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N-8-Substituted benztropinamine analogs as selective dopamine transporter ligands

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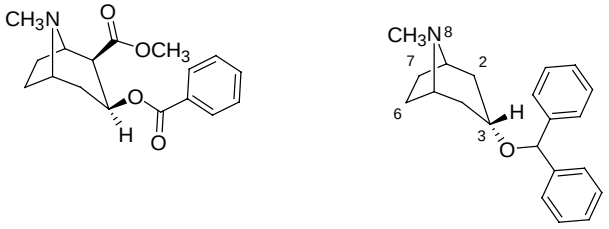
Abstract—A series of N-8-substituted benztropinamines was synthesized and evaluated for binding at the dopamine (DAT), serotonin (SERT), norepinephrine (NET) transporters, and muscarinic M1 receptors. In general, the isosteric replacement of the C-3 benzhydrol ether of benztropine by a benzhydryl amino group was well tolerated at the DAT. However, for certain N-8 substituted derivatives, selectivity over muscarinic M1 receptor affinity was reduced.

Cocaine (**1**) is an effective psychomotor stimulant drug that is subject to widespread abuse. Among the multiple actions of cocaine, the inhibition of dopamine uptake via the dopamine transporter (DAT) is thought to be the primary mechanism underlying its stimulant and reinforcing effects.[1–4]

The dopamine uptake inhibitor benztropine (**2**, BZT) has structural similarities to cocaine (**1**), however, the behavioral effects of BZT and many of its analogs differ from those of cocaine.[5] Structure–activity relationships (SAR) for benztropine-based analogs have been developed in an attempt to provide insight into dopamine transporter function and the neurobiological substrates involved in cocaine abuse[6,7] and these efforts may also provide leads for the discovery of medications for the treatment of cocaine abuse (see Fig. 1).

A variety of structural analogs of BZT have been synthesized to improve affinity for the DAT and selectivity among the sites at which the analogs bind. For example, the effect of various substituents on the C-3 benzhydrol ether moiety has been studied,[8,9] an ester function has been introduced into the 2-position,[10,11] and the consequence of substituents at the 6,7-bridgehead has been reported.[12–15] Furthermore, the SAR of analogs bearing different substituents on the N-8 nitrogen have been explored.[16–19]



1: cocaine **2:** BZT (benztropine)

Figure 1. Structures of cocaine and benztropine (BZT).

In continuation of the efforts to explore the SAR of BZT analogs, this report examines the isosteric replacement of the benzhydrol ether moiety by a benzhydryl amino group. If such a modification is tolerated at the DAT, it is expected that, compared to their corresponding BZT analogs, the resulting benztropinamine derivatives **4a–m** might be more chemically stable and the water solubility might be improved.

The synthesis of the N-8 substituted benztropinamine analogs is depicted in Scheme 1. In short, the α-tropin-

