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DIFFERENTIAL BINDING AND DOPAMINE UPTAKE ACTIVITY OF COCAINE ANALOGUES MODIFIED AT NITROGEN

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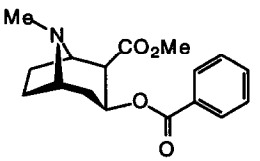
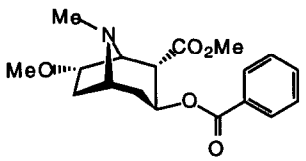
Abstract: Syntheses of a variety of new N-modified cocaine analogues are reported together with their binding affinities and dopamine uptake inhibition activities. Compounds **3** and **13**, which exhibit discrepancies in their binding and uptake potencies, were tested for their ability to antagonize cocaine's inhibition of dopamine reuptake.

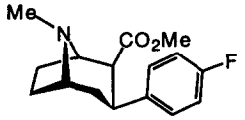
In our continuing efforts to examine the structure-activity relationships (SAR) of a broad variety of cocaine derivatives,[1] we have reported recently that methoxylation of cocaine reduces binding affinity and produces compounds of differential binding and dopamine reuptake activity, with several of these compounds being more active in the binding experiments than in dopamine uptake inhibition.[1a] These results suggested that it may indeed be possible to design a *functional antagonist* of cocaine through structural modifications of the cocaine molecule. Since the nitrogen atom in cocaine is important for potent binding to the cocaine recognition site associated with the dopamine transporter, and the substituent at nitrogen can be modified readily, we felt that it would be worthwhile to synthesize various new N-modified cocaine analogues and to examine both their binding affinities and their ability to inhibit dopamine reuptake.



Cocaine



7α-Methoxypseudococaine



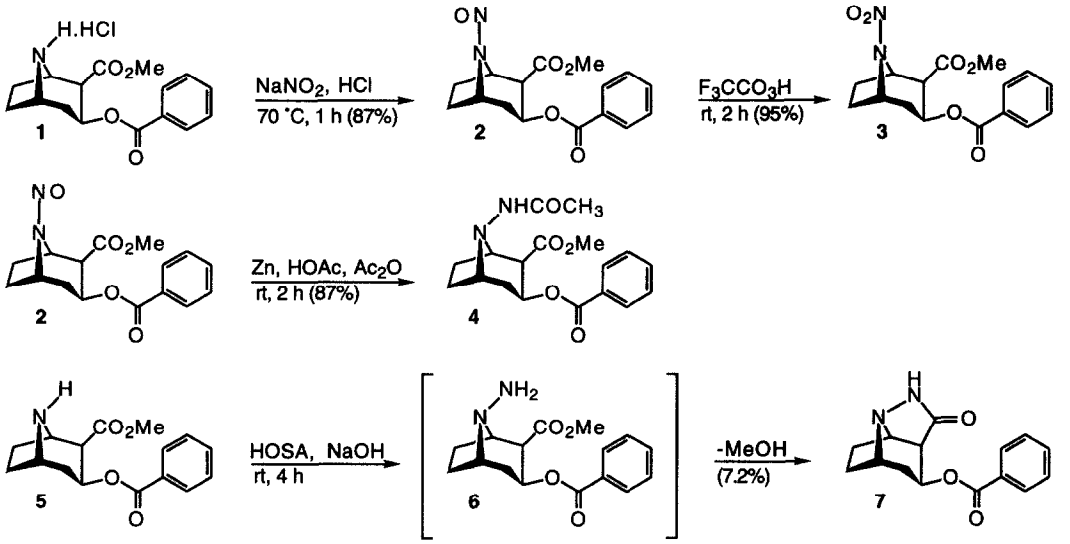
WIN 35,428

As has become evident from earlier studies, substitution at nitrogen can have a large effect on activity, in particular when changes of the electron density at nitrogen are involved.[2] This was demonstrated by the conversion of the cocaine N-methyl group to its methiodide salt bearing a formal positive charge on nitrogen which resulted in 100-fold reduction in activity. Replacement of the N-methyl group with an electron withdrawing acetyl group resulted in a compound of 30-fold reduced

binding affinity. The use of electronically similar but sterically larger groups such as propyl or benzyl in the replacement of the N-methyl group of cocaine reduced the activity by only 0-7-fold. Additionally, Reith *et al.* found that both norcocaine **1** and *N*-allyl-*N*-norcocaine had only slightly reduced binding affinities relative to cocaine as measured in the cortex or striatum.[3] The binding properties of three N-modified 4-fluorophenyl tropanes ("Win" series, allyl, propyl and hydrogen in place of N-methyl) were previously reported by Madras *et al.* who showed that all three compounds were more potent than cocaine in displacing [^{3}H]cocaine from its binding sites. Comparison with the parent 2β-carbomethoxy-3β-(4-fluorophenyl)tropane (Win 35428), however, showed that the potency was decreased.[4] Thus, these studies reveal that replacement of the N-methyl group of cocaine with sterically larger groups has relatively small effects on the binding potency, unless the electron density on nitrogen is decreased, which then results in a greater loss in activity.

