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LETTER

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# Synthesis and Evaluation of Dimeric Derivatives of 5-HT$_{2A}$ Receptor (5-HT$_{2A}$R) Antagonist M-100907

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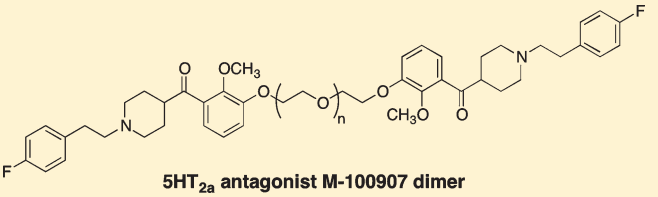
Supporting Information

**ABSTRACT:** It is now well accepted that at least some serotonin receptors exist in dimeric and oligmeric forms. The linking of receptor ligands has been shown to have potential in the development of selective agonists and antagonists for traditionally refractive receptors. Here we report the development of a dimeric version of the known 5-HT$_{2A}$R antagonist, M-100907. Derivatives of M-100907 were synthesized to determine an appropriate site for the linker connection. Then, homodimers with polyether linkers of different lengths were functionally tested in a bioassay to determine the optimal linker length. Attachment at the catechol of M-100907 with linkers between 12 and 18 atoms in length proved to be optimal.




5HT$_{2a}$ antagonist M-100907 dimer

**KEYWORDS:** Serotonin, 5-HT2AR, antagonist, dimers, addiction

Serotonin (5-hydroxytryptamine, 5-HT) and its receptors (particularly the 5-HT$_2$ subgroup) are implicated in a variety of different functions in the CNS ranging from appetite control, learning and memory, depression, and anxiety, to playing a role in impulsivity.[1] A significant amount of work has been carried out in an effort to develop ligands that exhibit selectivity for one of the many different subtypes of 5-HT receptors.[1,2] Given that all these receptor subtypes have evolved to bind the same agonist and that 5-HT$_2$ receptors share a high degree of homology, this has been a daunting task.[3] Recent evidence indicates that at least some 5-HT receptors exist as dimers, and possibly oligomers.[4] Consequently, one approach to improve affinity and selectivity of ligands for a given receptor would be the development of multivalent ligands.[5] The synthesis of dimeric ligands has been used in a number of G protein-coupled receptors to probe the role of receptor dimerization as well as to improve binding selectivity.[6] Additionally, there have been reports of differences between the functional properties of bivalent ligands versus monomeric ligands.[5a] This paper reports the synthesis of dimeric 5-HT$_{2A}$R antagonists and the optimization of such molecules.

The design of a bivalent ligand begins with the selection of an appropriate monomeric ligand. The piperidine M-100907 (**1**) has been shown to bind to 5-HT$_{2A}$R with high affinity (IC$_{50}$ = 3.3 nM) and is greater than 100 times more selective for the 5-HT$_{2A}$R over either the 5-HT$_{2C}$R or 5-HT$_{2B}$R (Figure 1).[7] M-100907 has been used as a selective 5-HT$_{2A}$R antagonist in a wide variety of in vitro and in vivo studies.[8] Despite this work, few studies have examined the structure−activity relationships with analogues of this molecule to optimizing the attachment of fluorescent tags, bioaffinity labels, or other molecules to the basic M-100907 structure.[9] Such derivatives would offer the potential to develop molecular probes with a variety of moieties attached, thus allowing imaging or the delivery of other molecules.[10] We sought to determine the proper location for attachment of a tether, without significantly decreasing functional activity, by synthesizing derivatives with alkyl chains or ethylene glycol groups attached at opposite ends of the parent molecule (**2**, **3**). We additionally examined a highly active precursor of M-100907, in which the secondary alcohol has been replaced as a ketone. Relative to M-100907, this molecule lacks a chiral center, thus eliminating the diastereomers obtained when linking a racemic mixture of monomers.

