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Synthesis and activity of functionalizable derivatives of the serotonin (5-HT) 5-HT_{2A} receptor (5-HT_{2A}R) antagonist M100907

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ABSTRACT

The approach of tethering together two known receptor ligands, to be used as molecular probes for the study of G protein-coupled receptor (GPCR) systems, has proven to be a valuable approach. Selective ligands that possess functionality that can be used to link to other ligands, are useful in the development of novel antagonists and agonists. Such molecules can also be attached to reporter molecules, such as fluorophores, for the study of GPCR dimerization and its role in signaling. The highly selective serotonin (5-HT) 5-HT_{2A} receptor (5-HT_{2A}R) antagonist M100907 (volinanserin) is of clinical interest in the treatment of neurological and mental health disorders. Here, we synthesized the most active (+)-M100907 enantiomer as well as a series of derivatives that possessed either an alkyne or an azide. The triazole resulting from the dipolar cycloaddition of these groups did not interfere with the ability of the bivalent ligand to act as an antagonist. Thus, we have synthesized a number of compounds which will prove useful in elucidating the role of the 5-HT_{2A}R in the central nervous system.

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M100907 (**1**), also known as volinanserin, is a highly selective 5-HT_{2A}R receptor antagonist developed initially by Sanofi-Aventis for the treatment of schizophrenia[1] and sleep disorders.[2] Our group reported that M100907 derivatives substituted at the methoxy group of the catechol ring retain the 5-HT_{2A}R antagonist activity with either the ketone (**2**) or racemic hydroxyl group (**3**) at the benzylic position.[3,4] Reported here is the installation of a polyethylene glycol (PEG) linker substituted at the methoxy group of M100907 and the chiral resolution of the molecule to provide a version of M100907 possessing an ether tether that is terminated with an azide (**4**) or an alkyne (**5**). The azide on (**4**) will be used for future connection to other molecules such as fluorophores, affinity tags and other receptor ligands (Fig. 1).[5,6]

The synthetic route to M100907 developed by Rice[7] was utilized, however, the chiral resolution was carried out at an earlier stage to provide the possibility of introducing different substituents onto the piperidinyl group. The conditions for this resolution were different from previously reported.[7]

The route to M100907-azide **4**, began with the protection of commercially available guaiacol (Scheme 1). Guaiacol was reacted with TBDPSCl, imidazole and catalytic amount of DMAP at room temperature for 24 h to generate compound **6** in 98% yield. The silyl protected **6** was regioselectively *ortho*-lithiated by *n*-butyl lithium with TMEDA for 2 h at room temperature. Then Weinreb amide **7** was added at -70 °C and the mixture was stirred at room temperature for 21 h to produce the ketone **8**. Following reaction with the lithiated **6**, the Boc group of **8** was removed with TFA to give **9**. Sodium borohydride reduction then provided the racemic alcohol **10** (Scheme 1).

Weinreb amide **7** was synthesized from isonipecotic acid (Scheme 2). BOC protection of isonipecotic acid by reaction of ditertbutyldicarbonate, in a mixed solvent of 1,4-dioxane, acetonitrile and water in the presence of 1 N NaOH gave compound **12** which was then reacted with *N,O*-dimethylhydroxylamine hydrochloride, and the coupling reagent HBTU, in the presence of DIPEA to give the desired Weinreb amide **7** in ~80% yield over the two steps.

For the chiral resolution of a later synthetic intermediate by the Rice group,[7] methanol was used as the solvent to obtain the resolved salt with (*R*)-mandelic acid. However, the solubility of the diastereomeric salt formed from compound **10** and (*R*)-mandelic acid was sufficiently high in methanol that the yield of recovered material was low. Using a 1:1 ratio of acetonitrile:methanol for the first recrystallization and 1:2 ratio for the second

