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The synthesis and biological evaluation of 2-(3-methyl or 3-phenylisoxazol-5-yl)-3-aryl-8-thiabicyclo[3.2.1]octanes

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ABSTRACT

Cocaine, a potent stimulant of the central nervous system, owes its reinforcing and stimulant properties to its ability to inhibit monoamine uptake systems such as the Dopamine Transporter (DAT), and the Serotonin Transporter (SERT) located on presynaptic neurons in the striatum. The search for pharmacotherapies for cocaine addiction has focused on the design of compounds that bind selectively to the DAT and manifest slow onset of stimulatory action with long duration of action. We had reported that 3-aryl-2-carbomethoxy-8-thiabicyclo[3.2.1]octanes are potent and selective inhibitors of the DAT. In this Letter we report on the effects of replacement of the 2-carbomethoy group by a 2-isoxazole. This new class of 8-thiabicyclo[3.2.1]octanes provides potent and selective inhibitors of the DAT. The 3β-aryl compounds are particularly potent inhibitors of DAT (IC_{50} = 7–43 nM) with substantial selectivity versus inhibition of SERT.

Cocaine addiction continues to present a global problem. Notwithstanding substantial research, a clinically useful candidate to address this addiction has yet to emerge and physicians confronted with addicted patients continue to lack appropriate medications. Nonetheless, a substantial understanding of this disease has emerged, and the directions that discovery research may take in order to uncover a useful medication have made considerable advances.

Cocaine is a potent stimulant of the central nervous system. It owes its reinforcing and stimulant properties to its ability to inhibit monoamine uptake systems such as the Dopamine Transporter (DAT), and the Serotonin Transporter (SERT) located on presynaptic neurons in the nucleus accumbens and striatum.[1–8] Cocaine inhibition of these monoamine uptake systems reduces the removal of excess dopamine from the synapse. The resultant increase in synaptic dopamine concentration increases activation of postsynaptic dopamine receptors. This hyperstimulation of postsynaptic receptors is responsible for cocaine's stimulant activity. The reinforcing and addictive properties of cocaine are postulated to be related to its pharmacokinetic profile[9] characterized by its immediate effect (<15 sec) and short duration of action (10–15 min). Therefore, the search for replacement therapeutic agents has focused on the design of compounds that bind selectively to the DAT, but manifest slow onset of stimulatory action with long duration of action.[9]

The class of bicyclo[3.2.1]octanes,[10,11] of which cocaine (Fig. 1) is a member, has offered a springboard for the discovery of DAT selective inhibitors. Since 1973 when Clarke et al.[12] disclosed WIN35,428, a 2β-carbomethoxy-3β-(4-fluorophenyl)-8-azabicyclo[3.2.1]octane, a large number of 8-azabicyclo[3.2.1]octane (tropane) analogs have been prepared and evaluated and some have offered intriguing possibilities as potential medications for cocaine abuse, as well as imaging agents[13] for diagnosis of diseases that are correlated with dopamine neuron compromise. Of particular relevance to the work that we now describe is Carroll's DAT inhibitor, RTI336, currently in clinical trials.[14] This compound is a 3β-(4-chlorophenyl)tropane substituted in the 2β-position by a 3-(4-methylphenyl)isoxazole in place of the C2β-methyl ester present in WIN35,428.[15]

In 1997, we presented evidence[16] that the 8-**aza** functionality within the 8-azabicyclo[3.2.1]octane series was not a prerequisite for potent inhibition of monoamine uptake systems. We proposed that the topological properties of tropane-like ligands (bicyclo[3.2.1]octanes) that bind to monoamine uptake systems was more important than the presence, or absence, of specific functionality, and we reported that 8-oxabicyclo[3.2.1]octanes (8-oxatropanes)[16] and 8-thiabicyclo[3.2.1]octanes (8-thiatropanes)[11] also manifested substantial binding potency at the DAT as well as selectivity versus SERT inhibition. The biological liability of the C2-ester became apparent to us in our early design of potential SPECT

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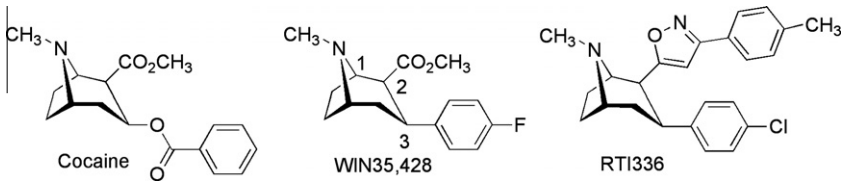


