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Design and Synthesis of an Irreversible Dopamine-Sparing Cocaine Antagonist†

Peter C. Meltzer,[a,*] Shanghao Liu,[a] Heather S. Blanchette,[a] Paul Blundell[a] and Bertha K. Madras[b,‡]

[a]*Organix Inc., 240 Salem Street, Woburn, MA 01801, USA*
[b]*Department of Psychiatry, Harvard Medical School and New England Regional Primate Research Center, Southborough, MA 01772, USA*

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Abstract—Cocaine is a powerful reinforcer and stimulant that binds to specific recognition sites associated with monoamine transporters in the mammalian brain. The search for a functional antagonist to the addictive properties of cocaine has focused on the discovery of a molecule that can inhibit cocaine binding to the dopamine transporter (DAT) but continue to allow dopamine transport by the DAT. No such dopamine-sparing cocaine antagonist has been reported and it is becoming evident that dopamine-sparing antagonism of the pharmacological effects of cocaine by a classical antagonist may not be possible. Herein we present a new concept for the design of dopamine-sparing cocaine antagonists. A unique approach is utilized to deliver an inhibitor that binds irreversibly to the DAT, then cleaves and leaves behind a small fragment attached to the DAT that blocks access by cocaine but permits dopamine transport. The design of these compounds takes advantage of a cysteinyl sulfhydryl group in the DAT. This group is hypothesized to attack the incoming inhibitor and lead to selective inhibition of the cocaine binding site while sparing dopamine transport. This concept of a mechanism based irreversible dopamine-sparing cocaine antagonist has now been demonstrated to be viable and, as example, the unsaturated **6** showed inhibition of cocaine (63%) at the DAT after 24 h incubation, while at that point considerably less inhibition of dopamine is manifested (23%). In contrast, the epoxide **7** showed a greater inhibition of dopamine reuptake than cocaine binding at 24 h (68% versus 18%).

Introduction

The widespread abuse of cocaine has led to considerable societal problems. It is a powerful reinforcer and stimulant that binds to specific recognition sites associated with monoamine transporters in the mammalian brain.[1–6] The search for a functional antagonist to the addictive properties of cocaine has been the focus of considerable research, and the dopamine transporter hypothesis has guided much of this work.[2,7,8] Accumulating evidence supports the view that the addictive properties of cocaine derive from inhibition of dopamine reuptake by the presynaptic dopamine transporter (DAT) (Fig. 1). Such inhibition leads to an increase of dopamine (DA) concentration within the synapse, and this increase in available DA then causes activation of postsynaptic DA receptors. Excess stimulation of postsynaptic DA receptors then leads to the well-known sequellae of cocaine pharmacology. It has therefore been a goal of research to discover a molecule that can inhibit cocaine binding to the DAT but continue to allow DA transport by the DAT. While the cocaine inhibitor would still bind to the DAT, it would not affect the rate of reuptake of DA by this transporter. In this manner, the concentration of DA in the synapse would remain at normal physiological levels.

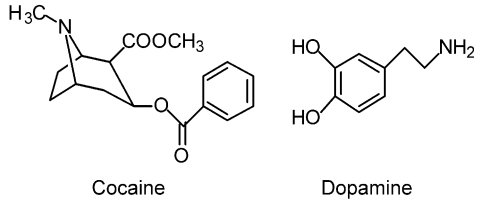

Figure 1. Cocaine and dopamine.

†A communication describing the biology of these compounds is being submitted concurrently to *Molecular Pharmacology*.

*Corresponding author. Tel.: +1-781-932-4142; fax: +1-781-933-6695; e-mail: meltzer@organixinc.com
‡Corresponding author, biology.

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The search for a cocaine antagonist, or cocaine replacement, has been dominated by an exploration of six main classes of compounds.[9,10] These include the tropanes,[11–19] GBR analogues,[20,21] methylphenidate analogues,[22,23] cocaine analogues,[24–26] mazindol,[27] and diarylmethoxy tropanes.[17,28,29] To date there has been no discovery of a 'dopamine-sparing cocaine antagonist'.[30] Indeed, dopamine-sparing antagonism of the pharmacological effects of cocaine by a classical antagonist may not be possible. A consideration of the difference between antagonism of a receptor versus antagonism of a transporter, such as the DAT, is instructive. A receptor *agonist* relies upon binding of the agonist to the biological macromolecule. Once bound, the *agonist* activates the receptor and this activation leads to the biological and pharmacological sequellae. A classical receptor *antagonist* binds to the receptor, but cannot activate it. In analogy, DA activates postsynaptic receptors by first binding and then activating those receptors. The DA excess within the synapse is removed by the DAT. Cocaine binds to the DAT and inhibits this DA reuptake. Activation of postsynaptic DA receptors caused by the inability to remove DA from the synapse then leads to cocaine pharmacology. Therefore, inhibition of DA reuptake may be a necessary and sufficient cause of cocaine pharmacology. Consequently a cocaine antagonist is not simply a molecule that will inhibit cocaine binding at the DAT, but more importantly, it will permit transport of synaptic DA to the presynaptic neuron.

In recognition of this, much research has focused on the design of DAT inhibitors that differentiate between dopamine reuptake and cocaine inhibition.[31] Deutsch and Schweri[32] have defined the *discrimination ratio* (DR) to differentiate between dopamine reuptake and cocaine inhibition. This is the ratio of the IC_{50} for the test compound for inhibition of [^{3}H]dopamine reuptake divided by the corresponding IC_{50} of [^{3}H]WIN 35,428 binding inhibition to the DAT. Since small differences (DR <10) could be accounted for in terms of assay difficulties, it was proposed that a ratio of greater than 10 may signal a potential cocaine antagonist. While the discrimination ratio has been a useful tool, it is becoming apparent that it does not necessarily indicate the potential for pharmacological antagonism. Numerous compounds that manifest a DR greater than 10 have been prepared and none have proven to be dopamine-sparing cocaine antagonists. We have prepared compounds that manifest DRs of 10–137 but none have proven to be antagonists of the in vivo pharmacological effects of cocaine.

