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Receptor affinities of dopamine D1 receptor-selective novel phenylbenzazepines

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Abstract

We prepared a series of 18 novel substituted phenylbenzazepine congeners of the dopamine D1/D5 receptor partial-agonist SKF-83959 (*R*,*S*-3-methyl-6-chloro-7,8-dihydroxy-1-[3′-methylphenyl]-2,3,4,5-tetrahydro-1H-benzazepine) and characterized their potency and selectivity in assays of dopamine, 5-HT and adrenoceptors in rat brain tissue or membranes of genetically transfected cells. The *R*-enantiomer of SKF-83959 (MCL-202) and three other novel racemic 1-phenyl-7,8-dihydroxybenzazepines (MCL-204, -203, and -207) showed very high dopamine D5 receptor affinity; MCL-209 displayed the greatest dopamine D5 receptor affinity. These five potent novel ligands also had >100-fold selectivity for dopamine D1 over dopamine D2, D3, serotonin 5-HT-2A receptors and α2-adrenoceptors. They require further functional testing to characterize their intrinsic activity, and for potential stimulant-antagonist actions, as observed with SKF-83959 and MCL-202.

Keywords: Dopamine; Dopamine D1 receptor; Partial-agonist; Phenylbenzazepine

1. Introduction

Phenylbenzazepines are benzodiazepine analogs that include the first dopamine D1 receptor-selective agents reported (Fig. 1). The dopamine D1 receptor antagonist SCH-23390 (*R*[+]-3-methyl-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-benzazepine) and partial-agonist SKF-38393 (*R*[+]-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol) have had broad application as experimental probes of dopamine D_1 (or D1A) receptors and the similar, but much less abundant dopamine D5 (or D1B), receptors (Neve and Neve, 1997). Phenylbenzazepines have contributed to searches for dopamine D1 receptor-based therapeutic agents including candidate antihypertensive (Singh and Goyal, 1999; Mathur, 2003) and antiparkinsonism agents (Neumeyer et al., 2003) among agonists, and candidate antipsychotics among antagonists (Karlsson et al., 1995).

Dopamine D1 receptor-selective agents also include candidate treatments for psychostimulant abuse and dependence (Bergman et al., 2000). D1 receptor *partial-agonists* can reduce abuse-related behavioral effects of stimulants and may have less likelihood of producing either hypotension associated with full-agonists or the behavior-disrupting effects of antagonists. For example, the D1 partial-agonist SKF-83959 (*R*,*S*-3-methyl-6-chloro-7,8-dihydroxy-1-[3′-methylphenyl]-2,3,4,5-tetrahydro-1H-benzazepine; Fig. 1) has moderately high cerebral dopamine D1 receptor affinity and can reduce abuse-related behavioral effects of cocaine, including its self-administration, in monkeys (Bergman and Goldberg, 1998; Khroyan et al., 2000). Moreover, such effects of SKF 83959, in contrast to other dopamine D1 receptor agonists or antagonists occur at doses with only minor disruptive effects on other behaviors (Rosenzweig-Lipson and Bergman, 1994; Platt et al., 2000).

Based on previously developed phenylbenzazepines, relatively minor changes in the 3- and 3′-alkyl, and 6-halo substituents of the molecule appears to have important consequences for dopamine D1 activity (Fig. 1; Table 1). In the present study, we tested the hypothesis that changes in

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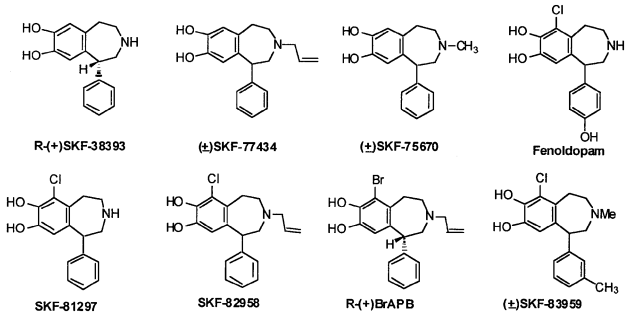


Fig. 1. Chemical structures of dopamine D1 receptor-selective phenylbenzazepines.

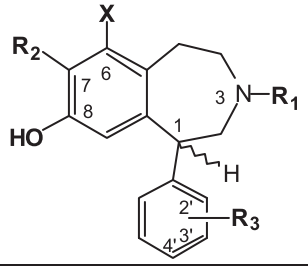
the 3- and 3′-alkyl, and 6-halo substituents of SKF-83959 would affect their potency and selectivity for dopamine D1 and D5 receptors.

