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SAR Studies of Piperidine-Based Analogues of Cocaine. Part 3: Oxadiazoles

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Abstract—The synthesis of novel 4β-aryl-1-methyl-3α-(3-substituted-1,2,4-oxadiazol-5-yl)piperidines, bioisosteres of ester (+)-**1**, is described. The synthesized oxadiazoles were evaluated for their affinity to the DAT and their ability to inhibit monoamine reuptake at the DAT, NET, and 5HTT. The results show that affinity to the DAT and ability to inhibit the reuptake at the DAT, NET, and 5HTT is a function of the size of the substituent in the oxadiazole ring. (+)-(3*R*,4*S*)-4β-(4-Chlorophenyl)-1-methyl-3α-(3-methyl-1,2,4-oxadiazol-5-yl)piperidine [(+)-**2a**], which is structurally and pharmacologically most similar to the ester (+)-**1** in this series, showed at least a 2-fold longer duration of action when compared to ester (+)-**1**.

Cocaine continues to be a widely abused drug. The development of pharmacotherapies that could assist the patient in initiating and maintaining abstinence and in relapse prevention is important. Compounds that partially mimic the effects of cocaine (substitute agonists) and have a long duration of action could potentially be the best candidates for relapse prevention treatment.[1]

Cocaine possesses complex pharmacological properties, which include the interaction with different neurotransmitter targets such as the dopamine (DA), serotonin (5-HT), and norepinephrine (NE) transporters, cholinergic and σ receptors, and sodium and calcium channels, and others.[1–3] Despite the variety of these pharmacological properties, the predominant model for the origin of the reinforcing properties of cocaine is the blockade of the dopamine transporter (DAT),[4] while its effect on serotonergic systems also has been implicated in cocaine-seeking behavior in an animal model.[5–7] The complexity of the mechanisms mediating the addictive properties of cocaine is such that it is difficult to define appropriate targets for medication development. For these purposes, compounds that only partially mimic the actions of cocaine may be viable candidates. That is, since cocaine inhibits the transport of all three biogenic amines with roughly equal potency, compounds that selectively inhibit the uptake of DA and 5-HT[8] or DA and NE deserve to be evaluated in this context.

Recently, the synthesis, pharmacology, and locomotor studies of some 3,4-substituted piperidine-based cocaine analogues have been reported.[8–11] Despite the lack of the tropane nucleus in these compounds, they represent a series of potent DA, NE, and 5-HT reuptake inhibitors with K_i values in the nanomolar range. Moreover, the results of different animal behavioral tests strongly suggest that ester (+)-**1**, a modestly DAT/NET selective compound, could be a possible medication for relapse prevention during the treatment of cocaine addiction.[12] In refining this approach, it is thought that the development of compounds with a longer duration of action remains desirable.

Oxadiazoles commonly serve as bioisosteric equivalents of esters with increased stability to biochemical degradation.[13,14] Herein we describe the synthesis of new 4β-(4-chlorophenyl)-3α-(3-substituted-1,2,4-oxadiazol-5-yl)-piperidines, and we also report the results of pharmacological and preliminary behavioral studies.

The synthesis of the oxadiazoles is outlined in Scheme 1 and follows a previously reported procedure.[15,16]

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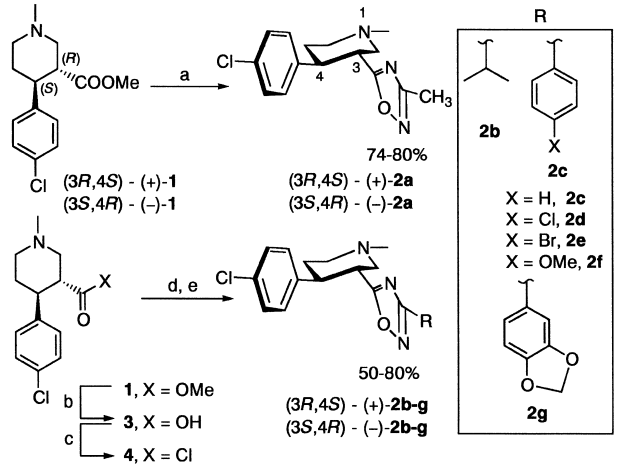

Scheme 1. Reagents and conditions: (a) $CH_3C(=NOH)NH_2$, NaH, molecular sieves, THF, reflux, 74%; (b) 6 N HCl, reflux; (c) oxalyl chloride, 1 μL of DMF, CH_2Cl_2; (d) $RC(=NOH)NH_2$, pyridine, $CHCl_3$, reflux; (e) AcOH, reflux.

