Supplementary Material

Design, synthesis and characterization of novel gamma-aminobutyric acid type A receptor ligands

Kamal P. Pandey,¹ Md Zubair Ahmed Khan,¹ Lalit K. Golani,² Prithu Mondal, Md Yeunus Mian, Farjana Rashid, V. V. N. Phani Babu Tiruveedhula, Daniel E. Knutson, Dishary Sharmin, Taukir Ahmed, Sepideh Rezvanian, Nicolas M. Zahn, Leggy A. Arnold, Jeffrey M. Witkin, and James M. Cook*¹

* These authors contributed equally to this research

Department of Chemistry and Biochemistry, Milwaukee Institute for Drug Discovery, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin 53211, USA

E-mail: capncook@uwm.edu

Table of Contents

1. Figure S1. Showing halogen bond interactions ..........................................................S2
2. Method of molecular docking ..............................................................................S2
3. Chiral HPLC analysis report of oxazolines 5c and 5d ..............................................S3
4. Chiral HPLC analysis report of oxazoline 5c ..........................................................S6
5. Chiral HPLC analysis report of oxazoline 5d ..........................................................S7
6. ¹H NMR spectra of oxazoline 5 .............................................................................S8
7. ¹³C NMR spectra of oxazoline 5 .............................................................................S9
8. ¹H NMR spectra of oxazoline 5a ...........................................................................S10
9. ¹³C NMR spectra of oxazoline 5a ...........................................................................S11
10. ¹H NMR spectra of oxazoline 5b .........................................................................S12
11. ¹³C NMR spectra of oxazole 5b ...........................................................................S13
12. ¹H NMR spectra of oxazole 5c ............................................................................S14
13. ¹³C NMR spectra of oxazoline 5c ........................................................................S13
14. ¹H NMR spectra of oxazoline 5d ........................................................................S14
15. ¹³C NMR spectra of oxazoline 5d ........................................................................S15
16. ¹H NMR spectra of oxazoline 6 ...........................................................................S16
17. ¹³C NMR spectra of oxazoline 6 ...........................................................................S17
18. ¹H NMR spectra of oxazoline 6c .........................................................................S18
19. ¹³C NMR spectra of oxazole 6c ...........................................................................S19
20. References ...........................................................................................................S19
1. **Figure S1.** Showing halogen bond interactions

![Figure S1. Showing halogen bond interaction in complex of alprazolam (orange) with α1β3γ2L GABA<sub>A</sub> receptor (PDB: 6HUO) at α+γ interface benzodiazepine binding site [α1 (represented as chain D, color: aquamriane) and γ2 (represented as chain C, color: orchid)], dashed lines indicate halogen and hydrogen bonds.](image)

2. **Method of molecular docking:** The Ligand-protein interactions were analyzed by molecular docking using AutoDock Vina 1.5.6. The PDB file of the CryoEM structure of the human full-length α1β3γ2L GABA<sub>A</sub> receptor in the complex with alprazolam (6HUO) was downloaded from the Protein Data Bank and was prepared for docking by fixing missing bonds or atoms, adding polar hydrogens and assigning charges by AM1-BCC, and removing water molecules. The proteins were validated by first removing the bound ligand (alprazolam) and this was followed by docking it in the same binding site. The compounds were drawn, and energy minimized in Chimera.\(^1\) A grid size of 17 Å x 17 Å x 17 Å and centered at coordinates 152.80 (x), 163.02 (y), and 161.14 (z) were used. Illustrations of the 3D models were generated using Chimera and Python.\(^1\)\(^2\) The dockings were performed with standard search parameters and poses with the best scores were selected for the analysis.
3. Chiral HPLC analysis report of the racemic mixture of oxazolines 5c and 5d (10 % EtOH in Hexane)

![Analysis Report]

**<Sample Information>**

- **Sample Name**: racemic
- **Sample ID**: racemic_chiral 3-11-2020.lcm_10-12-20_002.lcd
- **Data Filename**: chiral 3-11-2020.lcm
- **Method Filename**: 10-12-20.lcb
- **Vial #**: Vial 2
- **Injection Volume**: 5 µL
- **Date Acquired**: 10/12/2020 3:26:34 PM
- **Date Processed**: 10/12/2020 7:42:38 PM

**<Chromatogram>**

![Chromatogram]

