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Discovery of novel and selective tertiary alcohol containing inhibitors of the norepinephrine transporter

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Abstract—A novel series of tertiary alcohol containing 2-substituted benzyl morpholines have been discovered as potent and selective inhibitors of the norepinephrine transporter. Efficient synthetic routes were developed featuring a highly diastereoselective nucleophilic addition of benzyl Grignard reagents to enantiopure (4-benzylmorpholin-2-yl)phenylmethanone (**11**) as the key synthetic step. In vitro binding affinity for the norepinephrine, dopamine and serotonin transporters and in vivo examination of a select compound (**16**) in a pharmacodynamic animal model for norepinephrine reuptake inhibition are presented.

Monoamine reuptake inhibitors are widely used for the treatment of major psychiatric disorders[1] (Fig. 1).

Selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine (Prozac™), have been widely used to treat patients with depression. Selective norepinephrine reuptake inhibitors (SNRIs) are also used for the treatment of depression (reboxetine **3**, Edronax™)[2] and the treatment of attention deficit hyperactivity disorder (atomoxetine **2**, Strattera™).[3] More recently, a compound with a dual activity profile, i.e., exhibiting reuptake inhibition of both serotonin and norepinephrine, has been approved by the FDA for treatment of depression and the management of pain associated with diabetic peripheral neuropathy (duloxetine **4**, Cymbalta™).[4] Given the high degree of validation of monoamine reuptake inhibition as a clinically proven mode of action, novel compounds with improved metabolic

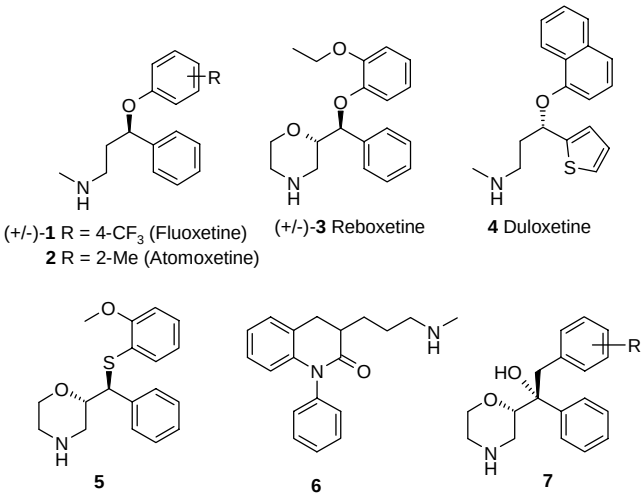

Figure 1. Mono and dual amine reuptake inhibitors.

Keywords: Depression; Atomoxetine; Attention deficit hyperactivity disorder; ADHD; Norepinephrine; Serotonin; Dopamine; Reuptake; Inhibitor; Selective norepinephrine reuptake inhibitor; α-MMT; Metariminol; Morpholine; Tertiary alcohol.
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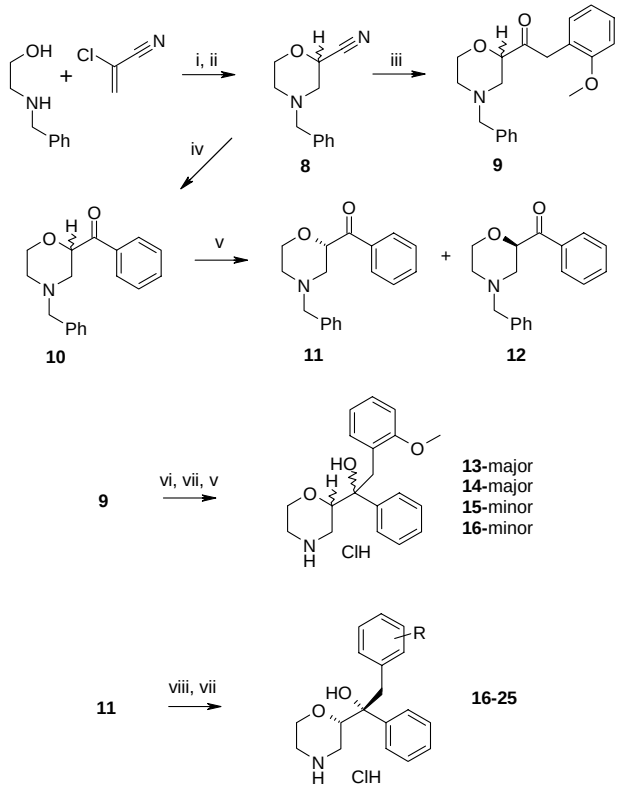
and/or pharmacological properties are of great interest to industrial and academic researchers alike.

