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The effect of 6-substituted-4′,4″-difluorobenztropines on monoamine transporters and the muscarinic M1 receptor

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Abstract—A series of racemic 6-hydroxy and carboalkoxy substituted-4′,4″-difluorobenztropines was synthesized and evaluated for binding at the dopamine (DAT), the serotonin (SERT), the norepinephrine (NET) transporters, and the muscarinic M1 receptor. Each of the analogues displaced [^{3}H]WIN 35,428 (DAT) with a range of affinities from 5.81 to 175 nM and [^{3}H]pirenzepine (M1), with a range of affinities (K_i = 27.0–8430 nM). Binding affinities at the SERT and the NET were generally low.
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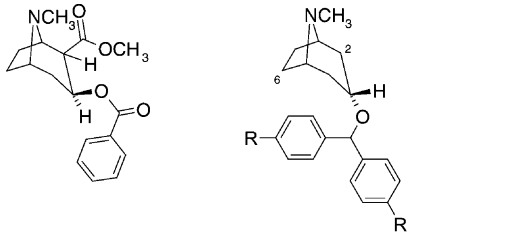
Cocaine is a potent psychostimulant and drug of abuse. The primary mechanism by which cocaine affects its psychomotor stimulant and reinforcing actions is by inhibiting dopamine reuptake through blockade of the dopamine transporter (DAT).[1–4]

Benztropine (BZT) has structural similarities to cocaine (Fig. 1), but the behavioral effects of BZT and many of its analogues differ from those of cocaine.[5] Structure–activity relationships (SAR) for benztropine-based analogues have been developed for both the DAT and the muscarinic M1 receptor, in an effort to characterize binding profiles that might lead to the development of a cocaine abuse therapeutic agent. To improve the affinity at the DAT and selectivity among the sites at which the analogues bind, different series of structural analogues of BZT have been synthesized. For example, the effect of various substituents on the benzhydrol moiety has been studied,[6] an ester function has been introduced into the 2-position[7,8] and several analogues with different substituents on the nitrogen have been made.[9–12] Both N-substitution and (S)-2-substitution in this series, significantly decrease muscarinic receptor binding, while retaining high affinity for DAT. Recent investigations into substitution at the 6,7-bridgehead of the tropane based DAT inhibitors has been limited to hydroxylation or methoxylation.[13–15] The 6β-methoxy-4′,4″-difluorobenztropine reported by Simoni et al. was generally well tolerated at the DAT.[13] In contrast, the 6β- and 7β-hydroxylated analogues of difluoropine, which has a 2-methylester moiety on the tropane ring, showed significant reduction in DAT affinity.[14] DAT affinities for both 6- and 7-hydroxylated 3-phenyltropanes were comparable to the unsubstituted parent compounds[14,15] and one of these analogues was reported to attenuate cocaine-induced locomotor activity, providing support to further investigate the 6,7-bridgehead for chemical modification. Hence the synthesis and binding evaluation of a series of racemic-6-hydroxy and



Cocaine **R=H: Benztropine, BZT**
R=F: AHN 1-055, 4',4''-diF-BZT

Figure 1. Structures of cocaine, BZT, and **AHN 1-055**.

Keywords: Monoamine transporters; Muscarinic M1 receptor; Benztropine; Ligand binding.