2. Methods

2.1. Materials

Receptor sources include brain tissue of adult, male Sprague–Dawley rats (Charles River Laboratories, Burlington, MA) for all assays, except that dopamine D3 and D5 receptor assays used cell membranes from Sf9 (dopamine D3 receptors) or Chinese hamster ovary (CHO; dopamine D5 receptors) cell lines transfected to express human receptor genes selectively and obtained from Sigma-RBI (Natick, MA). In addition, (+)-butaclamol, cinanserin, (−)-eticlopride, haloperidol, and phentolamine mesylate were from Sigma-RBI; *cis*-flupenthixol was donated by Lundbeck (Copenhagen, Denmark). Radioligands from Perkin Elmer/NEN (Boston, MA) were [^{3}H]ketanserin (63 Ci/mmol), [^{3}H]MK-912 (76.5 Ci/mmol), [^{3}H]nemonapride

Table 1
Receptor potencies of phenylbenzazepines



Compound	Isomer	X	R_1	R_2	R_3	Receptor potencies ($K_i \pm$ S.E., nM)					
						D1	D2	D3	D5	5-HT-2A	α2
MCL-204	*RS*(±)	Br	$CH_2CH{=}CH_2$	OH	3′-CH_3	0.11 ± 0.03	83.8 ± 7.8	283 ± 81	12.0 ± 1.0	598 ± 86	709 ± 37
SCH-23390[a]	*R*(+)	H	CH_3	Cl	H	0.12 ± 0.01	1210 ± 13	>10,000	–	10.8 ± 1.6	208 ± 11
MCL-203	*RS*(±)	Br	CH_3	OH	3′-CH_3	0.19 ± 0.02	440 ± 67	>10,000	2.47 ± 0.28	224 ± 37	461 ± 37
MCL-207	*RS*(±)	Cl	CH_3	OH	2′-CH_3	0.46 ± 0.03	226 ± 32	177 ± 37	2.32 ± 0.35	70.6 ± 6.1	66.6 ± 4.2
MCL-202	*R*(+)	Cl	CH_3	OH	3′-CH_3	0.49 ± 0.38	515 ± 45	374 ± 67	1.53 ± 0.06	88.6 ± 2.6	≥ 3000
MCL-210	*RS*(±)	Cl	$CH_2CH{=}CH_2$	OH	3′-CH_3	0.52 ± 0.10	119 ± 17	334 ± 70	9.94 ± 1.45	995 ± 332	877 ± 63
MCL-209	*RS*(±)	Cl	H	OH	3′-CH_3	0.60 ± 0.14	≥ 5000	>10,000	0.88 ± 0.18	1202 ± 240	240 ± 48
MCL-214	*RS*(±)	Br	CH_3	OH	2′-CH_3	1.10 ± 0.10	409 ± 43	467 ± 59	3.42 ± 0.26	45.8 ± 3.5	186 ± 18
SKF-83959	*RS*(±)	Cl	CH_3	OH	3′-CH_3	1.18 ± 0.18	920 ± 114	399 ± 64	7.56 ± 2.47	266 ± 21	295 ± 37
MCL-216	*RS*(±)	Br	H	OH	2′-CH_3	1.81 ± 0.18	19.5 ± 0.5	20.4 ± 1.1	1.95 ± 0.23	398 ± 59	133 ± 6
SKF-81297	*R*(+)	Cl	H	OH	OH	1.90 ± 0.10	1272 ± 102	>10,000	–	995 ± 332	509 ± 111
MCL-206	*RS*(±)	Cl	CH_3	OH	4′-CH_3	1.93 ± 0.20	362 ± 78	>10,000	3.96 ± 0.26	147 ± 11	250 ± 35
R(+)-BrAPB[b]	*R*(+)	Br	$CH_2CH{=}CH_2$	OH	H	2.29 ± 0.26	209 ± 23	–	–	–	–
MCL-205	*RS*(±)	Br	H	OH	3′-CH_3	4.41 ± 0.28	1072 ± 221	>10,000	13.7 ± 1.2	1295 ± 137	338 ± 14
SKF-82958	*R*(+)	Cl	CH_3	OH	H	4.56 ± 0.15	264 ± 30	77.3 ± 24.7	–	1612 ± 313	558 ± 75
MCL-211	*RS*(±)	Br	CH_3	OH	3′-CF_3	8.66 ± 1.04	1048 ± 144	356 ± 56	–	68.9 ± 9.0	365 ± 11
SKF-77434	*R*(+)	H	$CH_2CH{=}CH_2$	OH	H	10.5 ± 0.5	1000 ± 100	–	–	≥ 3000	632 ± 172
MCL-212	*RS*(±)	Br	CH_3	OH	4′-CH_3	19.3 ± 1.7	1031 ± 98	≥ 10,000	4.36 ± 0.23	124 ± 22	493 ± 62
MCL-201	*S*(−)	Cl	CH_3	OH	3′-CH_3	21.3 ± 2.2	2136 ± 573	659 ± 38	≥ 3000	1001 ± 115	632 ± 87
SKF-38393	*R*(+)	H	H	OH	H	26.6 ± 1.3	>10,000	>10,000	–	>10,000	248 ± 13
MCL-215	*RS*(±)	Br	$CH_2CH{=}CH_2$	OH	2′-CH_3	37.1 ± 2.1	239 ± 53	41.5 ± 5.3	–	742 ± 64	414 ± 45
MCL-213	*RS*(±)	Br	$CH_2CH{=}CH_2$	OH	4′-CH_3	128 ± 9	1705 ± 265	385 ± 40	–	977 ± 120	1091 ± 150
MCL-218	*RS*(±)	Br	H	OH	3′-CF_3	1238 ± 163	>10,000	>10,000	–	992 ± 95	776 ± 89
MCL-230A	*RS*(±)	Cl	CH_3	H	3′-CH_3	>10,000	–	>10,000	–	–	–
MCL-217	*RS*(±)	Br	$CH_2CH{=}CH_2$	OH	2′-CF_3	>30,000	>30,000	>10,000	–	>30,000	>10,000