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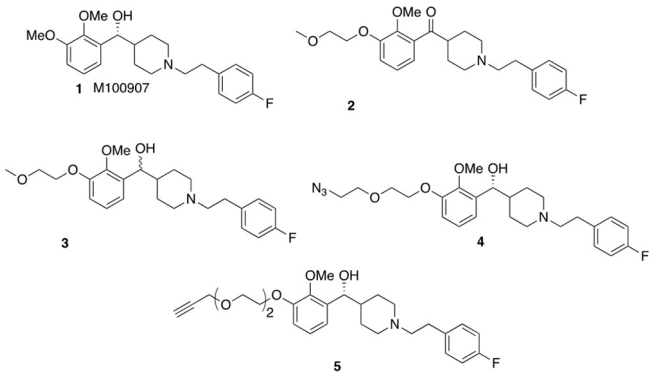


Fig. 1. Structures of M100907 (**1**) and derivatives (**2**–**5**) which retained 5-HT_{2A}R antagonist properties.

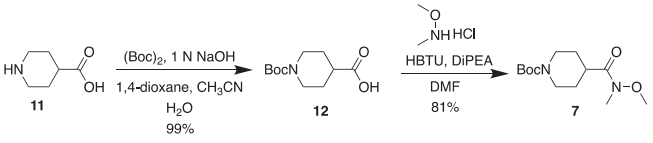
recrystallization, provided one diastereomeric salt in 36% overall yield as white crystals (Scheme 3). Aqueous workup of the diastereomeric salt with ammonium hydroxide afforded the enantiomer in a purity of >95% *ee* (Fig. 2).

The optical purity was evaluated with (*R*)-(−)-1,10- binaphthyl-2,2′-diyl hydrogen phosphate [(−)-BNP] as a ^{1}H NMR shift reagent (Fig. 2).[8] The benzylic proton adjacent to the hydroxyl group gave a doublet near 4.6 ppm that was resolved from all other aliphatic signals. This peak was followed on adding (−)-BNP. When one equivalent of (−)-BNP was added to the racemic **10** in $CDCl_3$, the benzylic signal separated into 2 doublets, with *R*-enantiomer at 4.4 ppm and the S-enantiomer at 4.5 ppm. Chemical shift changes of this signal were linear relative to the concentration of amine and (−)-BNP. Higher concentrations of (−)-BNP resulted in more significant proton shifting but with broadening of the proton signal.[8]

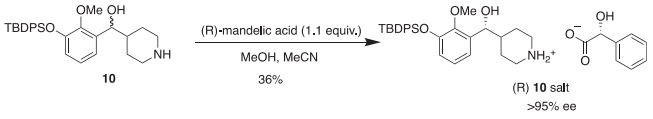
The resolved diastereomeric salt was partitioned between ammonium hydroxide and dichloromethane to obtain a single enantiomer amine (***R*)-10**, which underwent *N*-alkylation with (2-tosylethyl)-4-fluorobenzene **11** to generate compound **12**. Removal of the TBDPS group and reaction with linker **13** provided azide-terminated M100907 (**4**) (Scheme 4).

The alkyne needed to form the homobivalent **15** was synthesized from intermediate **12** through cleavage of the silyl group and alkylation with the tosylated PEG-alkyne **14** (Scheme 5). The bivalent was then synthesized by formation a 1,2,3-triazole ring generated from the dipolar cycloaddition between the azide and the alkyne.[5] This reaction was carried out by adding copper sulfate with sodium ascorbate in a mixture of **4** and **5** DMF and water at room temperature to form the triazole homodimer **15**.

Inhibition of 5-HT_{2A}R-mediated signaling was determined by measuring the reduction of 5-HT (1 μM) stimulated intracellular calcium (Ca^{2+}) release in CHO-K1 cells stably expressing the 5-



Scheme 2. Synthesis of compound **7**.



Scheme 3. Chiral resolution of compound **10**.