**<Peak Table>**

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret Time</th>
<th>Area%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.491</td>
<td>55.793</td>
</tr>
<tr>
<td>2</td>
<td>24.435</td>
<td>44.207</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100.000</td>
</tr>
</tbody>
</table>

**<Notes>**

- **Sample Type**: Unknown
- **Level**: 1
- **Acquired by**: Kamal Pandey
- **Processed by**: Kamal Pandey

© AUTHOR(S)
4. Chiral HPLC analysis report of oxazoline 5c (KPP-III-96B in 10 % EtOH in Hexane)

**Analysis Report**

**<Sample Information>**

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>KPP-III-96B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample ID</td>
<td></td>
</tr>
<tr>
<td>Data Filename</td>
<td>KPP-III-96B_chiral 3-11-2020.lcm_10-12-20_003.lcd</td>
</tr>
<tr>
<td>Method Filename</td>
<td>chiral 3-11-2020.lcm</td>
</tr>
<tr>
<td>Batch Filename</td>
<td>10-12-20.lcb</td>
</tr>
<tr>
<td>Vial #</td>
<td>Vial 3</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>5 µL</td>
</tr>
<tr>
<td>Date Acquired</td>
<td>10/12/2020 4:07:34 PM</td>
</tr>
<tr>
<td>Date Processed</td>
<td>10/13/2020 11:34:52 AM</td>
</tr>
<tr>
<td>Sample Type</td>
<td>Unknown</td>
</tr>
<tr>
<td>Level</td>
<td>1</td>
</tr>
<tr>
<td>Acquired by</td>
<td>Kamal Pandey</td>
</tr>
<tr>
<td>Processed by</td>
<td>Kamal Pandey</td>
</tr>
</tbody>
</table>

**<Chromatogram>**

![Chromatogram Graphic]

**<Peak Table>**

<table>
<thead>
<tr>
<th>Peak</th>
<th>Ret. Time</th>
<th>Area%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.551</td>
<td>0.005</td>
</tr>
<tr>
<td>2</td>
<td>20.786</td>
<td>0.004</td>
</tr>
<tr>
<td>3</td>
<td>24.499</td>
<td>99.992</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100.000</td>
</tr>
</tbody>
</table>

(3D)DAD Ch1 254nm KPP-III-96B_chiral 3-11-2020.lcm_10-12-20_003.lcd
5. Chiral HPLC analysis report of oxazoline 5d (KPP-IV-09 in 10 % EtOH in Hexane)

Analysis Report

<Sample Information>
Sample Name: KPP-IV-09
Sample ID: 
Data Filename: KPP-IV-09_chiral_3-11-2020.Icm_10-12-20_004.Icd
Method Filename: chiral_3-11-2020.Icm
Batch Filename: 10-12-20.Icb
Vial #: Vial 4
Injection Volume: 5 ul
Date Acquired: 10/12/2020 4:48:37 PM
Date Processed: 10/12/2020 7:09:47 PM
Sample Type: Unknown
Level: 1
Acquired by: Kamal Pandey
Processed by: Kamal Pandey

<Chromatogram>

![Chromatogram Image]

<Peak Table>

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.752</td>
<td>0.267</td>
</tr>
<tr>
<td>2</td>
<td>19.412</td>
<td>99.733</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100.000</td>
</tr>
</tbody>
</table>

(3D)DAD Ch1 254nm
KPP-IV-09_chiral_3-11-2020.Icm_10-12-20_004.Icd
6. $^1$H NMR of oxazoline 5 (CDCl$_3$)
7. $^{13}$C NMR of oxazoline 5 (CDCl3)
8. $^1$H NMR of oxazoline 5a (CDCl₃)
9. $^{13}$C NMR of oxazoline 5a (CDCl3)
10. $^1$H NMR of oxazoline $5b$ (CDCl$_3$)
11. $^{13}$C NMR of oxazoline 5b (CDCl3)
21. $^1$H NMR of oxazoline 5c (CDCl3)
22. $^{13}$C NMR of oxazoline 5c (CDCl$_3$)
14. $^1$H NMR of oxazoline 5d (CDCl$_3$)
15. $^{13}$C NMR of oxazoline 5d (CDCl₃)
16. $^1$H NMR of oxazole 6 (CDCl$_3$)
17. $^{13}$C NMR of oxazole 6 (CDCl3)
18. $^1$H NMR of oxazole 6c (CDCl3)
19. $^{13}$C NMR of oxazole 6c (CDCl3)

References