The structures of our newly synthesized *N*-modified cocaine derivatives can be divided into two groups: *N*-norcocaine compounds with a nitrogen-nitrogen bond (Scheme 1), and cocaine derivatives with a functionalized N-methyl group (Scheme 2). The cocaine analogues with an N-N bond were

Scheme 1. Synthesis of N-N-functionalized cocaine analogues.

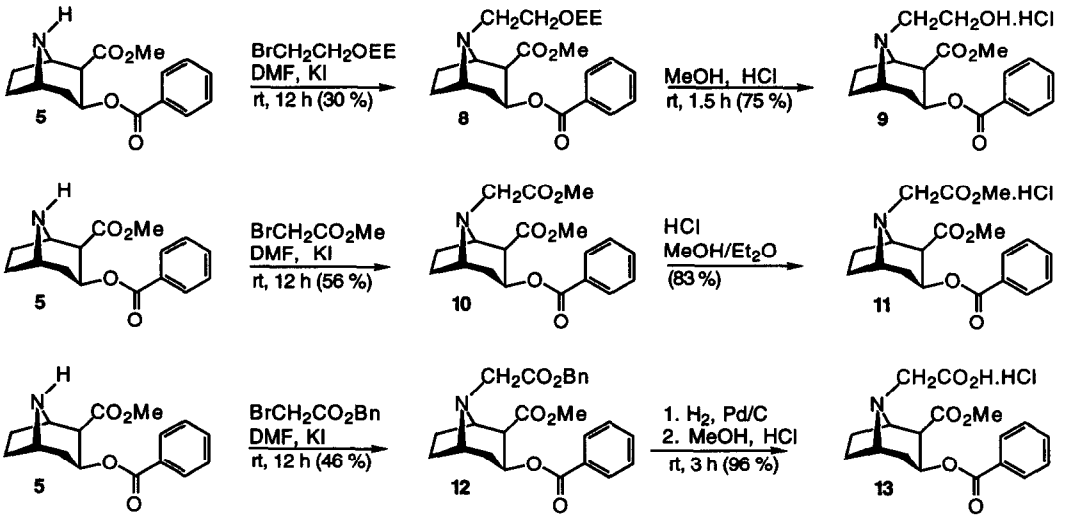


prepared from *N*-norcocaine **1** (Scheme 1).[5] Treatment of **1** with nitrous acid yielded *N*-nitroso-*N*-norcocaine **2**, which was easily oxidized to the N-nitro compound **3** by the action of hydrogen peroxide in trifluoroacetic acid.[6] Attempts to introduce an amino group by reduction of the nitroso group of **2** were unsuccessful, for *N*-acetylamino-*N*-norcocaine **4** was formed instead.[7] Direct introduction of the N-amino group in **5** with hydroxylamine-*O*-sulfonic acid (HOSA)[8] resulted in

formation of the tricyclic lactam **7**, most likely by initial formation of hydrazine **6**, followed by intramolecular attack on the carbomethoxy group.

The cocaine derivatives **9**, **11**, and **13** were all prepared by direct alkylation of the secondary amino group of *N*-norcocaine **5**. Accordingly, alkylation with *O*-ethoxyethyl protected 2-bromoethanol yielded intermediate **8**, which upon treatment with hydrochloric acid was deprotected to give compound **9**. Compound **11** was prepared by alkylation with methyl bromoacetate, and compound **13** was prepared by alkylation with benzyl bromoacetate followed by hydrogenolytic removal of the benzyl group.

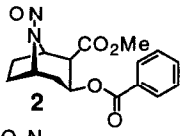
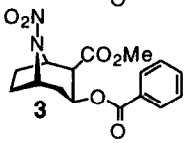
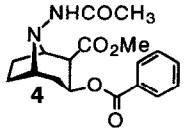
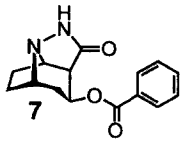
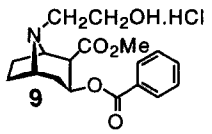
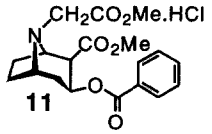
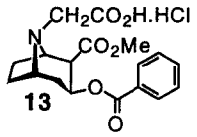
Scheme 2. Synthesis of N-methyl functionalized cocaine analogues.