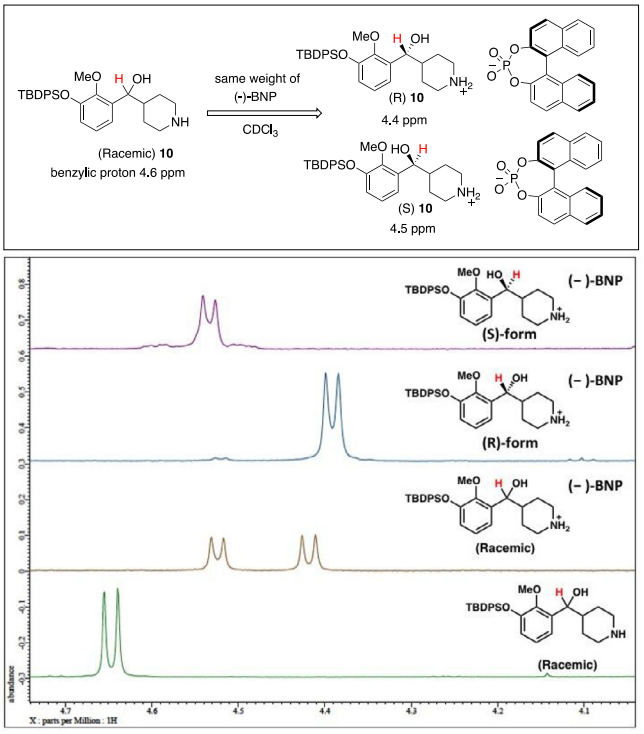
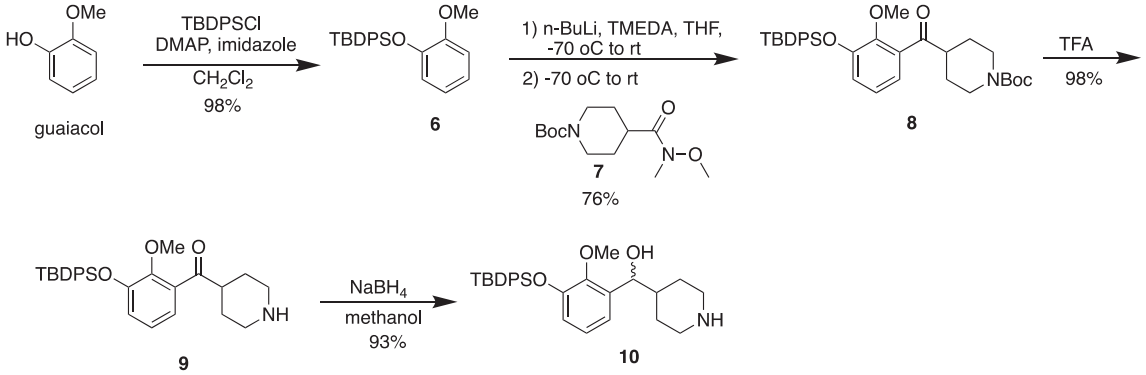
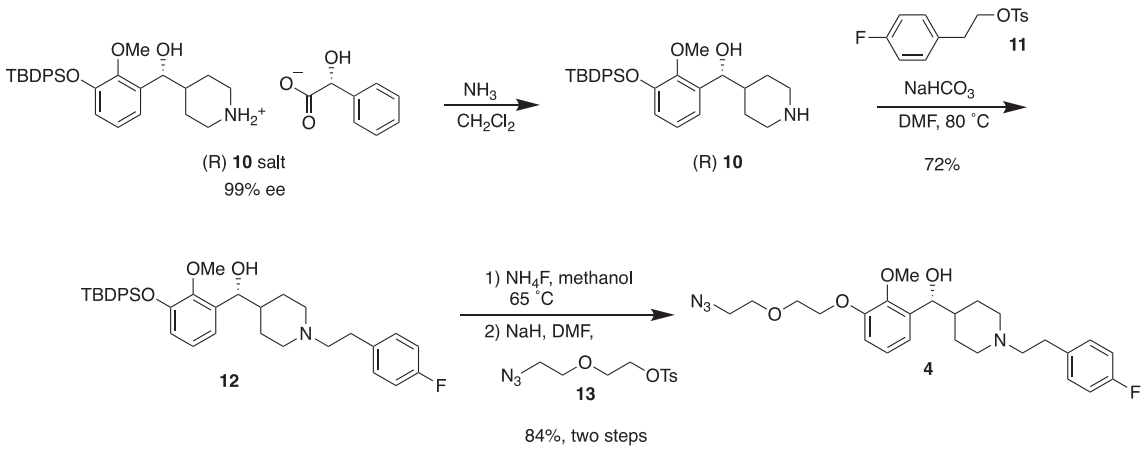


Fig. 2. Determination of the optical purity of **10**. The proton NMR shift of benzylic proton of compound **10** after mixing with same weight of (−)-BNP in $CDCl_3$.

