# *Notes*

## Tolerance in the Replacement of the Benzhydrylic O Atom in 4-[2-(Diphenylmethoxy)ethyl]-1-benzylpiperidine Derivatives by an N Atom: Development of New-Generation Potent and Selective N-Analogue Molecules for the Dopamine Transporter

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The replacement of the benzhydrylic oxygen atom of our previously developed dopamine transporter (DAT)-specific ligands 4-[2-(diphenylmethoxy)ethyl]-1-[(4-fluorophenyl)methyl]-piperidine, **1a**, and 4-[2-(bis(4-fluorophenyl)methoxy)ethyl]-1-benzylpiperidine, **1b**, by a nitrogen atom resulted in the development of the N-analogues 4-[2-((diphenylmethyl)amino)ethyl]-1-[(4-fluorophenyl)methyl]piperidine, **4a**, and 4-[2-((bis(4-fluorophenyl)methyl)amino)ethyl]-1-benzylpiperidine, **4b**. Biological evaluation of these compounds in rat striatal tissue and in HEK-293 cells expressing the cloned human transporters demonstrated high potency and selectivity of these compounds for the DAT. Thus the potency of the compound **4a** for the DAT was 9.4 and 30 nM in rat striatal tissue and in the cloned transporter cells, and its binding selectivity for the DAT compared to the serotonin transporter (SERT) for these two systems was 62 and 195, respectively. The compound **4b** similarly exhibited high potency and selectivity for the DAT. Thus, the replacement of the O atom in **1a**,**b** by an N atom in **4a**,**b** only had small effects on potency and selectivity. In comparison with GBR 12909 [1-[2-(bis(4-fluorophenyl)methoxy)ethyl]-4-(3-phenylpropyl)piperazine] and WIN 35,428 [3$\beta$-(*p*-fluorophenyl)-2$\beta$-carbomethoxytropane] binding, these two novel N-analogues were slightly more potent and far more selective for the DAT. Thus, these novel N-analogues represent more polar new-generation piperidine congeners of GBR 12909. They might have useful potential application in developing a pharmacotherapy for cocaine dependence.

Naturally occurring cocaine is a potent stimulant and strong reinforcer in humans.[1] Since cocaine abuse has reached an alarming level in the United States, the development of an effective medication to treat cocaine addiction is a national priority. Cocaine binds to several neurotransporter systems in the brain, but its binding to the dopamine transporter (DAT) is implicated in cocaine addiction and reinforcement.[2−5]

Structure−activity relationship (SAR) studies in the development of drugs for the DAT has led to structurally different classes of molecules including the recently discovered 8-oxa analogues of *N*-norcocaine as non-nitrogen-containing inhibitors for the DAT.[6−8] Compounds related to GBR 12909 and GBR 12935 were demonstrated to have good potency and selectivity for the DAT.[9−11] SAR studies in this class of compounds have shown that various cyclic and acyclic diamine moieties can be introduced by altering the piperazine ring and also that bioisosteric replacement of the phenyl ring in these molecules can be achieved without compromising the affinity and selectivity in many of these analogues.[12,13] Some of the principle findings from these SAR studies include (1) the benzhydryl moiety as a headgroup in these molecules is essential for activity and (2) the central piperazine ring can be modified.[13]

In our SAR studies on these GBR 12909-related compounds, we have shown that the piperazine moiety can be altered into a piperidine ring without compromising activity provided that the N atom is in the correct position.[14] We also have explored different alkyl chain lengths at the 1- and 4-positions of the piperidine ring and discovered the requirement of *N*-benzyl substitution for optimum activity and selectivity in these analogues, contrasting with the results found in the case of conventional GBR molecules.[15−17] Furthermore, the introduction of different substituents on the *N*-benzylic aromatic ring and incorporation of a bioisosteric replacement in these molecules resulted in the development of quite selective and potent compounds for the DAT.[16]

In our further efforts to discover new molecular determinants and also to obtain further insight into the molecular characterization of binding to the DAT, compounds **4a**,**b** were designed. In compounds **4a**,**b** the O atom of the benzhydryl headgroup in our earlier developed DAT-specific compounds **1a**,**b**[15,16] is replaced by a more basic N atom. Although the presence of a

