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Synthesis and Octopaminergic-agonist Activity of 3-(Substituted Phenyl)imidazolidine-2-thiones and Related Compounds

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3-(Substituted phenyl)imidazolidine-2-thiones (SPITs) and related compounds were synthesized by cyclizing monoethanolamine hydrogen sulfate with arylisothiocyanates in the presence of sodium hydroxide. The activity for stimulating adenylate cyclase prepared from thoracic nerve cords of the American cockroach, *Periplaneta americana* L., was examined with these compounds. A SPIT with a 2,6-diethylphenyl group (48) was the only full agonist, the other SPIT derivatives being partial agonists. Greater enzyme activation appeared to result from short-chain alkyl rather than halogen substitution at the 2,6-positions of the aromatic ring of SPITs. Increasing the chain length from methyl to ethyl in 2,6-disubstituted SPIT caused an increase in the enzyme activation. Meanwhile, further increase of the chain length from ethyl to isopropyl in 2,6-disubstituted SPIT caused a decrease in the enzyme activation. Superimposition of energy-minimized octopamine and 48 revealed structural and conformational similarities that account for the higher Vmax value of 48. There was a marked decrease in the enzyme activation after alkylating at C_4 or C_5 of the imidazolidine ring of the potent SPITs. Thus, a certain degree of bulkiness and hydrophobicity at the 2- and 6-positions on the phenyl ring of a SPIT and the *N*-terminal was favorable for activating adenylate cyclase.

Key words: 3-(substituted phenyl)imidazolidine-2-thiones; octopaminergic agonist; adenylate cyclase; molecular modeling; *Periplaneta americana*

In our recent works,[1-9] octopaminergic-agonist activity was found in 2-(arylimino)thiazolidines (AITs). Of the AIT compounds, the 2,6-diethyl derivative was the most active for stimulating adenylate-cyclase activity in the thoracic nerve cord of *P. americana*, being followed by the 4-chloro, 2-methyl and 2,6-diisopropyl derivatives.[1] The effect of heteroatoms on the activity of octopaminergic agonists by substituting N or O for S in AIT was also compared,[2] and some 2-(arylimino)oxazolidines were very active for stimulating adenylate-cyclase activity.[2,4-11] The effect of substituents on the phenyl ring, as well as heteroatoms, was important for the activity of octopaminergic agonists.[3] However, information on the structural requirements of AITs and related compounds to act as octopaminergic agonists is still limited. Meanwhile, 3-(substituted phenyl)imidazolidine-2-thiones (SPITs) were obtained as isomers of AITs and their octopaminergic-agonist activities were found. This report deals with the synthesis and octopaminergic-agonist activity SPITs in order to better understand the structure-activity relationships.

Materials and Methods

Chemicals. Octopamine [OA, 2-amino-1-(4-hydroxyphenyl)ethanol], theophylline (1,3-dimethylxanthine), and ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) were purchased from Nacalai Tesque (Kyoto, Japan); GTP was from Sigma Chemical Co. (St. Louis, MO, U.S.A.); ATP disodium salt was from Kohjin Co. (Tokyo, Japan); and various isothiocyanates were from Lancaster Synthetic Div. (Morecambe, England).

Radiochemical. The cAMP radioimmunoassay (RIA) kit (code RPA 509) was purchased from Amersham International (Buckinghamshire, England).

Analyses. All melting point (mp) data were measured with MP-S3 micro-melting point apparatus (Yanako, Kyoto, Japan) and are uncorrected. ^{1}H-NMR was measured with a JEOL JNM-FX100 spectrometer at 100 MHz, TMS being used as an internal standard. Mass spectra were obtained by a JEOL JMS-DX300 spectrometer connected to a JEOL-JMA3500 data processing system at an ionizing voltage of 30 eV.