We have recently disclosed novel monoamine reuptake inhibitors containing an arylthiomorpholine motif as in **5** or based on a quinolone containing scaffold as in **6**.[5,6] Herein, we would like to report the discovery, synthesis and biological activity of a series of tertiary alcohol containing benzyl morpholines as exemplified by generic structure **7**.[7]

The synthesis of the target molecules **13**–**25** is detailed in Scheme 1.[8] Reaction of 2-chloro acrylonitrile with *N*-benzyl ethanolamine, followed by treatment with potassium *tert*-butoxide, afforded the *N*-benzyl protected 2-cyanomorpholine **8** reliably in a one-pot procedure.[9] Treatment of this nitrile with 2-methoxybenzyl magnesium chloride afforded the ketone **9** in good overall yield. Similarly, addition of phenyl magnesium chloride to the nitrile **8** provided the racemic ketone **10**; this was readily resolved by chiral HPLC or chiral SFC affording the ketones **11** and **12** in quantities of up to several hundred grams.[10,11] Addition of phenyl magnesium chloride to ketone **9** produced a mixture of all possible isomers (de ~80%). After silica gel chromatographic separation of the diasteromers and removal of the *N*-benzyl protecting group by catalytic phase transfer hydrogenolysis, each of the single enantiomers (**13**–**16**) was obtained by chiral HPLC.[12]



Scheme 1. Reagents and conditions: (i) Et_2O or neat 15 °C to rt, 24 h. (ii) THF, *t*-BuOK 0 °C 3 h; 70–80%. (iii) 1-Methyl magnesium chloride-2-methoxy benzene 0 °C to rt, 1.5 h, then 5 N HCl, 0 °C, 58%. (iv) Phenyl magnesium chloride 0 °C to rt, 1.5 h, then 5 N HCl, 0 °C, 70%. (v) Chiral preparative HPLC resolution or chiral preparative SFC resolution.[10] (vi) Phenyl magnesium chloride −10 °C, 0.5 h; then SiO_2 chromatographic separation of diasteromers (vii) Pd/C, HCO_2NH_4, EtOAc; or ACE-Cl, THF followed by MeOH; 40–75%. (viii) Mg turnings, 1,2-dibromoethane, 1-halomethyl-substituted benzene, Et_2O 0 °C to rt, 0.5 h; or commercially available benzylic Grignard reagents, add compound **11** in Et_2O, −20 °C to rt, 2 h; 40–100%.

A key synthetic step in the preparation of **16**–**25** consisted of a highly diastereoselective addition of an appropriately substituted benzyl Grignard reagent to ketone **11** (de >95%). Debenzylation of the resultant adduct was achieved by hydrogenolysis, or carbamate exchange with α-chloro ethyl chloroformate (ACE-Cl) followed by methanolysis, to provide the target molecules **16**–**25** as single enantiomers in good overall yields.

X-ray crystallographic analysis of the 2-bromo-analogue **21** allowed determination of absolute stereochemistry (2*S*,2′*R*) (Fig. 2).[13] Assignment of the (2*S*,2′*R*) stereochemistry for products **16**–**20** and **22**–**25** was by analogy to this result. The high degree of diastereoselectivity in the addition step of benzyl Grignard reagents to (4-benzylmorpholin-2-yl)phenylmethanone (**11**) can be rationalised on the basis of a chelation-controlled nucleophilic attack of the organometallic reagent.

All compounds presented in Table 1 were tested as single enantiomers for their binding affinity to the norepinephrine (NET), serotonin (SERT) and dopamine (DAT) transporters.[14] All the 2-methoxy-substituted isomers **13**–**16** displayed binding affinity to the NET and poor affinity to the SERT and DAT. The most active isomer, **16**, was also the single enantiomer resulting from the synthetic route described from ketone **11**; this therefore suggests that **16** also contained the (2*S*,2′*R*) stereochemistry. Compounds **17** and **18**, which contain methoxy substitution in the 3- and 4-position, respectively, displayed reduced binding affinity to the transporters. These results along with the binding affinity for the NET of the unsubstituted construct **19** indicated a preference for substitution in the 2-position for the NET (within this select set of compounds).

