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# Piperidine-Based Nocaine/Modafinil Hybrid Ligands as Highly Potent Monoamine Transporter Inhibitors: Efficient Drug Discovery by Rational Lead Hybridization

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## Abstract

Some piperidine-based nocaine/modafinil hybrid ligands have been designed, synthesized, and found to display an improved potency at all three monoamine transporters and particularly for DAT and/or NET. Some highly active and selective monoamine transporter inhibitors with low nanomolar to subnanomolar potency were identified. Ligands of this type may find important applications as positron emission tomography imaging tools and in the treatment of central nervous system disorders such as depression and sleep apnea.

In the central nervous system (CNS), monoamines such as dopamine (DA), serotonin (5-HT), and norepinephrine (NE) have an important modulatory role in neurotransmission and are intimately involved in a variety of physiological functions and pathological conditions.[1,2] Selective monoamine transporter (DAT, SERT, or NET) inhibitors (e.g., GBR 12909, mazindol, fluoxetine, paroxetine, reboxetine, amoxapine, desipramine, imipramine, bupropion, and nisoxetine) have been developed to treat a number of psychological and neurological disorders such as depression, drug abuse, attention deficit hyperactivity disorder (ADHD), anxiety, mood problems, Parkinson's disease, Alzheimer's disease, schizophrenia, bipolar disorder, chronic pain, migraine, epilepsy, multiple sclerosis, stroke, trauma, mania, obesity, and narcolepsy.[3–8] However, important issues regarding the selectivity and mechanisms of action of these drugs remain unresolved. Research with novel ligands that vary in their selectivity profiles and potency at each of these three transporter sites may be of value to unraveling the relevant pharmacological mechanisms and thus aid in the discovery of new medications with fewer side effects.

Previously, we reported on the synthesis and pharmacology of some 3,4-substituted piperidine-based cocaine analogues.[9] After extensive in vitro studies, together with assays of locomotor activity, and self-administration and drug discrimination studies, the DAT/NET selective ligand (+)-methyl 4*β*-(4-chlorophenyl)-1-methylpiperidine-3α-carboxylate [(+)-CPCA, **1,** nocaine] was chosen for advancement to human clinical studies for the treatment of cocaine addiction[10,11] Nocaine has lower potency and efficacy compared with cocaine in increasing locomotor activity in rodents and produces partial methamphetamine-like discriminative

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**Supporting Information Available:** Experimental details, spectral data, and ClogP data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org

stimulus effects, although it is fully cocaine-like in cocaine-trained animals. Moreover, it shows reduced reinforcing effects in nonhuman primates. Nocaine dose-dependently antagonizes cocaine-induced locomotor activation and potentiates the discriminative stimulus effects of a low dose of cocaine. On the other hand, modafinil (provigil) **2**, a non-amphetamine-like wake-promoting agent, has been approved recently for the treatment of narcolepsy. However, modafinil has been linked to side effects that include nausea, infection, nervousness, anxiety, and insomnia. It shows a low but selective affinity to the DAT in canine brain membranes.[12] Modafinil's mode of action is complex and still uncertain, although studies suggest that it increases wakefulness by activating α-1 noradrenergic transmission[13] or hypothalamic cells that contain the peptide hypocretin[14] or that it may work by modulating GABAergic tone.[15] Other research suggests that the presynaptic activation of DA transmission is a key pharmacological event in mediating the wake-promoting effects of currently available CNS stimulants and that it is critical for the pharmacological control of wakefulness, while activation of the NE system is critical for rapid eye movement sleep regulation.[16] Experiments using DAT knock-out mice appear to support an important role for the DAT in sleep regulation and thus in the wake-promoting action of modafinil.[17]

Since valuable therapies have emerged from compounds exhibiting varying levels of transporter selectivity, we sought to examine the effect of creating hybrid molecules combining structural features of nocaine and modafinil. Specifically, we decided to explore the effect of replacement of the hydrolyzable ester function of nocaine with the same type of sulfur-containing side chain as found in modafinil. This alteration to one of the key pharmacophore elements of nocaine was anticipated to further reduce its reinforcing properties while possibly improving its half-life (Chart 1).

Our synthetic approach to these hybrid structures starting from arecoline[9] is outlined in Schemes 1 and 2. The requisite steps are quite straightforward and based in part on the methods devised for the synthesis of nocaine itself. In the course of oxidation of the sulfide intermediates to the corresponding sulfoxides, an additional chiral center is created at the sulfur atom. Typically, two diastereoisomers are generated in a ratio of approximately 1:1, which is readily observable by NMR. Possible separation of the sulfoxides by HPLC was attempted but was unsuccessful. However, in light of the fact that the sulfoxides have substantially lower activity compared with the sulfides (see Table 1) and that the commercial drug modafinil itself is used in racemic form, further efforts to bring about the preferential formation of one stereoisomer over the other by optimizing the oxidation conditions using selective oxidizing agents have not been made.