Figure 1. Cocaine, WIN35,428, and RTI336

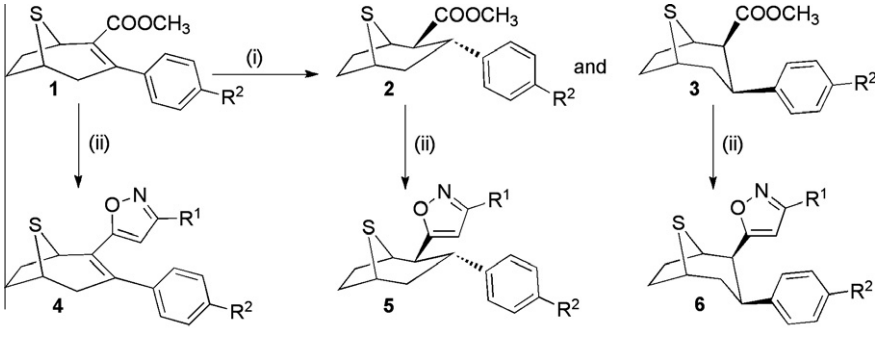
imaging agents[13,17] when we had speculated that rapid in vivo hydrolysis of the C2-ester may have been a contributing factor toward erratic imaging results. We therefore replaced that ester with a hydrolysis-stable ethyl ketone and developed an improved agent, Fluoratec.[13] This led us to reconsider how we might stabilize C2-functionality in the 8-oxa and 8-thiatropane series. Kotian's and Carroll's reports[15,18] of a comparison of 8-aza tropane C2-bioisosteres prompted us to introduce a C2-isoxazole in our 8-thiatropane series.

We now report the synthesis and biological evaluation of a series of 2-(3-methyl or 3-phenylisoxazol-5-yl)-3-aryl-8-thiabicyclo[3.2.1]octanes and oct-2-enes. These compounds have proved potent and selective inhibitors of DAT.

The synthesis of this new class of 2-isoxazolyl-3-aryl-8-thiabicyclo[3.2.1]oct-2-enes **4** and -2-anes, **5** and **6**, is presented in Scheme 1. The starting unsaturated C2-methyl esters **1** provided the isoxazoles **4** as described below. These esters **1** were then reduced with samarium iodide to obtain both the 3α-aryl (**2**) and 3β-aryl (**3**) configured compounds, as we have described previously.[11] Separation of the 3α-**2** and the 3β-**2** was cumbersome and time consuming. Since the C2-isoxazoles were the target compounds, it proved much more convenient to convert a mixture of the isomers **2** and **3** to their corresponding isoxazoles **5** and **6** and then conduct separation of the mixture at the isoxazole stage. Thus *n*-BuLi was added to a mixture of **2** and **3** in anhydrous THF at 0 °C containing either acetophenone oxime to obtain the 3-methylisoxazoles (**5a–e** or **6a–e**) or benzophenone oxime to obtain the 3-phenylisoxazoles (**5f–j** or **6f–j**) in anhydrous THF at 0 °C. The reaction was allowed to warm to room temperature and the final ring closure and dehydration were effected at reflux after addition of sulfuric acid. The products were extracted into methylene chloride and purified by flash column chromatography to provide pure **5a–e** and **6f–j**. While the unsaturated isoxazoles **4** were obtained in reasonable yields for both the 3-methyl- and 3-phenylisoxazoles (40–80%), the saturated 3-methylisoxazoles (**5a–e** and **6f–j**) were generally obtained in poor yields (7–45%).

Configurational assignments of compounds **4**, **5**, and **6** were readily achieved by ^{1}H NMR. In particular, the resonance positions and multiplicity observed for the H_2, H_3, $H_{4\alpha}$, and $H_{4\beta}$ protons proved diagnostic. Thus the 2,3-enes, exemplified for **4a**, manifested characteristic double multiplets between δ 2.96–3.04 for $H_{4\beta}$ that reflected gem coupling ($J_{4\alpha,4\beta}$ = 19 Hz) as well as vicinal ($J_{4,5}$ = ~3 Hz) and w-long range coupling ($J_{4,6}$ = ~3 Hz). $H_{4\alpha}$ resonated at δ 2.48 ($J_{4\alpha,5}$ = ~2.5 Hz). The vicinal coupling constants of J = ~2.5–3 Hz for both $H_{4\alpha}$ and $H_{4\beta}$ to H_5 implied approximately equal dihedral angles ($H_{4\alpha}$ or $H_{4\beta}$–C_4–C_5–H_5 of 55–60°) thus indicating that C_1–C_2–C_3–C_4–C_5 are approximately coplanar. The 3α-aryl boat configured **5a** manifested diagnostic resonances for H_2 δ 3.03, H_3 δ 3.16 (ddd), and $H_{4\alpha}$ δ 1.37 (ddd). The coupling constant between H_3 and H_2 and H_4 respectively ($J_{3,2}$ = 11.5 Hz, $J_{3,4\alpha}$ = 13 Hz) confirmed transdiaxial interactions that can only be achieved in a boat conformation. Furthermore, the double double doublet for $H_{4\alpha}$ ($J_{3,4\alpha}$, = 13 Hz, $J_{4\alpha,4\beta}$ = 13 Hz, $J_{4\alpha,5}$ = 2 Hz) evident in these C2-isoxazoles had proved diagnostic among boat conformers in all tropane-like classes of [3.2.1]bicyclooctanes.[16,19,20,11] The 3β-aryl chair-configured **6a** had H_2 δ 3.66 (dd), H_3 δ 3.43, $H_{4\alpha}$, δ 1.36 (ddd), and $H_{4\beta}$ δ 2.50 (ddd).