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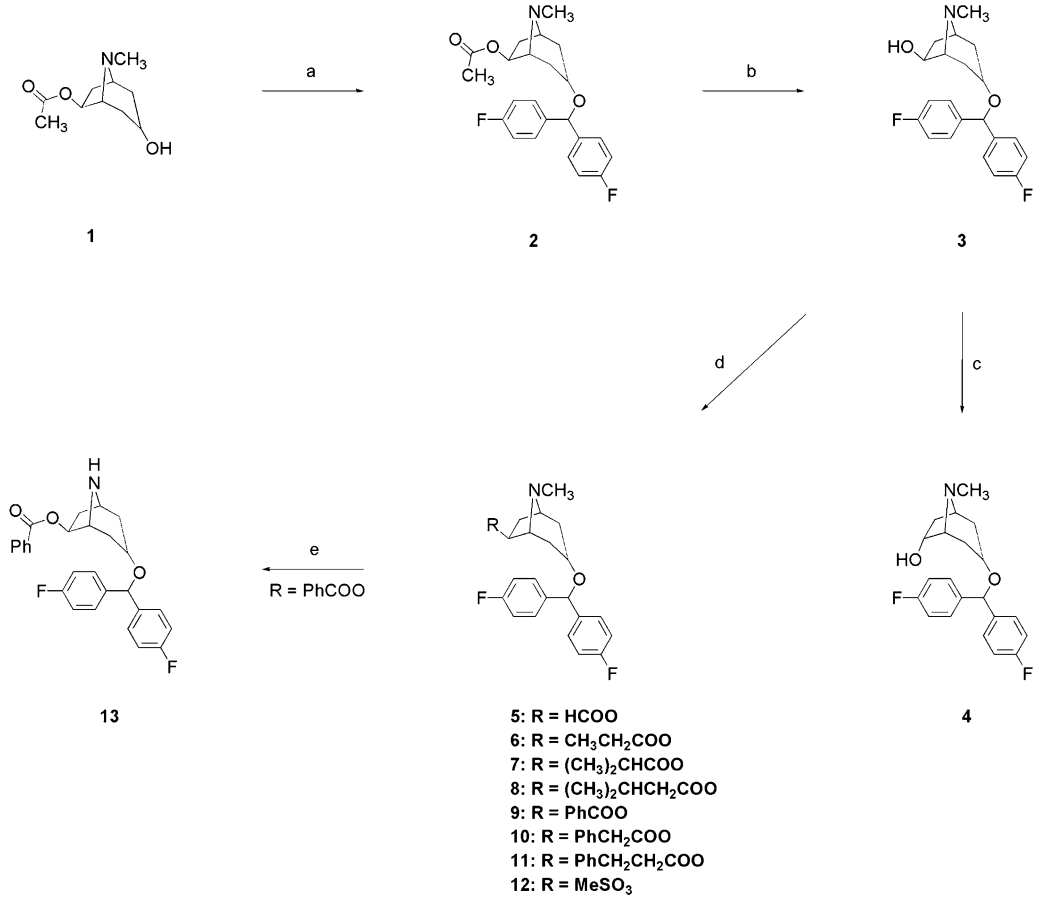
carboalkoxy-substituted 4′,4″-difluorobenztropines was initiated in order to determine the effects of these substitutions on DAT, serotonin transporter (SERT), norepinephrine transporter (NET), and muscarinic M1 receptor binding.

The synthesis of these novel 6-substituted 4′,4″-difluorobenztropines is depicted in Scheme 1. In short, 6β-acetyltropine (**1**)[16] was alkylated with 4,4′-difluoro- benzhydryl chloride following an adapted procedure described earlier[6] to yield the acetyl derivative **2**. Since a saponification procedure with base proved to be unreliable, the β-hydroxy derivative **3** was obtained by reduction of the acetyl moiety of **2** with lithium aluminum hydride. Esterification under standard conditions employing the corresponding acid chloride or acid anhydride in the presence of triethylamine as the base generated the derivatives **5**–**11**. The benzoic acid nor derivative **13** was prepared from **9** by N-demethylation with 1-chloroethyl chloroformate followed by methanolysis. To test the impact of the configuration of the 6-hydroxy group the α-derivative **4** was prepared stereoselectively by an oxidation–reduction sequence.[17]