The newly synthesized ligands were evaluated for the ability to inhibit high affinity uptake of DA, 5-HT, and NE using rat synaptosomal nerve endings.[6a] From the uptake inhibition data (Table 1), it is surmised that the replacement of the hydrolyzable ester function of nocaine with the sulfur appendage found in modafinil leads in general to a substantial enhancement in DAT/NET inhibitory potency for many of the ligands. In addition, some of the ligands display unique levels of transporter selectivity and potency.

The conversion of the carboxylic ester of nocaine into the intermediate alcohol **4** and iodide **5** improves activity at the SERT without causing any significant alteration in potency at the DAT or NET. However, the introduction of the sulfur appendage into nocaine significantly enhances the inhibitory activity at the DAT and NET. Thus, the key intermediate **6**, a hybrid ligand, displays fairly good activity at the NET ($K_i$ = 25 nM) and at the DAT ($K_i$ = 80 nM) while at the same time exhibiting moderate activity at the SERT ($K_i$ = 208 nM). The oxidation of sulfide **6** to sulfoxide **7** leads to a reduced transporter activity. Alcohol **8** exhibits a remarkable potency at the NET ($K_i$ = 0.94 nM) and at the DAT ($K_i$ = 16 nM) as well as a 170-fold and 10-fold selectivity vs the SERT, respectively. The corresponding alcohol **9** containing

a sulfoxide function has a lower potency ($K_i$ = 42 nM) at the NET and a 50-fold selectivity vs the SERT, but it displays a higher potency ($K_i$ = 12 nM) at the DAT and a 180-fold selectivity vs the SERT. In comparison to alcohol **8**, the methyl ether analogues **10** and **11** exhibit a lower but still fairly good DAT/NET activity. Among the inverse acyl and benzoyl esters, ligands **12**, **13**, and **15** are good DAT/NET inhibitors while ligand **14** is interestingly a good SERT and NET inhibitor with potencies of 6.7 and 4.5 nM, respectively. Among the amide analogues, ligands **16a–d** display a similar and fairly potent NET activity while **16c** is also a good DAT inhibitor. Interestingly, ligand **16e** exhibits outstanding activity with approximately 1 nM potency at all three monoamine transporters. Ligand **16f** is an outstanding SERT and NET inhibitor with potencies of 4.5 and 0.68 nM, respectively. In comparison to the sulfide ligands **16a–f**, the corresponding sulfoxide-amide analogues **17a–f** generally display a reduced activity except compound **17b**, which exhibits a slightly improved potency at the NET ($K_i$ = 12 nM) and the DAT ($K_i$ = 55 nM) and a much better selectivity vs the SERT ($K_i$ = 1795 nM). While oxadiazole analogues are of interest because of their anticipated prolonged action and high metabolic stability, ligands **18** and **19** display only a moderate to low activity at the NET. Interestingly, the N-demethylated ligands **20–22** exhibit a remarkable activity at the NET with $K_i$ down to 0.56 nM (**20**), a result that likely reflects the change from a tertiary to a secondary amine.

In regard to their druglike properties, many of these ligands have ClogP values (see the Supporting Information) in the range deemed suitable for penetration of the blood–brain barrier, as per Lipinski's "rule of five".[19] On the basis of results in hand, we intend to focus on ligands displaying improved NET activity with $K_i$ values less than 1 nM. Specifically, given the fact that ligands **8** and **16f** exhibit remarkable NET inhibitory activity($K_i$ values for NET are 0.94 and 0.68 nM, respectively) while also exhibiting suitable lipophilicity (ClogP in the range of 2–4), we believe that they may be good NET-selective ligands primed for C-11 labeling for advancement to the small-molecule-directed brain imaging and therapy program. To assess the bioavailability and initial cocaine-like behavioral activity of **8**, we compared two doses of cocaine and **8** in an open-field locomotor activity study (Figure 1). Ligand **8** was more potent than cocaine and appeared to be somewhat more efficacious as well. Although much more extensive testing is needed to fully characterize the behavioral effects of **8**, these differences are consistent with its 17-fold NET vs DAT selectivity and its virtual lack of activity at SERT.[20] Further behavioral testing of some of the ligands in this series is currently underway.