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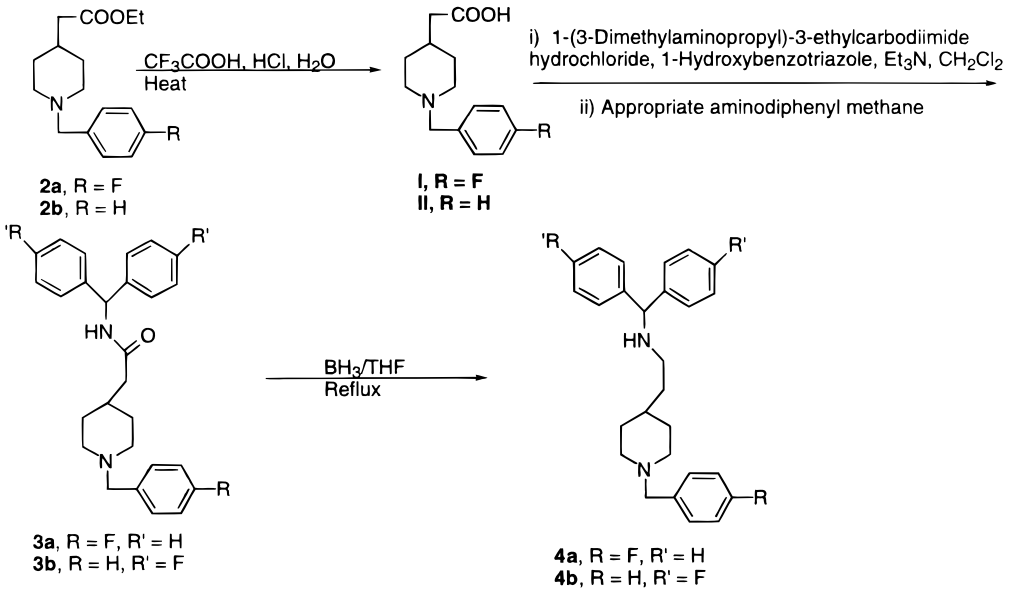
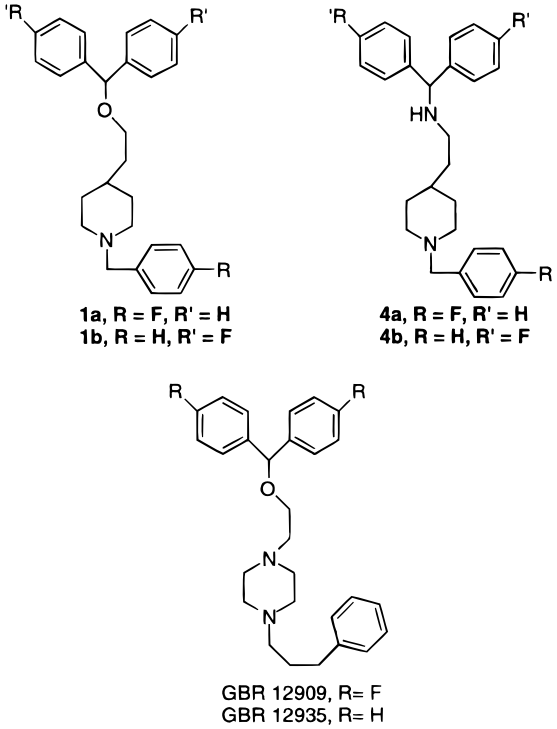
**Scheme 1**




benzhydryl group is essential for activity in GBR 12909-related compounds,[13] it is not known whether the O atom in the benzhydryl moiety is required for activity. These new N-analogues could be suitably functionalized at the secondary N atom for different pharmacological purposes which include using them as molecular probes[18] and in the development of radiotracers for single photon emission computed tomography (SPECT) and positron emission tomography (PET) imaging studies of DAT.[19,20] Results from such studies will help to further characterize the cocaine binding domains on the DAT and also to develop diagnostic tools for neurodegenerative diseases. It will be of interest to compare the behavioral pharmacological profile of these drugs with those of GBR 12909, which has been proposed to have cocaine antagonizing properties.[21−23] The pharmacokinetic properties of these N-analogues could be different from those of GBR 12909 because they are expected to be more polar and less lipophilic, an issue addressed in this study along with DAT and SERT activity.




