Structure–Activity Relationship Studies of *N*-Sulfonyl Analogs of Cocaine: Role of Ionic Interaction in Cocaine Binding

Alan P. Kozikowski,*,† M. K. Eddine Saiah,‡ John S. Bergmann,§ and Kenneth M. Johnson§

Trophix Pharmaceuticals, Inc., 40 Cragwood Road, South Plainfield, New Jersey 07080, Mayo Foundation, 4500 San Pablo Road, Jacksonville, Florida 32224, and Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas 77555-1031

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Six new *N*-sulfonylated analogs of cocaine have been prepared, and these compounds have been evaluated for their ability to inhibit [^{3}H]mazindol binding and [^{3}H]dopamine uptake into striatal synaptosomes. The *N*-sulfonyl compounds still inhibited binding and uptake at low micromolar concentrations despite the neutral character of the tropane nitrogen, thus suggesting that the binding of cocaine to the dopamine transporter may not require protonation of its nitrogen and ionic interaction with its recognition site.

Introduction

Cocaine is a powerful central stimulant with potent reinforcing properties.[1] It produces abuse and withdrawal patterns that are uniquely different from those of other major drugs of abuse.[2] The dopamine transporter (DAT) plays a pivotal role in the regulation of dopaminergic transmission. The DAT is also a target for cocaine, PCP, and neurotoxins such as 1-methyl-4-phenylpyridinium (MPP^+) as well as drugs widely used in the clinic for the treatment of central nervous system disorders.[3,4] The human DAT has been cloned, and its primary structure has been elucidated.[5] No crystallographic data on the structure of DAT or, for that matter, any other member of the family of neurotransmitter transporters have been obtained to date. However, site-directed mutagenesis studies have recently been reported, and these results have revealed that aspartate and serine residues lying within the first and seventh hydrophobic putative transmembrane regions are crucial for cocaine binding and dopamine uptake.[6] These findings suggest that the carboxyl group of aspartic acid residue 79 may engage in an ionic interaction either with dopamine's protonated amino group or with the protonated nitrogen of cocaine in binding to the transporter (see Figure 1).

To further explore this point, we investigated the activity of six new cocaine analogs in which the methyl group on the nitrogen was replaced by a sulfonyl group, thus rendering the bridge nitrogen neutral and incapable of protonation under physiological conditions.

Chemistry

The new cocaine analogs **1a,c–f** (Figure 2) were prepared by treating norcocaine with the corresponding sulfonyl chloride in triethylamine as solvent. The excess triethylamine was used to trap the acid generated during the reaction. Lower temperatures (−20 °C) were found to give cleaner reactions. Compound **1b** was prepared by treating norcocaine with trifluoromethanesulfonic anhydride at −78 °C. Not surprisingly, attempts to isolate salts of these compounds by treating them with hydrogen chloride gas failed, thus confirming

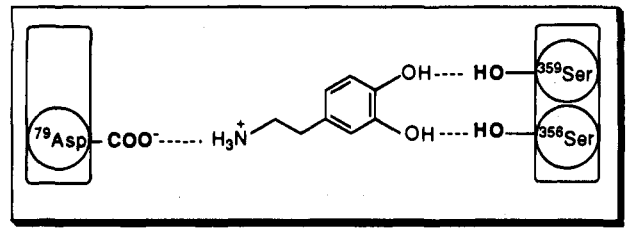

Figure 1. Model of the recognition site of dopamine on the DAT between the first and seventh putative transmembrane domains.

the neutral character of their nitrogen. The ^{1}H NMR spectra reveal deshielding of protons 1-H and 5-H compared to their counterparts in cocaine. The average chemical shift of proton 1-H in the sulfonamide series was 4.6 ppm (4.23 ppm in the case of cocaine), and the average chemical shift of 5-H was 4.5 ppm (4.02 ppm in the case of cocaine). Since the chemical shift of a nucleus is proportional to the electron density around it, the downfield resonance of these protons reflects the low electron density of the N—C1 and N—C5 bonds and, indirectly, the bridge nitrogen.

Pharmacology

The six analogs were tested for their ability to displace [^{3}H]mazindol binding from rat striatal membranes as well as to inhibit high-affinity uptake of [^{3}H]-dopamine into striatal nerve endings (synaptosomes) in accordance with protocols previously described.[7] Mazindol was selected since it has been shown to label the cocaine binding site on the dopamine transporter of rat striatal membranes.[3a,8] The results of this study are presented in Table 1.

