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Synthesis and monoamine transporter affinity of 3-aryl substituted trop-2-enes

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Abstract—A series of new 3-aryl-tropanes have been synthesized, and their affinities and selectivities were evaluated for monoamine transporters. (1*RS*)-3-(Fluoren-2-yl)-8-methyl-8-azabicyclo[3.2.1]oct-2-ene exhibited the highest affinity for the human serotonin transporter (IC_{50} = 14.5 nM). It is also 52-fold and 230-fold selective over human dopamine and norepinephrine transporters, respectively.

The transporter proteins involved in the neuronal monoamine uptake are attractive targets for the treatment of cocaine abuse and other psychiatric disorders.[1–4] Pharmacological treatment for cocaine abuse and addiction, however, remains elusive. The focus on the dopamine transporter (DAT) as the primary therapeutic target for cocaine addiction has been proposed on the basis of self-administration studies, in which reinforcing properties of cocaine and related drugs correlate with their potencies at inhibiting [^{3}H]mazindol binding of dopamine (DA), but not to binding of serotonin (5-HT) or norepinephrine (NE) to their respective transporters.[5] The 'dopamine hypothesis' assumes that behaviors associated with cocaine addiction result from the accumulation of dopamine in the synapse and its actions on one or more dopamine receptor subclasses.[6]

However, cocaine blocks several monoamine transporters, including those for serotonin (SERT) and norepinephrine (NET). Recent evidence showed that double DAT–SERT knockout mice failed to develop the conditional place preference.[7] In contrast, single DAT or SERT knockouts developed place preference for cocaine.[8] Altogether, these data suggest that the 5-HT system could interact with the DA system and contribute to the reinforcing actions of cocaine. Conversely, in double knockout mice that lack both SERT and NET, the rewarding properties of cocaine were significantly enhanced, indicating that the actions of cocaine on the NET might result in aversive effects.[9]

Among the many structurally diverse classes of monamine uptake inhibitors, 3-aryl-tropanes have renewed interest due to their strong affinities and selectivities.[10–13] Recent conformational and binding studies revealed definitive roles for the orientations of the aryl substituent and the electron lone pair of the bridge head nitrogen in determining their biological activities.[10,12] SAR studies on a number of rigid phenyl tropanes, in which the electron lone pair was fixed in either orientation (*equatorial* or *axial*, relative to the piperidine ring), revealed that the SERT favored *equatorial* orientation, while the DAT showed a slightly higher preference for the *axial* conformation.[12] On the other hand, the selectivity of the NET was not significantly affected.

Herein, we report on the activity of a series of semi-rigid 3-aryl-8-methyl-8-aza-bicyclo-[3.2.1]oct-2-enes (3-aryl-

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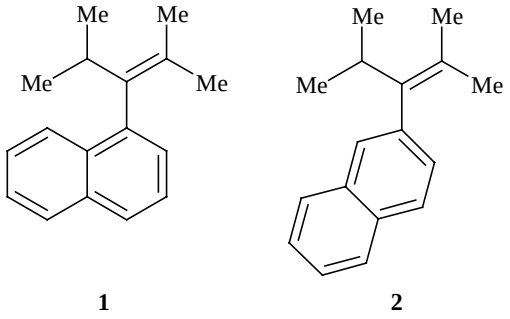
trop-2-enes) with the aim to probe the spatial requirements of the aryl binding pocket of the human monoamine transporters and to correlate the orientation of the aryl moieties to the biological activity. As a reference, a few selected 3-aryl-8-methyl-8-azabicyclo[3.2.1]octane-3-ols, lacking the 2,3-double bond, are also evaluated for their activity. The solution structures of the ligand molecules have been determined by NMR spectroscopy to establish orientational preferences of the aryl and the *N*-methyl groups in relation to the tropene skeleton (published elsewhere). These ligands can be viewed either as cocaine derivatives lacking the 2-substituent, with the α,β-vinylene group incorporated into the tropane skeleton (Fig. 1), or as the semi-rigid analogs of styrene with spectroscopically detectable rotational isomers.[14,15] The nature of the selected aryl groups confers various rotational degrees of freedom resulting in distinctive conformations and overall molecular shapes as a result of the hindered rotation about the single bond (Fig. 2).