## Chemistry

The synthesis of compounds **4a**,**b** is shown in Scheme 1. Starting materials **2a**,**b**, synthesized and characterized earlier,[15,16] were converted into intermediate acids **I** and **II** by treatment with a mixture of trifluoroacetic acid, HCl, and water. The acid was not purified and was used in the next step directly. Treatment of the crude acid with the water-soluble coupling agent 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide to form an active ester intermediate followed by treatment with the appropriate aminodiphenylmethane resulted in the production of amides **3a**,**b**.[24] Reduction with borane/THF complex provided the final compounds **4a**,**b** which were converted to their water-soluble dihydrochloride salts.

## Biology

Biological studies of the newly synthesized compound **4a** were carried out with rat brain striatal membrane tissue as described earlier.[15−17] The compound **4a** and all other compounds were also tested in cells expressing the cloned human (h) DAT and SERT.[25−27]

Our initial biological evaluation of **4a** was carried out with rat brain striatal tissue as shown in Table 1, showing appreciable potency and selectivity for the DAT. It was approximately twice as potent as GBR 12909 in displacing [$^3$H]Win 35,428 ($IC_{50}$ = 9.4 vs 19.3 nM) and about 14 times more selective for the DAT relative to the SERT (SERT/DAT = 62 vs 4.5). Furthermore, a comparison of the affinity of the new analogue **4a** to that of its benzhydrylic ether counterpart **1a** showed **4a** to be more potent (9.4 vs 17.2) but less selective (62 vs 112) than **1a** for the DAT, when tested under similar assay conditions.[16] On the other hand, another closely related analogue (**1b**) was equipotent with **4a**, albeit less selective.

**Table 1.** Affinity and Selectivity of Drugs at the Dopamine and Serotonin Transporter in Rat Striatum

| | $IC_{50}$ (nM) | | |
|---|---|---|---|
| compound | DAT, [$^3$H]Win 35,428[a] | SERT, [$^3$H]citalopram[a] | SERT/DAT |
| GBR 12909 | 19.3 ± 7.3 | 87 ± 10 | 4.5 |
| **4a** | 9.4 ± 2.6 | 585 ± 101 | 62 |
| **1a** | 17.2 ± 4.7[b] | 1920 ± 233[b] | 112 |
| **1b** | 9.7 ± 0.4[c] | 198 ± 7[c] | 20 |

[a] The DAT was labeled with [$^3$H]WIN 35,428 and the SERT with [$^3$H]citalopram. Results are average ± SEM of three independent experiments assayed in triplicate. [b] See ref 15. [c] See ref 14.

**Table 2.** Affinity and Selectivity of Drugs at the Cloned Human Dopamine and Serotonin Transporters

| | $IC_{50}$ (nM) | | | | |
|---|---|---|---|---|---|
| compound | hDAT, [$^3$H]Win 35,428[a] | hSERT, [$^3$H]citalopram[a] | hDAT, [$^3$H]DA | SERT/DAT | ClogP[d] |
| Win 35,428 | 34 ± 7 | 463 ± 61 | 11 ± 3 | 13.6 | |
| GBR 12909 | 43 ± 10 | 741 ± 56 | 26 ± 7 | 17 | 6.88 |
| **4a** | 30 ± 5 | 5848 ± 792 | 32 ± 11[b] | 195 | 6.21 |
| **4b** | 33 ± 4 | 10657 ± 650 | 58 ± 4[b] | 322 | 6.35 |
| **3a** | 3213 ± 962 | 49179 ± 4899 | ND | 15 | |
| **1a** | 37.6 ± 1.1 | ND[c] | ND | | 6.62 |
| **1b** | 35 ± 2 | ND | ND | | 6.76 |

[a] The hDAT was labeled with [$^3$H]WIN 35,428 and the hSERT with [$^3$H]citalopram. Results are average ± SEM of 3−5 independent experiments assayed in triplicate. [b] The experiments were done in duplicate. [c] ND, not done. [d] See ref 28.