Results and Discussion

The six sulfonamides described in this report can be classified in two groups according to their affinity. The first set of compounds includes **1a–c** which display micromolar to submicromolar K_i values in inhibiting [^{3}H]mazindol binding and [^{3}H]dopamine uptake in striatal synaptosomes. Compound **1a** was 4-fold less potent than cocaine in the mazindol-binding experiment and 6 times less potent in inhibiting dopamine uptake. Replacement of the methanesulfonyl group by trifluoromethanesulfonyl gave analog **1b** which is comparable

† Trophix Pharmaceuticals, Inc.
‡ Mayo Foundation.
§ University of Texas Medical Branch.
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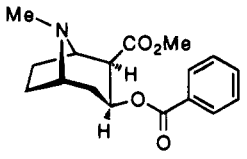
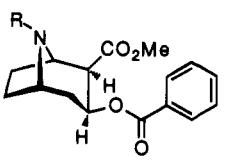
Table 1. [^{3}H]Mazindol Binding and [^{3}H]Dopamine Uptake of Cocaine and Analogs **1a–f**

compound	K_i (μM), mean ± SE (n = 3)[a]	
	[^{3}H]mazindol binding	[^{3}H]dopamine binding
(−)-cocaine	0.28 ± 0.06	0.32 ± 0.01
1a	1.29 ± 0.08	1.97 ± 0.07
1b	0.33 ± 0.03	0.76 ± 0.02
1c	0.12 ± 0.01	0.16 ± 0.01
1d	20.8 ± 3.5	61.0
1e	5.72 ± 1.14[b]	18.8 ± 0.9[c]
1f	6.82 ± 0.58	16.4 ± 1.4

[a] n = 3 except for compound **1d** where n = 2 for [^{3}H]mazindol binding and n = 1 for dopamine uptake and for cocaine where n = 4 for [^{3}H]mazindol binding and n = 12 for dopamine uptake. [b] Maximal inhibition of 80% at 100 μM. [c] Maximal inhibition of 72% at 300 μM.



(-)-Cocaine



1a, R = CH_3SO_2
1b, R = CF_3SO_2
1c, R = $OCNSO_2$
1d, R = $PhSO_2$
1e, R = p-NO_2PhSO_2
1f, R = p-$MeOPhSO_2$

Figure 2. Structure of cocaine and its N-sulfonyl analogs.

in potency to cocaine in the mazindol-binding and dopamine uptake experiments. This result is somewhat surprising since the trifluoromethanesulfonyl group is known to be one of the strongest electron-withdrawing groups,[9] thereby rendering the lone pair of nitrogen unavailable for protonation. Most interestingly, the sulfonyl isocyanate **1c** was 2-fold more potent than cocaine in both mazindol-binding and dopamine uptake experiments. To our knowledge, this is the first example of a simple modification to the nitrogen bridge of cocaine that leads to an analog more potent than cocaine. The second set of compounds was active in the high micromolar range with a drop in affinity varying from 20 times compared to cocaine (compounds **1d,f**) to 75 times (compound **1e**). It is possible that steric factors may largely be responsible for the decreased activity of compounds **1d–f** as compared to compounds **1a–c**.

In previous reports,[10a,b] it has been suggested that a basic nitrogen atom is required for binding to the cocaine recognition site. An attractive explanation for this observation is that in the physiological medium, the nitrogen is protonated and recognized by the negatively charged carboxylate[6] of aspartate 79 of the transporter molecule. However, the data presented above indicate that a neutral nitrogen bridge can also provide high-affinity ligands and, accordingly, that a basic nitrogen is not an absolute requirement for binding.

Moreover, the methiodide salt **2** of cocaine (Figure 3) has been reported[10] to possess an IC_{50} of 35 μM which corresponds to a 111-fold decrease in binding. If it is assumed that cocaine and its analogs bind through an ionic interaction between the protonated nitrogen and the negatively charged carboxylate of aspartate 79, then the low affinity of compound **2** is difficult to rationalize, since this type of interaction is spatially isotropic, i.e., it does not depend on geometric parameters such as spatial overlap or interplanar angles.[11] Because drug–receptor interactions involving an ionic bond are esti-

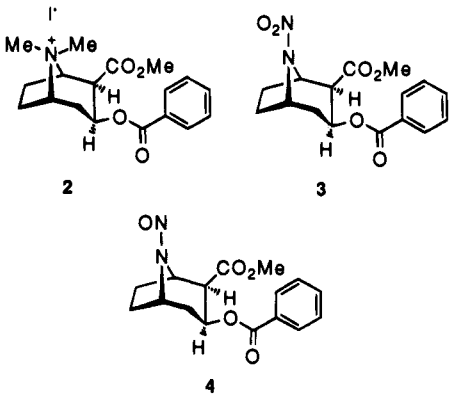


Figure 3.