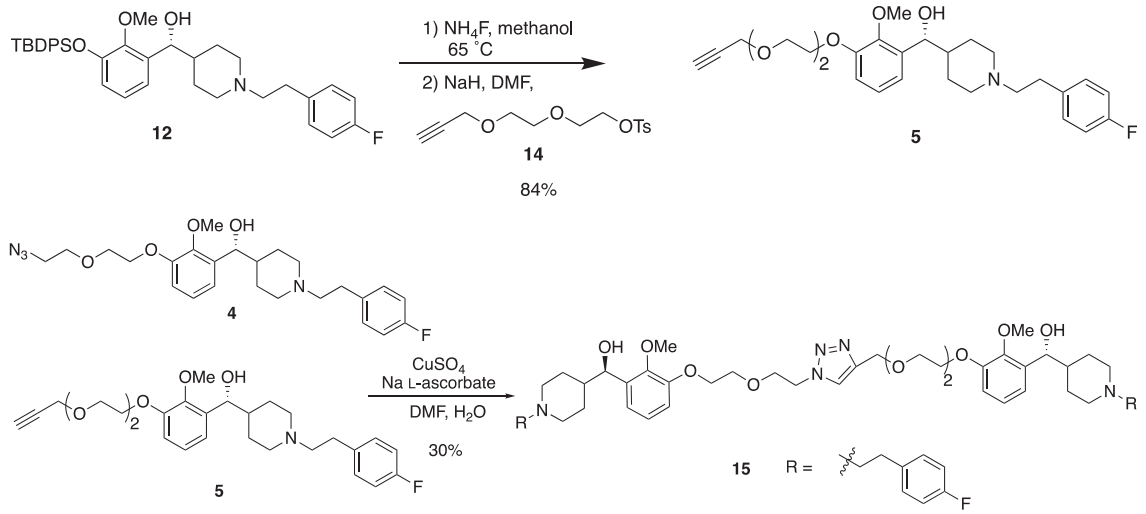
HT_{2A}R.[3,4] Serotonin induces a concentration-dependent increase in Ca_i^{2+} release with an EC_{50} of 4.2 nM ($pEC_{50} = 8.38 \pm 0.10$) and 1 μM of 5-HT exhibited maximal intracellular Ca_i^{2+} release



Scheme 1. Synthesis of the precursor **10** of M100907 derivative **4**.



Scheme 4. Synthesis of the target molecule, M100907-azide **4** from the chiral resolved **10**.



Scheme 5. Synthesis of M100908-alkyne **5** and M100907 homodimer **15** with the heterocyclic 1,2,3-triazole ring.

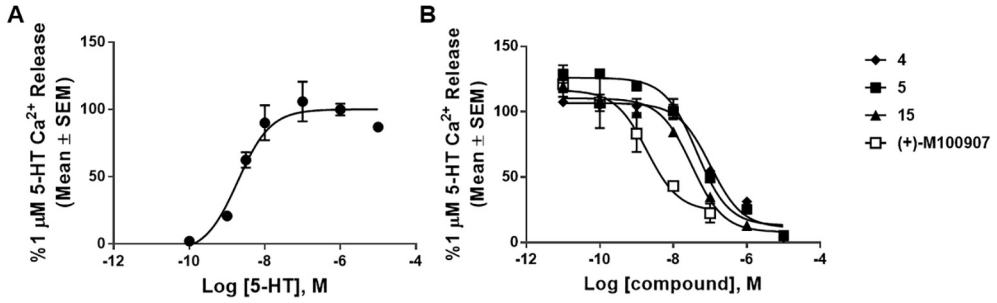
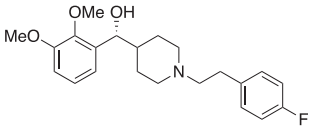
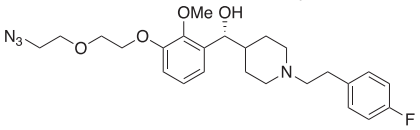
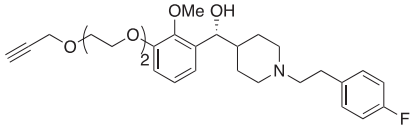
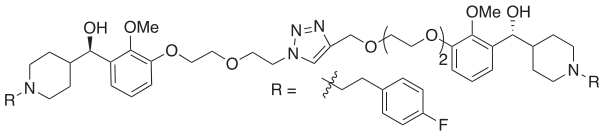
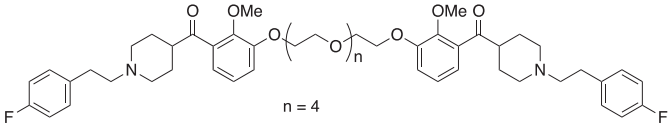
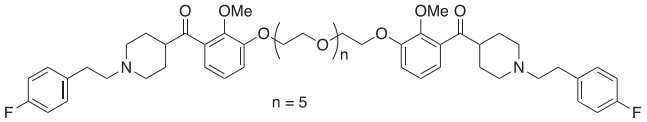


