HHS Public Access

Author manuscript

Bioorg Med Chem Lett. Author manuscript; available in PMC 2019 September 01.

Published in final edited form as:

Bioorg Med Chem Lett. 2018 September 01; 28(16): 2697–2700. doi:10.1016/j.bmcl.2018.04.003.

Agonists of the γ-Aminobutyric Acid Type B ($GABA_B$) Receptor Derived from β-Hydroxy and β-Amino Difluoromethyl Ketones

Munia F. Sowaileh[a], **Amy E. Salyer**[b], **Kuldeep K. Roy**[a,c], **Jinu P. John**[b,d], **James R. Woods**[b,e], **Robert J. Doerksen**[a], **Gregory H. Hockerman**[b], and **David A. Colby**[a]

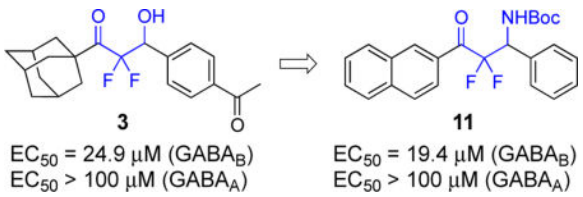
[a]Department of BioMolecular Sciences, University of Mississippi, University, Mississippi 38677, United States

[b]Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, Indiana 47907, United States

Abstract

β-Hydroxy difluoromethyl ketones represent the newest class of agonists of the $GABA_B$ receptor, and they are structurally distinct from all other known agonists at this receptor because they do not display the carboxylic acid or amino group of γ-aminobutyric acid (GABA). In this report, the design, synthesis, and biological evaluation of additional analogues of β-hydroxy difluoromethyl ketones characterized the critical nature of the substituted aromatic group on the lead compound. The importance of these new data is interpreted by docking studies using the X-ray structure of the $GABA_B$ receptor. Moreover, we also report that the synthesis and biological evaluation of β-amino difluoromethyl ketones provided the most potent compound across these two series.

Graphical abstract



Keywords

GABA; receptor; fluorine; agonist; $GABA_B$; gamma-aminobutyric acid

[c]Present address: National Institute of Pharmaceutical Education and Research, 4, Raja S. C. Mullick Road, Jadavpur, Kolkata 700 032, WB, India

[d]Present address: AmbioPharm, Inc., North Augusta, South Carolina 29842, United States

[e]Present address: Alkermes, Inc., Waltham, Massachusetts 02451, United States

Supplementary Material

Experimental procedures; molecular modeling procedures and supporting results; and ^{1}H, ^{19}F, and ^{13}C NMR spectra. Supplementary data associated with this article can be found in the online version.

The inhibitory neurotransmitter, γ-aminobutyric acid (GABA), reduces the excitability of neurons and assists in the regulation of other neurotransmitters, especially in the central nervous system. The two major types of GABA receptors are $GABA_A$ and $GABA_B$ and both are validated targets for drug discovery.[1] The $GABA_A$ receptors are ion channels and can be controlled by three classes of pharmaceuticals, the barbiturates, the benzodiazepines, and the newer non-benzodiazepine sedatives, such as zaleplon and zopiclone.[2] The $GABA_B$ receptors are metabotropic G-protein-coupled receptors, and serve as the target of the muscle relaxant, baclofen.[3] Moreover, the $GABA_B$ receptors are the focus of drug discovery efforts in muscle spasticity disorders,[3] schizophrenia,[4] pain,[5] and gastroesophageal reflux disease (GERD).[6] Agonists,[7] antagonists,[8] positive allosteric modulators,[9,10] and negative allosteric modulators[11] of the $GABA_B$ receptor are known, and in 2013, the X-ray structures of this receptor in the ligand-free state and in the presence of agonists and antagonists were reported.[12]

Nearly all of the known agonists of the $GABA_B$ receptor display the structure of GABA, and, for example, baclofen is the 3-*para*-chlorophenyl analogue of GABA (Figure 2).[13] Although the pharmaceutical formulation of baclofen is a racemic mixture, the (*R*)-(−)-enantiomer is significantly more active. The 3-phenyl derivative is the agent, phenibut, and in a similar fashion, (*R*)-(−)-phenibut is the more active enantiomer.[14] Also, the 2-chlorothienyl group is a surrogate for the *para*-chlorophenyl group of baclofen and is displayed in $GABA_B$ agonist **1**.[15] Other key analogues of baclofen, which are also agonists of the $GABA_B$ receptor, are the pyridinyl methoxy derivative **2**,[16] (*R*)-(−)-GABOB,[17] and CGP44532.[18] The only known agonist of the $GABA_B$ receptor that does not display the backbone of GABA or baclofen is the difluoromethyl ketone **3**.[19]

The β-hydroxy difluoromethyl ketones are a distinct group of ligands of the $GABA_B$ receptor and were first reported in 2013.[19] The structure–activity relationships for the lead compound **3** in this series are that the fluorines and the β-hydroxy substituent are required for activity (Scheme 1).[19] Specifically, the non-fluorinated analogue of **3** is inactive and methyl ether derivative created from methylation of the β-hydroxy substituent of **3** is also inactive. The bulky, lipophilic adamantyl group or naphthyl group is common in other active derivatives. On the other hand, the *para*-acetyl phenyl group of **3** tolerates many other structures such as alkyl, alkenyl, and aryl groups. Although these data are insightful, the agent **3** presents additional opportunities to identify new structure-activity relationships for agonist activity for the $GABA_B$ receptor. In the present study, we have prepared new β-hydroxy difluoromethyl ketones and characterized the β-amino difluoromethyl ketones as another complementary scaffold.