mated to be in the order of 8–10 kcal/mol,[12] the sulfonamides described herein would be expected to have very low potency compared to cocaine, since they are unable to participate in this type of interaction. The binding of these compounds to the transporter is therefore more likely to be due to hydrogen bonding or weak polar interactions. In fact, a close look at the sulfonamide series indicates that compounds presenting the possibility of additional hydrogen bonding (i.e., in addition to the -SO_2- group itself) or polar interactions (like **1b** or **1c** as opposed to **1a**) exhibited higher affinity for the dopamine transporter. Replacement of the trifluoromethyl group or isocyanate group by phenyl led to a sharp reduction in potency, as in **1d**, whereas some restoration of activity was observed when a methoxy or nitro group was appended to the 4-position of the benzenesulfonyl group. The residue of the transporter which is responsible for the recognition of the cocaine nitrogen may be one which can act either as a hydrogen bond donor or acceptor (e.g., the hydroxyl group of tyrosine[13]). This hypothesis explains why cocaine analogs which are not capable of additional hydrogen bonding, such as **1d** or **2**, display lower potencies. This notion would also explain in part an anomaly we reported in a recent paper[16] where the N-nitro derivative **3** (Figure 3) was found to be 13 times more potent than the nitroso derivative **4**; the former compound has one additional hydrogen bond acceptor site compared with compound **4**.

In conclusion, we describe a new series of N-modified sulfonyl analogs of cocaine which bind to the dopamine transporter with good affinity. These results demonstrate that a basic amino group is not needed for high-affinity binding as previously suggested. Since Asp 79 of the DAT has been found to be crucial for dopamine uptake, our data lend support to the hypothesis that the recognition sites of cocaine and dopamine, while probably overlapping, are not comprised identically of the same amino acids. This conclusion is in agreement with our previous report wherein we showed that cocaine was able to fully protect the cocaine binding site from alkylation by a high concentration of N-ethylmaleimide while dopamine did not afford significant protection.[17] Thus, it seems possible theoretically to identify compounds which bind to the cocaine site on the DAT without blocking simultaneously the reuptake of dopamine. Correlation of the present results with those described previously for other cocaine analogs modified on nitrogen is underway in order to more precisely define the structural requirements for cocaine binding.

Experimental Section

^1H and ^{13}C NMR spectra were recorded at 300 and 75 MHz (Bruker WH-300), respectively, in $CDCl_3$. ^1H chemical shifts (δ) were reported with Me_4Si (δ = 0.00 ppm) or $CHCl_3$ (δ = 7.26 ppm) as internal standards. ^{13}C chemical shifts (δ) were reported with $CHCl_3$ (central peak, δ = 77.00 ppm) as internal standard. The following abbreviations are used, br = broad, d = doublet, m = multiplet, q = quartet, s = singlet, t = triplet. Low- and high-resolution mass spectra were determined on a VG 70-SE double-focusing magnetic sector spectrometer.

General Procedure for the Synthesis of the *N*-Sulfonylated Cocaine Analogs. Norcocaine (0.5 mmol, 144.5 mg) was dissolved in 4 mL of anhydrous triethylamine, and the mixture was cooled at −20 °C under argon. After 15 min, 0.55 mmol of the corresponding sulfonyl chloride was added slowly. After 1 h, TLC analysis indicated completion of the reaction. The reaction mixture was evaporated under reduced pressure, and the crude product was purified by column chromatography using CH_2Cl_2–MeOH (99–1) to give a clear oil which crystallized when triturated with ether.

***N*-(Methylsulfonyl)norcocaine (1a).** Via the general procedure, 148.8 mg (81%) of white crystals was obtained. **1a**: mp 158–160 °C; $[\alpha]^{25}_D$ −31.6° (*c* 1.0, CH_2Cl_2); ^1H NMR δ 1.65–2.04 (m, 4H), 2.45–2.62 (m, 1H), 3.06–3.13 (m, 1H), 3.62 (s, 3H), 4.35–4.45 (m, 1H), 4.62–4.66 (m, 1H), 5.39–5.43 (m, 1H), 7.37–7.96 (m, 5H). Anal. ($C_{17}H_{21}NO_6S$) C, H, N, S.