The DAT has been cloned[33–35] and the binding site for dopamine described.[30] The hDAT is comprised of 620 amino acids putatively arranged in 12 interconnected helices. However the tertiary structure is unknown. In 1997 we proposed a working model for the DAT as a transmembrane channel, comprised of the 12 surrounding helices, that provides an active channel through which DA is transported intracellularly.[13] Each helical domain presents its polar (amide) linkage toward the core of the helix. This places the amino acid side chain on the outside of the helix facing the internal structure of the channel. Consequently, any ligand passing into this channel is confronted by the side-chain face of the relevant helices. Dopamine is conceived to pass into, and through, this channel into the presynaptic neuron. Ligands (e.g., cocaine) are conceived to pass into the channel, bind across transmembrane domains and block entry (binding) of any other potential ligands or substrates (including DA). Blockade by ligand binding will thus prevent binding of any competing ligand or substrate (DA) whether or not the competing ligand or substrate binds at the identical molecular acceptor site or not. Therefore *any* molecule that can enter and bind to the 12-membered transmembrane channel and is not transported, may inhibit dopamine uptake. This implies that it may not be possible to develop a dopamine-sparing cocaine antagonist that is of molecular dimensions and topology sufficient to obstruct physical passage of dopamine itself. Therefore, notwithstanding the probability that different ligands bind at different acceptor sites within the DAT, each ligand that blocks DA reuptake will be unable to function as a dopamine-sparing cocaine antagonist. A new design of an antagonist is required.

Herein we present a different concept for dopamine-sparing cocaine antagonists. It utilizes a unique approach to deliver a small non-tropane irreversible DAT inhibitor that blocks DAT access by cocaine but permits DA transport. These compounds are based on the premise that to obtain cocaine antagonism without dopamine inhibition, *the bulk of the antagonist should be removed from the binding site after an interaction that renders the dopamine transporter unavailable for cocaine, but readily available for dopamine transport*. Thus selective and potent compounds bind to the dopamine transporter but, once bound, interact covalently with a proximate amino acid. The bulk of the parent guiding tropane dissociates, leaving a small residue attached.

Although a binding site for dopamine has been proposed [(Asp^{79}) in the transmembrane domain 1 (TMD 1) and at two serine residues in the transmembrane domain 7 (TMD 7)],[30] it is still unclear where cocaine and the tropane analogues bind on the DAT. SAR studies have indicated that it is probable that the disparate DAT ligands (cocaine, 3-aryltropanes, 8-oxa-3-aryltropanes, 8-carba-3-aryltropanes, GBR compounds, methyl phenidate, mazindol) occupy different acceptor sites within the DAT. Consequently the design of an irreversible mechanism-based cocaine antagonist of the DAT has to rely on the probable availability of amine (e.g., lysine) or sulfhydryl (e.g., cysteine) groups near the cocaine acceptor site. This first generation of mechanism-based cocaine antagonists is designed to utilize available cysteine residues on the DAT.

There are 13 cysteine residues in the hDAT. Six of these are located within the putative helices of the DAT while seven are in the connecting amino acid chains.[36] Therefore, it is likely that cysteine residues are available in the vicinity of the cocaine acceptor site on the DAT. Cao et al.[37] pointed out that the cocaine receptor has an active nucleophilic thiol, and showed that *N*-ethylmaleimide, an excellent sulfhydryl trap, inhibited [^{3}H]dopamine

uptake totally but [^{3}H]cocaine binding only partially. Schweri showed that *N*-ethylmaleimide irreversibly inhibits binding of methylphenidate to the stimulant recognition site[38] and Reith later showed[39] that cocaine and dopamine protect different sites on the DAT from *N*-ethylmaleimide alkylation. Therefore not only are cysteine residues important to DAT function, but it is likely that different cysteines are proximate to the dopamine and cocaine binding sites. Consequently, our design of an irreversible antagonist takes advantage of a reactive cysteinyl sulfhydryl group in the region of the cocaine binding site. While cysteine can provide a unique opportunity for design of mechanism-based antagonists of the DAT, it also presents substantial pharmacological challenges. This is because cysteine is ubiquitous in proteins. As an example, glutathione, present in large amount in vivo, may preempt the delivery of a thiol sensitive ligand to the actual site of interest. Consequently, we sought to design a molecule that would be activated to thiol attack primarily within the site of interest in a pseudo-intramolecular interaction with the available sulfhydryl moiety of cysteine. While sulfhydryl nucleophile-accepting moieties such as maleimides, α,β-unsaturated systems, and isothiocyanates, may be appropriate functionality for this concept, these reactive functional groups may also be expected to interact irreversibly with amino acid residues in unrelated biological structures. Consequently careful control of the reactivity of these sulfhydryl acceptors is required here. Interaction of the sulfhydryl acceptor with the cysteine residue at the DAT should preferably be triggered once it is localized within the DAT acceptor site. We initially selected an epoxide as the sulfhydryl nucleophile acceptor, but subsequently found that a terminal double bond provided the opportunity via free radical initiated chemistry.