Variations of M-100907 were synthesized (Scheme 1) in which one of the methoxy groups on the catechol was replaced (**2**) or in which the fluoro group on the opposite end of the molecule was modified (**3**). The derivatives were synthesized by a route similar to the approach of Rice and Ullrich.[11] Metalation of the triisopropylsilyl protected catechol **6** followed by reaction with Weinreb amide **19** provided the necessary ketone **7**. Removal of the Boc protecting group followed by alkylation with the tosylate of *p*-fluorophenethanol yielded the basic structure **9**. Deprotection of the triisopropylsilyl protecting

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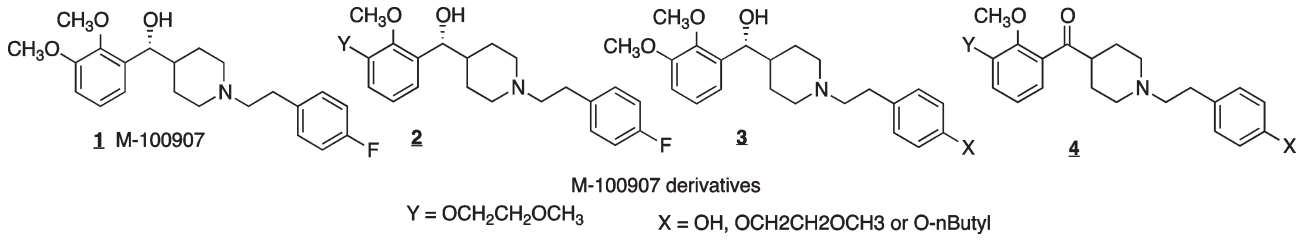
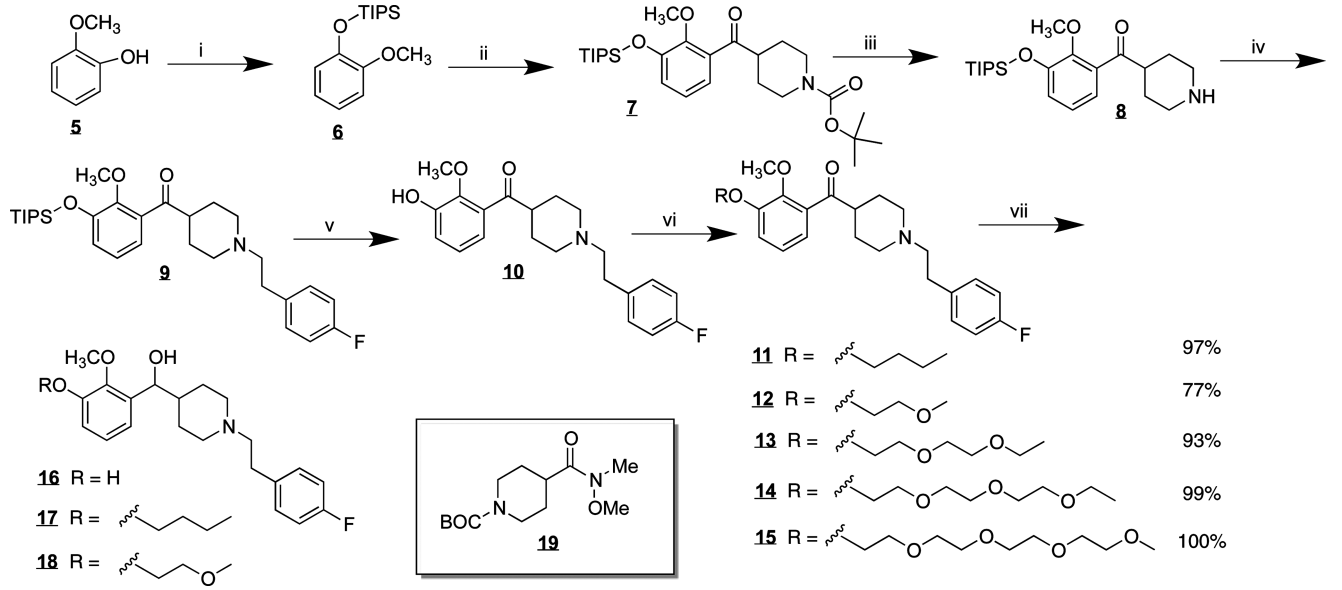