The affinities (IC_{50} values) for the DAT and SERT were determined in competition studies with tritium labeled ligands (Table 1).[21] The DAT was labeled with [^{3}H]3β-(4-fluorophenyl)tropane-2β-carboxylic acid methyl ester ([^{3}H]WIN35,428 or [^{3}H]CFT (4 nM)) and non-specific binding was defined as the difference in

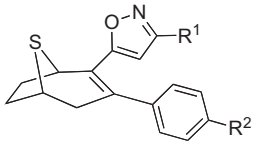


Reaction conditions: (i) SmI_2, MeOH, THF, -78 °C; (ii) nBuLi, acetone oxime (or benzophenone oxime), THF, -78 °C to R.T.; reflux with H_2SO_4 / H_2O/ THF solution

	R^1	R^2		R^1	R^2
a	CH_3	H	**f**	C_6H_5	H
b	CH_3	F	**g**	C_6H_5	F
c	CH_3	Cl	**h**	C_6H_5	Cl
d	CH_3	Br	**i**	C_6H_5	Br
e	CH_3	3,4-Cl_2	**j**	C_6H_5	3,4-Cl_2

Scheme 1.

Table 1
Inhibition of [^{3}H]WIN35,428 binding to the human dopamine transporter (hDAT) and [^{3}H]citalopram binding to the human serotonin transporter (hSERT) for **4**–**6**[a]



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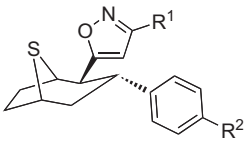

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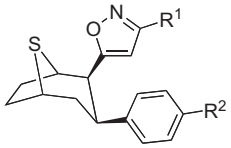

6

Compd	R^1	R^2	**4**	IC_{50} (nM)		**5**	IC_{50} (nM)		**6**	IC_{50} (nM)	
			Compd #[b]	DAT	SERT	Compd #	DAT	SERT	Compd #	DAT	SERT
Cocaine										670 ± 92	540 ± 35
2-Ester[e]	—	F	O-2643	220	>10 μM	O-3856	59	>10 μM	O-3876	38	494
2-Ester[e]	—	Cl	O-2683	13	>10 μM	O-3768	11	>1 μM	O-3806	9.6	33
a	CH_3	H	O-4165	>2 μM	>10 μM	O-3961	65 ± 4	>10 μM	O-4315	12 ± 1	>10 μM
b	CH_3	F	O-3990	>1 μM	>10 μM	O-3991	22 ± 3	>10 μM	O-4210	7.0 ± 2.0	>1 μM
c	CH_3	Cl	O-3978	155 ± 54	>10 μM	O-3936	32.7 ± 2.8	>7 μM	O-6257	7.2 ± 1.8	>1 μM
d	CH_3	Br	O-3992	139 ± 32	>10 μM	O-3977	46.6 ± 9.5	>4 μM	O-3994	14 ± 1.4	755 ± 110
e	CH_3	3,4-Cl_2	O-3911	69.2 ± 17.9	>10 μM	O-3976	101.3 ± 14.4	>2 μM	O-3979	43.5 ± 15.9	766 ± 327
f	C_6H_5	H	O-4144	>5 μM	>10 μM	O-3887	>1 μM	>10 μM	O-4028	157 ± 42	>10 μM
g	C_6H_5	F	O-4070	>2 μM	>10 μM	O-4204	456 ± 71	>10 μM	O-4209	49 ± 10	>10 μM
h	C_6H_5	Cl	O-4004[c]	66.5	>10 μM	O-3888[c]	130	>10 μM		[d]	[d]
i	C_6H_5	Br	O-3993[c]	126	>10 μM	O-3909[c]	238	>10 μM		[d]	[d]
j	C_6H_5	3,4-Cl_2	O-3995[c]	332	>10 μM	O-3912[c]	497	>10 μM	O-3913[c]	269	>10 μM