Synthesis of SPITs and related compounds. The SPIT compounds were synthesized by the cyclization of monoethanolamine hydrogen sulfate[12] with arylisothiocyanates in the presence of sodium hydroxide. Conc. sulfuric acid was added dropwise to monoethanolamine at 40°C. The reaction mixture was then heated under vacuum at 100°C for 30 min, and the resulting solid was recrystallized from MeOH to give the desired hydrogen sulfate.[1] A solution of 2-equivalent molar sodium

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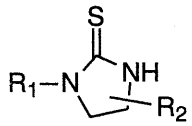
Abbreviations: AIT, 2-(arylimino)thiazolidine; SPIT, 3-(substituted phenyl)imidazolidine-2-thione; OA, octopamine; EGTA, ethylene glycol bis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; RIA, radioimmunoassay; BFGS, Broyden-Fletcher-Goldfarb-Shanno; SEAL, steric and electrostatic alignment

Table I. Structure, Analytical Data, and Octopaminergic-agonist Activity of 3-(Substituted Phenyl)imidazolidine-2-thiones and Related Compounds on Adenylate Cyclase from a Homogenate of American Cockroach Nerve Cords



Compound			Mp (°C)	Yield (%)	Molecular formula	Found (calcd.) %			Adenylate-cyclase activity[a]		
No.	R_1	R_2				C	H	N	Vmax (%)	K_a (μM)	K_a(OA)/K_a(comp.)
1	*n*-Bu	5-Ph	93–94	10	$C_{13}H_{20}N_2OS$[b]	61.81(61.87)[b]	7.86(7.99)[b]	11.03(11.10)[b]	2.1±0.2	—	—
2	Ph	4-Me	140–141	55	$C_{10}H_{12}N_2S$	62.37(62.47)	6.29(6.29)	14.55(14.57)	1.6±0.3	—	—
3	Ph	5-Ph	176–177	9	$C_{15}H_{14}N_2S$	70.56(70.83)	5.51(5.55)	10.96(11.01)	3.8±0.2	—	—
4	2-Br-Ph	H	145–146	53	$C_9H_9BrN_2S$	42.03(42.04)	3.57(3.53)	10.91(10.89)	16.9±0.2	—	—
5	2-CF_3-Ph	H	137–138	20	$C_{10}H_9F_3N_2S$	48.75(48.78)	3.64(3.68)	11.59(11.37)	9.0±0.1	—	—
6	2-Et-Ph	H	155–156	19	$C_{11}H_{14}N_2S$	63.68(64.04)	6.82(6.84)	13.51(13.58)	3.0±0	—	—
7	2-SCF_2CHClF-Ph	H	140–141	24	$C_{11}H_{14}ClF_3N_2S_2$	40.57(40.43)	3.12(3.08)	9.00(8.57)	3.9±0.2	—	—
8	2-isoPr-Ph	H	156–157	24	$C_{12}H_{16}N_2S$	65.36(65.42)	7.28(7.32)	12.63(12.71)	4.6±0	—	—
9	3-NO_2-Ph	H	97–98	86	$C_9H_9N_3O_2S$	48.26(48.42)	4.07(4.06)	18.61(18.82)	1.9±0	—	—
10	4-Cl-Ph	H	167–168	29	$C_9H_9ClN_2S$	51.02(50.82)	4.29(4.27)	13.19(13.17)	4.2±0.2	—	—
11	4-F-Ph	H	119–120	38	$C_9H_9FN_2S$	54.58(55.08)	4.61(4.62)	14.06(14.27)	5.6±0.2	—	—
12	4-Me-Ph	H	112–113	44	$C_{10}H_{12}N_2S$	61.78(62.47)	6.31(6.29)	14.38(14.57)	7.4±0.1	—	—
13	4-Me-Ph	5-Et	148–149	16	$C_{12}H_{16}N_2S$	65.09(65.42)	7.27(7.32)	12.64(12.71)	2.7±0.1	—	—
14	4-CF_3-Ph	H	116–117	43	$C_{10}H_9F_3N_2S$	48.54(48.78)	3.76(3.68)	10.91(11.37)	11.4±1.1	—	—
15	4-CN-Ph	H	210–211	36	$C_{10}H_9N_3S$	57.44(56.58)[c]	4.55(4.75)[c]	19.56(19.80)[c]	8.3±0.6	—	—
16	4-MeS-Ph	H	150–151	43	$C_{10}H_{12}N_2S_2$	53.54(53.57)	5.39(5.39)	12.43(12.49)	3.7±0	—	—
17	4-Et-Ph	H	127–128	37	$C_{11}H_{14}N_2S$	63.68(64.04)	6.82(6.84)	13.51(13.58)	3.3±0.2	—	—
18	4-EtO-Ph	H	84–85	59	$C_{11}H_{14}N_2OS$	59.34(59.43)	6.43(6.35)	12.56(12.60)	4.9±0.7	—	—
19	4-isoPr-Ph	H	140–141	39	$C_{12}H_{16}N_2S$	65.38(65.42)	7.31(7.32)	12.70(12.71)	3.7±0.1	—	—
20	4-*n*-Bu-Ph	H	104–105	41	$C_{13}H_{18}N_2S$	66.52(66.63)	7.69(7.74)	11.80(11.95)	14.0±0	—	—
21	4-Et_2N-Ph	H	189–190	23	$C_{13}H_{19}N_3S$	61.83(61.61)	7.62(7.68)	16.54(16.85)	3.3±0.1	—	—
22	4-BzO-Ph	H	170–171	48	$C_{16}H_{16}N_2OS$	67.90(67.58)	5.78(5.67)	9.58(9.85)	7.8±0.2	—	—
23	2,3-Cl_2-Ph	H	145–146	42	$C_9H_8Cl_2N_2S$	43.40(43.74)	3.35(3.26)	11.25(11.33)	3.6±0.1	—	—