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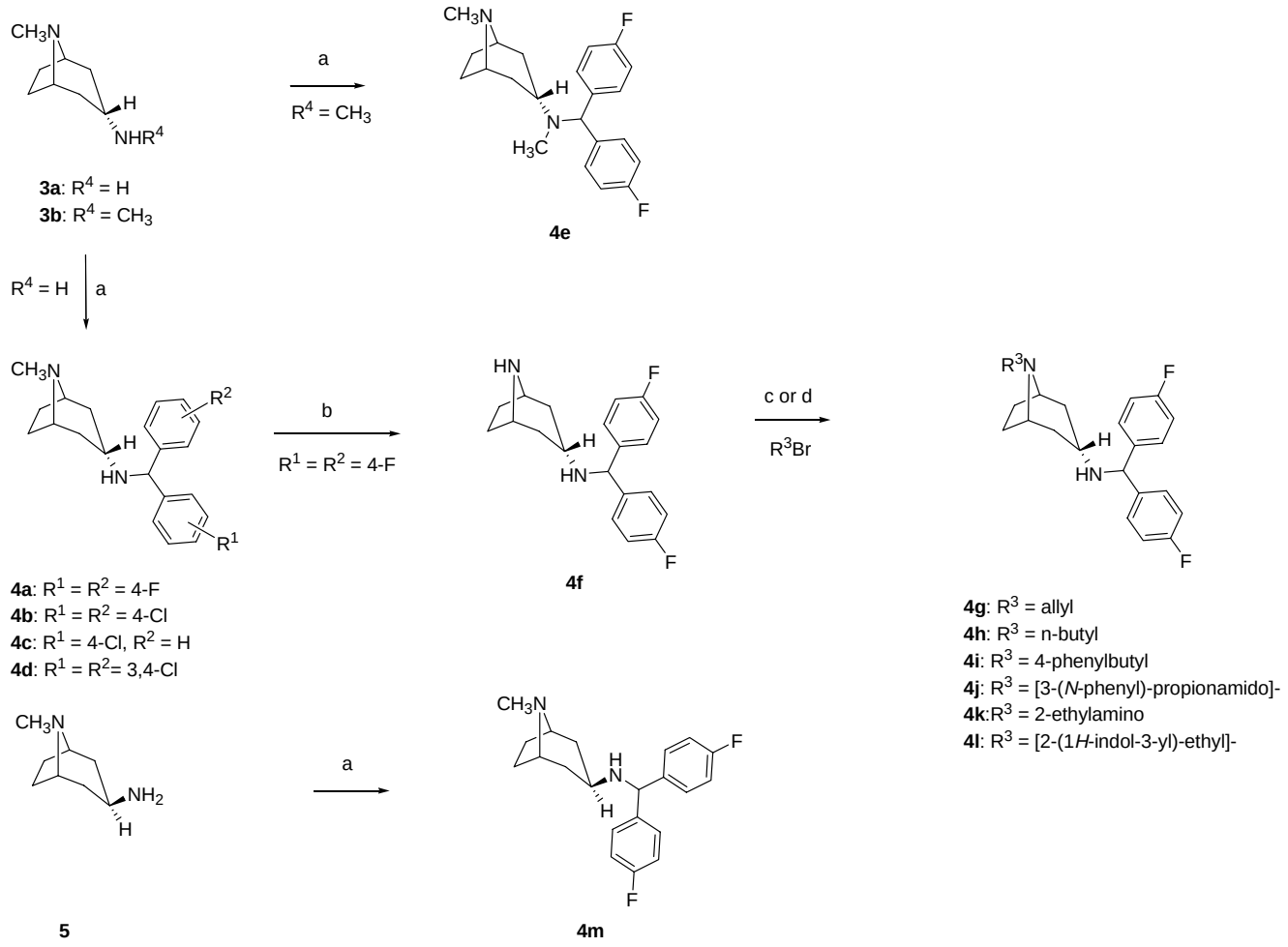


Scheme 1. Synthesis of the benztropinamine analogs. Reagents and conditions: (a) 1—1 eq. benzhydryl chloride analogue, acetonitrile, reflux, 34–71%. (b) 1—1-Chloroethyl chloroformate, 1,2-dichloroethane, Na_2CO_3, reflux. 2—Methanol, reflux, 92%. (c) 1.0 eq. alkyl bromide, $NaHCO_3$, acetonitrile, sealed tube, 120 °C, 58–65%; **4k**: 1. 2-(2-bromoethyl)-isoindoline-1,3-dione, 2. hydrazine, 51%. (d) **4l**: 1—2-indoleacetic acid, DCC, HOBt, 2—$LiAlH_4$, 67%.

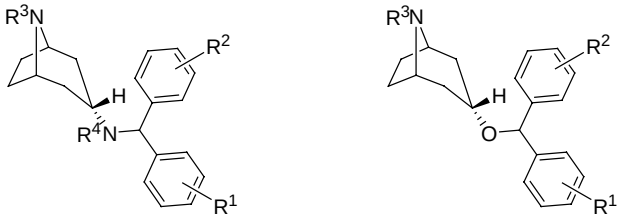
amines **3**[20] were alkylated with the appropriate benzhydryl chloride to give the benztropinamines **4a–e** in low to moderate yields. The N-nor-derivative **4f** was prepared in excellent yield from **4a** by N-demethylation with 1-chloroethyl chloroformate followed by methanolysis. The N-8 substituted derivatives **4g–j** were prepared from **4f** by alkylation with the appropriate alkylbromide. Side products resulting from potential alkylation at the benzhydryl nitrogen were not observed, presumably due to steric hindrance. Compounds **4k** and **4l** were prepared using standard procedures. In order to test the influence of the configuration at C-3 of the tropane moiety on binding at the monoamine transporters and the M1 receptors, the β analog **4m** was synthesized from the β-tropinamine **5**.[21] All final compounds were purified by column chromatography and crystallization of their oxalate or tartrate salts.[22]

Binding affinities of the novel benztropinamines **4** and selected BZT analogs **6** at the DAT in rat brain membranes are depicted in Table 1. The benztropinamine derivative **4a** is the amine analog of the benzhydrol ether derivative **6a**. Compound **4a** (K_i = 11.3 nM) demonstrates a ~3-fold lower affinity at the DAT than the ether derivative **6a** (K_i = 4.11 nM). Under these assay conditions **6a** exhibits DAT over SERT and DAT over NET selectivities of 794- and 148-fold, respectively.[14] For **4a** the binding selectivities of DAT over SERT and DAT over NET (Table 2) appear to be preserved. **4a** (K_i = 7.81 nM) exhibits a very similar binding affinity at the M1 receptor as **6a** (K_i = 11.6 nM).[14] Thus, due to the lower binding affinity of **4a** at the DAT, the DAT over M1 receptor selectivity for this compound is somewhat reduced as compared to **6a**.