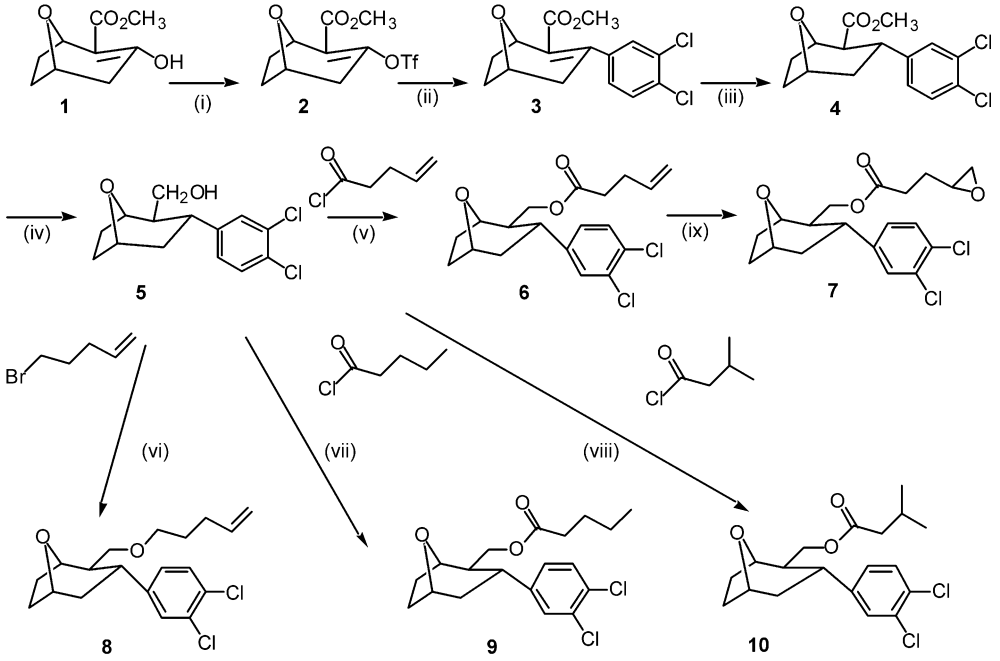
Results

Chemistry

The synthetic route to the target compounds is presented in Scheme 1. Thus the 8-oxabicyclo[3.2.1]octene **3**[13] served as precursor for the 3α-aryl target compounds **7**–**10**. Reduction of the 2,3-ene **3** with samarium iodide provided both the 3α-aryl and 3β-aryl compounds. The 3α-aryl compound **4** was used here since 3α-aryl compounds are more DAT selective than are the 3β-aryl analogues.[40] Reduction of the ester with lithium aluminum hydride in THF then provided the alcohol **5**. Reaction of the alcohol, under basic conditions with selected acid chlorides and alkyl or alkenyl bromides, provided the esters **6**, **9** and **10** and ether **8** in good yield. The epoxide **7** was obtained by oxidation of the unsaturated ester **6** with *m*-chloroperbenzoic acid in methylene chloride.

Biology

The affinities (IC_{50}) of the compounds for the dopamine and serotonin transporters were determined in competition studies using [^{3}H]2β-carbomethoxy-3β-(4-fluorophenyl)-8-methyl-8-azabicyclo[3.2.1]octane ([^{3}H]WIN 35,428) to label the dopamine transporter and [^{3}H]citalopram to label the serotonin transporter. Binding data for the compounds are presented in Table 1. Competition studies were conducted with a fixed concentration of radioligand and a range of concentrations of the test compound. All compounds inhibited [^{3}H]WIN 35,428 and [^{3}H]citalopram binding in a concentration-dependent manner. The six compounds inhibit [^{3}H]WIN 35,428 binding to the DAT with nM potency (17–75 nM) and are reasonably selective versus the SERT (7-fold to 30-fold). In our assay of potential functional antagon-



Scheme 1. Synthesis of 2-substituted 8-oxabicyclo[3.2.1]octanes. Reagents and conditions: (i) $Na(TMS)_2N$, $Ph(Tf)_2N$, THF, −78 °C; (ii) $ArB(OH)_2$, Pd_2dba_3, Na_2CO_3, LiCl; (iii) SmI_2, methanol, −78 °C; (iv) LAH, THF, 100%; (v) Et_3N, RCOCl, 51%; (vi) THF, NaH, RBr, 30%; (vii) RCOCl, Et_3N, CH_2Cl_2, 12%; (viii) Et_3N, RCOCl, CH_2Cl_2, 76%; (ix) *m*CPBA, CH_2Cl_2, 54%.

ism, the hDAT was incubated with each of the compounds 6–10 for a period of 24 h. The cells were then exhaustively washed to remove non-covalently bound ligand and binding of cocaine (20 nM) and dopamine uptake (V_{max}) were compared.

The controls selected for this study, WIN 35,428 and (−)-cocaine did not bind irreversibly to the DAT since inhibition of both DA uptake and cocaine binding after 24 h incubation was 90% or more in both cases (Table 1). Therefore, washout was essentially complete. The compounds of this study may be expected to have similar lipophilicity and were therefore expected to be washed out similarly. In contrast to WIN 35,428 and (−)-cocaine, the sulfhydryl acceptor, *N*-ethylmaleimide, did manifest irreversible binding within the same time frame (35% inhibition of both functions). The epoxide **7** showed a greater inhibition of dopamine reuptake than cocaine binding at 24 h (68% vs 18%). Compounds **6** [racemic and enantiomerically pure (1*R*)-**6**] significantly inhibited cocaine binding to the DAT after 24 h (63 and 80%, respectively). However, inhibition of dopamine was much reduced. Unsaturated ether **8** manifested a preferred inhibition of cocaine binding to dopamine uptake (80% vs 40%) and therefore bound irreversibly and allowed substantial dopamine uptake. Saturated ester **9** showed no inhibition of binding or uptake; therefore no irreversible binding took place. In contrast, saturated ester **10** showed similar and substantial inhibition of DA uptake and cocaine binding after 24 h (60%). This latter result is surprising, and compound **10** is currently under further investigation in our laboratories.

Discussion

To date, no cocaine antagonists have been reported, although a significant effort has been directed toward their discovery.[9,10,18,26,28,31,41–43] The fundamental difficulty arises from the fact that cocaine is a dopamine antagonist at the DAT: it blocks dopamine reuptake. Consequently, any other ligand that blocks cocaine uptake at the DAT, may also block dopamine. Therefore it can have the same, or similar pharmacological effects as cocaine itself, that is, increase dopamine concentration in the synapse. There has been a significant effort to discover compounds that bind potently and selectively to the DAT but that bind allosterically in the hope that such compounds would inhibit cocaine uptake *but not dopamine* reuptake. To date this approach to dopamine-sparing cocaine antagonists has failed.