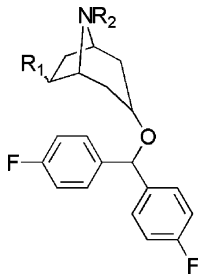
The ligands were evaluated in competition binding assays[10,18] in rat brain membranes (Table 1). Two of the 6-substituted compounds, the 6β-hydroxy derivative **3** and the 6β-formyl ester **5**, showed similar affinities to the parent compound, **AHN 1-055**, in binding to the DAT. These compounds were highly selective (>1000-fold) for the DAT over the SERT and showed a >200-fold selectivity for the DAT over the NET. The derivatives **3** and **5** exhibited an 8-fold selectivity for the DAT over muscarinic M1 sites. Derivative **3** ($K_i = 46.8$ nM) as well as ligand **5** ($K_i = 48.3$ nM) showed a ~4-fold decrease in M1 binding affinity as compared to the unsubstituted parent compound, **AHN 1-055** ($K_i = 11.6$ nM). The anticholinergics atropine ($K_i = 1.98$ nM) and the 6,7-substituted scopolamine ($K_i = 0.89$ nM) were equipotent at the M1 receptor. Neither of those tropane based compounds bind with high affinity to the DAT.[19]



a: 1.2 eq. 4,4'-difluorobenzhydryl chloride, neat, 160 °C, 15min, 24%.
b: 1.75 eq. $LiAlH_4$, THF, room temperature, 2h, 82%.
c: 1. Swern oxidation, 31%; 2. 2.5 eq. $NaBH_4$, EtOH, -78°C/room temperature, 16 h, 74%.
d: 1.2 eq. RCOCl or $(RCO)_2O$, 1.2 eq. NEt_3, $CHCl_3$, room temperature, 38-79%.
e: 1. 2 eq. 1-chloroethyl chloroformate, 4 eq. $NaHCO_3$, 1,2-dichloroethane, reflux, 4h; 2. MeOH, room temperature, 16h, 41%.

Scheme 1. Synthesis of the 6-hydroxy and carboalkoxy-substituted derivatives of 4′,4″-difluorobenztropine. Reagents and conditions: (a) 1.2 equiv 4,4′-difluorobenzhydryl chloride, neat, 160 °C, 15 min, 24%; (b) 1.75 equiv $LiAlH_4$, THF, rt, 2 h, 82%; (c) (1) Swern oxidation, 31%; (2) 2.25 equiv $NaBH_4$, EtOH, −78 °C/rt, 16 h, 74%; (d) 1.2 equiv RCOCl or $(RCO)_2O$, 1.2 equiv NEt_3, $CHCl_3$, rt, 38–79%; (e) (1) 2 equiv 1-chloroethyl chloroformate, 4 equiv $NaHCO_3$, 1,2-dichloroethane, reflux, 4 h; (2) MeOH, rt, 16 h, 41%.

Table 1. Inhibition constants (K_i) at the monoamine transporters and the muscarinic M1 receptor in rat brain membranes[a]



