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Trishomocubane as a scaffold for the development of selective dopamine transporter (DAT) ligands

Samuel D. Banister [a], Iman A. Moussa [a], Corinne Beinat [a], Aaron J. Reynolds [a], Paolo Schiavini [a], William T. Jorgensen [a], Michael Kassiou [a,b,c,*]

[a] School of Chemistry, The University of Sydney, Sydney, NSW 2006, Australia
[b] Brain and Mind Research Institute, Sydney, NSW 2050, Australia
[c] Discipline of Medical Radiation Sciences, The University of Sydney, Sydney, NSW 2006, Australia

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ABSTRACT

In our continued exploration of trishomocubane derivatives with central nervous system (CNS) activity, *N*-arylalkyl-8-aminopentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecanes (**10**–**13**) displaying affinity for the sigma (σ) receptor were also found, in several cases, to interact with the dopamine transporter (DAT). Compound **12** was identified as the first trishomocubane-derived high affinity DAT ligand (K_i = 1.2 nM), with greater than 8300-fold selectivity over the monoamine transporters NET and SERT, and only low to moderate affinity for σ_1 and σ_2 receptors.

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The dopamine transporter (DAT) is a member of the Na^+/Cl^--dependent neurotransmitter transporter family, and plays a key role in terminating dopamine (DA) neurotransmission.[1,2] Like the structurally related monoamine carriers, the norepinephrine transporter (NET) and the serotonin transporter (SERT), the DAT is a presynaptic, membrane-bound protein comprised of 12 transmembrane domains, with amino and carboxy termini located intracellularly.[3–5]

Biochemical and pharmacological studies have linked DAT dysfunction to a number of major central nervous system (CNS) disorders, including Parkinson's disease (PD), attention deficit hyperactivity disorder (ADHD), and substance abuse, thereby identifying the DAT as a potential therapeutic target.[2,6] Inhibition of DAT-mediated DA uptake, as well as DA efflux via the DAT, are mechanisms of action for many psychostimulants, most notably cocaine, methamphetamine, and methylphenidate.[7,8] Indeed, many stimulants of abuse effect changes in DAT regulation in vivo, and such alterations of DAT function are implicated in the neurotoxicity and dependence liability of these substances.[9]

Although DA, norepinephrine (NE), and serotonin (5-hydroxytryptamine, 5-HT) generally act as specific substrates for their respective transporters, there is evidence that both DA and NE can be transported by either DAT or NET in vivo,[10–12] underscoring the high structural homology between these carriers and the difficulty of designing potent and selective DAT ligands. Several structurally disparate classes of DAT ligand have been reported, however, many display cross-reactivity with the SERT and/or NET.[13–15]

Cocaine itself possesses submicromolar affinity for the DAT, NET, and SERT, but modification of the pendant functional groups around the tropane scaffold has produced a myriad of compounds with improved selectivity for the DAT over other monoamine transporters. Since the dual DAT/NET activity of cocaine-analog WIN 35,065-2 (**1**, Fig. 1) was first reported, structural alteration of the 3β-phenyl, 2β-ester, and amine groups of **1** have produced increasingly potent and selective DAT ligands, including RTI-229 (**2**), one of the most promising members of a sizeable library of congeners.[16] Tropane **3**, an analog of benzatropine, showed high affinity for the DAT (K_i = 12.5 nM), and moderate selectivity over the NET (284-fold) and SERT (872-fold), but, like benzatropine itself, retained affinity for M1 muscarinic acetylcholine receptors (K_i = 2.11 μM).[17–19]

Tropane derivative PE2I (**4**) was identified as a high affinity DAT ligand (K_i = 17 nM), with much lower affinity for either the NET (K_i >1000 nM) or the SERT (K_i = 500 nM),[20] and LBT-999 (**5**) similarly possesses high affinity for DAT (IC_{50} = 2.4 nM) with good selectivity over the NET and SERT (IC_{50} >1000 nM in both cases).[21] Both **4** and **5** have been labeled with carbon-11, providing some of the most recent molecular probes for in vivo imaging of the DAT by positron emission tomography (PET).[22–24]

* Corresponding author.
E-mail address: m.kassiou@usyd.edu.au (M. Kassiou).