In contrast to this approach, the molecules presented here are based on the premise that cocaine antagonism without dopamine inhibition can be achieved if the bulk of the antagonist is removed from the binding site after an interaction that renders the site unavailable for cocaine, but available for dopamine trafficking. To this end we have designed molecules that manifest high potency and selectivity for the DAT. They possess a *ligand* that directs the agent to the binding site on the DAT. The *ligand* is attached, via a cleavable *tether*, to an acceptor moiety (*barb*) that can bind covalently to an amino acid residue (e.g., thiol of cysteine) in the vicinity of the binding site. The attack of the receptor site based thiol is then followed by release of the *ligand*. The *barb* remains to perturb, or block, the site locally to cocaine. The *barb* should be sufficiently small so as not to block 'incoming' dopamine and should also not cause extensive perturbation of the receptor site. This process is depicted in Figure 2.

The selection of appropriate *barb* functionality is complicated by the relative kinetics required in order for the delivery, binding, reaction, release, and washout to occur in sequential order. Specifically, the rate of interaction of the *barb* with a thiol must be slower than the rate of delivery of the ligand to the site of action itself as well as binding to that acceptor site. However, once within the binding site, the attack upon the incoming ligand should be efficient in order to avoid washout of the ligand prior to covalent binding. The subsequent cleavage of the *ligand* should be slower than the inter-

Table 1. Comparison of affinity of compounds for the dopamine transporter (DAT) and serotonin transporter (SERT) and the effects on dopamine transport and cocaine binding[a]

Compound	Dopamine transporter IC_{50} (nM)	Serotonin transporter IC_{50} (nM)	Selectivity SERT/DAT	Inhibition of DA uptake after 24 h (%)	Inhibition of cocaine binding after 24 h (%)
	[^{3}H]WIN 35,428	[^{3}H]citalopram			
WIN 35,428	11	160	15	2	12
(−)-Cocaine[b]	95	270	2		
N-Ethylmaleimide	>100,000	>100,000	—	35	35
6, O-1893 (1*R*/*S*)	20	593	30	23	63
6, O-2185 (1*R*)	24	326	14	48	80
7, O-1834	17	292	17	68	18
8, O-2153	58	755	13	40	80
9, O-2102	71	917	13	0	0
10, O-2059	75	515	7	60	60

[a]Effects of 24-h pre-incubation of the test compounds on [^{3}H]dopamine transport and [^{3}H]cocaine (20 nM) bound in HEK-293 cells transfected with the human dopamine transporter. The cells were extensively washed prior to conducting binding and transport assays. Errors generally do not exceed 15% between replicate experiments. Highest doses tested were generally 10–100 μM. Results are expressed as % of control values and are the means of 2–5 determinations, each conducted in triplicate.
[b]Cocaine washes out completely within minutes.

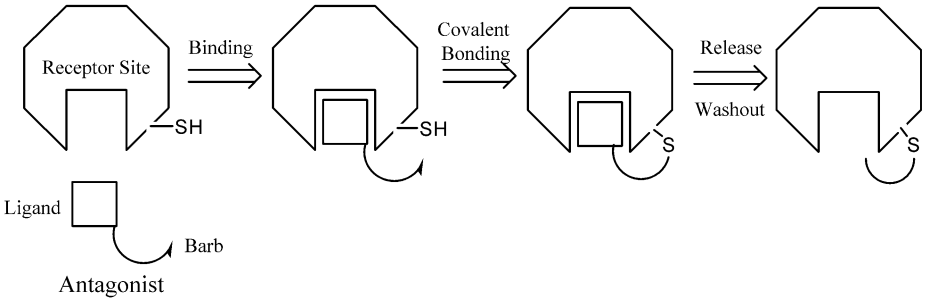


Figure 2. The approaching antagonist binds and leaves the *barb* behind, covalently bound to the receptor.

action with the cysteine in order to avoid cleavage of the molecule prior to delivery, binding, and covalent attachment at the active site. However, this cleavage, once within the DAT acceptor site, should be complete, with release of the tropane moiety. The washout rate of the released tropane moiety will then determine the onset time of the dopamine sparing cocaine antagonist as well as its efficacy. The duration of action of the successful cocaine antagonist will then be determined by the relative turnover rate of the DAT itself. These considerations have guided our selection of a suitable *barb* and *ligand*.

The *ligand*

The *ligand* serves as a 'honing' device to guide the molecule specifically and selectively to the dopamine transporter. The 8-oxabicyclo[3.2.1]octane family has provided an array of DAT binding agents.[13] Herein we used 2β-carbomethoxy-3α-(3′,4′-dichlorophenyl)-8-oxabicyclo[3.2.1]octane, a potent (DAT $IC_{50} = 2.34$ nM) and reasonably selective (SERT $IC_{50} = 31$ nM) compound, for this first generation of irreversible dopamine-sparing cocaine antagonists.

The *tether*

The C2-position of the 8-oxabicyclo[3.2.1]octanes provides an excellent opportunity for introduction of our *tether*. An ester function was initially selected as the cleavable link.

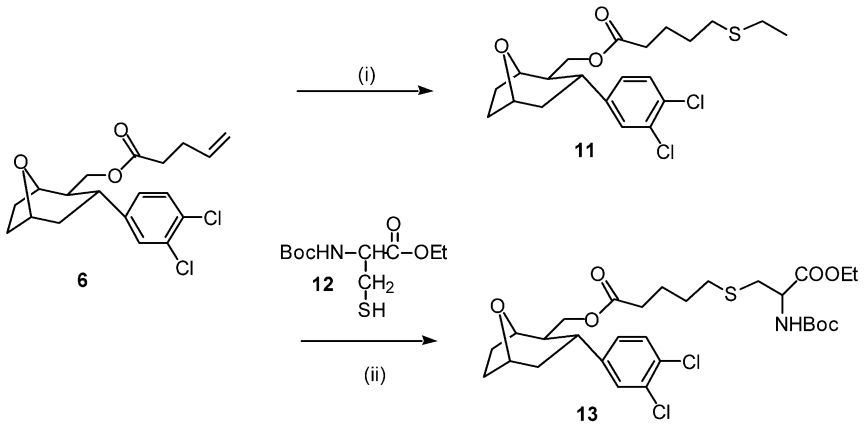
The *barb*

Our initial design of a *barb* was based upon the premise that nucleophilic attack by a cysteinyl sulfhydryl group on an epoxide (possibly catalyzed by aspartic acid) would cause a cascade of reactions to form a five-membered lactone attached to the cysteine. We had anticipated acceleration of this reaction in the pseudo-intramolecular situation in which the ligand is tightly held in close proximity to the nucleophile. Therefore, the first *barb* selected was an epoxide. However, the epoxide **7** manifested a greater inhibition of dopamine reuptake than of cocaine binding. Apparently compound **7** does not guide the ligand to the cocaine binding site, but prefers binding to the dopamine site, much as observed by Cao[37] in his experiments with *N*-ethylmaleimide. Notwithstanding, the idea of a 'honing device' had succeeded, albeit to the undesired site.