In the next experiments, we assessed the binding of **4a**,**b** and **3a** to the cloned human transporters expressed in HEK cells (Table 2). Not only the source of transporter but also the assay conditions were quite different from those in the rat striatal experiments.[25,27] The new analogues **4a**,**b** exhibited high potency in inhibiting [$^3$H]Win 35,428 binding to the hDAT. The potencies of compounds **4a**, **1a**,**b**, and GBR 12909 for the cocaine binding sites maintained the same rank order (Table 2) as in rat striatum (Table 1). Compounds **4a**,**b** were almost equipotent, while **3a**, the amide precursor to **4a**, was inactive. Inactivity of **3a** may be due to the lack of conformational flexibilities of the diphenyl moiety and the highly polar nature of the amide or its inability to act as an H-bond acceptor with the receptor.

Interestingly, the compound **4a** was much more selective but slightly less potent at the cloned hDAT than at the rat DAT in striatal tissue ($IC_{50}$ = 30 vs 9.4 nM, SERT/DAT = 195 vs 62), and **4b** was the more selective of the two (SERT/DAT = 322, Table 2). Thus, under the current assay conditions these novel N-analogues (**4a**,**b**) were much more selective than either GBR 12909 or Win 35,428 (195 and 322 vs 17 and 13.6). Compounds **4a**,**b** can be considered lead compounds for new-generation piperidine congeners of GBR 12909 with high potency and selectivity for the DAT. In the dopamine uptake experiments, GBR 12909 and Win 35,428 were more potent than **4a**,**b** (26 and 11 vs 32 and 58). Furthermore, a direct comparison of the affinities of the novel compounds **4a**,**b** with their oxa analogues **1a**,**b** demonstrated almost equal inhibition potencies at the DAT.

## Conclusion

Our current results demonstrate that the benzhydrylic O atom of **1a**,**b** can be replaced by an N atom with only minor changes in binding activity and selectivity for the DAT. Our present study, thus, describes the development of the potent and selective N-analogues **4a**,**b** for the DAT as lead compounds for the development of new-generation piperidine congeners of GBR 12909. Furthermore, these compounds are a little less lipophilic than GBR 12909 and our previously synthesized compounds as evidenced from the calculated ClogP values (Table 2).[28] Comparison of the calculated partition coefficient values of GBR 12909 and **4a** indicates a 4-fold higher lipid solubility of GBR 12909 compared to **4a** ($7.6 \times 10^6$ vs $1.6 \times 10^6$). Our future SAR studies in this class of molecules with proper functionalization of the newly introduced secondary amine and the aromatic rings should produce more polar molecules. It is also interesting to note that the hydrochlorides of **4a**,**b** are water-soluble (22 and 20 mg/mL for **4a**,**b**, respectively), whereas GBR 12909 and its derivatives are only partially water-soluble. We are currently exploring the importance of this newly introduced N atom in the binding interaction through more detailed SAR studies. In the future, it will be interesting to observe the in vivo effect of a suitable compound from this class in antagonizing cocaine effects as compared with that of GBR 12909.

## Experimental Section

Analytical silica gel-coated TLC plates (Si 250F) were purchased from Baker, Inc., and were visualized with UV light or by treatment with phosphomolybdic acid (PMA). Flash chromatography was carried out on Baker silica gel, 40 $\mu$M. $^1$H NMR spectra were routinely obtained at 100 MHz on a Bruker WP-100-SY spectrometer. The NMR solvent used was $CDCl_3$ as indicated. TMS was used as an internal standard. Elemental analyses were performed by Atlantic Microlab, Inc., and were within ±0.4% of the theoretical value.

[$^3$H]Win 35,428 (83.5 Ci/mmol), [$^3$H]citalopram (85.7 Ci/mmol), and [$^3$H]dopamine (60.0 Ci/mmol) were obtained from DuPont-New England Nuclear (Boston, MA). Cocaine hydrochloride was purchased from Mallinckrodt Chemical Corp. (St. Louis, MO). Win 35,428 naphthalenesulfonate was purchased from Research Biochemicals, Inc. (Natick, MA).