Commercially available tropine-3-one, **14** (Scheme 1), was used as a starting material in all the reactions. The intermediate alcohols (**3**, **5**, **7**, **9**, and **11**) result from the *exo* attack of the carbanions generated from the organometallic reagent on **14**, under an inert nitrogen atmosphere and at different temperatures and solvents. The mixtures were allowed to warm up to room temperature and then quenched with a 1 M solution of the cold hydrochloric acid. The alcohols **3**, **5**, **7**, **9**, and **11** were then refluxed in neat trifluoroacetic acid for variable reaction times, depending on the steric hindrance of the tertiary alcohol, to give the alkenes **4**, **6**, **8**, **10**, and **12** (Scheme 1) in quantitative yields.[16]

Aryllithium reagents were used for **5**, **7**, **9**, and **11**, whereas arylmagnesium bromide was used for **3**. Aryllithium reagents were synthesized starting from either the appropriate arylbromides or *n*-butyllithium at −78 °C. There are several reported reactions for the deprotonation of the unsubstituted fluorene, among which deprotonation with *n*-butyllithium at the 0 °C produced the highest yield for **5**.[17] The aryllithium reagent of **7** was obtained by the deprotonation of 2-bromofluorene at −25 °C, the optimal conditions found for this reaction. The Grignard reagent was generated for alcohol **3** from commercially available 2-bromofluorene. Methylene bridge protons have pK_a of 22 and so the deprotonation becomes a competing reaction with the transmetalation even at low temperature.[18] Self-condensation prevails



1 **2**

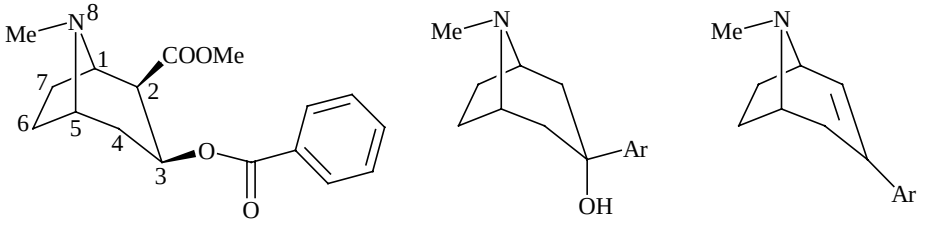
Figure 2. Structures of α,β-naphthylethylenes.[15]

and no nucleophilic attack occurs. The two generated carbanions can also lead to two possible addition products.

The compound **10a** was formed during the isolation of **10** following the dehydration reaction as a result of quaternization of N8 by dichloromethane (Scheme 1). Tertiary amines were known to be quaternized readily by dihalomethanes.[19] However, the formation of **10a** as a result of carbene addition to the tertiary amine cannot be completely ruled out, since among the six aryltropenes synthesized, an alternative product was formed only in the case of **10**.[20] In addition, high pressure conditions are generally required for the quaternization of tertiary amines by dichloromethane. The preferential alkylation of N8 over the double bond may be due to the steric hindrance introduced by the orthogonal benzene ring.

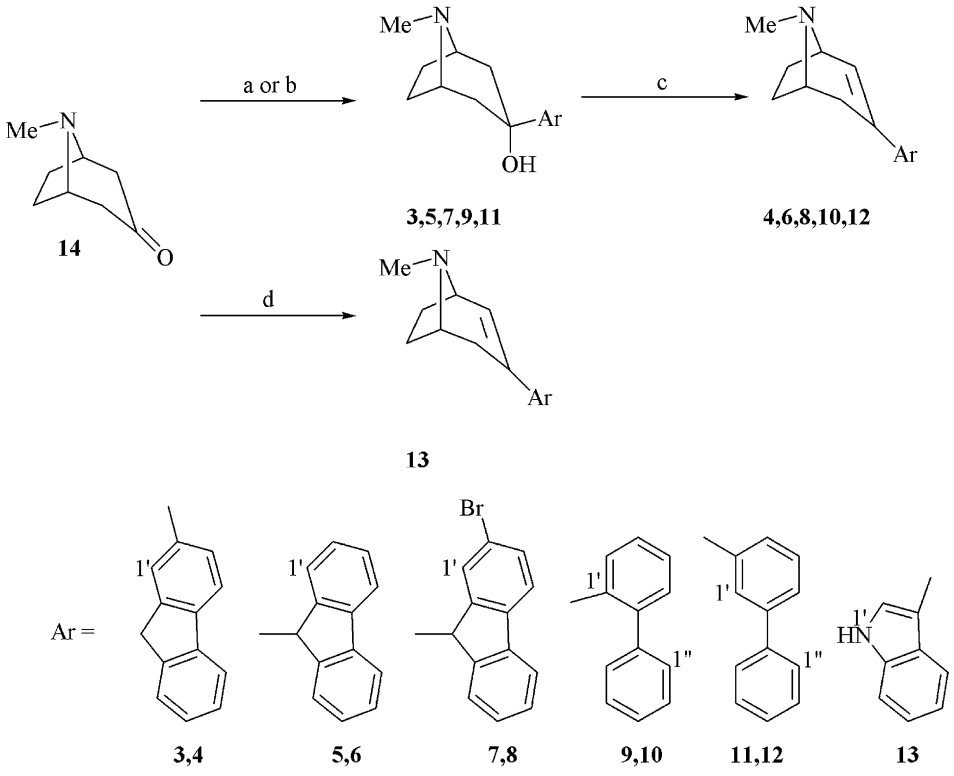
Indole derivative **13** (Scheme 1), a rigid analog of serotonin, was synthesized by refluxing **14** with indole and NaOMe in MeOH. The direct route to **13** was chosen over the two-step nucleophilic addition–dehydration pathway to eliminate the protection–deprotection step of the indole nitrogen.[21]