***N*-[(Trifluoromethyl)sulfonyl]norcocaine (1b).** Norcocaine (0.5 mmol, 144.5 mg) was dissolved in 4 mL of anhydrous pyridine, and the mixture was cooled at −78 °C under argon. After 10 min, 0.55 mmol of trifluoromethanesulfonic anhydride was added slowly. After 30 min, TLC indicated the completion of the reaction. Purification by column chromatography gave 160 mg (76%) of a clear oil. All attempts to crystallize this oil were unsuccessful. **1b**: $[\alpha]^{25}_D$ −45.4° (*c* 1.0, CH_2Cl_2); ^1H NMR δ 1.99–2.29 (m, 4H), 2.66–2.75 (m, 1H), 3.21–3.24 (m, 1H), 3.75 (s, 3H), 4.46–4.51 (m, 1H), 4.62–4.65 (m, 1H), 5.40–5.48 (m, 1H), 7.41–8.00 (m, 5H), 8.35–8.39 (m, 4H); ^{13}C NMR δ 28.5, 29.0, 34.8, 49.6, 52.2, 58.0, 59.9, 65.4, 123.8, 128.6, 129.8, 133.5, 165.8, 168.8; ^{19}F NMR ($CDCl_3$, $CFCl_3$ as internal standard) δ −6.15 (s). Anal. ($C_{17}H_{18}F_3NO_6S$) C, H, N, S.

***N*-(Isocyanatosulfonyl)norcocaine (1c).** Via the general procedure, 115 mg (59%) of white crystals was obtained. **1c**: mp 121–123 °C; $[\alpha]^{25}_D$ −38.1° (*c* 1.0, CH_2Cl_2); ^1H NMR δ 1.72–1.89 (m, 2H), 2.01–2.07 (m, 2H), 2.17–2.30 (m, 1H), 2.46–2.51 (m, 1H), 3.18–3.22 (m, 1H), 3.67 (s, 3H), 4.43–4.45 (m, 1H), 4.58–4.61 (m, 1H), 5.37–5.45 (m, 1H), 7.42–8.00 (m, 5H); ^{13}C NMR δ 25.8, 26.0, 32.4, 47.7, 51.0, 55.5, 58.4, 66.3, 132.8, 134.0, 138.0, 172.7, 175.0. Anal. ($C_{17}H_{18}N_2O_7S$) C, H, N, S.

***N*-(Phenylsulfonyl)norcocaine (1d).** Via the general procedure, 135.3 mg (63%) of white crystals was obtained. **1d**: mp 189–191 °C; $[\alpha]^{25}_D$ −27.8° (*c* 1.0, CH_2Cl_2); ^1H NMR δ 1.75–1.97 (m, 4H), 2.49–2.54 (m, 1H), 3.10–3.19 (m, 1H), 3.54 (s, 3H), 4.41–4.42 (m, 1H), 4.60–4.62 (m, 1H), 5.28–5.33 (m, 1H), 7.32–7.96 (m, 5H). Anal. ($C_{22}H_{23}NO_6S$) C, H, N, S.

***N*-[(*p*-Nitrophenyl)sulfonyl]norcocaine (1e).** Via the general procedure, 187.4 mg (79%) of pale yellow crystals was obtained. **1e**: mp 209–211 °C; $[\alpha]^{25}_D$ −11.8° (*c* 1.0, CH_2Cl_2); ^1H NMR δ 1.85–2.05 (m, 4H), 2.46–2.54 (m, 1H), 3.16–3.19 (m, 1H), 3.57 (s, 3H), 4.40–4.42 (m, 1H), 4.68–4.71 (m, 1H), 5.31–5.38 (m, 1H), 7.40–8.14 (m, 5H), 8.35–8.39 (m, 4H); ^{13}C NMR δ 27.8, 28.8, 34.3, 48.8, 51.3, 56.3, 58.3, 65.4, 123.8, 128.0, 128.3, 129.1, 129.2, 132.9, 145.5, 149.7, 165.3, 168.7. Anal. ($C_{22}H_{22}N_2O_8S$) C, H, N, S.

***N*-[(*p*-Methoxyphenyl)sulfonyl]norcocaine (1f).** Via the general procedure, 158 mg (74%) of white crystals was obtained. **1f**: mp 199–201 °C; $[\alpha]^{25}_D$ −13.2° (*c* 1.0, CH_2Cl_2); ^1H NMR δ 1.73-2.01 (m, 5H), 2.49–2.58 (m, 1H), 3.12–3.15 (m, 1H), 3.60 (s, 3H), 3.87 (s, 3H), 4.36–4.40 (m, 1H), 4.60–4.61 (m, 1H), 5.28–5.36 (m, 1H), 6.95–7.01 (m, 2H), 7.39–7.58 (m, 3H), 7.78–7.86 (m, 2H), 7.97–8.01 (m, 2H); ^{13}C NMR δ 27.6, 28.0, 34.5, 49.1, 55.2, 58.1, 65.8, 113.7, 127.9, 129.2, 129.3, 130.8, 132.8, 162.6, 165.4, 168.7. Anal. ($C_{23}H_{25}NO_7S$) C, H, N.

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