24	2-Cl,4-Br-Ph	H	139–140	45	$C_9H_8BrClN_2S$	37.13(37.07)	2.80(2.77)	9.47(9.61)	7.4±0	—	—
25	2,4-Cl_2-Ph	H	155–156	19	$C_9H_8Cl_2N_2S$	43.35(43.74)	3.30(3.26)	11.14(11.33)	17.8±0.1	23	0.26
26	2,4-F_2-Ph	H	108–109	45	$C_9H_8F_2N_2S$	50.42(50.46)	3.82(3.76)	12.98(13.08)	4.0±0.1	—	—
27	2-Br,4-Me-Ph	H	125–126	59	$C_{10}H_{11}BrN_2S$	44.42(44.29)	4.08(4.09)	10.28(10.33)	7.7±0.4	—	—
28	2-Me,4-Cl-Ph	H	118–119	16	$C_{10}H_{11}ClN_2S$	52.81(52.98)	4.82(4.89)	12.22(12.35)	30.6±0.4	2.6	2.3
29	2-Me,4-Cl-Ph	4-Me	114–115	32	$C_{11}H_{13}ClN_2S$	54.99(54.88)	5.52(5.44)	11.64(11.63)	8.4±0.3	—	—
30	2-CF_3,4-Cl-Ph	H	144–145	10	$C_{10}H_8ClF_3N_2S$	42.55(42.79)	3.00(2.87)	10.48(9.98)	9.9±0.1	—	—
31	2-CF_3,4-Br-Ph	H	190–191	40	$C_{10}H_8BrF_3N_2S$	37.02(36.94)	2.49(2.48)	8.62(8.61)	3.3±0.1	—	—
32	2-NO_2,4-Me-Ph	H	135–136	64	$C_{10}H_{11}N_3O_2S$	50.50(50.62)	4.75(4.67)	17.29(17.71)	3.6±0.1	—	—
33	2-NO_2,4-MeO-Ph	H	157–158	42	$C_{10}H_{11}N_3O_3S$	47.79(47.42)	4.46(4.38)	16.20(16.59)	4.5±0.2	—	—
34	2-NO_2,4-CF_3-Ph	H	95–96	39	$C_{10}H_8F_3N_3O_2S$	41.17(41.24)	2.51(2.77)	13.63(14.43)	9.3±0.1	—	—
35	2,4-Me_2-Ph	H	162–163	29	$C_{11}H_{14}N_2S$	63.74(64.04)	6.74(6.84)	13.57(13.58)	10.5±0.5	2.7	2.2
36	2,4-$(MeO)_2$-Ph	H	162–163	25	$C_{11}H_{14}N_2O_2S$	55.01(55.44)	5.91(5.92)	11.51(11.75)	3.4±0.2	—	—
37	2,5-Cl_2-Ph	H	175–176	38	$C_9H_8Cl_2N_2S$	44.05(43.74)	3.53(3.26)	11.21(11.33)	7.8±0.5	—	—
38	2,5-F_2-Ph	H	52–53	91	$C_9H_8F_2N_2S$	50.45(50.46)	3.85(3.76)	13.43(13.08)	11.9±0.6	—	—
39	2-Me,5-Cl-Ph	H	190–191	50	$C_{10}H_{11}ClN_2S$	52.85(52.98)	4.94(4.89)	12.13(12.35)	10.8±0.7	25	0.24
40	2-Cl,5-CF_3-Ph	H	127–128	25	$C_{10}H_8ClF_3N_2S$	43.14(42.79)	2.94(2.87)	9.42(9.98)	4.6±0.2	—	—
41	2-MeO,5-Cl-Ph	H	214–215	37	$C_{10}H_{11}ClN_2OS$	49.49(49.49)	4.63(4.53)	11.47(11.54)	3.1±0	—	—
42	2-MeO,5-Me-Ph	H	121–122	65	$C_{11}H_{14}N_2OS$	59.37(59.43)	6.37(6.35)	12.39(12.60)	15.4±2.1	—	—
43	2,5-$(MeO)_2$-Ph	H	127–128	67	$C_{11}H_{14}N_2O_2S$	55.44(55.44)	5.98(5.92)	11.45(11.75)	2.2±0.2	—	—
44	2,6-Cl_2-Ph	H	121–122	65	$C_9H_8Cl_2N_2S$	43.81(43.74)	3.29(3.26)	11.34(11.33)	2.4±0.1	—	—
45	2,6-F_2-Ph	H	161–162	63	$C_9H_8F_2N_2S$	50.32(50.46)	3.69(3.76)	12.93(13.08)	3.6±0.4	—	—
46	2-Cl,6-Me-Ph	H	195–196	24	$C_{10}H_{11}ClN_2S$	52.88(52.98)	4.89(4.89)	12.25(12.35)	8.9±0.8	—	—
47	2,6-Me_2-Ph	H	210–211	37	$C_{11}H_{14}N_2S$	63.68(64.04)	6.74(6.84)	13.47(13.58)	3.9±0.1	—	—
48	2,6-Et_2-Ph	H	71–72	16	$C_{13}H_{81}N_2S$	66.39(66.63)	7.71(7.74)	11.65(11.95)	104.0±1.6	8.5	0.71
49	2,6-Et_2-Ph	4-Me	136–137	21	$C_{14}H_{20}N_2S$	67.13(67.70)	8.09(8.12)	11.11(11.28)	16.1±1.3	—	—
50	2-Et,6-isoPr-Ph	H	162–163	34	$C_{14}H_{20}N_2S$	67.60(67.70)	8.12(8.12)	11.29(11.28)	3.6±0.1	—	—
51	2,6-isoPr$_2$-Ph	H	115–116	10	$C_{15}H_{22}N_2S$	68.42(68.66)	8.53(8.45)	10.30(10.67)	23.8±0.3	2.8	2.1
52	3,4-Cl_2-Ph	H	90–91	71	$C_9H_8Cl_2N_3S$	44.35(43.74)	3.36(3.26)	11.47(11.33)	3.9±0.2	—	—
53	3-NO_2,4-Cl-Ph	H	101–102	22	$C_9H_8Cl_2N_2O_2S$	41.24(41.95)	2.98(3.13)	15.70(16.31)	3.6±0.1	—	—
54	3-Cl,4-Me-Ph	H	162–163	42	$C_{10}H_{11}ClN_2S$	52.46(52.98)	4.93(4.89)	11.92(12.35)	3.4±0	—	—
55	3-CF_3,4-Cl-Ph	H	78–79	71	$C_{10}H_8ClF_3N_2S$	41.07(41.46)[b]	2.98(3.13)[b]	10.07(9.67)[b]	7.2±0.2	—	—
56	3,4-$(MeO)_2$-Ph	H	99–100	52	$C_{11}H_{14}N_2O_2S$	55.78(55.44)	6.10(5.92)	11.10(11.75)	3.1±0.1	—	—
57	3,5-Me_2-Ph	H	145–146	50	$C_{11}H_{14}N_2S$	63.46(64.04)	6.85(6.84)	13.35(13.58)	3.7±0.1	—	—