In general, the yields in these reactions range from 5% to 43%. The lowest yield (cumulative overall yield) was observed for **4** and is likely either due to the self-alkylation or the reprotonation of the carbanions generated during the course of the reaction. The same reasoning is applicable to the transmetalation reaction. Lower yields observed with **9**, in comparison to **11**, are attributed to the increased steric hindrance in the 2-lithiumbiphenyl carbanion that also influences its generation.



cocaine **I** **II**

Figure 1. The structures of cocaine (**1**) and the tropane-based ligands (**I** and **II**). The numbering scheme of the tropane system is shown for cocaine.



Scheme 1. Synthetic route to 3-aryl-8-methyl-8-aza-bicyclo[3.2.1]oct-2-enes **3**–**13**: Reagents and conditions: (a) ArBr, *n*-BuLi/THF, −78 °C to rt, (b) ArBr (Ar = fluorenyl), Mg, MeI, Et_2O, hν, reflux, (c) TFA, reflux, (d) indole, NaOMe, MeOH, reflux. The overall isolated yields of the products are as follows: **4**, 8%; **5**, 19%; **6**, 17%; **7**, 5%; **9**, 15%; **10**, 15%; **10a**, 4%; **11**, 43%; **12**, 42%; **13**, 11%. The yields are not listed for **3** and **8**; **3** was used without purification in the dehydration step, while **8** was obtained in minimal quantities. Atom numbering for each 3-aryl substituent is also shown.

The fluorenyl group in C3 position of **3** and its alkene derivative **4** (Scheme 1) has two structural effects: (a) projects the extended sp^2 functionality into the binding pocket and (b) fixes the orientation of the methylene bridge in such a way that the aromatic ring is almost planar. Compounds **5**, **6**, **7**, and **8** (Scheme 1) are the positional isomers of **3** and **4**. These molecules can adopt at least two conformations, as dictated by the sp^3 hybridized carbon at C9′. The fluorenyl ring can be oriented either in plane or orthogonal to the double bond. The latter orientation positions the benzene rings above and below the plane of the tropane ring. The biphenyl substituted tropanes **9**, **10** and **11**, **12** (Scheme 1) can be viewed as *ortho* and *meta* substituted biphenyls. The lack of methylene linker makes them more conformationally flexible than fluorenes **3** and **4**. The smaller pyrrole ring adjacent to the tropane ring, on the other hand, is expected to decrease the steric barrier for rotation and increase torsional flexibility of the indole portion in **13**.

The transporter-binding affinities were measured by using competitive binding assays against standard ligands in accordance with the NIDA protocol.[22] The tritiated (^{3}H-labeled) WIN-35,428, Citalopram®, and Nisoxetine® were used as standard ligands for the DAT, SERT, and NET, respectively.