Fig. 3. Representative Ca_i^{2+} release in 5-HT_{2A}R-CHO cells. [A] 5-HT evokes a concentration-dependent elevation of Ca_i^{2+} release (pEC_{50} = 8.38 ± 0.10; EC_{50} = 4.2 nM) and 1 μM of 5-HT induces maximal Ca_i^{2+} release. [B] M100907 derivatives induce a concentration-dependent inhibition of 1 μM of 5-HT. pIC_{50} and IC_{50} values are listed in Table 1.

(Fig. 3A). As expected, the active isomer (+)-M100907[7] displayed low nanomolar potency in inhibiting 5-HT (1 μM)-evoked Ca_i^{2+} release (IC_{50} = 4.8 nM; pIC_{50} = 8.32 ± 0.40; Fig. 3B). None of the compounds displayed activity in the absence of 5-HT (data not shown) and all compounds retained sub-micromolar antagonist activity as shown in Table 1. Compound **4** retained antagonist activity suggesting that tethering diethylene-azido linker on the catechol ring of M100907 does not disrupt antagonist properties. Although compounds **5** and **15** displayed slightly lower potency when compared to parent (+)-M100907, these compounds retained comparable potency to a previously published series of 5-HT_{2A}R bivalent ligands which contain 14 and 17 ethylene glycol linkers.[3,4] These results indicate that the 1,2,3-triazole ring formed in the click reaction does not significantly affect 5-HT_{2A}R antagonist activity.

Derivatives of (+)-M100907 that possess either an alkyne or an azide have been synthesized. The most active enantiomer was obtained by resolution of a relatively early intermediate in the synthesis. The ability of these molecules to maintain 5-HT_{2A}R antagonist properties as (+)-M100907 was demonstrated together with

Table 1
Effect of M100907 derivatives on Intracellular Calcium Release in 5-HT_{2A}R-expressing cells.

Compound Number	Structure	pIC_{50} ± SEM[a] (IC_{50} nM)
1 (+)-M100907		8.32 ± 0.33 (4.8 nM)
4		7.33 ± 0.18 (46.7 nM)
5		7.25 ± 0.13 (56.6 nM)
15		7.44 ± 0.24 (36.5 nM)
16[c]		7.53 ± 0.18[b] (29.5 nM)
17[c]		7.40 ± 0.28 (39.8 nM)

[a] pIC_{50} is presented as mean ± SEM (n = 3–5 independent experiments), except for **16**; IC_{50} values were calculated from averaged pIC_{50} values.
[b] Compound **16** was run in two independent experiments; pIC_{50} reported ± SD.
[c] Data previously published.3,4

the ability to use dipolar cycloaddition between the alkyne and azide to link these molecules to form bivalent antagonists. The 1,2,3-triazole ring generated in the dipolar cycloaddition used to link to the M100907 derivatives does not interfere with the ability of the bivalent ligand to act as an antagonist. Thus, functional versions of M100907 have been synthesized with tethers possessing azides or alkynes, allowing the application of these molecules in the synthesis of dimeric ligands as well as conjugation of the 5-HT_{2A}R antagonist to reporter molecules, such as fluorophores, for the study of GPCR dimerization and their role in signaling.