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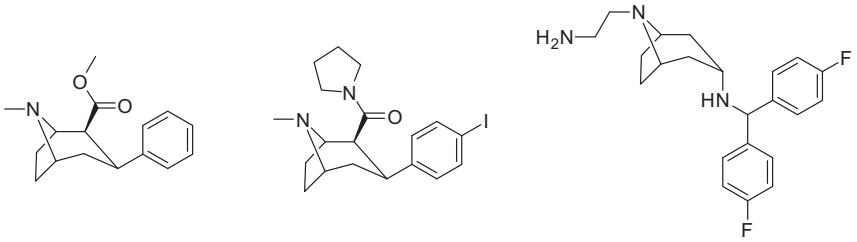
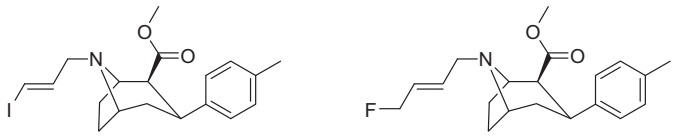


1: WIN 35,065-2

2: RTI-229
DAT IC_{50} = 0.37 nM
NET IC_{50} = 1728 nM
SERT IC_{50} = 991 nM

3
DAT K_i = 12.5 nM
NET K_i = 3550 nM
SERT K_i = 10900 nM
M1 K_i = 2110 nM



4: PE2I
DAT IC_{50} = 0.37 nM
SERT IC_{50} = 991 nM
NET IC_{50} = 1728 nM

5: LBT-999
DAT IC_{50} = 2.4 nM
SERT IC_{50} > 1000 nM
NET IC_{50} > 1000 nM

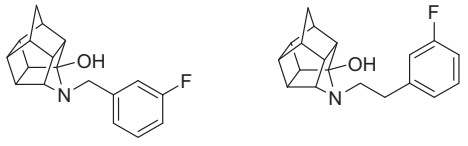
6
DAT K_i = 14 nM

7
DAT K_i = 7.2 nM
NET K_i = 44 nM
SERT K_i = 57 nM

Figure 1. Polycyclic ligands with affinity for the DAT.

Aside from tropane, few other bicyclic frameworks have delivered compounds with high DAT affinity. Several 2,3-disubstituted quinuclidines were reported to possess high DAT affinity, including **6**, which displayed a K_i value of 14 nM for the DAT, although its selectivity for the DAT over other monoamine transporters was not reported.[25] Nanomolar affinity for the DAT was shown for several bicyclo[2.2.2]octanes, including **7** (K_i = 7.2 nM), although selectivity over the NET and SERT was low; approximately six and eightfold, respectively.[26]

We report here a new class of polycarbocyclic DAT ligand, with excellent selectivity over the NET and SERT, arising from our continued exploration of trishomocubane derivatives with affinity for the sigma (σ) receptor. We recently confirmed that isomerization of the hemiaminal bridge of prototypic trishomocubane σ receptor ligands **8** and **9** (Fig. 2) leads to reduced σ receptor affinity and introduces off-target affinity for other CNS sites.[27] To further delineate the role of the hemiaminal functionality of **8** and **9**, *N*-arylalkyl-8-aminopentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecanes entirely lacking the hemiaminal bridge (**10**–**13**, Scheme 1) were synthesized.

