Published in final edited form as:

Bioorg Med Chem Lett. 2011 January 1; 21(1): 48–51. doi:10.1016/j.bmcl.2010.11.076.

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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Keywords

Tropanes; Dopamine transporter; Monoamine transporter ligands; Cocaine medications

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 profile⁹ characterized by its immediate effect (<15 seconds) and short duration of action (10–15 minutes). 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

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Supplementary Information

Supplementary information associated with this article can be found, in the online version, as doi: . Synthetic procedures, spectral data, and biological assays are provided.

The class of bicyclo[3.2.1]octanes,^{10,11} of which cocaine (Figure 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 2 β -carbomethoxy-3 β -(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 agents¹³ 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 3 β -(4-chlorophenyl)tropane substituted in the 2 β -position by a 3-(4-methylphenyl)isoxazole in place of the C2 β -methyl ester present in WIN35,428.¹⁵

In 1997 we presented evidence¹⁶ 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 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 3 α -aryl (**2**) and 3 β -aryl (**3**) configured compounds, as we have described previously.¹¹ Separation of the 3 α -**2** and the 3 β -**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 ¹H-NMR. In particular, the resonance positions and multiplicity observed for the H₂, H₃, H_{4 α} and H_{4 β} protons proved diagnostic. Thus the 2,3-enes, exemplified for **4a**, manifested characteristic double multiplets between δ 2.96-3.04 for H_{4 β} that reflected gem coupling ($J_{4\alpha,4\beta}$ = 19 Hz)

as well as vicinal ($J_{4,5} = \sim 3$ Hz) and w-long range coupling ($J_{4,6} = \sim 3$ Hz). $H_{4\alpha}$ resonated at $\delta 2.48$ ($J_{4\alpha,5} = \sim 2.5$ Hz). The vicinal coupling constants of $J = \sim 2.5$ –3 Hz for both $H_{4\alpha}$ and $H_{4\beta}$ to H_5 implied approximately equal dihedral angles ($H_{4\alpha}$ or $H_{4\beta}$ -C4-C5- H_5 of 55° – 60°) thus indicating that C1-C2-C3-C4-C5 are approximately coplanar. The 3α -aryl boat configured **5a** manifested diagnostic resonances for H_2 $\delta 3.03$, H_3 $\delta 3.16$ (ddd), and $H_{4\alpha}$ $\delta 1.37$ (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 $\delta 3.66$ (dd), H_3 $\delta 3.43$, $H_{4\alpha}$ $\delta 1.36$ (ddd), and $H_{4\beta}$ $\delta 2.50$ (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 [3H]3 β -(4-fluorophenyl)tropane-2 β -carboxylic acid methyl ester ([3H]WIN 35,428 or [3H]CFT (4 nM)) and non-specific binding was defined as the difference in binding in the presence and absence of (–)-cocaine (100 μ M). [3H]Citalopram was used to label the SERT and non-specific binding was measured in the presence of fluoxetine (10 μ M). Competition studies were conducted with a fixed concentration of radioligand and a range of concentrations of the test drug. All drugs inhibited [3H]WIN 35,428 and [3H]citalopram binding in a concentration-dependent manner. Inhibition constants (IC_{50}) are presented in Table 1.

The goal of this preliminary study was two-fold. 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 to 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**, **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 2 β -(3-methylisoxazol-5-yl)-3 β -(4-chlorophenyl)-8-thiabicyclo[3.2.1]octane ($IC_{50} = 0.59$ nM) reported by Carroll¹³ 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 3-5 fold 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 3 β -aryl compounds proved particularly potent inhibitors of DAT (IC₅₀ = 7-43 nM) with substantial selectivity versus inhibition of SERT. In both the 3 α -aryl and 3 β -aryl series, the 3-methylisoxazole manifested superior DAT inhibitory potency and selectivity compared with the 3-phenylisoxazoles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Institute on Drug Abuse (PM: DA11542; AJ: DAO18165; BKM: DA06303) and by the VA Merit Review and Research Career Scientist Programs (AJ).

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Figure 1.
Cocaine, WIN35,428 and RTI336

Reaction conditions: (i) Sml_2 , MeOH, THF, -78°C ; (ii) $n\text{BuLi}$, 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 ₆ H ₅	H
b	CH ₃	F	g	C ₆ H ₅	F
c	CH ₃	Cl	h	C ₆ H ₅	Cl
d	CH ₃	Br	i	C ₆ H ₅	Br
e	CH ₃	3,4-Cl ₂	j	C ₆ H ₅	3,4-Cl ₂

Scheme 1.

General Synthetic Route to 2-(Isoxazol-5-yl)-3-aryl-8-thiabicyclo[3.2.1]octanes

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

Table 1

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

^aCompounds are racemic.

^bEach value is the mean ± SEM of at least three independent experiments, each conducted in triplicate.

^cExcept: 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.

^dNot available.

^eData taken from Pham-Huu et al. Bioorg & Med. Chem. 15 (2007) 1067.