These unique compounds were discovered following the development of a new synthetic protocol that uses pentafluoro-*gem*-diols to produce difluoroenolates for aldol reactions[20,21] and was later extended to imino-aldol reactions.[22,23] The first objective was to define the role of substituents on the *para*-acetylphenyl group of **3**. Compounds **4**–**6** were synthesized,[24] and each displays a change to the *para*-acetyl group on the phenyl ring (Figure 3). Compound **7** was prepared to understand if a heteroaromatic group could replace the adamantyl group. The analogue **8** bears a larger isoindolinedione to replace the *para*-acetyl phenyl group. Next, the β-amino difluoromethyl ketones **10**–**14** were synthesized using the

literature methods.[22,23] The naphthyl and adamantyl groups were conserved (i.e., **10**–**11** and **12**, respectively) and the *N*-phenylsulfonyl and *N*-*tert*-butylcarbamate groups were selected as substituents for the amine.

In 2018, we reported a procedure in which unactivated imines bearing *N*-benzyl, aryl, or alkyl group reacted with difluoroenolates generated from pentafluoro-*gem*-diols in the presence of magnesium salts.[25] This process enables the creation of additional β-amino difluoromethyl ketones displaying a *N*-benzyl group to complement those bearing *N*-phenylsulfonyl and *N*-*tert*-butylcarbamate group (Figure 4). The naphthyl derivative **15** completes the series from **10** and **11**, and compound **16** and **17** were prepared to obtained additional structure-activity data.[25]

Compounds **4**–**17** were tested for agonist activity at the $GABA_A$ and $GABA_B$ receptors according to the previously reported procedures.[19] The screen for agonist activity at the $GABA_B$ receptor is conducted in a HEK293/Cre-luc cell line expressing both subunits of the human $GABA_B$ receptor. Activation of the $GABA_B$ receptor accesses an endogenous signaling pathway and results in the inhibition of cAMP production. In this assay, forskolin is applied to stimulate cAMP production, and inhibition of the forskolin-stimulated cAMP production is measured (assays are performed in triplicate). All of the compounds that display activity at the $GABA_B$ receptor are also screened for agonist activity at the $GABA_A$ receptor using a whole-cell voltage-clamp method with HEK293 cells transfected with the $\alpha_1\beta_2\gamma_2$ subunits of the rat $GABA_A$ receptor. The pharmaceutical, baclofen, is a racemic mixture and selective agonist for the $GABA_B$ receptor; however, most of its pharmacological activity results from the (−)-baclofen enantiomer.[19] In a similar fashion, the lead compound **3** also has more agonist activity from a single enantiomer (i.e., the (+)-**3**-enantiomer); however, an absolute stereochemical assignment of secondary alcohol was not reported.[19] The fluorinated compounds **4**–**17** were all tested as racemic mixtures. The β-hydroxy difluoromethyl ketones **4**–**8** do not display any observable activity, even at concentrations up to 100 μM. These results demonstrate the low tolerance for changes to the *para*-acetyl phenyl group, especially as the replacement of the acetyl with an ethyl group produces an inactive compound (i.e., compound **4**). On the other hand, some structural variation is allowed because our prior studies validated that the styrene **9** displays activity of 40 μM compared to the lead compound **3** at 24.9 μM at the $GABA_B$ receptor.[19] The β-amino difluoromethyl ketone **11** was characterized as the most active agent across the entire difluoromethyl ketone series. The β-amino difluoromethyl ketone **11** displays EC_{50} = 19.4 μM, and, notably, when the *N*-Boc group in **11** is replaced with *N*-SO_2Ph in **10** or with *N*-Bn in **15**, agonist activity decreases to EC_{50} = 32.7 μM or EC_{50} = 91 μM, respectively. All three of these molecules display no activity at the $GABA_A$ receptor, which correlates well with the β-hydroxy difluoromethyl ketones, which also preferential for the $GABA_B$ receptor (over the $GABA_A$ receptor). The other β-amino analogues **12**–**14** and **16** were inactive at the $GABA_B$ receptor. The para-chlorophenyl derivative **17** displays moderate activity at the $GABA_B$ receptor.

Additional biological characterizations of compounds **3** and **11** were performed using a radio-ligand binding assay screen with cloned neurotransmitter receptors and transporters (Table 2).[26] Difluoromethyl ketones are known in the literature to display activity at many

targets,[27,28] so these screens provide an important perspective on the relative role of these ligand for the $GABA_B$ receptor over other neurotransmitter receptors. Adrenoreceptors and biogenic amine, dopamine, opioid, histamine, muscarinic, serotonin, and sigma receptors were examined, and interestingly, both **3** and **11** display less than 30% inhibition and are considered inactive in all of these assays. These data strengthen the knowledge of the preference of this class of compounds (i.e., β-hydroxy difluoromethyl ketone and β-amino difluoromethyl ketone) for the $GABA_B$ receptor.

