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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], David A. Colby [a,*]

[a] Department of BioMolecular Sciences, University of Mississippi, University, MS 38677, United States
[b] Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907, United States

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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.

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 (Fig. 1).[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

* Corresponding author.
E-mail address: dacolby@olemiss.edu (D.A. Colby).
[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, SC 29842, United States.
[e] Present address: Alkermes, Inc., Waltham, MA 02451, United States.

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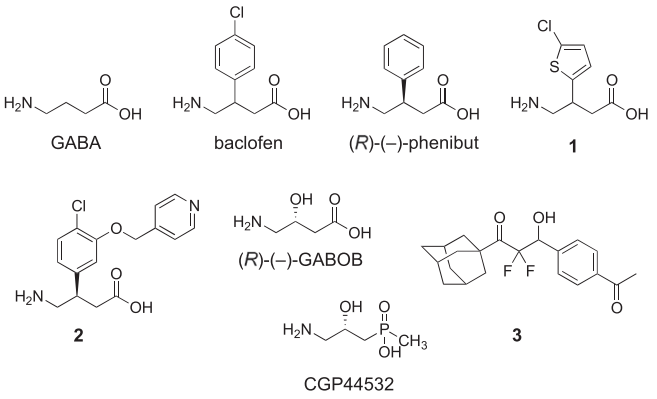
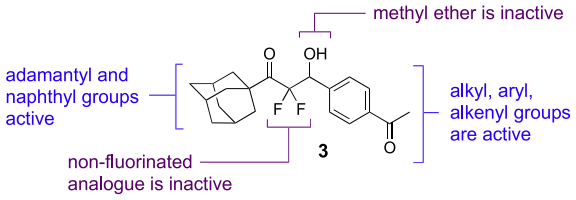


Fig. 1. Structures of some known agonists of the $GABA_B$ receptor.



Scheme 1. Structure-activity relationships for compd **3** and agonist activity at the $GABA_B$ receptor.

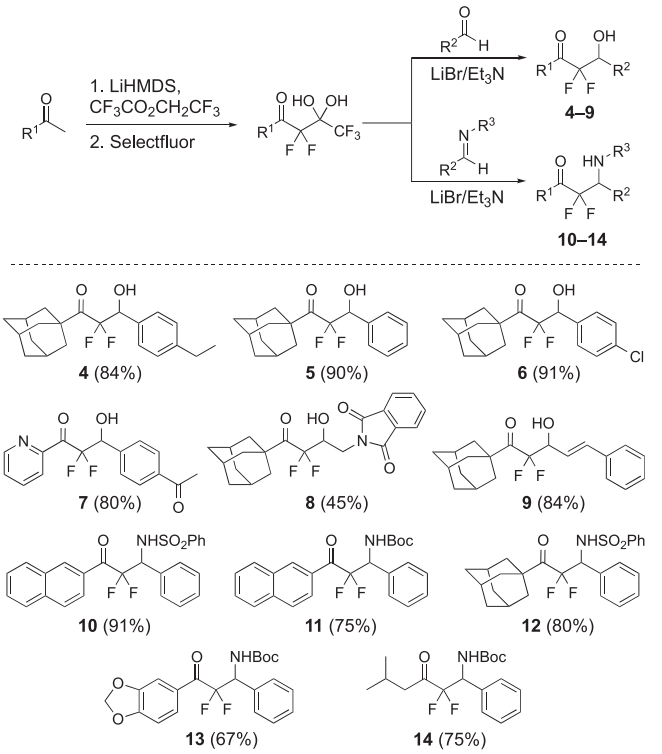


Fig. 2. Preparation of β-hydroxy difluoromethyl ketones **4**–**9** and β-amino difluoromethyl ketones **10**–**14**. Isolated yields for the aldol and imino-aldol reactions are given in parenthesis.

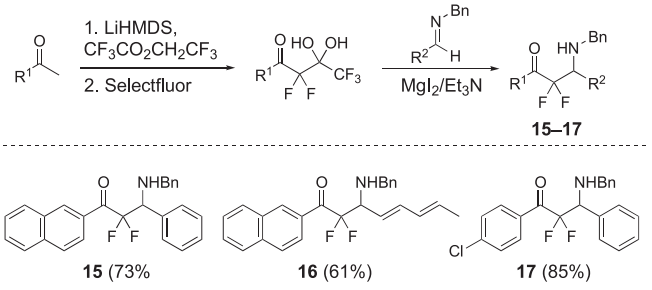


Fig. 3. Synthesis of β-amino difluoromethyl ketones **15**, **16**, and **17**. Isolated yields for the imino-aldol reactions are given in parenthesis. See Ref. 25.

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 (Fig. 2). 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, *N*-aryl, or *N*-alkyl groups 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 (Fig. 3). The naphthyl derivative **15** completes the series from **10** and **11**, and compounds **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 (See Table 1).[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