Compound	R1	R2	DAT [^{3}H]WIN 35,428 $K_i \pm$ SEM (nM)	SERT [^{3}H]citalopram $K_i \pm$ SEM (nM)	NET [^{3}H]nisoxetine $K_i \pm$ SEM (nM)	M1 Receptor [^{3}H]pirenzepine $K_i \pm$ SEM (nM)
3	β-HO	CH_3	6.28 ± 0.45	9090 ± 615	1300 ± 142	48.3 ± 6.86
4	α-HO	CH_3	31.3 ± 2.27	11,700 ± 977	6390 ± 486	27.0 ± 3.43
5	β-HCOO	CH_3	5.81 ± 0.31	8290 ± 279	1380 ± 190	46.8 ± 6.56
2	β-CH_3COO	CH_3	57.1 ± 5.56	16,500 ± 1610	6910 ± 573	432 ± 47.8
6	β-CH_3CH_2COO	CH_3	75.4 ± 3.01	14,000 ± 1130	3720 ± 457	142 ± 21.1
7	β-$(CH_3)_2CHCOO$	CH_3	151 ± 9.42	14,800 ± 1850	10,200 ± 948	784 ± 86.4
8	β-$(CH_3)_2CHCH_2COO$	CH_3	148 ± 23.3	7960 ± 1010	6610 ± 754	723 ± 66.5
9	β-PhCOO	CH_3	107 ± 10.0	3730 ± 533	1370 ± 82.3	552 ± 52.9
10	β-$PhCH_2COO$	CH_3	119 ± 14.9	5850 ± 682	6206 ± 394	2270 ± 307
11	β-$PhCH_2CH_2COO$	CH_3	171 ± 11.7	7960 ± 671	5690 ± 276	3480 ± 409
12	β-$MeSO_3$	CH_3	81.0 ± 3.27	13,800 ± 380	6880 ± 805	617 ± 66.5
13	β-PhCOO	H	175 ± 17.5	4440 ± 148	3170 ± 408	8430 ± 461
AHN 1-055	H	CH_3	4.11 ± 0.50	3260 ± 108	844 ± 56.5	11.6 ± 0.93
Cocaine	n/a		74.3 ± 5.42	293 ± 30.0	3302 ± 167	61,397 ± 10,932

[a] Each K_i value represents data from at least three independent experiments, each performed in triplicate. K_i values were analyzed by PRISM. A detailed description of the binding assay methods have been previously published.[10,18]

The impact of the orientation of the hydroxy group (α or β) on binding and selectivities was examined to reveal that the β-derivative **3** had a 5-fold higher affinity at the DAT and at the NET than the α-derivative **4**, though DAT over NET selectivity was retained (>200-fold). Ligand **3** was also 8-fold more selective at the DAT over the M1 receptor than compound **4**, which was essentially nonselective at these two binding sites. Affinity at the SERT was not significantly affected by the configuration at C-6.

Increasing the alkyl chain of the ester (ligands **2**, **6**–**8**) beyond a simple formyl group (derivative **5**) resulted in overall loss of affinity for the DAT, the NET, and the M1 receptor. Even though, as depicted in Figure 2, the modest DAT over M1 selectivity generally appeared to be retained. For example, the formyl ester derivative **5** had a >9-times higher affinity at both the DAT and the M1 receptor than the acetyl ester **2** while the DAT over M1 selectivity were not affected: both compounds showed an 8-fold selectivity ratio. This correlation between affinities at the DAT and affinities for the M1 receptor might indicate similar structure–activity relations within this series of 6-substituted benztropines (Fig. 2). This finding further suggests some structural overlap between the two binding sites, although the nitrogen or 2-substituted benztropines do not show this DAT/M1-relationship.[8,11]

In contrast to the less sterically bulky esters (ligands **2**, **5**–**9**) and the mesylate **12**, a significantly improved

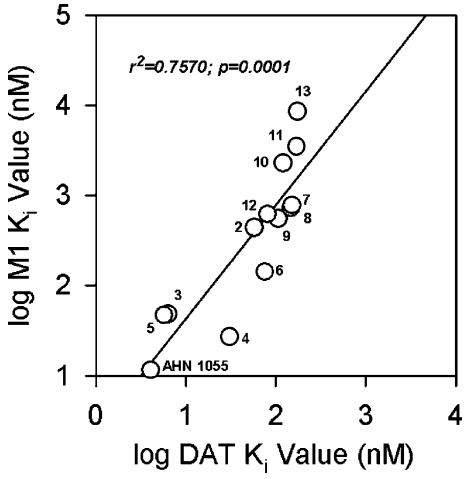


Figure 2. Correlation of affinities for muscarinic M1 receptor with the DAT affinities.

selectivity (20-fold) at the DAT over the M1 receptor was recorded for the phenylacetic acid ester **10** and the hydrocinnamyl acid ester **11**. However, ligands **10** and **11** had (>30-fold) lower affinity for the DAT than the parent compound **AHN 1-055**.