In order to integrate the new structure–activity relationships with the prior data, both enantiomers of the lead compound **3** were docked into the (*R*)-baclofen-bound $GABA_B$ receptor complex (PDB ID: 4MS4),[12] using the Induced Fit Docking protocol of the Schrödinger small molecule drug discovery suite.[24] The difluoromethyl ketone **3** is hydrated in the model, because difluoromethyl ketones are known to exist in the hydrated (*gem*-diol) form at physiological pH.[19] The respective *gem*-diol, in turn, occupies a similar site as the carboxylate of baclofen. Hydrogen-bonding is conserved with Ser130 and Gly171 (as observed with GABA-bound X-ray structure, PDB-ID:4MS3) and the *gem*-diol maintains a close proximity to the Ser153 (Figure 5). The β-hydroxy substituent in both the (*R*)-**3** and (*S*)-**3** alcohols cannot occupy the same site as the amino group of baclofen due to the shorter intramolecular distance from the difluoromethyl ketone group. However, the *para*-acetylphenyl group of both the (*R*)-**3** and (*S*)-**3** compounds employs a similar site as the *para*-chlorophenyl group of baclofen and maintains key interactions with the Trp278 residue. Lastly, docking studies present potential docking poses for **3** that accommodate the bulky adamantyl group within the ligand binding site of the $GABA_B$ receptor.

We attempted to dock both enantiomers of **10** and **11** into the $GABA_B$ receptor using both SP and XP Induced Fit Docking, and the methods gave similar results, so we report the XP ones. The sulfonamide group of (*S*)-**10** is engaged in hydrogen-bonding with Ser130 whereas the *gem*-diol of (*S*)-**11** is hydrogen-bonded to the Ser130 and Gly151, and both these functional groups lie in close proximity to Ser153 (Figure 5). The phenyl group attached to the stereogenic carbon in (*S*)-**10** occupies the same site as the amino group of baclofen. By contrast, the naphthalene ring of (*S*)-**11** occupies this site. Moreover, the naphthyl group of (*S*)-**10** is in the site occupied by the *para*-chlorophenyl group of baclofen, whereas for (*S*)-**11** this site is occupied by its phenyl group (Figure 6). (*R*)-**10** failed to dock into the binding site in several trials with different grid settings. The docked pose of (*R*)-**11** (GlideScore = −8.500) is provided in the supplementary information. Hence, we predict that the (*S*)-enantiomers are the more active for **10** and **11**.

The difluoromethyl ketones represent the newest class of agonists of the $GABA_B$ receptor, and further investigations through synthesis, biological evaluation, and computational analysis have not only revealed that enhancements in potency are possible but also that the structure–activity relationships are distinct from other classes of agonists that display the structure of GABA. The critical nature of the substituent on the aromatic group of the β-hydroxy difluoromethyl ketone was characterized. Also, the identification of the β-amino difluoromethyl ketones as potent agonists of the $GABA_B$ receptor is another significant advance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge funding from the University of Mississippi, the Ralph W. and Grace M. Showalter Research Trust, and the National Institute of General Medical Sciences (Grant P20GM104932). Its contents are solely the responsibility of the authors and do not necessarily represent the official view of the National Institutes of Health (NIH). Receptor binding profiles was generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2013-00017-C (NIMH PDSP). The NIMH PDSP is Directed by Bryan L. Roth, MD, PhD at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at NIMH, Bethesda MD, USA.