In summary, some piperidine-based nocaine/modafinil hybrid ligands have been synthesized and found to display an improved potency at all three monoamine transporters but particularly for DAT and/or NET. Some highly active and selective monoamine transporter inhibitors with low nanomolar to subnanomolar potency were identified including **8** and **20** (NET), **9**, **11**, **13**, **15**, **16c**, and **17b** (DAT/NET), **14** and **16f** (SERT/NET), and **16e** (DAT/SERT/NET). Because many of these ligands have ClogP values in the range deemed suitable for penetration of the blood–brain barrier, they can be examined for their in vivo actions and radiolabeled as possible positron emission tomography (PET) imaging tools. The potency and selectivity profiles of these hybrid molecules may allow us to gain a better understanding of the relation between transporter activity and biological effect. This knowledge may allow for an improved design of CNS therapeutics.

## Supplementary Material

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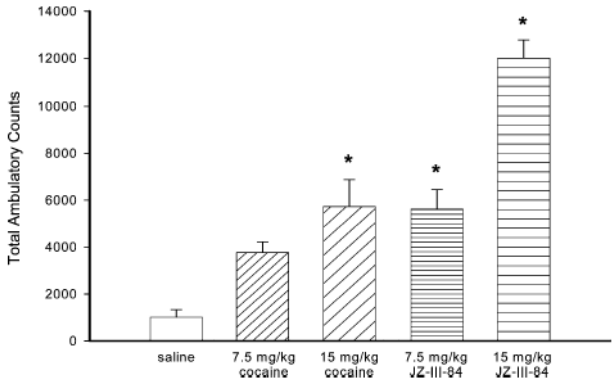



**Figure 1.**

Effect of cocaine and ligand **8** (JZ-III-84) on horizontal locomotor activity. Following a 30 min habituation period, saline (0.9%), cocaine, and ligand **8** were injected ip (1 $cm^3$/kg) in the doses indicated and total locomotor activity was automatically recorded for 90 min as infrared beam breaks in a 16 in. × 16 in. photobeam activity system (San Diego Instruments). Cocaine and ligand **8** significantly increased total activity counts as indicated by one-way analysis of variance ($F_{4,14} = 28.4$, $p < 0.001$). The asterisk (*) indicates that the individual treatment group is significantly different from saline control ($p < 0.05$, Tukey's post hoc test).

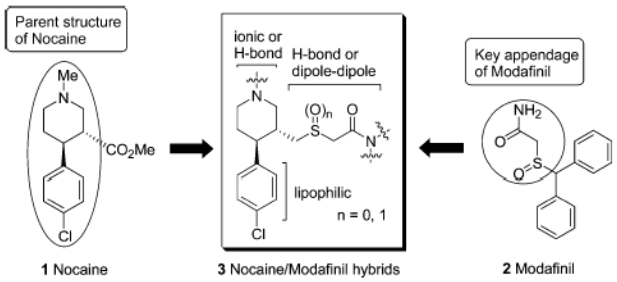
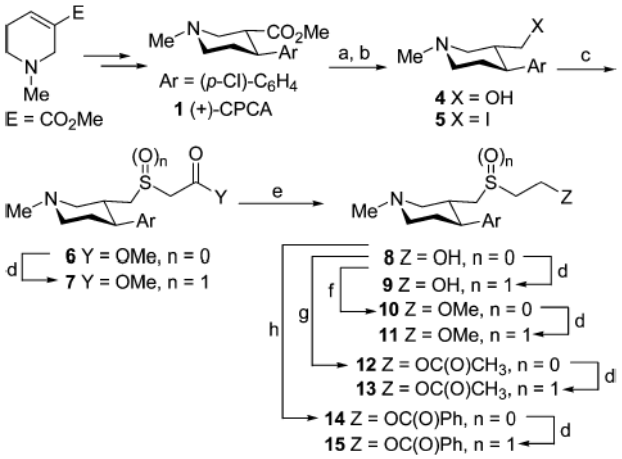
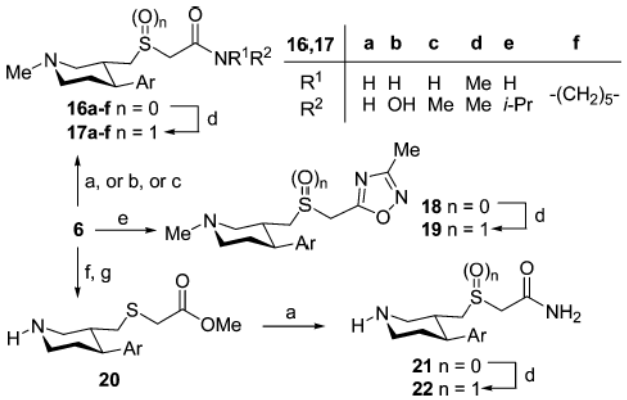