The benzoate **9** had a 15-fold higher affinity at the M1 receptor than its N-demethylated counterpart, the nor-derivative **13**. The effect of the nitrogen substitution was less profound on affinities at the monoamine transporters, for example, both compounds share the lowest

Table 2. Binding selectivities for the novel 6-substituted 4′,4″-difluorobenztropine analogues

Compound	SERT/DAT	NET/DAT	M1/DAT
3	1447	207	8
4	374	204	1
5	1427	238	8
2	289	121	8
6	186	49	2
7	98	68	5
8	54	45	5
9	35	13	5
10	49	52	19
11	47	33	20
12	170	85	8
13	25	18	48
AHN 1-055	793	205	3

selectivity ratios for the DAT over the NET (<20-fold) in this series. As such, the affinity of ligand **13** at the DAT ($K_i = 175$ nM) was low, it was the most selective compound in this series (48-fold) for the DAT over the M1 receptor (Table 2). N-demethylation of the parent compound **AHN 1-055** was previously shown to have similar effects on these affinities.[12]

In summary, these compounds show an interesting spectrum of activities and a novel structural approach to the development of DAT selective compounds. A comparison of M1 with DAT binding affinities suggests that within this series of compounds, binding at the two sites is related, however, these hydroxy and carboalkoxy substitutions on the 6,7-bridgehead may be exploited to provide selective and potent DAT ligands. In vivo studies and the synthesis and pharmacological evaluation of enantiomerically pure derivatives are currently underway.

Acknowledgements

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17. All new compounds have been fully characterized by spectroscopic means. Elemental analysis were within ±0.4% for C, H, N. Representative data: **3**: mp (oxalate, acetone/diethylether) 198 °C. R_f 0.11 ($CHCl_3/CH_3OH$ 10:1). IR (film): 3351. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.54 ('d', '*J*' 14.9, 1H), 1.68 ('d', '*J*' 14.8, 1H), 1.84 (dd, 13.2, 7.2, 1H), 1.90–1.98 (m, 2H), 2.47 (s, 3H), 2.67 (dd, *J* 13.2, 7.2, 1H), 2.97 (m, 1H), 3.00 (br, 1H), 3.21 (m, 1H), 3.52 (t, *J* 5.2, 1H), 4.61 (dd, *J* 7.1, 2.3, 1H), 5.32 (s, 1H), 6.93–6.98 (m, 4H), 7.20–7.25 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 29.44, 30.79, 37.22, 41.44, 59.25, 67.85, 69.48, 76.50, 79.94, 115.47 (J_{CF} 20), 115.49 (J_{CF} 21), 128.32 (J_{CF} 7), 128.33 (J_{CF} 8), 138.27 (J_{CF} 3), 161.79 (J_{CF} 244). Mass (EI): m/z 359.
Compound **5**: mp (DL-tartrate, acetone/diethylether) 148 °C. R_f 0.42 ($CHCl_3/CH_3OH$ 10:1). IR (film): 1718. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.70 (d, *J* 14.5, 1H), 1.90–2.01 (m, 4H), 2.46 (s, 3H), 2.66 (dd, 13.5, 7.6, 1H), 3.17 (m, 1H), 3.29 (m, 1H), 3.57 (t, *J* 4.5, 1H), 5.37 (s, 1H), 5.68 (dd, *J* 7.2, 2.5, 1H), 6.95–7.00 (m, 4H), 7.25–7.28 (m, 4H), 7.88 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 30.33, 31.97, 33.20, 36.96, 59.97, 65.96, 69.39, 79.53, 80.03, 115.42 (J_{CF} 20), 128.25 (J_{CF} 8), 128.40 (J_{CF} 8), 137.96 (J_{CF} 3), 137.99 (J_{CF} 3), 160.67, 161.71 (J_{CF} 243), 161.75 (J_{CF} 243). Mass (EI): m/z 387.
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