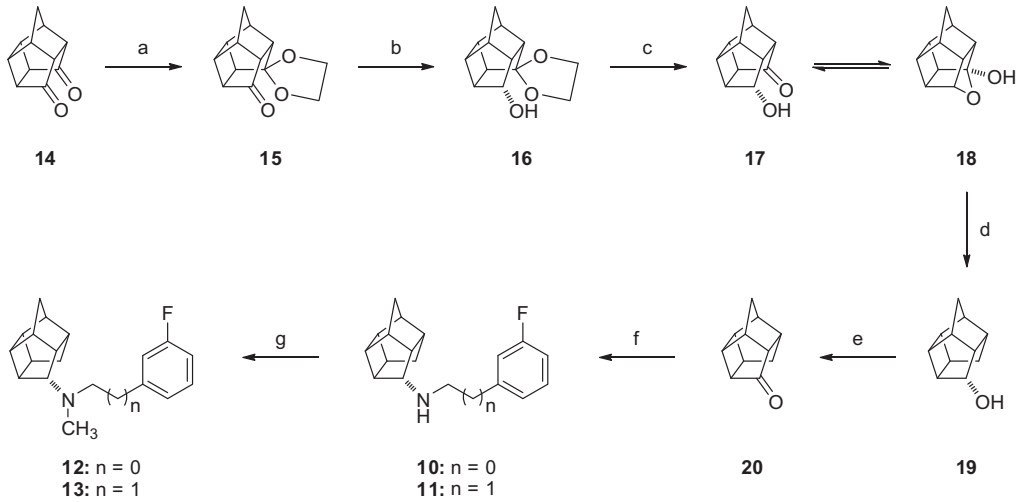


8 **9**

Figure 2. Prototypic trishomocubane-derived σ receptor ligands.

The synthesis commenced with commercially available Cookson's diketone (pentacyclo[5.4.0.0.^{2,6}.0^{3,10}.0^{5,9}]undecane-8,11-dione, **14**), which was mono-protected as the corresponding ethylene acetal **15**. The remaining ketone of **15** was reduced with lithium aluminum hydride to give exclusively racemic *endo*-alcohol **16**. Subsequent hydrolysis of **16** furnished hydroxy-ketone **17**, which exists in solution in equilibrium with the tautomeric internal hemiacetal **18**. Wolff–Kishner reduction of **17** under Huang-Minlon conditions gave alcohol **19**, followed by oxidation with pyridinium chlorochromate to yield ketone **20**.

Condensation of **20** with the appropriate primary amines, followed by sodium borohydride reduction of the intermediate imines, gave the racemic amines **10** and **11**. The sodium borohydride reduction of Schiff bases of **20** proceeds highly stereoselectively, with hydride delivered to the external face of the imine as steric occlusion by the polycyclic cage prevents internal hydride delivery. The newly installed *exo*-hydrogen of **10** (and **11**) appears



Scheme 1. Reagents and conditions: (a) ethylene glycol, *p*-TsOH (cat), PhMe, reflux, 5 h, 93%; (b) $LiAlH_4$, Et_2O, reflux, 2 h, 92%; (c) 6% aq HCl, rt, 3.5 h, 99%; (d) $NH_2NH_2{\cdot}H_2O$, diethylene glycol, 105 °C, 2.5 h, then KOH, 190 °C, 4 h, 96%; (e) PCC, CH_2Cl_2, rt, 2 h, 93%; (f) 3-$FPhCH_2(CH_2)_nNH_2$, EtOH, 100 °C, 18 h; (g) $NaBH_4$, EtOH, 0 °C to rt, 8 h; **10**, 79% over two steps; **11**, 92% over two steps; (h) 37% aq CH_2O, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, rt, 14 h, **12**, 92%; **13**, 93%.

as an apparent triplet in the ^1H NMR spectrum and displays vicinal coupling of a characteristic magnitude (^3J = 3.4 Hz) due to the dihedral angles imposed by the rigidity of the cage, entirely consistent with previous stereochemical assignments of related *endo*-8-substituted pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecanes.[28–30] Conversely, the *endo*-8-hydrogen of *exo*-8-substituted pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecanes exhibits a coupling constant of less than 1 Hz and displays little to no fine structure, typically appearing as a singlet.[28–30]

Reductive methylation of amines **10** and **11** using aqueous formaldehyde solution and sodium triacetoxyborohydride gave the corresponding tertiary amines **12** and **13**, respectively.

The amines thus synthesized (**10**–**13**) were subjected to protein binding assays against a comprehensive panel of CNS receptors, transporters, and ion channels (see Table S1 for full binding profiles). The K_i values for **10**–**13** at σ_1 and σ_2 receptors, and DA, NE and 5-HT transporters are shown in Table 1. Rat brain homogenates were used as a source of σ_1 receptors, whilst PC12 cells were used as a σ_2 receptor source. All transporter assays employed membranes from human embryonic kidney cells expressing the human forms of the transporters. The specific radioligands [^{3}H](+)-pentazocine and [^{3}H]DTG were used in the σ_1 and σ_2 receptor assays, respectively, whilst [^{3}H]WIN-35,428, [^{3}H]nisoxetine, and [^{3}H]citalopram were employed in the DAT, NET, and SERT assays, respectively.