References and notes

1. Froestl W. Future Med Chem. 2011; 3:163–175. [PubMed: 21428811]
2. Hanson SM, Morlock EV, Satyshur KA, Czajkowski CJ. Med Chem. 2008; 51:7243–7252.
3. Froestl W. Adv Pharmacol. 2010; 58:19–62. [PubMed: 20655477]
4. Mizukami K, Sasaki M, Ishikawa M, Iwakiri M, Hidaka S, Shiraishi H, Iritani S. Neurosci Lett. 2000; 283:101–104. [PubMed: 10739885]
5. Balerio GN, Rubio MC. Pharmacol Res. 2002; 46:281–286. [PubMed: 12220972]
6. Alstermar C, Amin K, Dinn SR, Elebrin T, Fjellström O, Fitzpatrick K, Geiss WB, Gottfries J, Guzzo PR, Harding JP, Holmén A, Kothare M, Lehmann A, Mattsson JP, Nilsson K, Sundén G, Swanson M, von Unge S, Woo AM, Wyle MJ, Zheng X. J Med Chem. 2008; 51:4315–4320. [PubMed: 18578471]
7. Froestl W, Mickel SJ, Hall RG, von Sprecher G, Strub D, Baumann PA, Brugger F, Gentsch C, Jaekel J. J Med Chem. 1995; 38:3297–3312. [PubMed: 7650684]
8. Froestl W, Mickel SJ, von Sprecher G, Diel PJ, Hall RG, Maier L, Strub D, Melillo V, Baumann PA. J Med Chem. 1995; 38:3313–3331. [PubMed: 7650685]
9. Guery S, Floersheim P, Kaupmann K, Froestl W. Bioorg Med Chem Lett. 2007; 17:6206–6211. [PubMed: 17884493]
10. Mugnaini C, Pedani V, Casu A, Lobina C, Casti A, Maccioni P, Porcu A, Giunta D, Lamponi S, Solinas M, Dragoni S, Valoti M, Colombo G, Castelli MP, Gessa GL, Corelli F. J Med Chem. 2013; 56:3620–3635. [PubMed: 23544432]
11. Chen L-H, Sun B, Zhang Y, Xu T-J, Xia Z-X, Liu J-F, Nan F-J. ACS Med Chem Lett. 2014; 5:742–747. [PubMed: 25050158]
12. Geng Y, Bush M, Mosyak L, Wang F, Fan QR. Nature. 2013; 504:254–259. [PubMed: 24305054]
13. Brown KM, Roy KK, Hockerman GH, Doerksen RJ, Colby DA. J Med Chem. 2015; 58:6336–6347. [PubMed: 25856547]
14. Dambrova M, Zvejniece L, Liepinsh E, Cirule H, Zharkova O, Veinberg G, Kalvinsh I. Eur J Pharmacol. 2008; 583:128–134. [PubMed: 18275958]
15. Pirard B, Carrupt P-A, Testa B, Tsai R-S, Berthelot P, Vaccher C, Debaert M, Durant F. Bioorg Med Chem. 1995; 3:1537–1545. [PubMed: 8634834]
16. Xu F, Peng G, Phan T, Dilip U, Chen JL, Chernov-Rogan T, Zhang X, Grindstaff K, Annamalai T, Koller K, Gallop MA, Wustrow DJ. Bioorg Med Chem Lett. 2011; 21:6582–6585. [PubMed: 21920749]
17. Hinton T, Chebib M, Johnston GAR. Bioorg Med Chem Lett. 2008; 18:402–404. [PubMed: 17981464]
18. Ong J, Bexis S, Marino V, Parker DAS, Kerr DIB, Froestl W. Eur J Pharmacol. 2001; 412:27–37. [PubMed: 11166733]
19. Han C, Salyer AE, Kim EH, Jiang X, Jarrard RE, Powers MS, Kirchhoff AM, Salvador TK, Chester JA, Hockerman GH, Colby DA. J Med Chem. 2013; 56:2456–2465. [PubMed: 23428109]
20. Han C, Kim EH, Colby DA. J Am Chem Soc. 2011; 133:5802–5805. [PubMed: 21443226]

21. Han C, Kim EH, Colby DA. Synlett. 2012; 23:1559–1563.
22. Xie C, Wu L, Mei H, Soloshonok VA, Han J, Pan Y. Tetrahedron Lett. 2014; 55:5908–5910.
23. Xie C, Wu L, Zhou J, Mei H, Soloshonok VA, Han J, Pan YJ. Fluorine Chem. 2015; 172:13–21.
24. See Supplementary Material for details
25. Nguyen AL, Khatri HR, Woods JR, Baldwin CS, Fronczek FR, Colby DA. J Org Chem. 2018; 83:3109–3118. [PubMed: 29446944]
26. Besnard J, Ruda GF, Setola V, Abecassis K, Rodriguiz RM, Huang XP, Norval S, Sassano MF, Shin AI, Webster LA, Simeons FR, Stojanovski L, Prat A, Seidah NG, Constam DB, Bickerton GR, Read KD, Wetsel WC, Gilbert IH, Roth BL, Hopkins AL. Nature. 2012; 492:212–220.
27. Fä C, Hardegger LA, Baitsch L, Schweizer WB, Meyer S, Bur D, Diederich F. Org Biomol Chem. 2009; 7:3947–3957. [PubMed: 19763297]
28. Moore CL, Leatherwood DD, Diehl TS, Selkoe DJ, Wolfe MS. J Med Chem. 2000; 43:3434–3442. [PubMed: 10978191]

Highlights

- β-Hydroxy difluoromethyl ketones have distinct SAR data at the $GABA_B$ receptor
- β-Amino difluoromethyl ketones are now characterized as $GABA_B$ agonists
- Docking studies at the $GABA_B$ receptor suggest similar binding modes to baclofen