Data are for 25 novel and comparison phenylbenzazepines, in descending order of dopamine D1 receptor potency. Chemical names are provided in text.

[a] Dopamine D1 receptor antagonist included for comparison.

[b] Adapted from Neumeyer et al. (1992).

(85 Ci/mmol), and [^{3}H]SCH-23390 (75 Ci/mmol). Novel compounds were synthesized at Natural Pharmacia International (NPI; Belmont, MA) and Organomed (Coventry, RI), and fully characterized chemically in the Medicinal Chemistry Laboratory (MCL), McLean Hospital. General synthetic methods for the preparation of these novel compounds have been reported previously (Neumeyer et al., 1992).

2.2. Dopamine receptors in rat forebrain tissue

Test agents were assayed for affinity at dopamine receptors in rat caudate–putamen tissue from adult male Sprague–Dawley rats. Fresh, rapidly dissected brain tissue was hand-homogenized in 50 mM Tris–HCl buffer pH 7.4, containing 150 mM NaCl, with 30–40 μg protein/tube. For dopamine D1 receptor assays, tissue was incubated with [^{3}H]SCH-23390 (0.30 nM) for 30 min at 30 °C, using excess *cis*-flupenthixol (10 μM) as a blank. For dopamine D2 receptor assays, [^{3}H]nemonapride (0.075 nM) was incubated with the same tissue preparation at 30 °C for 90 min, with excess haloperidol (10 μM) as a blank. These methods were reported previously (Faedda et al., 1989; Kula et al., 1992, 1997).

2.3. Dopamine receptors in transfected cell membranes

Assays for potency at human dopamine D3 and D5 receptors used transfected cell membranes prepared in 50 mM Tris–HCl (pH 7.4) buffer containing 150 mM NaCl to provide 50 and 2 μg/assay, respectively. dopamine D3 receptor assays used [^{3}H]nemonapride (0.10 nM), incubated for 60 min at 30 °C, with excess (−)-eticlopride (0.1 μM) as a blank. Dopamine D5 receptor assays used [^{3}H]SCH-23390 (0.80 nM) incubated for 90 min at 27 °C, with excess (+)-butaclamol (10 μM) as the blank. These methods were described previously (Kula et al., 1994, 1997).

2.4. 5-HT receptors and adrenoceptors

Rat whole-brain minus cerebellum was homogenized in 50 mM Tris–HCl buffer pH 7.7, to provide 100–200 μg of protein/assay (Kula et al., 1997). Assays of serotonin 5-HT-@A/2C receptors incubated the homogenate with [^{3}H]ketanserin (0.40 nM) for 15 min at 37 °C, with cinanserin (1 μM) used as a blank (Leysen et al., 1982). Assays of α2-adrenoceptors incubated the same amount of brain homogenate with [^{3}H]MK-912 (1.0 nM) at room temperature for 80 min, with phentolamine (10 μM) as blank (Pettibone et al., 1989).