**Chart 1.**
Rational Design of Nocaine/Modafinil Hybrid Ligands




[a] Reagents and conditions: (a) $LiAlH_4$, 92%; (b) $Ph_3P/I_2$/imidazole, 86%; (c) methyl thioglycolate, $Cs_2CO_3$, 88%; (d) 35% $H_2O_2$, HOAc, 61−80%; (e) $LiAlH_4$, 82%; (f) NaH, MeI, THF, 65%; (g) $Ac_2O$/pyridine, DMAP, 70%; (h) benzoyl chloride, $Et_3N$, DMAP, 68%.

**Scheme 1a.**




[a] Reagents and conditions: (a) (for **16a** and **21**) $NH_3$ (liquid), *t*-BuOH, 90−95%; (b) (for **16b**) hydroxylamine, KOH in MeOH, 76%; (c) (for **16c**−**f**) $HNR^1R^2$ in MeOH, 72−92%; (d) 35% $H_2O_2$, HOAc, 64−87%; (e) acetamide oxime, NaH, 4 Å molecular seives, THF, 79%; (f) 1-chloroethyl chloroformate, $CH_2Cl_2$; (g) MeOH, reflux, 80% (for two steps).

**Scheme 2a.**

## Table 1

Inhibition at Monoamine Transporters ($K_i$ ± SEM (nM))[a]

| compd | [$^3$H]-DA uptake | [$^3$H]-5-HT uptake | [$^3$H]-NE uptake |
|---|---|---|---|
| Cocaine | 423 ± 147 | 155 ± 0.4 | 108 ± 3.5 |
| (+)-CPCA[b] | 233 ± 62 | 8490 ± 1430 | 252 ± 43 |
| modafinil[c] | 3800 | | >10000 |
| **4**[b] | 497 ± 58 | 1550 ± 360 | 198 ± 53 |
| **5** | 376 ± 54 | 238 ± 49 | 424 ± 53 |
| **6** | 80 ± 23 | 208 ± 47 | 25 ± 6 |
| **7** | 231 ± 93 | 809 ± 36 | 53 ± 16 |
| **8** | 16 ± 5 | 158 ± 5 | 0.94 ± 0.27 |
| **9** | 12 ± 1 | 2183 ± 373 | 42 ± 16 |
| **10** | 50 ± 15 | 191 ± 57 | 6 ± 2 |
| **11** | 15 ± 5 | 469 ± 36 | 25 ± 6 |
| **12** | 35 ± 11 | 57 ± 18 | 3.6 ± 1.5 |
| **13** | 9 ± 3 | 87 ± 17 | 29 ± 4 |
| **14** | 68 ± 22 | 6.7 ± 1.5 | 4.5 ±1.2 |
| **15** | 32 ± 12 | 199 ± 14 | 18 ± 6 |
| **16a** | 159 ± 19 | 557 ± 150 | 39 ± 5 |
| **16b** | 85 ± 19 | 227 ± 7 | 15 ± 2 |
| **16c** | 13 ± 3 | 110 ± 45 | 25 ± 2 |
| **16d** | 116 ± 46 | 88 ± 22 | 27 ± 7 |
| **16e** | 1.0 ± 0.2 | 1.1 ± 0.4 | 0.8 ± 0.1 |
| **16f** | 83 ± 1 | 4.5 ± 0.8 | 0.68 ± 0.25 |
| **17a** | 209 ± 25 | 5790 ± 1712 | 178 ± 49 |
| **17b** | 55 ± 8 | 1795 ± 67 | 12 ± 1 |
| **17c** | 164 ± 28 | 2033 ± 573 | 86 ± 2 |
| **17d** | 2884 ± 654 | 924 ± 139 | 1249 ± 19 |
| **17e** | 248 ± 78 | 473 ± 60 | 20 ± 3 |
| **17f** | 379 ± 90 | 825 ± 99 | 460 ± 11 |
| **18** | 126 ± 15 | 221 ± 46 | 32 ± 10 |
| **19** | 1653 ± 56 | 1079 ± 269 | 218 ± 14 |
| **20** | 51 ± 16 | 13 ± 3 | 0.56 ± 0.09 |
| **21** | 114 ± 32 | 170 ± 6 | 10 ± 0.1 |
| **22** | 108 ± 29 | 775 ± 96 | 31 ± 3 |

[a] Data are the mean ± standard error of at least three experiments as described in ref [6a].

[b] Data taken from ref [18].

[c] Data taken from ref [16].