoimmune Encephalomyelitis</td>
</tr>
<tr>
<td>EI</td>
<td>Electron Impact</td>
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ES  Electrospray
FDG  Fluorodeoxyglucose
GABA  Gamma-aminobutyric Acid
GIT  Gastrointestinal Tract
h  Hour
HPLC  High Performance Liquid Chromatography
HRMS  High Resolution Mass Spectrometry
Hz  Hertz
IC\textsubscript{50}  Inhibition Constant at 50%
ID/g  Injected dose per gram
IMM  Inner mitochondrial membrane
LE  Lupus Erythmatosus
M  Molar
m  Multiplet
MBq  Megabequerel
min  Minute
mL  Millilitre
mmol  Milli mol
mp.  Melting Point
MS  Mass Spectrometry
m/z  Mass/charge ratio
NBS  \(N\)-Bromosuccinimide
OMM  Outer mitochondrial membrane
PBR  Peripheral Benzodiazepine Receptor
PE  Petroleum Ether
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PPA</td>
<td>Polyphosphoric Acid</td>
</tr>
<tr>
<td>q</td>
<td>Quartet</td>
</tr>
<tr>
<td>qC</td>
<td>Quaternary Carbon</td>
</tr>
<tr>
<td>RCY</td>
<td>Radiochemical Yield</td>
</tr>
<tr>
<td>RT</td>
<td>Room Temperature</td>
</tr>
<tr>
<td>s</td>
<td>Singlet</td>
</tr>
<tr>
<td>SAR</td>
<td>Structure-Activity Relationship</td>
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<tr>
<td>SPECT</td>
<td>Single Photon Emission Computed Tomography</td>
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<tr>
<td>StAR</td>
<td>Steroidogenic acute regulatory protein</td>
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<tr>
<td>t</td>
<td>Triplet</td>
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<tr>
<td>TFA</td>
<td>Trifluoroacetic Acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
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<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
</tr>
<tr>
<td>Tris</td>
<td>Tris(Hydroxymethyl)aminomethane</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>VDAC</td>
<td>Voltage Dependent Anion Channel</td>
</tr>
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</table>
Publications/Presentations

Publications

- **Homes, T.P.**, Keller, P.A., Katsifis, A., Mattner, F. (2006); Synthesis and in vitro binding of $N,N$-dialkyl-2-phenylindol-3-ylglyoxylamides for the peripheral benzodiazepine binding sites; Bioorganic and Medicinal Chemistry; 14; 3938-3946

- **Homes, T.P.** Mattner, F. Keller, P.A, Katsifis, A. (2007); Synthesis and in vivo evaluation of a novel $[^{123}\text{I}]$ indolglyoxylamide for the Peripheral Benzodiazepine Binding Sites; Journal of Labelled Compounds and Radiopharmaceuticals; 50; S307


Oral Presentations

- **Homes, T.P.**, Keller, P.A., Katsifis, A. Synthesis and peripheral and central benzodiazepine receptor binding affinity of $N,N$-dialkyl-2-phenylindol-3-ylglyoxylamides. RACI Young Chemist’s Symposium, 3rd July 2005, University of Sydney

Poster Presentations

- **Homes, T.P.,** Keller, P.A., Katsifis, A., Mattner, F. (2006); Synthesis and evaluation of *N,N*-dialkyl-2-phenylindol-3-ylglyoxylamides for the study of peripheral benzodiazepine binding sites; 4th France – Australia Symposium on Nuclear Medicine, Melbourne

- **Homes, T.P.,** Mattner, F., Keller, P.A., Katsifis, A. (2007); Synthesis and in vivo evaluation of a novel $[^{123}\text{I}]$ indolglyoxylamide for the peripheral benzodiazepine binding sites; 17th International Symposium on Radiopharmaceutical Science, 29th April-3rd May, Aachen, Germany
Abstract

Three classes of compounds were chosen for investigation to find a high affinity and selective iodinated peripheral benzodiazepine receptor (PBR) ligand; indol-3-ylglyoxylamides, pyrazolopyrimidines, and pyridopyrrolooxazepines and pyrrolobenzoxazepines. These compounds were chosen from a literature search for their high PBR affinity and selectivity, ease of synthesis, and the potential for radioiodination.

Fifteen new halogenated \(N,N\)-dialkyldiol-3-ylglyoxylamides were synthesised and tested for their PBR and central benzodiazepine receptor (CBR) affinity. The compounds IC\(_{50}\) values for the PBR ranged from 7.8 – 618 nM, and a structure activity relationship (SAR) was determined. Brominated compounds had higher binding affinities than their iodinated analogues, and indoles with a chloro substituent on position 5 had higher binding affinities than the non-chlorinated compounds. The optimum alkyl chain length was found to be two carbons. The highest affinity iodinated ligand, with a PBR IC\(_{50}\) of 8.2 nM, was radiolabelled with \(^{123}\)I in 55-60% radiochemical yield and evaluated \textit{in vivo} in Sprague-Dawley rats. Biodistribution studies revealed high uptake of the radiotracer in organs known to contain PBR, such as the kidneys, adrenals, heart, liver and lungs. Drug competition studies showed that the PBR drugs PK11195 and Ro5-4864, when injected into the rat 5 min prior to injection of the radiotracer, significantly decreased uptake of radiotracer into those organs. The CBR drug, flumazenil, did not decrease the uptake of the radiotracer. Metabolite studies showed that the radiotracer was > 95% intact in the heart, kidneys, adrenals, and brain after 3 h and was 65% intact in the plasma. This compound is the first radiolabelled...
PBR ligand of this class, and is an excellent candidate for future studies and may lead to a clinically useful imaging agent.

Three pyrazolopyrimidines were synthesised, with lengths of the alkyl chains being methyl, ethyl, or propyl groups. The highest affinity ligand, with the propyl groups, displayed an IC$_{50}$ of 7.9 nM, however, only the compound with ethyl groups displaying an IC$_{50}$ of 11.7 nM was radiolabelled with $^{123}$I in 95% radiochemical yield, and evaluated in vivo in rats. This compound showed high uptake into organs known to contain PBR, and also showed an interesting result in which pre-administration of Ro5-4864 did not cause any significant decrease of uptake of radiotracer in the kidney or heart, however PK11195 did cause of significant decrease in these organs. This compound provides the first radioiodinated PBR ligand of this class.

Two pyrrolopyridooxazepines and two pyrrolobenzoxazepines were synthesised and tested. One of the compounds was found to be inactive, while the others had moderate PBR IC$_{50}$ values of 24-39 nM. The moderate binding affinity for these compounds would unlikely lead to a successful imaging agent.
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