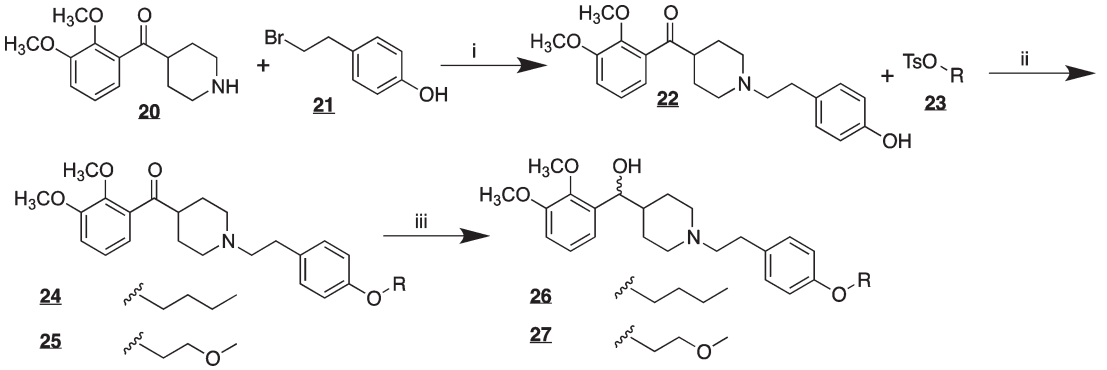

Figure 1

Scheme 1[a]




[a] Reagents and conditions: (i) TIPSCl, imidazole, DMF, rt, 24 h, 63%; (ii) *n*BuLi, −78 °C, reflux, 5 h, **19** rt, 18 h, 44%; (iii) TFA, rt, 2 h, 96%; (iv) (4-FPh) $C_2H_4OTs$, DIEA, $CH_3CN$, reflux, 24 h, 70%; (v) TBAF, THF, rt, 4.5 h, 60%; (vi) TsOR, $K_2CO_3$, acetone, reflux, 24 h; (vii) $NaBH_4$, EtOH, rt, 24 h, $K_2CO_3$, acetone, reflux, 2 h, **17**, 77% **18**, 74%.

Scheme 2[a]




[a] Reagents and conditions: (i) $NaHCO_3$, DMF, 110 °C, 48 h 53%; (ii) $K_2CO_3$, acetone, reflux, 18 h, (**20**, 75% **21**, 68%); (iii) $NaBH_4$, EtOH, rt, 1 h, (**26**, 77%, **27**, 74%).

group gave the free phenol **10** that could then be used to add the desired linker. In the case illustrated in Scheme 1, the alkylation proceeded with 1-bromobutane (**11**) or a 1-bromo-ether (**12**−**15**). In the case of **11** and **12**, the ketone was reduced with sodium borohydride to yield the racemic alcohols **17** and **18**. The derivatives in which the fluorophenyl group has been modified (Scheme 2) were accessed by alkylation of intermediate **20** with the bromide of *p*-hydroxyphenethanol and then alkylation of the phenol with the appropriate alkyl or ether tosylate (**23**).