A comparison between the α derivative **4a** and the β derivative **4m** reveals that configuration at C-3 plays a pivotal role for the binding affinity at the DAT and the NET, and to a somewhat lesser extent at the SERT and the M1 receptor. As such, compared to **4a**, the binding affinity of **4m** at the DAT is reduced by 58-fold. A similar SAR at the DAT concerning the configuration at C-3 has been previously described for the benzhydrol ether analogs.[8]

The introduction of a methyl group at the benzhydryl nitrogen, resulting in the tertiary amine **4e**, reduces the

Table 1. Binding data at the DAT for the benztropinamines **4** and selected BZT derivatives **6** in rat brain membranes[a]



4 **6**

Compound	R^1	R^2	R^3	R^4	DAT [^{3}H]WIN 35,428 K_i ± SEM (nM)
4a	4-F	4-F	CH_3	H	11.3 ± 1.61
4b	4-Cl	4-Cl	CH_3	H	38.1 ± 5.35
4c	4-Cl	H	CH_3	H	36.5 ± 0.66
4d	3,4-Cl	3,4-Cl	CH_3	H	5.35 ± 0.25
4e	4-F	4-F	CH_3	CH_3	123 ± 15.5
4f	4-F	4-F	H	H	8.45 ± 0.23
4g	4-F	4-F	Allyl	H	26.8 ± 3.43
4h	4-F	4-F	*n*-Butyl	H	21.5 ± 2.31
4i	4-F	4-F	4-Phenylbutyl	H	11.7 ± 0.39
4j	4-F	4-F	[3-(*N*-Phenyl)-propionamido]-	H	4.61 ± 0.54
4k	4-F	4-F	2-Ethylamino	H	12.5 ± 1.73
4l	4-F	4-F	[2-(1*H*-Indol-3-yl)-ethyl]-	H	64.5 ± 4.32
4m[b]	4-F	4-F	CH_3	H	661 ± 35
6a	4-F	4-F	CH_3	n/a	4.11 ± 0.50[14]
6b	4-F	4-F	[3-(*N*-Phenyl)-propionamido]-	n/a	6.22 ± 0.73
6c	4-F	4-F	2-Ethylamino	n/a	5.59 ± 0.62
6d	4-F	4-F	[2-(1*H*-Indol-3-yl)-ethyl]-	n/a	29.2 ± 3.24
Cocaine					74.3 ± 5.42

[a] Each K_i value represents data from at least three independent experiments, each performed in triplicate. K_i values were analyzed by GraphPad Prism. A detailed description of the binding assay methods has been previously published.[23]
[b] The configuration at C-3 is beta.

Table 2. Binding data and selectivities for the benztropinamines **4** at the SERT, NET and muscarinic M1 receptors

Compound	SERT[a] [^{3}H]Citalopram K_i ± SEM (nM)	NET[a] [^{3}H]Nisoxetine K_i ± SEM (nM)	M1[a] [^{3}H]Pirenzepine K_i ± SEM (nM)	SERT/DAT	NET/DAT	M1/DAT
4a	8685 ± 436	1810 ± 269	7.81 ± 1.17	769	160	0.7
4b	4180 ± 623	7580 ± 325	50.0 ± 7.10	110	199	1
4c	13,600 ± 1040	3170 ± 233	9.3 ± 0.27	373	87	0.3
4d	3010 ± 332	259 ± 33.6	17.8 ± 2.27	563	48	3
4e	17,200 ± 2460	8410 ± 724	18.6 ± 2.48	140	68	0.2
4f	4150 ± 368	997 ± 107	154 ± 19.6	491	118	18
4g	3920 ± 581	5580 ± 821	130 ± 10.5	146	208	5
4h	2640 ± 27.6	2920 ± 209	454 ± 36.7	123	136	21
4i	502 ± 68.1	1630 ± 115	438 ± 56.2	43	139	37
4j	608 ± 41	2470 ± 83.3	2540 ± 124	132	536	551
4k	10,900 ± 1090	3550 ± 222	2110 ± 109	872	284	169
4l	347 ± 41.3	8250 ± 939	413 ± 6.55	5	128	6
4m	48,500 ± 6370	15,2000 ± 12,300	59.4 ± 7.3	73	230	0.1
6a	3260 ± 108[14]	844 ± 56.5[14]	11.6 ± 0.93[14]	793	205	3

[a] A detailed description of the binding assay conditions have been previously published.[18] Binding data were analyzed as described in Table 1.

binding affinity at the DAT by 11-fold compared to the secondary amine **4a**. The binding affinities at the SERT and the M1 receptor (~2-fold lower) are far less affected by this modification than the binding affinity at the NET (~5-fold lower).