While we had clearly outlined the conceptual approach toward the design of these 'Trojan Horse' molecules, it is interesting that the molecules that have now become most exciting from this work were obtained serendipitously! We feel it instructive to describe the events that have led to this development in order to communicate not only the excitement of science, but also the sometime reality! An immediate synthetic precursor to the epoxide **7** was the ene **6**. While we initially had little expectation for **6** since reaction with a *nucleophile* is unprecedented, we submit all advanced intermediates for biological evaluation and **6** was similarly included. We were therefore incredulous when this compound showed attributes of a dopamine-sparing cocaine antagonist! In fact, the racemic compound **6** manifested a 23% inhibition of dopamine uptake compared with a robust 63% inhibition of cocaine binding after incubation for 24 h and extensive attempts to wash out any non-bound compounds. The enantiomerically pure (1*R*)-**6** inhibited cocaine binding by 80% and dopamine uptake by 48% after 24 h incubation. The (1*S*)-**6** analogue was anticipated to manifest weaker binding to the DAT and has therefore not been explored at this time.

We immediately explored the possibility that the terminal double bond in **6** acts as a sulfhydryl acceptor. Indeed, when **6** was stirred with ethanethiol (EtSH) in THF with either TsOH or with acetic acid at room temperature for 75 h, about 50% of it was consumed (Scheme 2). The product of addition, **11**, as well as the alcohol **5**, were obtained. Since **11** results from anti-Markownikoff addition of EtSH to the terminal double bond in **6**, the addition likely occurred via a free radical mechanism with O_2 (air) in the solvent as initiator. Indeed, when the above reactions were conducted under an N_2 atmosphere in an oxygen free solvent, no addition or cleavage occurred. The addition could also be facilitated by the free radical initiator AIBN. Thus, when **6** was stirred with diprotected cysteine **12** in acetonitrile in the presence of AIBN, **13** was obtained in 50% yield. Compound **5** was not detected in the crude product; in this case there was no acid in the reaction mixture to catalyze cleavage.

The fact that **6** reacts with a thiol supports the possibility that the sulfhydryl group of a cysteine in the DAT could also add to the terminal double bond in **6** under appropriate conditions such as the presence of oxygen or the presence of an hydroxyl radical. Furthermore, we



Scheme 2. Anti-Markownikoff addition of a thiol to **6a**. Reagents and conditions: (i) EtSH, THF, TsOH or CH_3COOH, 75 h, 22 °C, 12%; (ii) **12**, AIBN, CH_3CN, 4 h, 50%.

demonstrated that the ester in **6** is relatively stable prior to the radical attack by the thiol, and are therefore hopeful that this will be the case in vivo as well.

To evaluate this further we prepared the unsaturated ether **8** and the saturated esters **9** and **10**. Compound **8** was anticipated to bind irreversibly by radical chemistry but not to cleave. Surprisingly, it also proved to be a dopamine-sparing antagonist (Table 1). Compound **9**, which clearly cannot react with a sulfhydryl radical does not bind irreversibly as evidenced by the complete washout at the 24 h period. Compound **10**, in stark contrast, inhibits both dopamine uptake and cocaine binding equally notwithstanding the absence of a terminal double bond. These results are currently under investigation in our laboratories.

We suggest that irreversible inhibition of the cocaine (but not dopamine) binding site by **6** on the DAT may occur as follows. The first step is non-covalent binding of the ligand **6** to the acceptor site on the DAT. Since the ligand is now tightly bound, this is followed by pseudo-intramolecular attack by the sulfhydryl radical of a proximal cysteine, upon the double bond, to provide the covalently bound DAT–ligand complex. This complex then undergoes cleavage of the ester and releases 2-hydroxmethyloxabicyclo[3.2.1]octane for washout from the acceptor site and the DAT itself. The remaining *barb* on the binding site now serves to differentially inhibit dopamine uptake and cocaine binding. This explanation of the experimentally observed antagonism is entirely speculative at this time. It remains quite possible that these molecules effect conformational change of the DAT in such a way as to differentiate dopamine uptake from inhibition of cocaine binding.

Conclusion

Herein we have demonstrated that the concept of a mechanism based irreversible dopamine-sparing cocaine antagonist is viable: **6** shows inhibition of cocaine (63%) at the DAT up to 24 h while at that point only 23% inhibition of dopamine transport (V_{max}) is manifested. Future work will be focused on the optimization of lead compounds and the generalization of the concept to other templates. Finally, the mechanism by which unsaturated compounds are attacked by sulfhydryl radicals is well documented in the literature. Notwithstanding, at this time we cannot be confident that this mechanism pertains in the biological assays nor in vivo. We are currently investigating the biological fate of these compounds in an effort to better understand their mechanism of action.

Experimental

NMR spectra were recorded in $CDCl_3$ on a JEOL 300 NMR spectrometer operating at 300.53 MHz for ^1H, and 75.58 MHz for ^{13}C. Tetramethylsilane (TMS) was used as internal standard. Melting points are uncorrected and were measured on a Gallenkamp melting point apparatus. Thin layer chromatography (TLC) was carried out on Baker Si250F plates. Visualization was accomplished with either UV exposure or treatment with phosphomolybdic acid (PMA). Flash chromatography was carried out on Baker Silica Gel 40 mM or by radial chromatography on a Chromatotron. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA. All reactions were conducted under an inert (N_2) atmosphere. Coupling constants (J) are reported in Hz.