From the binding and dopamine uptake data presented in Table 1, it is clear that extension of the N-methyl group with a functionalized carbon chain as in **9**, **11**, and **13** leads to binding affinities not much different from that of cocaine. These data can be rationalized by the fact that the electron withdrawing functional groups are insulated from the nitrogen atom by one or two carbon atoms, and thus the effect of the hydroxyl group of **9**, the carbomethoxy group of **11**, and the carboxylic acid group of **13** is too small to have a pronounced influence on the binding affinity. Since the K_is for the binding of compounds **9**, **11**, and **13** are 2.2, 3.3, and 5.5 times smaller than the K_is for inhibition of dopamine reuptake, respectively, it is possible that such analogues may be capable of countering to some extent the effects of cocaine (however, see below).

As shown in Table 1, exchange of the N-methyl group with a functional group containing a nitrogen-nitrogen bond leads to a much more pronounced change in binding potency. All of the compounds **2**, **3**, **4**, and **7** show lower binding affinities in comparison to (-)-cocaine, ranging from a factor of more than one thousand for *N*-aminoacetyl-*N*-norcocaine **4** to a five-fold decrease in binding affinity for *N*-nitro-*N*-norcocaine **3**. In these cases the functional groups are directly connected to the

Table 1. K_i values (μM) ± S.E.M. for cocaine and compounds **2-13** on [^{3}H]dopamine uptake and [^{3}H]mazindol binding in rat corpus striatum P2 synaptosomes.

Compound	[^{3}H]Dopamine uptake; (n)	[^{3}H]Mazindol Binding; (n)
(-)-cocaine	0.32 ± 0.01; (12)	0.28 ± 0.0; (4)
 **2**	231.7 ± 39.5; (3)[a]	99.5 ± 12.3; (3)[b]
 **3**	21.2 ± 0.6; (3)	7.5 ± 0.9; (3)
 **4**	> 1000; (1)[c]	> 1000; (2)[d]
 **7**	115.0 ± 15.7; (2)	44.9 ± 6.2; (3)[e]
 **9**	1.6 ± 0.2; (3)	0.7 ± 0.1; (3)
 **11**	1.6 ± 0.1; (3)	0.48 ± 0.04; (3)
 **13**	2.1 ± 0.4; (3)	0.38 ± 0.02; (3)

(a) maximum inhibition at 1000 μM = 77 %; (b) maximum inhibition at 1000 μM = 82 %; (c) maximum inhibition at 1000 μM = 41 %; (d) maximum inhibition at 1000 μM = 42 %; (e) maximum inhibition at 1111 μM = 83 %.

nitrogen atom, therefore any electronic perturbation caused by these functionalities should have a much more pronounced effect on the binding affinity. Since the functional groups in the N-N series are all electron withdrawing, this explains the overall decrease in binding affinity. However, two of the analogues, with the N-methyl group replaced by the nitroso group (**2**) or the nitro group (**3**) were found to be of particular interest. We observed that replacement of the electron withdrawing nitroso group of **2** with the more electron withdrawing nitro group increases the binding affinity 13-fold. Since the opposite result was, in fact, expected, these data suggest that a decrease in electron density at the bridge nitrogen is not always responsible for a decrease in activity. The reason for this anomaly is presently unknown, however, our results indicate that besides the electron density at nitrogen there are other factors contributing to the overall binding affinity of these N-substituted analogues.

Of further interest is the discrepancy between the binding affinities and dopamine reuptake inhibition activity for **2** and **3**, with both compounds being more active in the binding experiments than in dopamine uptake. The K_i for the binding affinity of compounds **2** and **3** is 2.6 and 2.8 times smaller, respectively, than its K_i for inhibition of dopamine reuptake. Thus, the possibility exists that these compounds may be capable of countering to some extent the effects of cocaine, and therefore serve as functional "antagonists" of cocaine. [^{3}H]Dopamine uptake experiments were therefore conducted to examine whether **3** was capable of causing a concentration-dependent rightward shift of the cocaine inhibition curve. Surprisingly, no meaningful shift was observed with this compound. Additionally, no rightward shift beyond that expected for two inhibitors acting at the same site was detected when compound **13**, which shows a larger spread in binding and uptake potency than **3**, was tested in a similar fashion. In contrast, we had found that 7α-methoxypseudococaine, which shows a four-fold spread in binding and uptake potency, was capable of causing a three-fold rightward shift of the cocaine inhibition curve that was about 30 % greater than that expected for two inhibitors acting at the same site.[1a] While the interpretation of these data is not straightforward, it is clear that the identification of a cocaine antagonist can not be made by simply identifying discrepancies in the binding and uptake K_is. Hopefully, a better understanding of these issues will result from continued investigations of cocaine's chemistry and biology.

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