The impact on the binding affinity at the monoamine transporters and the M1 receptor of different substitution patterns on the benzhydryl moiety was evaluated with analogs **4b–d**. Compared to the 4,4′-difluoro analog, **4a**, the 4,4′-dichloro as well as 4-monochloro substitution, compounds **4b** and **4c**, reduced binding affinity at the DAT by approximately 3-fold. As with **4a**, the M1 over DAT selectivity was reduced compared to the corresponding ether derivatives. Only the derivative **4d**, with a 3,4-dichloro substitution on both phenyl rings,

has a somewhat higher binding affinity at the DAT (2-fold) and a higher DAT over M1 selectivity than **4a**. However, with **4d** DAT selectivity over NET was reduced by 3-fold.

In the benzhydrol ether (BZT) series, it has been shown that N-demethylation at the N-8 nitrogen and alkylation lead to higher DAT over muscarinc M1 selectivities. Likewise, in compounds **4f–k** this modification is generally well tolerated at the DAT and leads to a higher DAT over M1 selectivity profile than the parent compound **4a**. Furthermore, these N-8 modified compounds show a retained if not higher DAT over NET selectivity. However, with the exception of **4k**, all of these compounds demonstrate a somewhat lower DAT over SERT selectivity than **4a**.

As such, the anilino amide derivative **4j** exhibits the highest DAT binding affinity (K_i = 4.61 nM) and the highest DAT over NET and M1 selectivities in this series (535- and 551-fold, respectively). Incidentally, the corresponding ether derivative **6b** (K_i = 4.61 nM) has a similar binding affinity at the DAT and a similar selectivity profile over the NET and the muscarinic M1 receptor.[17] However, the benzhydrol ether derivative has a somewhat higher DAT over SERT selectivity than **4j**, (239-fold compared to 132-fold).[17]

The N-8 ethylamino derivative **4k** shows a similar binding affinity at the DAT as the parent compound **4a**. The DAT over SERT and NET selectivity profile is retained, if not somewhat improved. Compared to **4a**, the binding affinity at the muscarinic M1 receptor is greatly reduced (170-fold).

The indole derivative **4l** differs from the rest of the N-8 modified derivatives (compounds **4f–k**) in that its binding affinity at the DAT is 6-fold lower than that of the parent compound **4a**. Further, its SERT affinity is the highest within the series, presumably due to the indole moiety as a serotonin-like pharmacophore, resulting in only a 5-fold DAT over SERT selectivity. Indeed, the DAT/SERT dual activity has been suggested to also have promise as a cocaine-abuse medication target.[24] Interestingly, the corresponding benzhydrol ether derivative **6d** demonstrated a 5-fold lower binding affinity at the DAT compared to that of **6a**, but showed a 160-fold DAT over SERT selectivity (K_i (SERT) = 4600 nM).

In summary, these 3-amino derivatives of BZT show a novel structural approach to the development of DAT selective compounds. In general, the isosteric replacement of the benzhydrol ether moiety by an amino benzhydryl group is well tolerated at the DAT and compared to their ether counterparts, these derivatives show a similar SAR. In vivo studies are currently underway.

Acknowledgments

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22. Note: All new compounds have been fully characterized by spectroscopic means. Representative data: **4a**—IR: *v* 3303. ^{1}H NMR ($CDCl_3$): δ 1.57 (d, *J* 13.2, 2H), 1.96–2.05 (m, 6H), 2.25 (s, 3H), 2.74 (t, *J* 6.6, 1H), 3.09 (s, 2H), 4.85 (s, 1H), 6.93–6.97 (m, 4H), 7.18–7.22 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 26.97, 37.05, 40.92, 46.92, 60.84, 63.29, 115.31 (J_{CF} 21), 128.91 (J_{CF} 8), 139.67 (J_{CF} 3), 161.44 (J_{CF} 243). **4j**—IR: *v* 3313, 1674. ^{1}H NMR ($CDCl_3$): 1.44 (s, 1H), 1.72 (d, *J* 14.0, 2H), 2.00–2.08 (m, 4H), 2.18 (m, 2H), 2.45 (t, *J* 5.6, 2H), 2.69 (t, *J* 5.6, 2H), 2.90 (t, *J* 6.0, 1H), 3.33 (s, 2H), 4.89 (s, 1H), 7.19–7.25 (m, 6H), 7.47 (d, *J* 7.6, 2H), 7.98–7.25 (m, 5H), 11.53 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 26.99, 34.52, 37.27, 47.22, 48.78, 58.55, 63.27, 115.74 (J_{CF} 21),

119.78, 123.82, 129.30, 129.31 (J_{CF} 8), 139.14, 139.85 (J_{CF} 4), 162.1 (J_{CF} 245), 171.43.

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