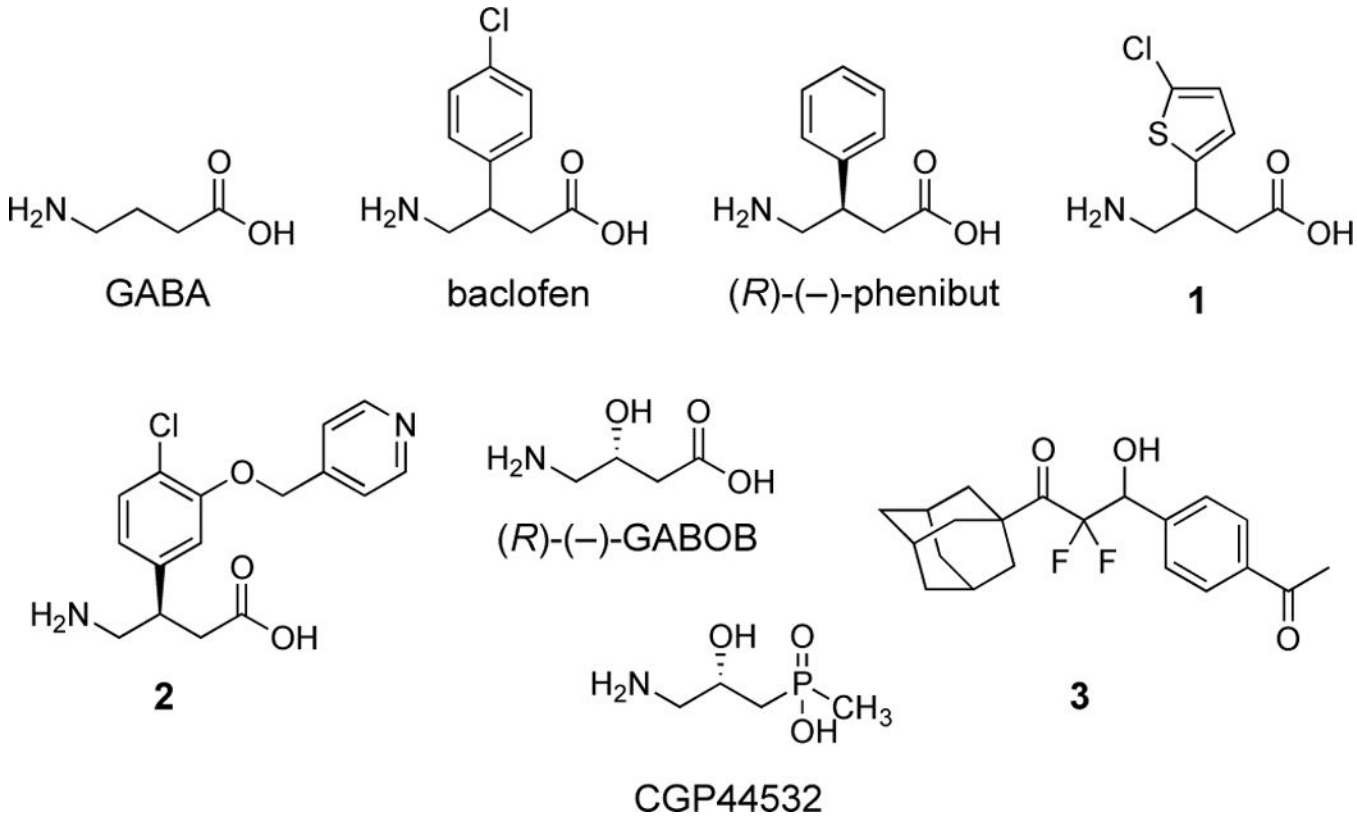


Figure 1.
Structures of some known agonists of the $GABA_B$ receptor.