Oxadiazoles (+)-**2a** and (−)-**2a** were synthesized in good yield from esters (+)-**1** and (−)-**1** and acetamide oxime.[15] In all other cases, esters (+)-**1** and (−)-**1** were converted to the corresponding acids (+)-**3** and (−)-**3**, and then to the acid chlorides (+)-**4** and (−)-**4**.

According to Carroll et al.,[15] the reaction between 3β-(substituted phenyl)tropane-2β-carboxylic acid chlorides and different amidoximes in a 3:1 mixture of pyridine and chloroform at reflux for 1.5 h led to the corresponding 1,2,4-oxadiazoles. In this one-pot protocol, the formation of amidoxime esters[16] was followed by their spontaneous cyclization with formation of the oxadiazoles. Surprisingly, however, these conditions afforded in our case only the amidoxime esters (according to ^1H and ^{13}C NMR). In order to effect cyclization, the solvent was removed, and the crude amidoxime esters were heated in glacial acetic acid[16] to give the desired 1,2,4-oxadiazoles (+)-**2b–g** and (−)-**2b–g**.[17] The chemical shifts and values of $^3J_{H-H}$ of the oxadiazoles **2a–g** and of the starting ester **1** indicate that all of these compounds exist, within the limits of error, in a single chair conformation of the piperidine ring where the 3α-oxadiazolyl and 4β-phenyl groups are equatorial.

Oxadiazoles (+)-**2a**, (+)-**2b**, (+)-**2d–g**, (−)-**2b**, and (−)-**2d–g** were tested for their affinity as inhibitors of [^{3}H]WIN 35,248 binding to the DAT in studies using rat striatal membranes. Compounds (+)-**2a–g** and (−)-**2a–g** were also tested for their ability to inhibit high affinity uptake of [^{3}H]DA, [^{3}H]5-HT, and [^{3}H]NE using rat nerve endings (synaptosomes) obtained from brain regions enriched in DAT, 5HTT, and NET, respectively, according to protocols described earlier.[8,18] The K_i data and selectivity profiles derived from the K_i values are provided in Table 1.[19]

To explore the behavioral consequences of replacing the ester with a bioisosteric oxadiazolyl group, we examined compound (+)-**2a** in the locomotor test in mice and in a drug-discrimination paradigm in rats according to protocols reported previously.[12] We selected oxadiazole (+)-**2a** for these studies because it was found to be the most similar to the lead ester (+)-**1** as regards its affinity for the three biogenic amine transporters. The results are shown in Figure 2.

In general, (−)-oxadiazoles (−)-**2a–g** with the 3*S*,4*R*-configuration of substituents in their piperidine ring show low potency at all transporters and do not bind to the DAT, which is consistent with the lower potency of (−)-ester **1** in comparison to (+)-ester **1** (see Table 1 and ref 9). Only oxadiazoles with relatively small substituents, such as methyl and *i*-propyl, possess residual activity at micromolar levels at the DAT [for (−)-**2a**] or at the DAT, NET, and 5HTT [for (−)-**2b**]. A dependence of potency on the size of the 3-substituent of the oxadiazole ring is observed for oxadiazoles (+)-**2a–g** in 3*R*,4*S*-series as well. Oxadiazoles (+)-**2d–g** with a rather large 3-aryl substituent bearing an additional substituent in its *para*-position have low potency at all transporters ($K_i > 3$ μM). It should be noted that our lead compound, ester (+)-**1**, and its bioisostere, the oxadiazole (+)-**2a**, exhibit similar pharmacological profiles.