58	3,5-$(MeO)_2$-Ph	H	111–112	4	$C_{11}H_{14}N_2O_2S$	55.56(55.44)	6.02(5.92)	11.39(11.75)	4.0±0.2	—	—
59	2,3,4-Cl_3-Ph	H	143–144	23	$C_9H_7Cl_3N_2S$	38.64(38.39)	2.58(2.51)	9.90(9.95)	8.5±0.3	—	—
60	2,4,5-Cl_3-Ph	H	210–211	40	$C_9H_7Cl_3N_2S$	38.72(38.39)	2.58(2.51)	10.00(9.95)	13.6±0.9	—	—
61	2,4,6-Br_3-Ph	H	152–153	24	$C_9H_7Br_3N_2S$	26.30(26.05)	1.76(1.70)	6.50(6.75)	3.3±0.2	—	—
62	2,4,6-Cl_3-Ph	H	160–161	75	$C_9H_7Cl_3N_2S$	38.39(38.39)	2.69(2.51)	9.74(9.95)	3.1±0.1	—	—
63	2,4,6-F_3-Ph	H	139–140	62	$C_9H_7F_3N_2S$	46.52(46.55)	3.04(3.04)	12.07(12.06)	1.4±0.1	—	—
64	2,6-Me_2,4-Br-Ph	H	209–210	25	$C_{11}H_{13}BrN_2S$	46.54(46.33)	4.57(4.59)	9.82(9.82)	8.9±0.8	—	—
65	2,4,6-Me_3-Ph	H	177–178	20	$C_{12}H_{16}N_2S$	65.19(65.42)	7.33(7.32)	12.57(12.71)	3.1±0.1	—	—
66	3,4,5-$(MeO)_3$-Ph	H	206–207	41	$C_{12}H_{16}N_2O_3S$	53.23(53.71)	6.29(6.01)	9.55(10.44)	14.5±0.5	—	—
67	2,3,4,5-Cl_4-Ph	H	150–151	38	$C_9H_6Cl_4N_2S$	34.62(34.20)	2.04(1.91)	9.10(8.86)	6.8±0.3	—	—
68	2,3,5,6-F_4-Ph	H	110–111	88	$C_9H_6F_4N_2S$	43.41(43.20)	2.43(2.42)	11.09(11.19)	11.4±1.3	—	—
69	1-Naphthyl	H	170–172	64	$C_{13}H_{12}N_2S$	68.15(68.39)	5.36(5.30)	12.04(12.27)	6.4±0.4	—	—
70	1-Naphthyl	4-Me	150–152	21	$C_{14}H_{14}N_2S$	68.66(69.39)	5.74(5.82)	11.25(11.56)	4.9±0.2	—	—
71	1-Naphthyl	4-Et	179–180	16	$C_{15}H_{16}N_2S$	70.17(70.28)	6.19(6.29)	10.98(10.93)	2.5±0.3	—	—
72	1-Naphthyl	5-Ph	210–211	25	$C_{19}H_{16}N_2S$	74.24(74.97)	5.35(5.30)	9.21(9.20)	2.8±0.1	—	—