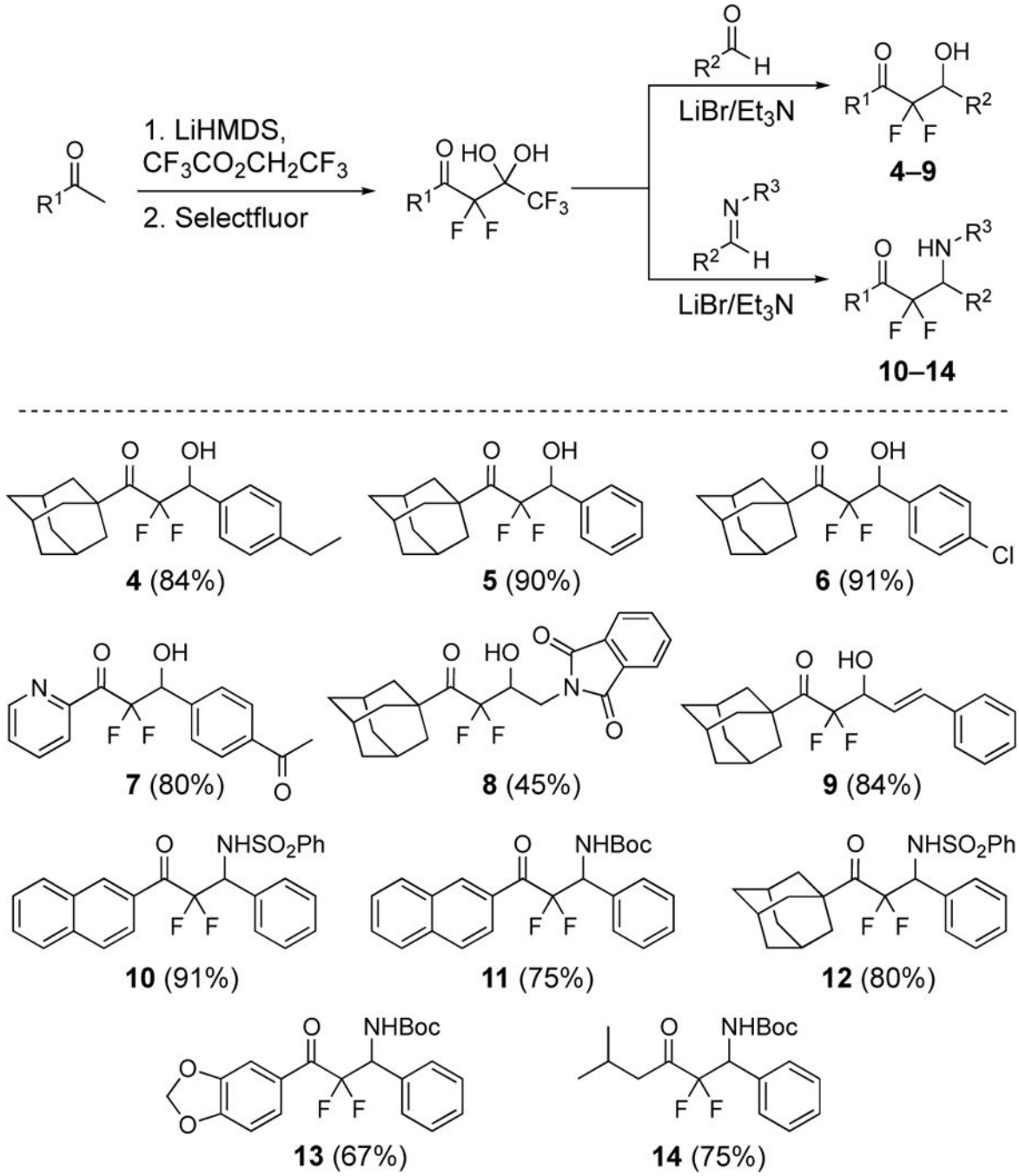


Figure 2.

Preparation of β-hydroxy difluoromethyl ketones **4**–**9** and β-amino difluoromethyl ketones **10**–**14**. Isolated yields for the aldol and imino-aldol reactions are given in parenthesis.

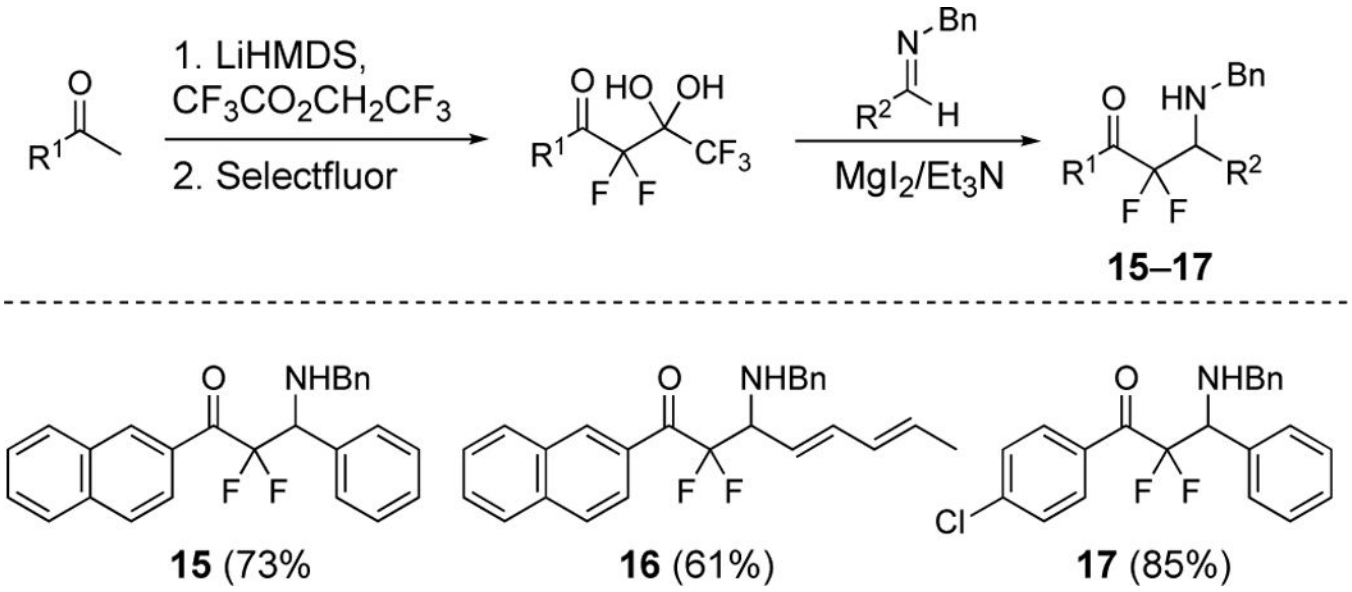


Figure 3.

Synthesis of β-amino difluoromethyl ketones **15**, **16**, and **17**. Isolated yields for the imino-aldol reactions are given in parenthesis. See ref. 25.