[3α-(3,4-dichlorophenyl)-8-oxabicyclo[3.2.1]oct-2β-yl] methanol (5). Lithium aluminum hydride (LAH) (3.64 g, 96 mmol) was cooled to 0 °C in anhydrous THF (40 mL). 2β-Carbomethoxy-3α-(3,4-dichlorophenyl)-8-oxabicyclo[3.2.1] octane, **4**[13] (8.0 g, 25.4 mmol) was dissolved in THF (60 mL) and added dropwise to the stirred reaction mixture. The reaction was warmed to 22 °C and stirred for 18.5 h. The slurry was cooled to 0 °C and the excess LAH was slowly decomposed by addition of water (25 mL) and refluxing for 20 min. The mixture was filtered and the solid collected was rinsed with ether. The filtrate was dried ($MgSO_4$), filtered and condensed in vacuo to a viscous clear oil (7.3 g, ca. 100%). A portion of the clear oil was purified by crystallization (methylene chloride/hexanes) yielding colorless cubic

crystals. Mp 75–76 °C; ^{1}H NMR δ 7.36 (d, 1H), 7.31 (d, 1H), 7.06 (dd, 1H), 4.44 (dt, 2H, J = 2.8, 8.3 Hz), 3.53 (dt, 2H, J = 2.8, 6.4 Hz), 2.61 (m, 1H), 2.36 (m, 1H), 2.22–2.14 (m, 1H), 2.11–1.90 (m, 1H), 1.75–1.62 (m, 3H), 1.47 (dt, 1H, J = 4.6, 5.2 Hz), 1.33 (ddd, 1H, J = 2.8, 11.0, 13.8 Hz); ^{13}C NMR δ 144.8, 132.2, 130.2, 130.0, 129.6, 127.3, 74.6, 71.9, 64.6, 51.0, 37.6, 35.2, 31.9, 30.8.

Pent-4-enoic acid-3α-(3,4-dichlorophenyl)-8-oxabicyclo [3.2.1]oct-2β-ylmethyl ester (6). The crude alcohol **5** (5.44 g, 18.9 mmol) was stirred with dry Et_3N (5 mL) in anhydrous CH_2Cl_2 (75 mL) under an N_2 atmosphere. Pentenoyl chloride (2.6 mL, 23.7 mmol) was added slowly via syringe. The reaction mixture was stirred for 15.5 h, filtered through a pad of silica and concentrated under vacuum to yield an orange oil. The crude product was purified by radial chromatography (6 mm, 20% ether/hexanes) to give a yellow oil (6.71 g, 96%). R_f 0.63 (20% ethyl acetate/hexanes). A portion of the crude oil (3.71 g, 10.0 mmol) was crystallized from hexanes to yield colorless plates (1.91 g, 51%). Mp 49–50 °C; ^{1}H NMR δ 7.34 (d, 1H), 7.29 (d, 1H), 7.04 (dd, 1H), 5.89–5.72 (m, 1H), 5.01 (ddd, 2H, J = 1.6, 8.8, 17.3 Hz), 4.45 (ddd, 1H, J = 2.48, 6.33, 8.8 Hz), 4.26 (d, 1H, J = 7.7 Hz), 3.95 (d, 2H, J = 6.1 Hz), 2.61 (m, 1H), 2.40–2.30 (m, 4H), 2.22–1.91 (m, 2H), 1.84–1.80 (m, 1H), 1.73–1.63 (m, 2H), 1.30 (ddd, 1H, J = 2.5, 11.3, 13.8 Hz); ^{13}C NMR δ 173.0, 144.2, 136.52, 132.4, 130.4, 130.3, 129.7, 127.3, 115.5, 74.2, 66.3, 47.9, 38.1, 36.0, 33.3, 32.0, 30.9, 28.7. Anal. ($C_{19}H_{22}O_3Cl_2$) C, H.

3-Oxiranyl-propionic acid 3α-(3,4-dichlorophenyl)-8-oxabicyclo[3.2.1]oct-2β-ylmethyl ester (7). Alkene **6** (3.4 g, 9.2 mmol) in dry CH_2Cl_2 (100 mL) was treated with *m*CPBA and stirred at 22 °C for 19 h. Excess *m*CPBA was quenched with $Na_2S_2O_3$ (3 g) and stirred with water (100 mL). The two phases were separated, and the organic phase was washed with saturated $NaHCO_3$ (2 × 100 mL). The aqueous phase was back extracted with methylene chloride, then the combined organic layers were dried ($MgSO_4$), filtered and condensed in vacuo to a yellow oil (4 g). The crude oil was purified by radial chromatography (6 mm, 20–30% ethyl acetate/hexanes) to give a pale-yellow oil (1.90 g, 54%). R_f 0.22 (30% ethyl acetate/hexanes); ^{1}H NMR δ 7.37 (d, 1H), 7.28 (d, 1H), 7.05 (dd, 1H), 4.45 (ddd, 1H, J = 2.5, 6.3, 8.8 Hz), 4.25 (d, 1H, J = 7.43 Hz), 3.95 (d, 2H, J = 6.0 Hz), 2.94, (ddd, 1H, J = 3.0, 6.9, 9.6 Hz), 2.75 (dd, 1H, J = 4.7, 4.1 Hz), 2.59 (dt, 1H, J = 6.9, 10.6 Hz), 2.49 (dd, 1H, J = 4.7, 5.0 Hz), 2.39–2.30 (m, 3H), 2.20–2.08 (m, 1H), 2.00–1.75 (m, 3H), 1.76–1.60 (m, 3H), 1.29 (ddd, 1H, J = 2.5, 11.3, 13.8 Hz); ^{13}C NMR δ 172.8, 144.2, 132.4, 130.4, 130.4, 129.7, 127.3, 74.2, 71.8, 66.5, 51.1, 47.9, 46.9, 38.1, 36.0, 32.0, 30.9, 30.2, 27.4. Anal. ($C_{19}H_{22}O_4Cl_2$) C, H.