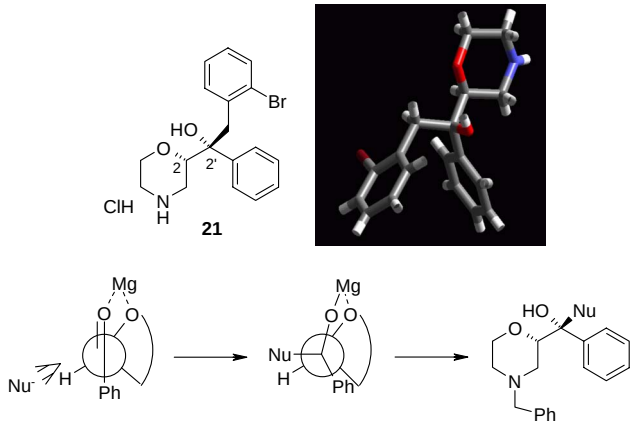
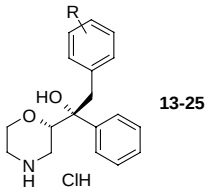


Figure 2. Structure derived from the X-ray diffraction[13] of **21** and rationalised stereochemical outcome of the addition reaction of benzyl Grignard reagents to (4-benzylmorpholin-2-yl)phenylmethanone, **11**.

Table 1. NET, SERT and DAT binding affinity for compounds **13–25**



13-25

Compound	R	K_i[a] (nM)		
		NET	SERT	DAT
13	2-OMe isomer 1	160.8 ± 28.4	(5 ± 5.7%)[b]	(4.7 ± 2%)[b]
14	2-OMe isomer 2	156 ± 25	(0.3 ± 0.7%)[b]	(4.6 ± 1.6%)[b]
15	2-OMe isomer 3	56 ± 6	(1.4 ± 0.7)[b]	(3.7 ± 2.5)[b]
16	2-OMe isomer 4	3.2 ± 0.4	(5.5 ± 0.7%)[b]	(3.8 ± 3.1%)[b]
17	3-OMe	(19.8 ± 0.8%)[b]	(2.8 ± 1.4%)[b]	(4.3 ± 2.2%)[b]
18	4-OMe	(25.2 ± 9.5%)[b]	(4.3 ± 4.7%)[b]	(6.2 ± 0.1%)[b]
19	H	148 ± 12.3	(4.2 ± 1.5%)[b]	(5.5 ± 3.5%)[b]
20	2-Cl	30 ± 9.7	(4.6 ± 0.7%)[b]	(20.8 ± 4.8%)[b]
21	2-Br	11.9 ± 2.4	(13 ± 3.6%)[b]	(24.1 ± 1.1%)[b]
22	2-OEt	5.2 ± 1	(2.4 ± 2.2%)[b]	(3.8 ± 3.2%)[b]
23	2-O*i*-Pr	7.7 ± 3.7	(2.7 ± 4%)[b]	(5.1 ± 3.5%)[b]
24	2-SCF3	54.4 ± 12.7	(14.8 ± 4.9%)[b]	(6.2 ± 2.1%)[b]
25	2-Ph	3.7 ± 1	(3 ± 0.6%)[b]	(2.9 ± 4%)[b]

[a] K_i values are means of at least three experiments.
[b] Values in parentheses represent the %-displacement of radioligand at 100 nM for NET and SERT, and 1000 nM for DAT. For experimental details of assay conditions, see reference section.[14] Minimum significant ratio (MSR) for binding assays: NET, 2.5; SERT, 3.0; DAT, 1.6.

A similar preference for 2-substitution has previously been observed and reported for a series of arylthiomethyl morpholine-based inhibitors of the NET (e.g., **5**).[5] Additional compounds with substitution in the 2-position of the benzylic functionality (**20**–**25**) also displayed measurable binding affinity to the NET and good selectivity versus the SERT and DAT. Introduction of 2-chloro or 2-bromo functionality (**20** and **21**, respectively) led to a significant increase in NET binding affinity compared to **19**. Alkoxy substitution in the 2-position was found to be particularly effective in yielding compounds with high binding affinity to the NET. The NET binding affinity of 2-methoxy-substituted analogue **16** was comparable to those of atomoxetine **2** (K_i (nM) = 5)[15] and reboxetine **3** (K_i (nM) = 1.1 ± 0.2).[1] Larger alkoxy substitution, as in **22** and **23**, also retained high binding affinity to the NET. The 2-trifluoromethylthio-substituted **24** had somewhat reduced binding affinity to the NET, while 2-phenyl substitution (**25**) afforded comparable binding to the most potent compound **16**.

Further in vitro characterization of **16** included evaluation of the extent of its metabolism in various species liver microsomes. Compound **16** was found to be extensively metabolised in rat liver microsomes, but significantly less in dog and human liver microsomes.[16] The benzylic tertiary alcohol functionality in **16** was found to be both chemically and configurationally stable under aqueous acidic conditions. After treatment of **16** with 0.1 N HCl at 40 °C, less than 5% loss of parent was observed after 72 h.[17]