[a] The adenylate-cyclase activity of *P. americana* was measured according to Nathanson's procedure, and the cAMP levels were measured by RIA. The basal (control) and OA (1 mM)-stimulated adenylate-cyclase activity values were 27.8±0.8(4.1±0.1% of OA) and 685.4±16.7 pmol cAMP/min/mg of protein, respectively. Vmax means the maximal response relative to OA and is expressed as the mean of 4 independent experiments, and K_a indicates the concentration giving half-maximal stimulation. The K_a values were calculated from at least 5 concentrations ranging from 10^{-7} M to 10^{-3} M with a Macintosh personal computer system, using the loglinear curve fitting method of KaleidaGraph (3.0.2J) and K_a for OA of 6.0 μM.

[b,c] Calculated as the [b]mono- and [c]hemi-hydrate, respectively.

hydroxide in 50% aq. EtOH was slowly added to a stirred equimolar mixture of monoethanolamine hydrogen sulfate obtained as just mentioned and arylisothiocyanate in 50% aq. EtOH. The mixture was refluxed for 3 hr to strip the ethanol, cold water being added to the residual mixture, which was then extracted several times with methylene chloride. The combined organic layer was dried over anhydrous sodium sulfate. Evaporation of the solvent and recrystallization of the residual solid from ether-hexane gave the desired compound. The structures of SPITs thus obtained were assigned by ^{1}H-NMR, MS (data not shown), and elemental analytical data (Table I).

Insect. The adenylate-cyclase assay was conducted on adult American cockroaches (*P. americana* L.). Males and females were used indiscriminately, as their nervous systems exhibited no gross structural or biochemical difference. The insects were reared under crowded conditions in this laboratory at 28°C with a photoperiod of 12 h light: 12 h dark and at a relative humidity of 65–70% for more than 5 years; they were provided with an artificial mouse diet (Oriental Yeast Co., Chiba, Japan) and water *ad libitum*.