3α-(3,4-Dichlorophenyl)-2β-pent-4-enyloxymethyl-8-oxabicyclo[3.2.1]octane (8). The alcohol **5** (200 mg, 0.69 mmol) and 5-bromo-1-pentene (114 mg. 0.76 mmol) were mixed in anhydrous THF (20 mL) at room temperature under N_2. Sodium hydride (113 mg, 60% in mineral oil, 2.82 mmol) was added. The resulting solution was heated at reflux for 3.5 h. The reaction solution was cooled and water (40 mL) was added. This was extracted with CH_2Cl_2 (2 × 50 mL). The extracts were dried (Na_2SO_4), combined and evaporated. The residue was purified by flash column chromatography yielding a light yellow oil (74 mg, 30%). R_f 0.32 (30% ethyl acetate/hexanes); ^{1}H NMR δ 7.35 (d, 1H), 7.29 (d, 1H), 7.04 (dd, 1H), 5.85–5.72 (m, 1H), 5.03–4.92 (m, 2H), 4.45–4.36 (m, 2H), 3.39–3.28 (m, 2H), 3.26–3.16 (m, 2H), 2.59–2.49 (m, 1H), 2.37–2.29 (m, 1H), 2.18–2.03 (m, 3H), 2.00–1.92 (m, 1H), 1.74–1.58 (m, 5H), 1.32–1.22 (m, 1H); ^{13}C NMR δ 145.0, 138.3, 132.3, 130.2, 130.0, 129.8, 127.4, 114.7, 74.8, 72.7, 71.7, 70.5, 49.4, 38.3, 35.8, 32.2, 31.0, 30.3, 28.7. Anal. ($C_{19}H_{24}Cl_2O_2$) C, H, Cl.

Pentanoic acid-3α-(3,4-dichlorophenyl)-8-oxabicyclo[3.2.1] oct-2β-ylmethyl ester (9). The crude alcohol **5** (0.70 g, 2.43 mmol) was dissolved in dry CH_2Cl_2 (20 mL) and treated with Et_3N (1.0 mL, 7.2 mmol) and valeryl chloride (0.6 mL, 5.1 mmol). The reaction solution was stirred under an N_2 atmosphere at 22 °C for 16 h. It was then diluted with CH_2Cl_2 (50 mL) and extracted with saturated $NaHCO_3$ solution (50 mL) and brine (50 mL). The organic phase was condensed and purified by radial chromatography (2 mm plate, 10% ethyl acetate/hexanes) to give a clear, colorless oil (0.70 g, 78%). The oil was crystallized from hexanes to yield colorless crystals (0.11 g, 12%). R_f 0.50 (20% ethyl acetate/hexanes). Mp 26 °C; ^{1}H NMR δ 7.36 (d, 1H), 7.29 (d, 1H), 7.06 (dd, 1H), 4.44 (ddd, 1H, J = 2.5, 6.6, 8.8 Hz), 4.27 (d, 1H, J = 7.7 Hz), 3.95 (d, 2H, J = 6.1 Hz), 2.59 (dt, 1H, J = 4.0, 6.9 Hz), 2.39–2.29 (m, 1H), 2.24–1.46 (m, 9H), 1.36–1.22 (m, 3H), 0.89 (t, 3H, J = 7.2 Hz); ^{13}C NMR δ 173.7, 144.2, 132.3, 130.3, 130.2, 129.6, 127.2, 74.2, 71.7, 66.1, 47.87, 38.1, 36.0, 33.8, 31.9, 30.8, 26.8, 22.1, 13.6. Anal. ($C_{19}H_{24}Cl_2O_3$) C, H.

3-Methylbutanoic acid-3α-(3,4-dichlorophenyl)-8-oxabicyclo[3.2.1]oct-2β-ylmethyl ester (10). The alcohol **5** (1.0 g, 3.48 mmol) was dissolved in dry CH_2Cl_2 (20 mL) and treated with Et_3N (1.0 mL, 7.2 mmol) and *i*-valeryl chloride (0.52 mL, 4.29 mmol). The reaction solution was stirred under N_2 at 22 °C for 16 h. It was then diluted with CH_2Cl_2 (50 mL) and extracted with saturated $NaHCO_3$ solution (50 mL) and brine (50 mL). The organic phase was condensed to a yellow oil, which was purified by radial chromatography (6 mm plate, 10–20% ethyl acetate/hexanes) to give a clear, colorless oil (0.98 g, 76%). R_f 0.40 (20% ethyl acetate/hexanes); ^{1}H NMR δ 7.37 (d, 1H), 7.29 (d, 1H), 7.06 (dd, 1H), 4.44 (ddd, 1H, J = 2.5, 6.3, 8.8 Hz), 4.27 (d, 1H, J = 7.7 Hz), 3.92 (d, 2H, J = 6.1 Hz), 2.62 (dt, 1H, J = 6.9, 11.0 Hz), 2.40–2.30 (m, 1H), 2.20–1.80 (m, 4H), 1.80–1.75 (m, 1H), 1.75–1.58 (m, 2H), 1.3–1.20 (m, 1H), 0.91 (d, 6H, J = 6.3); ^{13}C NMR δ 173.0, 144.1, 132.3, 130.3, 130.2, 129.7, 127.3, 74.2, 71.7, 66.0, 48.0, 43.2, 38.1, 35.9, 31.9, 30.9, 25.5, 22.3. Anal. ($C_{19}H_{24}O_3Cl_2$) C, H, Cl.