**Synthesis of 4-[((Diphenylmethyl)amino)carbonylmethyl]-1-[(4-fluorophenyl)methyl]piperidine (3a).** The starting material 1-[(4-fluorophenyl)methyl]-4-[(ethoxycarbonyl)methyl]piperidine (**2a**) (1.1 g, 3.9 mmol), which was synthesized and characterized earlier,[16] was dissolved in $CF_3COOH/HCl/H_2O$ (1:1:1). The solution was heated to reflux for 2.5 h, and the solvent was removed in vacuo to collect the dried intermediate acid **I** residue. The intermediate acid **I** was not purified and was dissolved in $CH_2Cl_2$ with excess triethylamine to liberate the free base. Into the methylene chloride solution was added 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (1.1 g, 5.7 mmol), followed by 1-hydroxybenzotriazole (0.9 g, 6.6 mmol). The solution was stirred at room temperature for 1 h, and into it was added aminodiphenylmethane (0.9 g, 4.9 mmol). The solution was stirred at room temperature overnight. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate. The organic layer was washed with 5% citric acid solution, followed by saturated $NaHCO_3$ solution, and was dried over $Na_2SO_4$. The organic extract was evaporated to collect a residue which was crystallized from ethyl acetate to give pure product **3a**, 0.85 g (53% yield), mp 174.5−175.1 °C. $^1$H NMR ($CDCl_3$): $\delta$ 1.24−1.93 (7H, m), 2.11 (2H, t, $J$ = 6 Hz, $-CH-(CH_2)-CONH-$), 2.74−2.86 (2H, m), 3.41 (2H, s, $-N-(CH_2)-PhF$), 5.93−6.29 (2H, m, $-(CH)-Ph_2$ + $-CO-(NH)-CH-Ph_2$), 6.87−7.63 (14H, m, 2Ph + Ph−F). Anal. ($C_{27}H_{29}N_2OF$) C, H, N.

**Synthesis of 4-[2-((Diphenylmethyl)amino)ethyl]-1-[(4-fluorophenyl)methyl]piperidine (4a).** Amide **3a** (0.24 g,

0.57 mmol) was dissolved in THF, and into it was added a solution of 1 M $BH_3$/THF complex (0.55 mL, 0.55 mmol). The solution was refluxed for 5 h, and after cooling (ice bath) unreacted $BH_3$/THF complex was quenched by careful addition of an excess amount of methanol. The solvent was removed in vacuo, and the residue was redissolved in 10% MeOH/HCl mixture and was heated to reflux for 1 h. Methanol was removed in vacuo, and the acidic solution was neutralized by careful addition of an excess amount of solid $NaHCO_3$. Product was extracted into the ethyl acetate layer and was dried over $Na_2SO_4$. Crude product was chromatographed, and the pure product **4a** was eluted with ether (0.1% $Et_3N$), 0.1 g (43% yield). $^1$H NMR ($CDCl_3$): $\delta$ 1.21−2.00 (7H, m), 2.57 (2H, t, $J$ = 6.5 Hz, −NH−$(CH_2)$−$CH_2$−), 2.74−2.86 (2H, m), 3.41 (3H, s, −N−$(CH_2)$−PhF), 4.79 (1H, s, −(CH)$Ph_2$), 6.88−7.44 (14H, m).

Free base was converted into its hydrochloride salt, mp 178.9−179.6 °C. Anal. ($C_{27}H_{31}N_2F$·2HCl·$H_2O$) C, H, N.

**Synthesis of 4-[((Bis(4-fluorophenyl)methyl)amino)-carbonylmethyl]-1-benzylpiperidine (3b).** Piperidine ester **2b** (0.9 g, 0.95 mmol) was reacted with aminobis(4-fluorophenyl)methane (1.29 g, 5.59 mmol) to produce **3b**, 0.46 g (31% yield), mp 162.5−163.4 °C. $^1$H NMR ($CDCl_3$): $\delta$ 1.17−1.95 (7H, m), 2.12 (2H, t, $J$ = 6.15 Hz, −CH−$(CH_2)$−CONH−), 2.77−2.90 (2H, m), 3.47 (2H, s, −$(CH_2)$−Ph), 5.86−6.25 (2H, m, −(CH)−$Ph_2$ + −CO−(NH)−CH−$Ph_2$), 7.00−7.61 (13H, m). HRMS: $m/z$ 434.2219 ($M^+$); calcd for $C_{27}H_{28}ON_2F_2$ 434.1891. Anal. ($C_{27}H_{28}ON_2F_2$) C, H, N.