The in vivo pharmacodynamic activity of compound **16** was investigated in an α-methyl-*m*-tyrosine (α-MMT)-induced cortical norepinephrine depletion model in rats. In this animal model, a subcutaneous dose of α-MMT produces through metabolic conversion the neurotoxin metaraminol which is subsequently transported via the NET into neurons resulting in a depletion of cortical norepinephrine.[18,19] Blockade of the NET by inhibitors prevents transport of metaraminol into neurons, thereby maintaining cortical NE levels. After a 3 mg/kg subcutaneous dose of compound **16**, a 70 ± 6% blockade of α-MMT-induced depletion of cortical norepinephrine was observed. Further, despite extensive in vitro metabolism in rat liver microsomes, compound **16** also showed significant dose-dependent activity after oral administration with a measured ED_{50} = 18 mg/kg (Fig. 3).[20]

In summary, we have discovered a novel series of highly selective and potent norepinephrine reuptake inhibitors containing a tertiary alcohol motif. An efficient synthetic

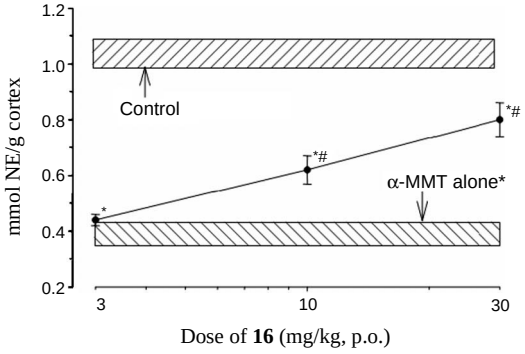


Figure 3. Blockade of α-MMT-induced depletion of cortical norepinephrine concentrations after oral administration of **16**.

route providing access to the targets in enantiomerically pure form has been developed. Compound **16** had the greatest binding affinity to the NET within the group of compounds presented and this inhibitor was found to be stable under aqueous acid conditions. Despite extensive metabolism in rat liver microsomes, **16** was found to have potent oral in vivo activity in the α-MMT pharmacodynamic model of NET inhibition. Further investigations and results with compound **16**, and other select compounds within this structural series of NET inhibitors, will be reported in future communications.

Acknowledgments

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10. Separation was achieved using one of two methods: (1) Chiral HPLC on a Diacel chiralpak AD 20 μM column with 100% ethanol/0.3% DMEA (dimethylethylamine) as eluent using a flow rate of 150 ml/min and UV-detection at 300 nm. (2) SFC Multigram II supplied by Mettler Toledo Autochem. Column: Chiralpak AD-H 25 cm × 21.0 mm i.d. 5 μ (micron); flow rate: 70 ml/min; modifier: 30% MeOH, 70% CO_2 with 0.2% DEMA (diethylmethylamine); temperature: 35 °C; outlet pressure: 100 bar; injection: 1 ml (500 mg); detection: UV at 260 nm.
11. Analytical data for (4-benzylmorpholin-2-yl)phenylmethanone, enantiomer 1: pale yellow solid, mp 80.1–82.2 °C, $[\alpha]_D^{20}$ −29.4° (MeOH, 12.40 g/l). 1H NMR (400 MHz, $CDCl_3$) δ 2.3 (m, 2H), 2.7 (dd, J = 11.5, 2 Hz, 1H), 3.1 (dt, J = 11.5, 2 Hz, 1H), 3.5 (d, J = 13 Hz, 1H), 3.6 (d, J = 13 Hz, 1H), 3.8 (td, J = 11, 2.5 Hz, 1H, 6), 4.0 (dt, J = 11, 2 Hz, 1H), 4.9 (dd, J = 10, 2.5 Hz, 1H), 7.2–7.3 (m, 5H), 7.4–7.5 (m, 2H), 7.6–7.7 (m, 1H), 7.9–8.0 (m, 2H). ESI-MS $C_{18}H_{19}NO_2$ MS (ES^+) m/z = 282 $[M+H]^+$. Analytical data for 4-(benzylmorpholin-2-yl)phenylmethanone, enantiomer 2: pale yellow solid, mp 82.9–83.6 °C, $[\alpha]_D^{20}$ +29.7° (MeOH, 11.78 g/l). Other data identical to enantiomer 1.
12. See Ref. 8 for detailed experimental procedures for Grignard addition and deprotection. Enantiomer separation was achieved using the following methods: isomers 1 and 2 chiral HPLC on a Chiralpak AD 10 μm column (supplied from Chiral technologies, Illkirch, France) with 85% heptane: 15% ethanol (0.2% DMEA) as eluent using a flow rate of 12 ml/min and UV detection at 260 nm. Isomers 3 and 4 chiral HPLC on a Chiralpak AD 10 μm column (supplied from Chiral technologies, Illkirch, France) with 85% heptane: 15% ethanol (0.2% DMEA) as eluent using a flow rate of 12 ml/min and UV detection at 260 nm.
13. CCDC 291659 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.
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