Adenylate-cyclase assay. Thoracic nerve cords of *P. americana* were homogenized at a concentration of 15 mg/ml in a 6 mM Tris-maleate buffer (pH 7.4) by using a chilled microtube homogenizer (S-203, Ikeda Sci., Tokyo, Japan). The homogenate was diluted (1 mg equivalent/ml) with 6 mM Tris-maleate, and then centrifuged at 120,000×*g* and 4°C for 20 min. The supernatant was discarded, the pellet being resuspended by homogenizing (1 mg equivalent/ml) in the buffer, and again centrifuged at 120,000×*g* and 4°C for 20 min. The resulting pellet (P2) resuspended in the buffer was equivalent to the starting amount (15 mg equivalent/ml). The adenylate cyclase activity was measured by Nathanson's procedure under optimal conditions[1-9,13-17] in a test tube containing 200 μl of 120 mM Tris-maleate (pH 7.4, including 15 mM theophylline, 12 mM $MgCl_2$, and 0.75 mM EGTA), 60 μl of the P2 fraction, and 30 μl of a solution of each synthesized compound in polyethylene glycol. An appropriate solvent control was run in parallel. The enzyme reaction (5 min at 30°C) was initiated by adding 10 μl of a mixture of 3 mM GTP and 60 mM ATP, stopped by heating at 90°C for 2 min, and then centrifuged at 1000×*g* for 15 min to remove the insoluble material. The cAMP level in the supernatant was measured by RIA.[1-9,14-17] Protein concentration was determined by the Lowry method,[18] using bovine serum albumin (Sigma, MO, U.S.A.) as the standard. Vmax means the maximal response relative to OA and is expressed as the mean of 4 independent experiments, and K_a indicates the concentration giving half-maximal stimulation. The K_a values were calculated from at least 5 concentrations ranging from 10^{-7} M to 10^{-3} M with a Macintosh personal computer system, using the loglinear curve-fitting method of Kaleida Graph (3.0.2J).

Single-crystal X-ray analysis. Three-dimensional intensity data for X-ray diffraction were collected with an Enraf-Nonius CAD 4 diffractometer, using Cu *K*a (λ=1.54284 Å) as the X-ray source. The structure was solved by direct methods and refined by the full-matrix least-squares method. The final R factor was 0.091 for 3864 observed reflections [I>s (I)]. All crystallographic calculations were performed on MicroVAX3100 hardware (DEC), using MolEN software to provide an interactive structural solution (Enraf-Nonius). Totally, 4604 reflections with $2\theta<149.84°$ were collected. Crystal data for **50** were as follows: molecular formula of $C_{14}H_{20}N_2S$, MW=248.39, triclinic system, space group P-1 (No. 2), a=11.805(2) Å, b=11.896(3) Å, c=8.433(3) Å, V=1081.95(49) Å^3, Z=4, D=1.63 g/cm^3, α=76.4 cm^{-1}, and crystal size of 0.04×0.035×0.035 cm.

Molecular modeling. Molecules were built with the Tektronix CAChe Work System™ package of programs

(Oxford Molecular Group, OR, U.S.A.) running in an Apple PowerMac 8100/112 (100 Mb RAM, OS J-7.5.1). Molecular geometry in cartesian coordinates of (*R*)-OA was obtained from available X-ray crystallographic data (Cambridge data base system) and used as one of the starting points for subsequent calculations. Starting from the characteristic average value angles of each structure, it was energy-minimized by using the Molecular Mechanics (MM2) and Molecular Orbital Package (MOPAC) PM3 program (version 94) included in the CAChe system (version 3.8) by connecting to an IBM RS6000 network server. The geometry of each energy-minimized molecule was checked by using the CAChe 3D visualization tools and then reoriented to compare the superimposition.

Energy-minimized molecules were generated. Firstly, a thorough conformational search was carried out with the molecular mechanics technique (MM2 force field), initially using the steepest descent minimization method followed by the conjugate gradient until the gradient was below 0.001 Kcal/mol in no more than 5000 steps. The unique minimum was then fully optimized with MOPAC (PM3 force field). Thus, a non-linear least-squares (NLLSQ) gradient minimization route by Bartel's method was applied for the gradient norm, while the Broyden-Fletcher-Goldfarb-Shanno (BFGS) method enabled the nearest minimum-energy geometry to be located for optimization. A full Mulliken population was checked to analyze the final restricted Hartree-Fock (RHF) wavefunction. The PRECISE keyword was also used in order to increase the geometric and electronic convergency criteria; in some cases, the GEO-OK keyword was used to prevent the BFGS routine from unexpected termination. Other settings were used as default.