Inhibition of 5-$HT_{2A}$R activity was determined by measuring the reduction in 5-HT (1 $\mu$M) stimulated intracellular calcium ($Ca^{2+}$) release in a line of CHO cells expressing 5-$HT_{2A}$R.[12]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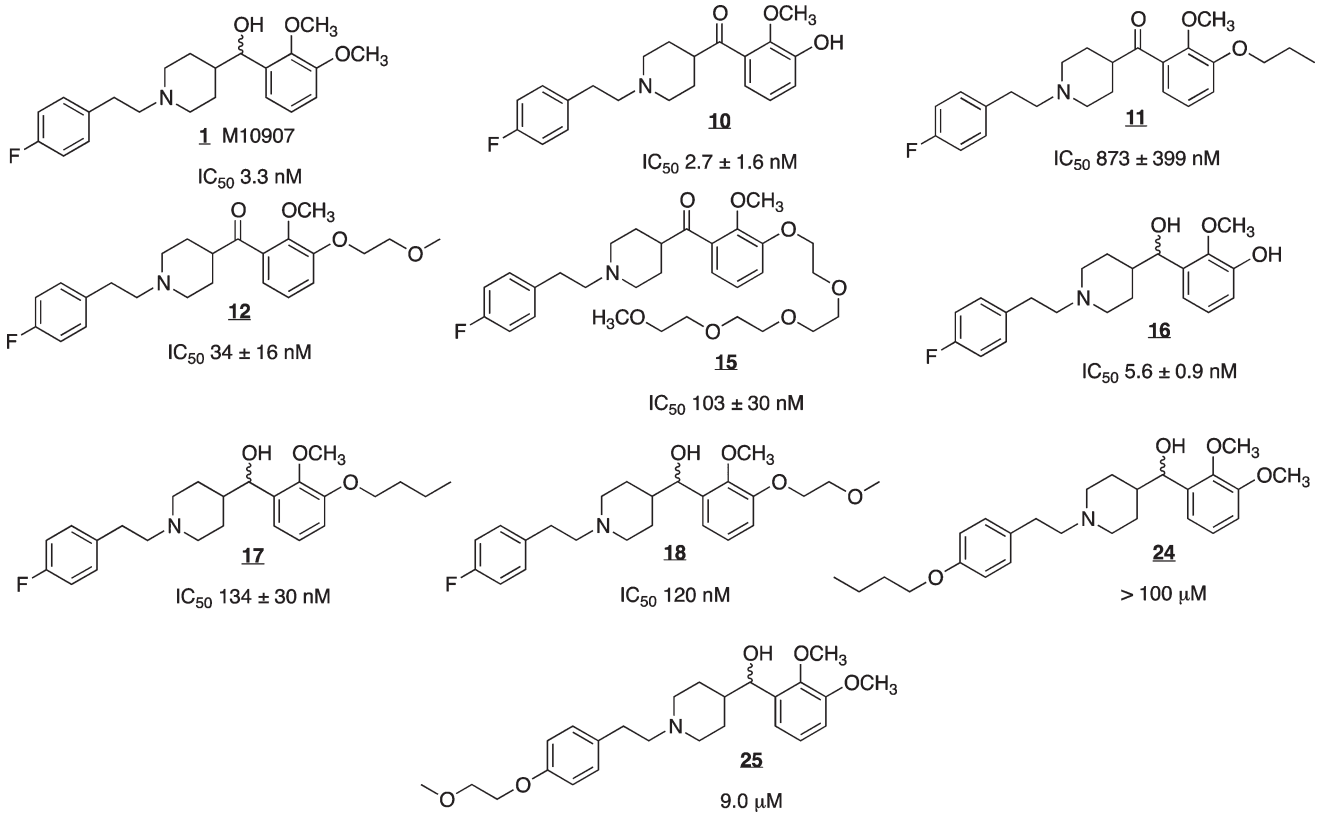
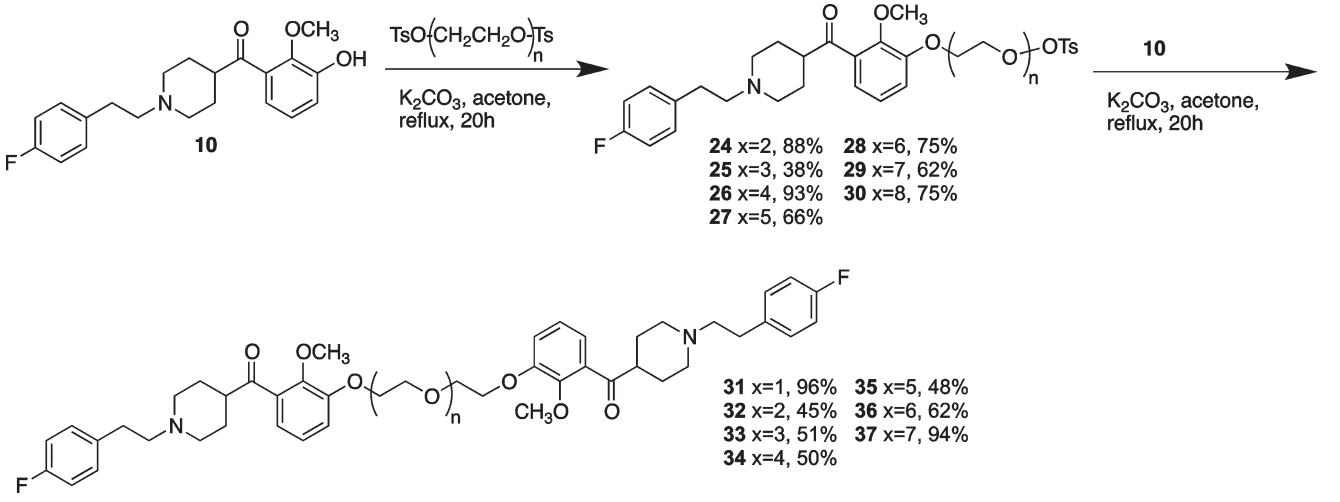