3. Results

Results of assays of potencies of 18 novel test phenylbenzazepines and seven known comparison compounds at dopamine as well as serotonin and adrenergic receptors are summarized in Table 1. The 24 phenylbenzazepines evaluated exhibited an extreme range of D1 potencies (0.11–30,000 nM), but as expected, the known comparison agents, D1 antagonist SCH-23390 and agonists SKF-38393, -77434, -81297, -82958, -83959, and *R*[+]-3-allyl-6-bromo-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-benzazepine (*R*[+]-6-BrAPB), all showed at least moderately high dopamine D1 receptor potency and dopamine D1/D2 receptor selectivity. Seven of the novel substituted catecholphenylbenzazepines had substantially higher dopamine D1 receptor affinity than the lead agent SKF-83959 ($K_i \leq 1.10$ nM; Table 1). Like MCL-204, the racemates MCL-203 and MCL-207 showed very high dopamine D1 receptor potency ($K_i = 0.19$ and 0.46 nM) that met or exceeded that of the *R*(+) enantiomer of SKF 83959, MCL-202 (D1 receptor $K_i = 0.49$ nM). Among novel compounds, racemic MCL-204 was the most potent dopamine D1 receptor ligand ($K_i = 0.11$ nM) and displayed high selectivity at dopamine D1 vs. D2 receptors (762-fold) and, also, at dopamine D1 vs. D5 receptors (109-fold). Racemic MCL-209 was the most potent novel compound at dopamine D5 receptors ($K_i = 0.88$ nM), yet it also retained high D1 receptor affinity ($K_i = 0.60$ nM). The greatest dopamine D1/D2 receptor-selectivity (8333-fold) was shown by MCL-209 and the greatest separation of dopamine D1/D5 receptor potencies (140-fold) was found with MCL-201 (dopamine D1 receptor $K_i = 21.3$ nM).

The correlation of potencies at different dopamine receptors for the 12 most potent novel agents with dopamine D1 receptor K_i values <25 nM was either weak (D1 vs. D2; $r_s = 0.522$, $p = 0.071$) or not significant (D1 vs. D5; Spearman rank-correlation $r_s = 0.385$, $p = 0.202$). For these same 12 novel agents, dopamine D1 receptor potency averaged 54.5-times greater than affinities at dopamine D5 receptor sites (255/4.68). All of the novel compounds lacked potency at dopamine D2 and D3 receptors, except MCL-216 ($K_i = 19.5$ and 20.4 nM, respectively). Similarly, all were relatively weak at 5-HT-2A serotonin receptors ($K_i \geq 71$ nM) and α2-adrenoceptors ($K_i \geq 67$ nM).

4. Discussion

The 25 substituted phenylbenzazepines evaluated are too few to support secure structure–activity assessments (Table 1). However, all of the novel agents with high dopamine D1 receptor potency had a halogen substituent at position 6: bromo- in MCL-204 and -203, chloro- in MCL-207 and -202, all of which were among the most potent dopamine D1 receptor ligands tested. Methyl-substitution on the accessory phenyl ring appeared to be optimal at position 3′ (*meta*), and a CF_3 substituent at the same position markedly reduced dopamine D1 receptor potency, suggesting that this position is important for dopamine D1 receptor interactions. The 3-*N*-substituent appeared to exert some effect as well: racemic MCL-209, which lacks an *N*-alkyl substituent, was the most potent novel compound at dopamine D5 receptors, whereas

compounds with either *N*-allyl (e.g., MCl-204) or *N*-methyl (e.g., MCL-203) substituents yielded the highest dopamine D1 receptor potencies observed.

These results indicate that the novel D1 receptor ligands MCL-204, -203, -207, -202, -210, -209, and -214 all had very high dopamine D1 receptor affinity ($K_i \leq 1$ nM) and high (230–8000-fold) D1/D2 receptor-selectivity. Racemic MCL-209 also had unusually high dopamine D5 receptor-potency (0.88 nM). MCL-204 is the most potent phenylbenzazepine dopamine D1 receptor ligand reported to date and its *R*(+)-enantiomer should be twice as potent. These findings present several substituted catecholphenylbenzazepines as promising leads to potent and selective D1 receptor ligands. The new lead agents require enantiomeric separation and further pharmacological characterization of their functional activity.

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