Secondary amines **10** and **11** showed high affinity for the σ_2 receptor (K_i = 19 and 6 nM, respectively) and greater than 10-fold selectivity over σ_1 receptors (σ_1/σ_2 = 10.4 and 11, respectively). Additionally, **10** and **11** also displayed micromolar affinity for the DAT (K_i = 3.44 and 1.68 μM, respectively) and the NET (K_i = 5.88 and 3.62 μM, respectively). The corresponding tertiary amines, **12** and **13**, retained a preference for σ_2 receptors, but possessed reduced affinity for both σ_2 and σ_1 sites. Compound **12**, comprised of a 3-fluorobenzyl group, showed only moderate affinity for σ_2 receptors (K_i = 117 nM) and—like nor-methyl parent compound **10**—greater than 10-fold selectivity over σ_1 receptors (σ_1/σ_2 = 13.4). However, of all CNS targets assayed, the affinity of **12** was greatest for the DAT (K_i = 1.2 nM). In a primary assay at each of the NET and SERT, compound **12**, at a concentration of 10 μM, failed to inhibit more than 50% of the specific binding of [^{3}H]nisoxetine or [^{3}H]citalopram, respectively, indicating that the K_i value for **12** at the NET and SERT is much greater than 10 μM. Thus, **12** represents a highly selective DAT ligand with greater than 8300-fold selectivity over the NET and SERT.

Extending the distance between the 3-fluorophenyl ring and the amine of **12**, to give **13**, resulted in an increase in σ_2 affinity (K_i = 39 nM), although **13** still retained a distinct preference for σ_2 rather than σ_1 receptors (σ_1/σ_2 = 29.2). Compared to **12**, compound **13** showed an 84-fold reduction in DAT binding (K_i = 101 nM), and similarly negligible affinity for either the NET (K_i >10 μM) or SERT (K_i >10 μM).

The lack of appreciable DAT affinity possessed by **10** and **11**, when compared with the moderate to high DAT affinity of **12** and **13**, indicates that the tertiary amine is essential for DAT interaction. Moreover, the improved selectivity for the DAT over the NET and SERT displayed by tertiary amines **12** and **13**, but not their nor-methyl counterparts, indicates that N-substituted *N*-methyl-8-aminopentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecanes may represent lead structures for the development of highly selective DAT ligands. The reduced DAT affinity of 3-fluorophenethyl derivative **13** compared

Table 1
Binding affinity of compounds **10**–**13** for σ receptors (σ_1, σ_2) and monoamine transporters (DAT, NET, and SERT), and predicted Log *P*

Compound	K_i[a] (nM)					Log *P*[b]
	σ_1	σ_2	DAT	NET	SERT	
10	198 ± 20	19.0 ± 0.8	3443 ± 590	5883 ± 927	>10,000	2.84
11	19.0 ± 0.8	6.0 ± 0.5	1684 ± 250	3623 ± 432	>10,000	3.12
12	1571 ± 157	117 ± 8	1.2 ± 0.1	NA	NA	3.21
13	1163 ± 89	39 ± 2	101 ± 5	NA	NA	3.49

[a] K_i values represent the mean ± SEM of four experiments.
[b] Log *P* calculated for unionized compounds using ChemBioDraw ver 12.0. NA = less than 50% inhibition of specific radioligand binding at primary assay concentration (10 μM).

to 3-fluorobenzyl analog **12** is suggestive of precise spatial requirements between certain pharmacophoric features within this class.

Having identified **12** as the first trishomocubane derivative to selectively interact with the DAT, further investigation of the structure–affinity relationships of 8-aminopentacyclo[5.4.0.0.^{2,6}.0^{3,10}.0^{5,9}]undecanes with DAT affinity is warranted, and may lead to the development of novel therapeutic agents with efficacy in diseases such as PD, ADHD, and drug addiction. The synthesis and CNS binding profiles of regioisomers and analogs of **12** will be reported in due course.

In addition, the radiolabeling of **12** with carbon-11 or fluorine-18 could provide molecular probes for the in vivo imaging of the DAT using PET.[31,32] Suitable radiotracers for PET imaging of the DAT may have utility in the early detection of PD, and as non-invasive probes to assess the efficacy of therapeutic intervention in PD progression.[33] Efforts are currently underway to synthesize carbon-11-labeled **12** via nor-methyl precursor **10**.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.075.

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