[a] Compounds are racemic.
[b] Each value is the mean ± SEM of at least three independent experiments, each conducted in triplicate.
[c] Except the data for compounds **h**–**j** were screening data used to determine which class to proceed with, the 3-methyl class or the 3-phenyl class. Errors do not generally exceed 15% between replicate experiments conducted in triplicate.
[d] Not available.
[e] Data taken from Ref. 11.

binding in the presence and absence of (−)-cocaine (100 μM). [^{3}H]Citalopram was used to label the SERT and non-specific binding was measured in the presence of fluoxetine (10 μM). Competition studies were conducted with a fixed concentration of radioligand and a range of concentrations of the test drug. All drugs inhibited [^{3}H]WIN35,428 and [^{3}H]citalopram binding in a concentration-dependent manner. Inhibition constants (IC_{50}) are presented in Table 1.

The goal of this preliminary study was twofold. First, we wished to explore whether the hydrolytically labile C2-ester of the parent 2-carbomethoxy analogs could be replaced within the 8-thiatropane series while maintaining DAT potency. Second, we wished to evaluate whether a small 3-methylisoxazole or the larger 3-phenylisoxazole would confer greater DAT inhibitory potency. Data in Table 1 show that introduction of a 2-isoxazole was not deleterious to DAT binding, indeed the C2-isoxazole resulted in substantial selectivity because these compounds had essentially no inhibitory potency at the SERT. In comparison with their progenitors, the 2-carbomethoxy analogs (**4**-, **5**-, and **6**-C2-methylesters), it is clear that exchange of an isoxazole for a carbomethoxy group at C2 resulted in similar, or even enhanced, potency for the 3α-boat **5** and 3β-chair **6** series, although reduced potency was evident in the 2,3-unsaturated isoxazole series **4**. Within the 2,3-ene-series **4**, the presence of a 3-methyl or a 3-phenyl on the C2-isoxazole had little, if any, impact on DAT potency. In contrast, within the 3α-boat **5** and 3β-chair **6** series, the 3-methylisoxazole compounds were between 5- and 20-fold more potent at DAT than the 3-phenylisoxazoles. Our on-going studies are consequently focused more heavily on the 3-methylisoxazole series. It is interesting that within the limited number of compounds within each structural class (**4**, **5**, and **6**) the 3β-aryl (chair-configured) compounds **6** were more potent DAT inhibitors than the 3α-aryl (boat-configured) class **5**, while the more planar compounds **4** had the least inhibitory potency at DAT. An exception to this was the family of 3-(3,4-dichlorophenyl) analogs **4e**, **5e**, **6e** and **4j**, **5j**, and **6j**. It is possible that the lipophilicity inherent in the 3,4-dichlorophenyl motif influenced the mode of interaction of these molecules with their binding site on the DAT. We had observed a similar effect previously in our exploration of 3,4-dichlorophenyl substituted tropanes[11,10] The replacement of the 8-aza functionality with the 8-thia functionality can lead to a reduction in DAT inhibitory potency. Thus the 2β-(3-methylisoxazol-5-yl)-3β-(4-chlorophenyl)-8-thiabicyclo[3.2.1]octane (IC_{50} = 0.59 nM) reported by Carroll[13] is about ten times more potent at DAT inhibition than the directly analogous 8-thia compound, **6c** (IC_{50} = 7.2 nM). Finally, within this class of 8-thia compounds, the influence of aromatic substituents on the 3-aryl group upon DAT potency or selectivity was small. Thus, the 4-fluorophenyl and 4-chlorophenyl analogs in each class (boat or chair) were similar to one another in potency (**5b**, **5c**: IC_{50} = 22–33 nM; **6b**, **6c**: IC_{50} = 7 nM). However, the chair compounds **6b**, **6c** were about three to fivefold more potent than the boat analogs **5b**, **5c**.

In conclusion, a series of 2-(3-methyl or 3-phenylisoxazol-5-yl)-3-aryl-8-thiabicyclo[3.2.1]octanes and oct-2-enes was synthesized. This new class of 8-thiabicyclo[3.2.1]octanes provided potent and selective inhibitors of the DAT. The C2-ester present in cocaine and the parent bicyclo[3.2.1]octanes could be replaced by a C2-isoxazole with retention of inhibitory potency at the DAT. The 3β-aryl compounds proved particularly potent inhibitors of DAT (IC_{50} = 7–43 nM) with substantial selectivity versus inhibition of SERT. In both the 3α-aryl and 3β-aryl series, the 3-methylisoxazole manifested superior DAT inhibitory potency and selectivity compared with the 3-phenylisoxazoles.

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Supplementary data

Synthetic procedures, spectral data, and biological assays are provided. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.076.

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