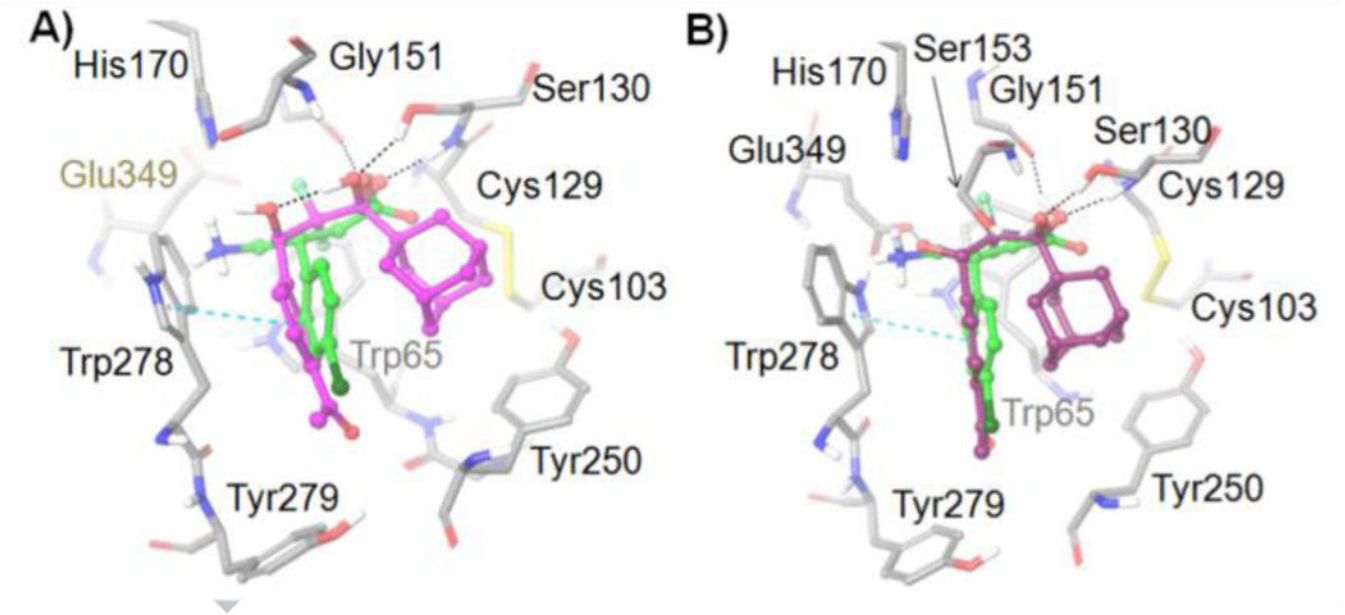


Figure 4.

Active site residues (gray) of the $GABA_B$ receptor from docking studies with: A) (*R*)-**3** (GlideScore = −10.080) (magenta) and B) (*S*)-**3** (GlideScore = −9.606) (purple). (*R*)-Baclofen (GlideScore = −11.063) is also shown in each image (green). Amino acids are depicted as tubes and ligands are represented as ball-and-stick. H-bonds and aromatic **π**–**π** stacking are shown as black and cyan dashed lines, respectively.