Replacement of the methyl group in (+)-**2a** with an *i*-propyl group in (+)-**2b** leads to a 2.5-fold reduction in DAT binding and to a 2.1- and 3.0-fold reduction in blocking the reuptake of DA and NE, respectively. The

Table 1. K_i's and selectivities for compounds (+)-**1**, (−)-**1**, (+)-**2a–g**, (−)-**2a–g**, and cocaine

Compd	K_i (nM)[b] ± SE (nM)				Selectivity		
	[^{3}H]WIN[a] Binding	[^{3}H]DA Uptake	[^{3}H]5-HT Uptake	[^{3}H]NE Uptake	5-HT/DA	NE/DA	NE/5-HT
Cocaine	146 ± 2.4	259 ± 19.9	155 ± 0.40	108 ± 3.50	0.37	0.20	0.70
(+)-**1**	315 ± 52.8	233.4 ± 62.0	8490 ± 1430	252 ± 43.4	36.4	1.08	0.03
(−)-**1**	2005 ± 610	2930 ± 580	930 ± 10	396 ± 112	0.32	0.14	0.43
(+)-**2a**	201 ± 2.00	187.2 ± 3.0	5960 ± 80	256 ± 16.9	29.5	1.27	0.04
(−)-**2a**	N/T[c]	954.9 ± 7.6	> 3000[d]	> 1000[d]	—	—	—
(+)-**2b**	494 ± 103	391 ± 64.8	2920 ± 190	778 ± 40.3	7.49	1.99	0.26
(−)-**2b**	2900 ± 70	3590 ± 80	1220 ± 230	2680 ± 600	0.34	0.75	2.19
(+)-**2c**	N/T	497.3 ± 95.1	> 3000[d]	> 10,000[d]	—	—	—
(−)-**2c**	N/T	1713.2 ± 141.5	> 3000[d]	> 3000[d]	—	—	—
(+)-**2d-g**, (−)-**2d-g**	> 3000	> 3000	> 3000	> 3000	—	—	—

[a]WIN 35,248.
[b]Data are mean ± standard error of at least three experiments as described in ref 8.
[c]Not tested.
[d]IC_{50} is shown; data do not allow the determination of K_i.

potency of (+)-**2b** in blocking 5-HT transport is 2.0-fold higher than the potency of (+)-**2a**. The pharmacological differences between (+)-**2c** and (+)-**2b** are even larger than those between (+)-**2b** and (+)-**2a**. With the introduction of an aromatic substituent, oxadiazole (+)-**2c** loses its activity at the 5-HT and NE transporters, while at the DAT, (+)-**2c** is only 2.6- and 1.3-fold less potent than (+)-**2a** and (+)-**2b**, respectively.

We observed large differences in the pharmacological properties of our 4β-(4-chlorophenyl)-3α-oxadiazolylpiperidines relative to those reported earlier by Carroll et al.[15] for 3β-aryl-2β-oxadiazolyltropanes. Even if differences in the assay conditions do not allow us to compare our data directly with those obtained by Carroll's group[15,20] it is clear that the introduction of aliphatic or aromatic substituents into the oxadiazole ring in the tropane series leads primarily to changes in selectivity, making the compounds more NET or 5HTT active with almost constant potency at the DAT. In the piperidine series such changes are not observed. For instance, the analogue of (+)-**2a** in the 3β-aryl-2β-oxadiazolyltropane series, 3β-(4-chlorophenyl)-2β-(3-methyl-1,2,4-oxadiazol-5-yl)tropane, was DAT and NET selective (binding to DAT/NET/5HTT, IC_{50} = 4.05, 363, and 2580 nM,[15] respectively), while the analogue of (+)-**2d** in the tropane series, 3β-(4-chlorophenyl)-2β-(3-methyl-1,2,4-oxadiazol-5-yl)tropane, was DAT and 5HTT selective (binding to DAT/NET/5HTT, IC_{50} = 4.06, 4070, and 404 nM,[15] respectively).[21] It should be noted, however, that the analogue of (+)-**2a** in the 3β-aryl-2α-oxadiazolyltropane series was poorly active at all transporters (binding to DAT/NET/5HTT, IC_{50} = 1030, 71,000, and 33,100 nM,[15] respectively).

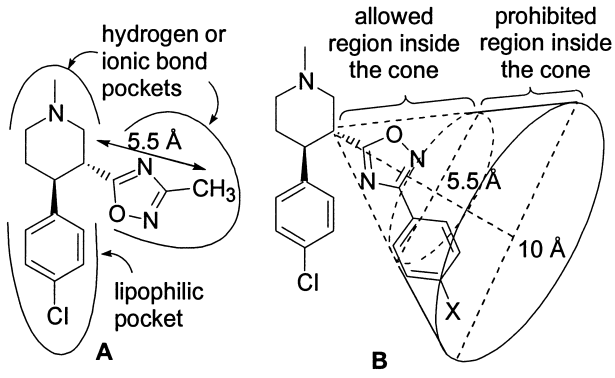

Figure 1. Pharmacophore model for 4β-aryl-3α-oxadiazolylpiperidines (A); depiction of the space occupied by the oxadiazolyl group during its rotation around the bond between C3 of the piperidine ring and C5 of the oxadiazole ring (B).