5-Ethylsulfanyl-pentanoic acid 3-(3,4-dichlorophenyl)-8-oxabicyclo[3.2.1]oct-2-ylmethyl ester (11). Compound **6** (100 mg, 0.26 mmol, 1.0 equiv) was mixed with TsOH (110 mg, 0.58 mmol, 2.2 equiv) in THF (4 mL). EtSH (1.2 mL, excess) was added. The resulting mixture was stirred at 22 °C for 75 h. Volatiles were removed under a

stream of N_2. The residue was dissolved in CH_2Cl_2 (5 mL) and washed with dilute NaOH (10 mL). The CH_2Cl_2 layer was separated and the aqueous layer extracted twice with CH_2Cl_2 (5 mL each). The CH_2Cl_2 solutions were dried over Na_2SO_4, combined and evaporated. The crude product was analyzed by ^{1}H NMR which showed 52% of **6** was consumed. Column chromatographic separation of the crude product gave three major compounds: **6** (recovered starting material, 25 mg, 25%), **11** (addition product, 14 mg, 12%) and **5** (11 mg, 15%). **11**: ^{1}H NMR δ 7.36 (d, 1H), 7.28 (d, 1H), 7.04 (dd, 1H), 4.48–4.42 (m, 1H), 4.25 (d, 1H), 3.94 (d, 2H), 2.61–2.55 (m, 1H), 2.51 (t, 4H), 2.40–2.30 (m, 1H), 2.23 (t, 2H), 2.17–2.06 (m, 1H), 2.01–1.92 (m, 1H), 1.87–1.78 (m, 1H), 1.74–1.52 (m, 5H), 1.34–1.25 (m, 2H), 1.25 (t, 3H); MS (CI, *m*/*z*): 431 $[M-H]^-$.

5-(2-*tert*)-Butoxycarbonylamino-2-ethoxycarbonlyethyl-sulfanyl)-pentanoic acid 3-(3,4-dichlorophenyl)-8-oxabicyclo[3.2.1]oct-2-ylmethyl ester (13). Compound **6** (56 mg, 0.15 mmol, 1.0 equiv), **12** (73 mg, 0.30 mmol, 2.0 equiv) and AIBN (4.0 mg, 0.024 mmol, 0.16 equiv) were mixed in a round bottom flask under N_2 for 10 min. CH_3CN (2.0 mL), which had been degassed with N_2 for 10 min, was added. The resulting solution was stirred and bubbled with N_2 for 10 min and then heated to 50–65 °C for 4 h. CH_3CN was removed and the crude product was analyzed by ^{1}H NMR, which showed 60% of **6** was consumed. Column chromatographic separation of this crude product gave 47 mg (50%) of **13**. ^{1}H NMR δ 7.36 (d, 1H), 7.28 (d, 1H), 7.04 (dd, 1H), 5.35 (d, 1H), 4.52–4.42 (m, 2H), 4.21 (q, 3H), 3.94 (d, 2H), 2.95 (d, 2H), 2.61–2.55 (m, 1H), 2.51 (t, 2H), 2.40–2.30 (m, 1H), 2.23 (t, 2H), 2.17–2.06 (m, 1H), 2.01–1.92 (m, 1H), 1.87–1.78 (m, 1H), 1.74–1.52 (m, 5H), 1.52 (s, 9H), 1.34–1.25 (m, 2H), 1.25 (t, 3H); MS (CI, *m*/*z*): 616 $[M-H]^-$.

Cell plating procedure. HEK-293 cells stably transfected with the hDAT plasmid were grown at 5% CO_2 in a 37 °C water-jacketed incubator until ready to plate. Media of dishes with 90% confluency was aspirated off and cells were rinsed once with 6 mL cold PBS (4 °C, pH 7.4). The rinse was aspirated and cells were harvested in 10 mL PBS and centrifuged for 5 min at 2000 rpm. The cells were then resuspended in an appropriate amount of media and the suspension was stirred. Tissue culture plates coated with poly-D-lysine were prepared by adding 500 μL of media to each well and then 300 μL of cell suspension. Each well contained 800 mL of media and cells. Each plate was shaken vigorously to distribute cells evenly in wells and care was taken to keep the media in the wells. Cells were grown overnight in the incubator and were about 40–50% confluent the next day.

Pre-incubation and rinse procedure. Novel compounds were dissolved, if necessary in an appropriate concentration of EtOH and then at a concentration of 1 mM in 37 °C assay buffer consisting of Tris (5 mM), Hepes (8.5 mM), NaCl (120 mM), KCl (5.4 mM), $CaCl_2$ (1.2 mM), $MgSO_4$ (1.2 mM), glucose (5 mM), and Tropolone (1 mM) at pH 7.4. The stock solution of 1 mM was diluted to 5–25 μM concentration. Cell buffer (pH 7.4 at 37 °C) containing the same final percentage of EtOH that was added to the drug solution was set aside to add to the plates. The 24-well plates were removed from the incubator and 200 μL of novel drug or control buffer was added to each well to a total volume of 1 mL. Plates were then placed in the incubator and incubated for appropriate times periods (1 h, 24 h). At the end of the preincubation, cell plates were removed from the incubator and media aspirated by suction. Each well was rinsed 5 times and a final rinse with buffer (pH 7.4 at 25 °C) was made for 1–2 min in preparation for the assay.

Cocaine binding assay. Serial dilutions of (−)-cocaine, mazindol (10 μM), [^{3}H]cocaine (20 nM) were made. The buffer was aspirated, rinsed from the plate and [^{3}H]cocaine (200 μL) was added to each well, followed by (−)-cocaine (200 μL) of the diluted stock, to a final total volume of 600 μL. Plates were incubated for 1 h, the drug solution was aspirated, each well was rinsed once with 1 mL ice-cold cell buffer (pH 7.4 at 4 °C) and SDS was added to each well. After removing the SDS scintillation fluid was added and radioactivity was measured by liquid scintillation spectrometry.

Dopamine uptake assay. Dopamine and mazindol were prepared in assay buffer (pH 7.4 at 37 °C). Serial dilutions of dopamine combined with 20 nM [^{3}H]dopamine, and mazindol were prepared to measure non-specific binding. Room temperature assay buffer was added to each well and buffer and mazindol were added to measure non-specific binding. In the dark, serially diluted [^{3}H]dopamine was added, incubation proceeded for 10 min, the incubation medium was aspirated and each well rinsed with assay buffer. SDS was added to each well following aspiration of last rinse, removed, scintillation fluid added and radioactivity measured with liquid scintillation spectrometry.

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