**Synthesis of 4-[((Bis(4-fluorophenyl)methyl)amino)-ethyl]-1-benzylpiperidine (4b).** Compound **3b** (0.09 g, 0.2 mmol) was converted to final compound **4b** by treatment with $BH_3$/THF, 0.07 g (80% yield). $^1$H NMR ($CDCl_3$): $\delta$ 1.25−2.00 (7H, m), 2.56 (2H, t, $J$ = 6.3 Hz, −NH−$(CH_2)$−$CH_2$−), 2.77−2.88 (2H, m), 3.46 (3H, s, −N−$(CH_2)$−Ph), 4.77 (1H, s, −(CH)$PhF_2$), 6.95−7.43 (13H, m).

Free base was converted into its hydrochloride salt, mp 250.8−254.5 °C. HRMS: $m/z$ 492.1906 [$(M - H)^+$]; calcd for $C_{27}H_{32}N_2F_2Cl_2$ 493.1304. Anal. ($C_{27}H_{30}N_2F_2$·2HCl·1.5$H_2O$) C, H, N.

**Biological Methods.** The rat DAT was labeled with [$^3$H]Win 35,428 and the rat SERT with [$^3$H]citalopram. Both binding assays were carried out under the same conditions with striatal tissue from male, young adult Sprague−Dawley rats, exactly as described in our previous work.[16,17] Briefly, rat striatal membranes were incubated with radioligand and inhibitor for 2 h on ice in a sodium phosphate buffer at a final $Na^+$ concentration of 30 mM, pH 7.4, at room temperature. The assays were terminated by filtration through glass fiber filtermats (Wallac Inc., Gaithersburg, MD), presoaked in 0.05% (v/v) poly(ethylenimine), with a MACH3-96 Tomtec harvester (Wallac Inc.). Filters were assayed for radioactivity in a Microbeta Plus liquid scintillation counter (Wallac Inc.).

The hDAT and hSERT were studied in the HEK-293 expression system of Eshleman et al.[25] and Eshleman et al.[26] The hDAT cDNA was cloned by Eshleman et al.,[25] the hSERT cDNA by Ramamoorthy et al.[27] The respective radioligands were [$^3$H]WIN 35,428 and [$^3$H]citalopram, and the general methods for working up and handling the cell preparations were as described by us previously.[29] Briefly, suspensions of cell membranes were incubated with inhibitor and [$^3$H]Win 35,428 for 15 min, or with inhibitor and [$^3$H]citalopram for 30 min, at room temperature in 122 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 10 mM glucose, 1 mM $CaCl_2$, 15 mM $NaH_2PO_4$ plus 7.5 mM $Na_2HPO_4$ mixed to achieve pH 7.4 at room temperature, and 0.1 mM tropolone for inhibition of COMT.[29] Termination of assays was as described above for rat striatal membranes.

Uptake of [$^3$H]dopamine into HEK-293 hDAT cells was measured in suspended, intact cells by general techniques as described by us previously.[30] Briefly, cells were preincubated with inhibitor for 15 min at room temperature in the same tropolone-containing buffer as described above; [$^3$H]dopamine was added, and the incubation continued for another 8 min. Termination of the assay consisted of addition of ice-cold buffer and rapid filtration through Whatman GF/C glass fiber filters, presoaked in 0.05% (w/v) poly(L-lysine), with a Brandel 24-pin harvester (Brandel Inc., Gaithersburg, MD). Radioactivity on filters was estimated by liquid scintillation counting (Beckman LS6000IC, Beckman Instruments, Inc., Fullerton, CA).

All compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted out in 10% (v/v) DMSO. Additions from the latter stocks resulted in a final concentration of DMSO of 0.5%, which by itself did not interfere with radioligand binding or uptake. After initial range-finding experiments, at least five concentrations of the test compound were studied spaced evenly around its $IC_{50}$ value. The latter was estimated by nonlinear computer curve-fitting procedures as described by us previously.[15]

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