Our results suggest that additional steric restrictions should be included in the pharmacophore models for the DAT,[3,22] NET, and 5HTT binding sites in the case of piperidine-based analogues of cocaine with certain 3α-substituents. We hypothesize that the binding pocket for the 3α-substituent is only about 5.5 Å long[23] as deduced from the distance between C3 of the piperidine ring and the carbon atom of the methyl substituent in the oxadiazole ring in compound (+)-**2a** (Fig. 1A).

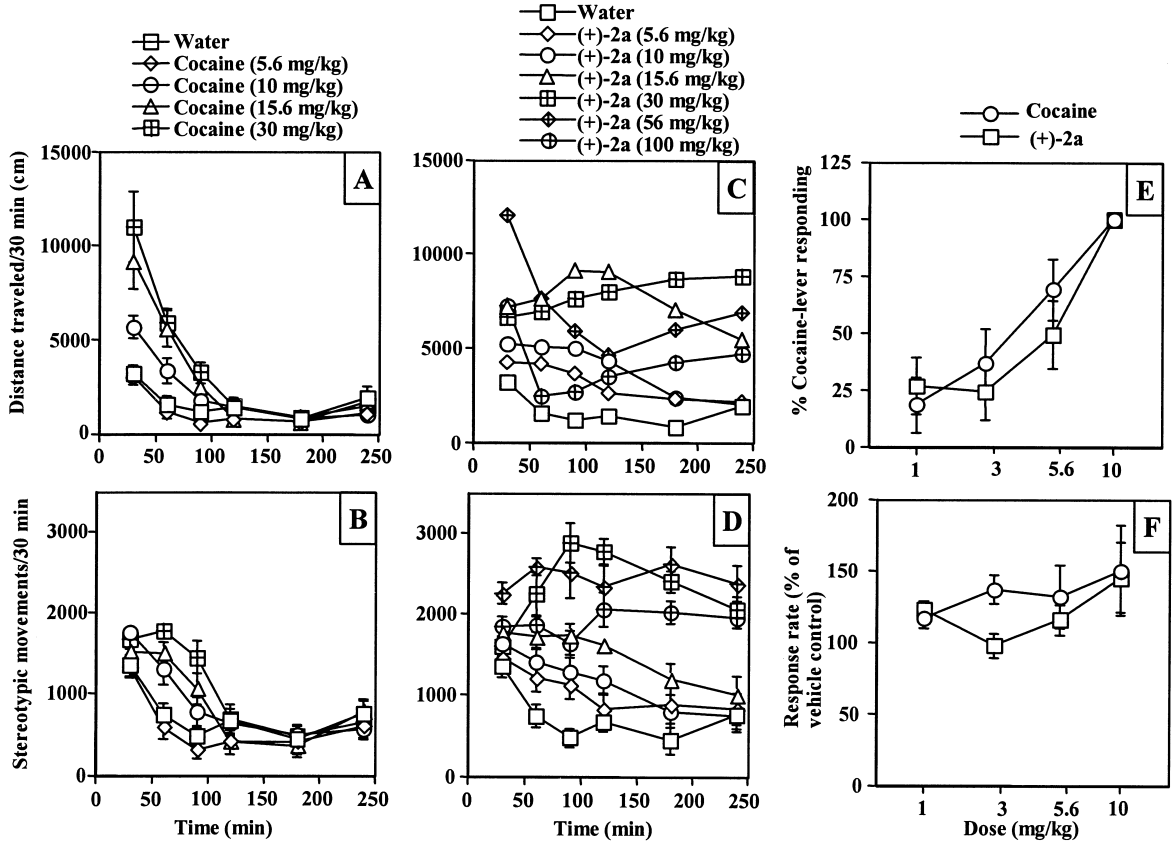

Figure 2. Comparison of (+)-**2a** and cocaine in locomotor stimulation (A–D) and drug discrimination (E and F) tests.