**Figure 2**

**Scheme 3**




Replacement of fluorine with an ether (**24**, **25**) resulted in significant loss of antagonist potency (Figure 2; see $IC_{50}$s below respective structures). Attachment of an aliphatic chain to the catechol (**11**, **17**) reduced the derivative's potency, while placement of an ethylene glycol group at that position (**12**, **15**, **18**) was less deleterious Inhibition of 5-HT-induced intracellular $Ca^{2+}$ release was maintained even with a 13-atom chain attached (**15**), indicating that this location is a potentially useful site for attachment of the necessary tether for the development of M-100907 derivatives.

Because our initial synthesis of M-100907 is racemic, linking racemic versions would result in the formation of diastereomers, complicating both purification and biological testing. Accordingly, the decision was made to use versions of the ketone intermediate (**4**) which was shown to be a reasonably potent ($IC_{50}$ = 34 nM) inhibitor of 5-$HT_{2A}$R in the initial $Ca^{2+}$ bioassays (**12**). Utilizing the benign tether location we had identified, a series of homodimers was synthesized. These dimers were synthesized by reaction of ketone **10** with an excess of the bis-tosylate of the appropriate polyethylene glycol. The monotosylate


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**Table 1. Dimer Potency as a Function of Length**

| compd | atoms in linker | $IC_{50}$ |
|---|---|---|
| **31** | 6 | 181 ± 71 nM |
| **32** | 8 | 56 ± 14 nM |
| **33** | 12 | 28 ± 16 nM |
| **34** | 14 | 32 ± 6 nM |
| **35** | 18 | 34 ± 10 nM |
| **36** | 21 | 154 ± 25 nM |
| **37** | 24 | 373 ± 153 nM |

products (**24**−**30**) were then reacted with excess ketone **10** to provide homodimers **31**−**37** (Scheme 3).

The homodimeric derivatives were tested in the same $Ca^{2+}$ release bioassay described earlier to determine whether they retained, or possibly had increased, potency. The ability of each derivative to inhibit 5-HT-induced intracellular $Ca^{2+}$ release was determined in three separate assays for each compound and reported in Table 1 as an $IC_{50}$. These data indicate that antagonist potency increased as tether length increased, with 12−18 atoms showing comparable inhibition, but fell off sharply by 24 atoms. While the potency of the effective dimers is not greater than the monomeric molecules, it is clear that increasing or reducing the length of the tether beyond the optimal range reduces antagonism. Additionally, compound **15** (Figure 2) demonstrated that the polyether did not affect the activity in a nonspecific manner by interaction with the membrane in which the receptor resides.

We have identified an appropriate site for dimerization of M-100907 analogues and have demonstrated that the dimeric molecules are antagonists at the $5\text{-}HT_{2A}R$ receptor. This work is the first step in the development of other homodimeric 5-HT ligands that will be evaluated as selective ligands for both homo- and heterodimeric receptor complexes. Molecules of this type have potential as probes for receptor dimerization as well as selective activation of dimeric ligands over their monomeric versions.

## ■ ASSOCIATED CONTENT

**Supporting Information.** Experimental section and NMR spectra of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

### Author Contributions

The design and synthesis of the molecules described in this paper was carried out by M.J.S. and S.R.G. K.A.C., P.K.S., A.M., T.S., and C.S.W. were responsible for assay development and testing.

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