Table 1 lists the affinities and selectivities of the 3-aryltropan-3-ols **5**, **7**, **9**, and **11** toward the hDAT, hSERT, and hNET. Generally, affinities in the micromolar levels were observed, in line with the earlier findings that correlated the effect of 2-substituents on the affinity toward rat DAT (IC_{50} of 5180 nM measured for 3-*exo*-benzoyloxytropane).[23] Steric bulk directly below the tropane ring, introduced by 3-*endo* OH, was tolerated to a certain extent only by the hSERT. Compound **5** is an exception with submicromolar activity (543 nM for the hDAT) and is also highly selective for the hDAT (65-fold over the hSERT). For all other compounds the selectivity was reversed from 1.6- to 6.5-fold in favor of the hSERT. The reversal of selectivity observed between **5** and **7** is attributed to the introduction of a bromine atom at 2-position of the fluorenyl moiety. Considering the aryl binding pockets on the hDAT and hSERT as narrow clefts of different sizes, it would be expected that the two compounds to have different biological activities.[13,24,25] The single bond connecting C3 and C9′ allows rotational flexibility sufficient to provide a better fit of the aryl group into the binding pocket, enabling bromine atom in **5** to engage in electrostatic and/or dipolar interactions with the amino acid residues above the plane of the tropane ring. Presumably these interactions are nonexistent with the hSERT. The steric bulk below the proximal benzene ring was not tolerated by any of the three human monoamine transporters, as observed with the compounds **9** and **11**. In the biphenyl series, the addition of a second benzene in the *meta* position, **11**, was particularly detrimental to the activity of the hDAT. In the *ortho* substituted isomer **9**, the rotation of the distal benzene ring is restricted relative to its counterpart in **11**.

Table 1. Affinities and selectivities of (1*RS*)-3-aryl-8-methyl-azabicyclo[3.2.1]octane-3-ols for human DAT, SERT and NET

Compound	IC_{50} (nM)[a,b]			Selectivity		
	hDAT	hSERT	hNET	hDAT/hSERT	hDAT/hNET	hSERT/hNET
5	543 ± 150	35,400 ± 7300	158,000 ± 5000	0.015	0.0034	0.22
7	16,800 ± 1300	2570 ± 1100	62,600 ± 3100	6.5	0.27	0.041
9	3980 ± 430	2520 ± 400	66,300 ± 4700	1.58	0.06	0.038
11	21,900 ± 4800	3770 ± 1690	41,500 ± 11,800	5.81	0.53	0.091

[a] IC_{50} is the concentration at which 50% inhibition occurs.
[b] IC_{50} values are means ± SEM from three independent experiments.

Much lower affinities were observed for hNET, as evidenced from the IC_{50} values for the displacement of [^{3}H]nisoxetine.

Table 2 lists the affinities and selectivities of the 3-aryl-substituted trop-2-ene ligands (**4**, **6**, **10**, **12**, and **13**) toward the hDAT, hSERT, and hNET. The ligands showed much lower activities for the hNET and were generally selective for the hSERT. Compounds **4**, **6**, **12**, and **13** showed submicromolar affinities for hSERT. Compounds **4** and **13** were the only ones with submicromolar affinities for the hDAT. DAT activity is sensitive to steric bulk at the *para* position of the phenyl ring.[26] Compound **4** was found to be the most active with an IC_{50} of 14.5 nM at the hSERT (Table 2) and was also highly selective, namely 52-fold and 230-fold selective for the hSERT over hDAT and hNET, respectively. The second most active compound, **13**, has reduced affinity for the hSERT, about 5-fold, with slightly improved activity at the hDAT compared to **4**. Other ligands largely remained inactive with IC_{50} for **10a** reaching the highest values of 67,000 and 16,100 nM for the hDAT and hSERT, respectively.

A multiplicity of factors can be attributed to the observed high affinity and selectivity of the aryl tropenes toward hSERT. First, the removal of the hydroxyl group (**4**, **6**, **10**, **12**, and **13**) through dehydration of the alcohols resulted in the flattening of the piperidine ring, making it more rigid and placing the aromatic moiety farther into the binding pocket.[13,24,25] The binding pocket, here, refers to the lipophilic pocket present in the transporter proteins that is expected to interact with the aryl group. The size of the aryl group has also been suggested to play a role in determining the selectivity of hSERT over hDAT.[24] The improved selectivity of **4** in comparison with the naphthyl analog reported in Appell et al.[10] is in line with the model proposed by Davis et al.[24] Second, the double bond hinders rotation around the single bond limiting the number of conformers. Third, flattening of the piperidine ring in combination with the nature of the aryl group influences the orientation of the *N*-methyl group. The quantitative nuclear Overhauser effect analysis of the ligand molecules at room temperature provided distance estimates of the various interproton pairs and the subsequent restrained energy minimization calculations revealed three-dimensional solution structures including the preferential orientations of the *N*-methyl group (*equatorial N*-methyl orientation is favored for **4**, **6**, **10**, and **12**, whereas *axial* conformation is favored for **13**). The preferential orientations of the *N*-methyl group in these molecules were also further confirmed by dynamic NMR and inversion magnetization transfer data. However, the low temperature dynamic NMR measurements revealed two populations for at least three sets of protons with the major conformers being *equatorial* for **4**, **6**, **10**, and **12**, and *axial* for **13**, respectively. In accordance with the results of Smith et al.[12] (the ligands are expected to be in the *axial* conformation to have higher activity and selectivity toward hSERT), it is tempting to speculate that the equilibrium is shifted to the conformer with the *axial* *N*-methyl orientation in the presence of the transporter proteins for **4**, **6**, **10**, and **12**.