Therefore compounds containing a 3α-substituent that are not able to adjust the position in space of that substituent and thereby minimize its unfavorable interaction with the binding pocket cannot effectively bind to the transporter and will have low potency. In compounds (+)-**2a** and (+)-**2b**, the 3α-substituent is small enough to fit into the pocket (Fig. 1A) and thus to bind to the transporter. In compounds (+)-**2c**–**g**, on the other hand, the 3α-substituent can only occupy positions within a conical region of the space extending for a distance of 10 Å from the piperidine ring and reaching into the prohibited region (Fig. 2B), thus making binding to the transporter difficult. On the other hand, dimeric piperidine-based esters and amides bearing a sufficiently long and flexible linker[10] can easily adjust the position in space of the 3α-substituent, thereby avoiding unfavorable steric interactions. The exact placement of the 3α-substituent in space in these models is unknown.

The oxadiazole (+)-**2a** is the most potent of the present compounds, and it is completely cocaine-like in both locomotor and drug-discrimination tests. Both cocaine (5.6–30 mg/kg, Fig. 2A and B) and oxadiazole (+)-**2a** (5.6–100 mg/kg, Fig. 2C and D) produced dose-dependent locomotor stimulation in mice. Both cocaine (56 mg/kg) and (+)-**2a** (156 mg/kg) produced convulsions within 5 min following drug injection. This suggests that (+)-**2a** readily enters the brain similar to cocaine. Within the range of nonconvulsant doses, there were significant differences in the duration of locomotor effects of cocaine versus (+)-**2a**. The locomotor stimulation by (+)-**2a** at moderate to high doses (15.6–100 mg/kg) lasted at least 4 h, while cocaine produced stimulation that lasted only 2 h at the maximal nonconvulsant dose (30 mg/kg). However, cocaine and compound (+)-**2a** had similar efficacies in increasing the distance traveled as indicated by their similar maximal effects. Oxadiazole (+)-**2a** appeared to have greater efficacy in increasing stereotypic behavior than cocaine.

In rats trained to discriminate cocaine from saline, both (+)-**2a** (1–10 mg/kg), and cocaine (1–10 mg/kg, Fig. 2E,F) produced dose-dependent and full substitution and had similar potencies. The doses of cocaine and (+)-**2a** necessary to produce 50 percent cocaine-appropriate responding were 3.98 (95% confidence limits: 3.16–5.13) mg/kg and 3.55 (95% confidence limits: 2.63–4.57) mg/kg, respectively.

Being DAT-NET selective, oxadiazole (+)-**2a** produces behavioral effects that are similar to those of the lead compound, ester (+)-**1**.[12] The results of the present behavioral tests are consistent with the compound's DA reuptake inhibitory activity, however, further studies will be required to evaluate the effects if any of the compound's lack of 5HTT activity.

In conclusion, the results of the SAR studies suggest that additional steric restrictions, such as the prohibited conical region for the 3α-substituent, should be included in the pharmacophore model for these oxadiazolylpiperidines. The greater duration of action of the oxadiazole (+)-**2a** in comparison to the ester (+)-**1** combined with its similar pharmacological and preliminary behavioral profile make it an interesting candidate for additional study. The longer duration of action of the oxadiazole (+)-**2a** may offer some therapeutic advantage.

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radioligand ([^{125}I]RTI-55) binding and [^{3}H]serotonin uptake by HEK cells expressing cDNA for the human serotonin transporter (HEK-hSERT cells), and its effect on radioligand ([^{125}I]RTI-55) binding and [^{3}H]norepinephrine uptake by HEK cells expressing cDNA for the human norepinephrine transporter (HEK-hNET cells). In these assays (+)-**2a** was highly selective for the dopamine transporter (IC_{50} = 183 nM; cocaine, IC_{50} = 263 nM) with no significant activity at the serotonin (K_i > 10 μM; cocaine IC_{50} = 471 nM) and norepinephrine transporters (IC_{50} = 1370 nM; cocaine, IC_{50} = 281 nM). The experiments were performed as described in Eshleman, A. J.; Carmolli, M.; Cumbay, M.; Martens, C. R.; Neve, K. A.; Janowsky, A. *J. Pharmacol. Exp. Ther*. **1999**, *289*, 877.

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