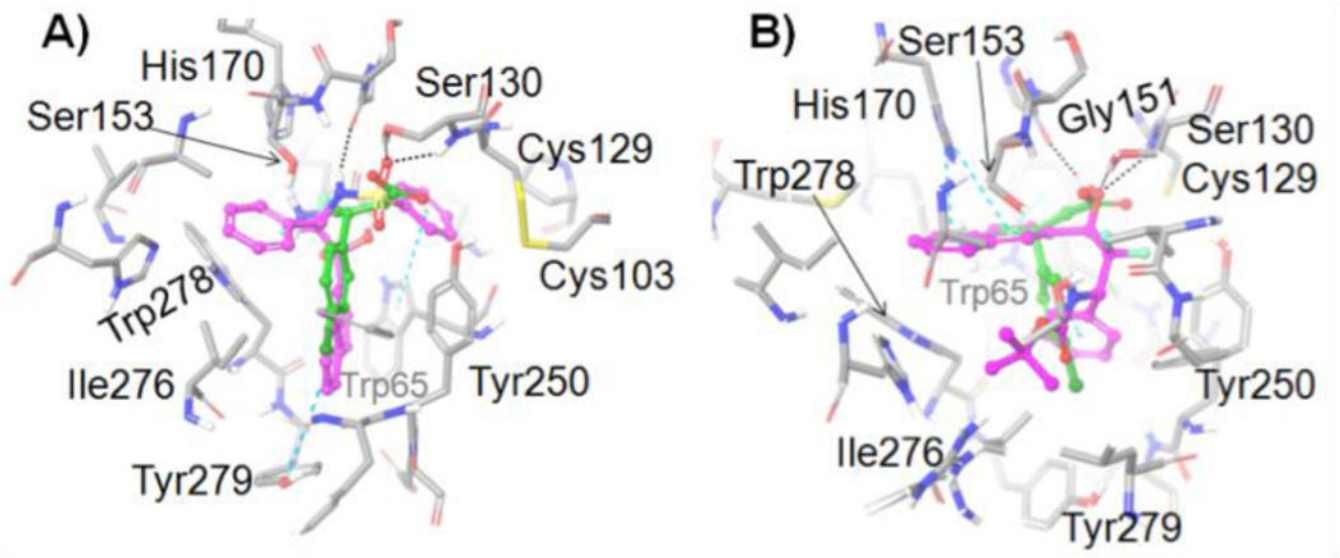
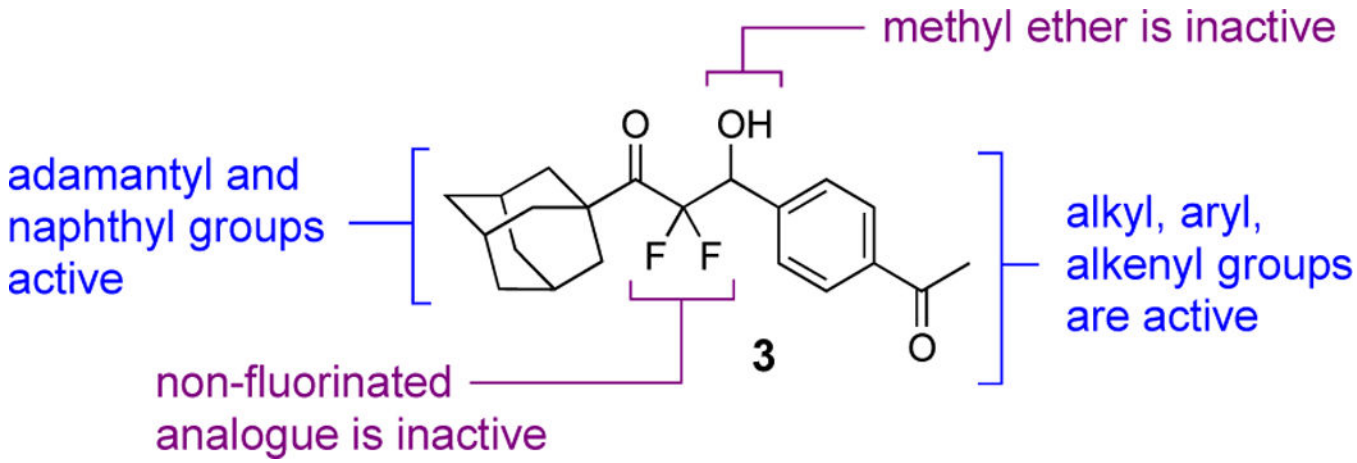


Figure 5.

Active site residues (gray) of the $GABA_B$ receptor from docking studies with: A) (*S*)-**10** (GlideScore = −11.037) (magenta) and B) (*S*)-**11** (GlideScore = −10.592) (magenta). (*R*)-Baclofen is also shown in each image (green). Amino acids are depicted as tubes and ligands are represented as ball-and-stick. H-bonds and aromatic **π**–**π** stacking are shown as black and cyan dashed lines, respectively.



Scheme 1.

Structure–activity relationships for compd **3** and agonist activity at the $GABA_B$ receptor.

Table 1

$GABA_A$ and $GABA_B$ assay data for baclofen and compds **3**–**17.**

compd	$GABA_B$ EC_{50} (μM)[a]	$GABA_A$ EC_{50} (μM)[a]
GABA[c]	0.53 ± 0.33	2.30 ± 0.59
(±)-baclofen[c]	1.7 ± 0.10	>100
(±)-**3**[b]	24.9 ± 1.30	>100
(−)-**3**[b]	37.8 ± 0.78	nd[c]
(+)-**3**[b]	15.9 ± 1.84	nd
4	>100[d]	nd
5	>100[d]	nd
6	>100[d]	nd
7	>100	nd
8	>100	nd
9[c]	40 ± 3.59	>100
10	32.7 ± 1.44	>100
11	19.4 ± 7.69	>100
12	>100	nd
13	>100	nd
14	>100	nd
15	91 ± 5.7	>100
16	>100	nd
17	57.4 ± 2.2	>100

[a] Values are given with the standard error.

[b] Ref 19.

[c] nd is not determined.

[d] Cytotoxic in assay at 50 μM and 100 μM doses.

Table 2

Radio-ligand binding assay screen for compds **3** and **11** in cloned receptors and transporters. Both **3** and **11** are inactive in all assays.

Adrenoreceptors	α_{1A}, α_{1B}, $\alpha_{1\Delta}$, α_{2A}, β_1, β_2, β_3
Biogenic amine transporters	DAT, NET, SERT
Dopamine receptors	D_1, D_2, D_3, D_4, D_5
Opioid receptors	DOR, KOR, MOR
Histamine receptors	H_1, H_2, H_3, H_4
Muscarinic receptors	M_1, M_2, M_3, M_4, MS
Serotonin receptors	5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_{5A}, 5-HT_6, 5-HT_7
Sigma receptors	r$Sigma_1$, r$Sigma_2$