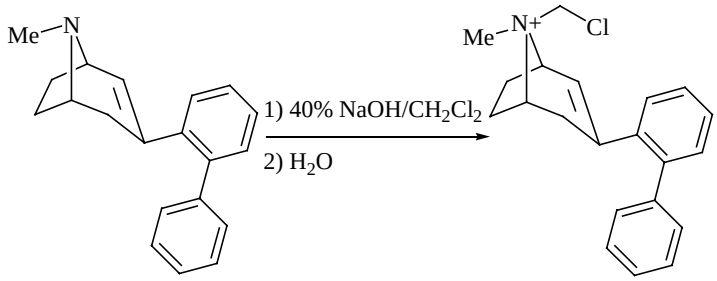
Even though the biological activities can be correlated satisfactorily to the conformational features of the ligands, the relative affinities cannot be discerned in terms of various contributions. Hence, we cannot make any definitive statements on whether the increased activity of **13** compared to **6**, **10**, and **12** or the decreased activity of **13** compared to **4** is a result of the differences in the conformational equilibria.

Compound **10** displayed only slight improvements over its alcohol precursor, most notably for the hNET

Table 2. Affinities and selectivities of (1*RS*)-3-aryl-8-methyl-azabicyclo[3.2.1]oct-2-enes for human DAT, SERT, and NET

Compound	IC_{50} (nM)[a,b]			Selectivity		
	hDAT	hSERT	hNET	hDAT/hSERT	hDAT/hNET	hSERT/hNET
4	757 ± 152	14.5 ± 2.5	3360 ± 560	52.2	0.23	0.0043
6	9350 ± 170	690 ± 120	82,700 ± 4800	13.6	0.113	0.0083
10	9240 ± 1370	1710 ± 700	6430 ± 1810	5.4	1.44	0.27
10a	67,000 ± 17,300	16,100 ± 1400	2020 ± 710	4.2	33.2	8.0
12	7220 ± 930	670 ± 142	23,000 ± 300	10.8	0.314	0.029
13	603 ± 34	72.6 ± 3.4	3956 ± 978	8.3	0.15	0.018

[a] IC_{50} is the concentration at which 50% inhibition occurs.
[b] IC_{50} values are means ± SEM from three independent experiments.



10 **10a**

Scheme 2. Reaction conditions leading to quaternary salt **10a**.

binding (10-fold). The second aromatic ring underneath the tropane ring disturbs its proper orientation at the binding sites of both the hDAT and hSERT. Repositioning of the aromatic ring in **12** led to a 3-fold increase in the activity at the hSERT.

Structurally, **10a** resembles its precursor **10**. The added *N*-chloromethyl group is positioned over the ethylene bridge (Scheme 2). In the case of **10a**, the steric bulk of the biphenyl group and the presence of the positive charge altogether had negative impact on the biological activity at the hDAT and hSERT. The presence of the positive charge on the nitrogen is detrimental to its biological activity; the methyl iodide salt of the cocaine is completely inactive at the DAT.[27] Alternatively, whether **10a** acts as an irreversible inhibitor of the monoamine transporters is not known.

The size and the orientation of the aryl moiety are important and **13** serves as a good example. Reducing the size of the aromatic ring from six-membered to five-membered relaxes the rotation around the single bond, thus decreasing the C2–C3–C2′–C3′ torsional angle. At the same time, conjugation between two π systems becomes feasible. A relatively smaller heterocycle, compared to the rest of the C3 substituents, has slightly improved affinity for the hDAT, but the affinity for the hSERT is five times lower than **4**.

In conclusion, 3-aryl-trop-2-enes were found to have good selectivity for the hSERT, and absence of the 2-substituent was found not to be critical for activity at the hSERT and to a certain level at the hDAT. The aryl binding pocket at the hSERT appears to be an elongated narrow cleft, tolerating a second additional phenyl ring, unlike the hDAT. The shifting of the conformational equilibrium toward the *axial N*-methyl conformer by hSERT may contribute to its high selectivity.

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