Materials and methods

Cell lines and cell culture

A CHO-K1 cell line stably transfected with the 5-HT_{2A}R (5-HT_{2A}-R-CHO cells; FA4 line) was a generous gift of K. Berg and W. Clarke (University of Texas Health Science Center at San Antonio). This line expresses transfected human (h)5-HT_{2A}R in the p198-DHFR-Hygro vector containing a hygromycin resistance gene.[9] Reverse transcription of RNA followed by a quantitative real time PCR assay confirmed that 5-HT_{2A}R-CHO cells expressed 5-HT_{2A}R mRNA (estimated to be approximately 3–4% of the mRNA level of the housekeeping gene cyclophilin), but not mRNA for other members of the 5-HT_2R family (i.e., 5-HT_{2B}R or 5-HT_{2C}R) (data not shown). Levels of 5-HT_{2A}R protein expression (200 fmol/mg protein) which approximates physiological levels in brain, have been reported[9] and immunoblot analysis in our hands confirmed moderate 5-HT_{2A}R protein expression (data not shown). Cells were grown at 37 °C, 5% CO_2 and 85% relative humidity in GlutaMax α-MEM (Invitrogen, Carlsbad CA), 5% fetal bovine serum (Atlanta Biologicals, Atlanta GA), 100 μg/ml hygromycin (Mediatech, Manassas VA) and were passaged when they reached 80% confluence.

Intracellular calcium assay

Intracellular calcium (Ca_i^{2+}) release was monitored using FLIPR Calcium 4 Assay Kit (Molecular Devices) according to previously published protocols with minor modifications.[3,4,10–13] Cells were plated in serum-replete medium at 16–20 K cells in 150 μl in black-sided, clear bottom 96-well tissue culture plates. Cells were fed 24 h later with serum-free medium and, following 3 h incubation, medium was removed and replaced with 40 μl of fresh serum-free medium plus 40 μl of Calcium 4 dye solution supplemented with 2.5 mM of water-soluble probenicid (Invitrogen, Carlsbad CA) to inhibit extracellular transport of the dye. Plates were incubated for 50 min at 37 °C followed by 60 min at room temperature in the dark. Fluorescence (λ_{ex} = 485 nm, λ_{em} = 525 nm) was measured with a FlexStation 3 (Molecular Devices). Baseline was established for 17 s before addition of 20 μl of vehicle (HBSS without calcium of magnesium) or 5× concentrated test compound and fluorescence was recorded every 1.7 s for a total of 60 s to detect any intrinsic activity of the test compounds. Fifteen minutes after test compound addition, a second 17 s baseline was recorded again immediately before addition of 25 μl of 5 μM 5-HT (final concentration = 1 μM). Fluorescence was subsequently measured for an additional 60 s. Maximum peak height was determined by the FlexStation software (SoftMax Pro 5.2) for each well. Concentration-response curves (10^{-10} to 10^{-5} M) were performed for each compound. A full 5-HT concentration response curve (10^{-10}–10^{-5} M) was performed in each experiment to establish assay and cell performance.

Data analysis

Peak responses from each well are expressed as a percent of the maximum Ca_i^{2+} response obtained with 1 μM of 5-HT. The pIC_{50} value for Ca_i^{2+} release was determined using 3-parameter nonlinear regression analysis (GraphPad Prism 7.02) and calculated from at least three independent experiments, each conducted in technical replicates of 3–8, and are presented as the mean ± SEM.

Author contributions

Y-C.C. and K.C.R. performed the chemical syntheses and analyses; Y-C.C. drafted the manuscript; C.A.S. and N.C.A. conducted pharmacological analyses; N.C.A., K.A.C. and S.R.G. conceptualized the project, oversaw experimental design/interpretation/analyses, and wrote/edited the manuscript.

Funding sources

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Conflicts of interest

The authors declare no competing financial interests.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2018.02.058.

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