A similarity comparison of the molecules was made with the atom-based rigid-fit method offered by PowerFit 1.0 from MicroSimulations (Mahwah, NJ, U.S.A.). PowerFit uses the well-validated steric and electrostatic alignment (SEAL) fitting potential as the objective fitting criterium, and it utilizes the "global search of best fit" from simulated annealing.[19,20] With this potential, fitting is scored by the volume-based steric overlay, Columbus-type electrostatic match, atom-type matching, distance constraints, and conformational energy.

Results and Discussion

SPIT with 2,6-diethylphenyl (**48**) was modeled on the phenyl substitution pattern of imidazolidine NC-5[21] as an established octopaminergic agonist. SPIT **48** stimulated OA-sensitive adenylate cyclase with Vmax (104±2% of OA) not significantly different from the value produced by an optimal concentration (1 mM) of OA and is thus a full agonist. No other derivatives had a higher Vmax value than OA, suggesting that they were partial agonists. SPIT **28** stimulated adenylate cyclase with only 31% (Vmax) of OA, although with higher potency in terms of K_a (2.6 μM):K_a(OA)/K_a(**28**)=2.3. Greater enzyme activation appeared to result from an alkyl chain of medium length (especially the diethyl compound; *i.e.*, **48**). The halogen-substituted derivatives (**44** and **45**) in the 2,6-positions of the aromatic ring were not effective at all (Table I): they showed Vmax values not significantly different from the value produced by the control, and their K_a values could not be measured due to their low potency. The 2-chloro, 6-methyl (**46**) derivative gave a slightly larger Vmax value than the 2,6-dimethyl derivative (**47**). Increasing the chain length from methyl (**47**), whose K_a value could not be measured due to its low potency, to ethyl (**48**) of the 2,6-disubstituted phenyl SPIT resulted in an increase in the Vmax value and potency (K_a). However, the Vmax value of the 2,6-diisopropyl (**51**) SPIT was considerably less than that of **48**. Thus, excessive bulkiness and/or hydrophobicity at the 2- and 6-positions of the phenyl ring appears to have created interference with the activation of the adenylate cyclase, although this hypothesis does not seem to explain why 2-ethyl, 6-isopropyl (**50**) SPIT, whose K_a value could not be measured due to its low potency, showed a lower Vmax value than that of **51**.

2-Methyl, 4-chloro SPIT (**28**) had a higher Vmax value and potency in terms of K_a than the positional isomer 2-methyl, 5-chloro (**39**), 2-chloro, 6-methyl (**46**), and 3-chloro, 4-methyl (**54**) SPITs. Substituting the methyl group of **28** by a chloro atom decreased the Vmax value and potency, leading to the 2,4-dichloro derivative (**25**). Substituting the chloro atom of **28** by a methyl group decreased the Vmax value, although K_a was not significantly affected, leading to the 2,4-dimethyl SPIT (**35**). Thus, among the 2,4-disubstituted SPITs, the presence of an alkyl group at the *o*-position and a halogen at the *p*-position seemed particularly important for OA agonism. Experiments with multi-substituted derivatives revealed that the 2,4,5-trichloro (**60**) and 2,3,5,6-tetrafluoro (**68**) derivatives had Vmax values significantly larger than that of the control, although their K_a values could not be measured due to their low potency. Adding a bromo atom at the *p*-position of inactive **47** increased the Vmax value slightly, leading to **64**. Hence, because of the high Vmax value of **48**, it is possible that 2,6-diethyl SPITs substituted with a halogen at the 3-, 4-, and/or 5-positions could each show a high Vmax value, although the number of test compounds is still limited and it is still too early to draw any conclusions.

The computer-generated perspective drawing of the

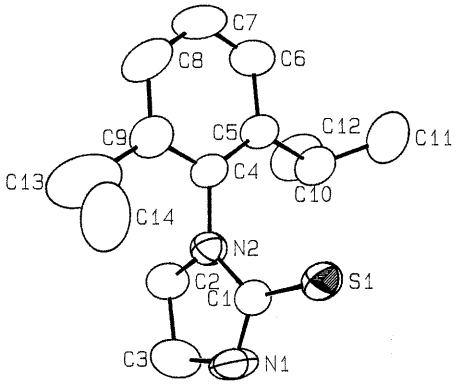


Fig. 1. Computer-generated Perspective Drawing of the Final X-Ray Model for **50**.

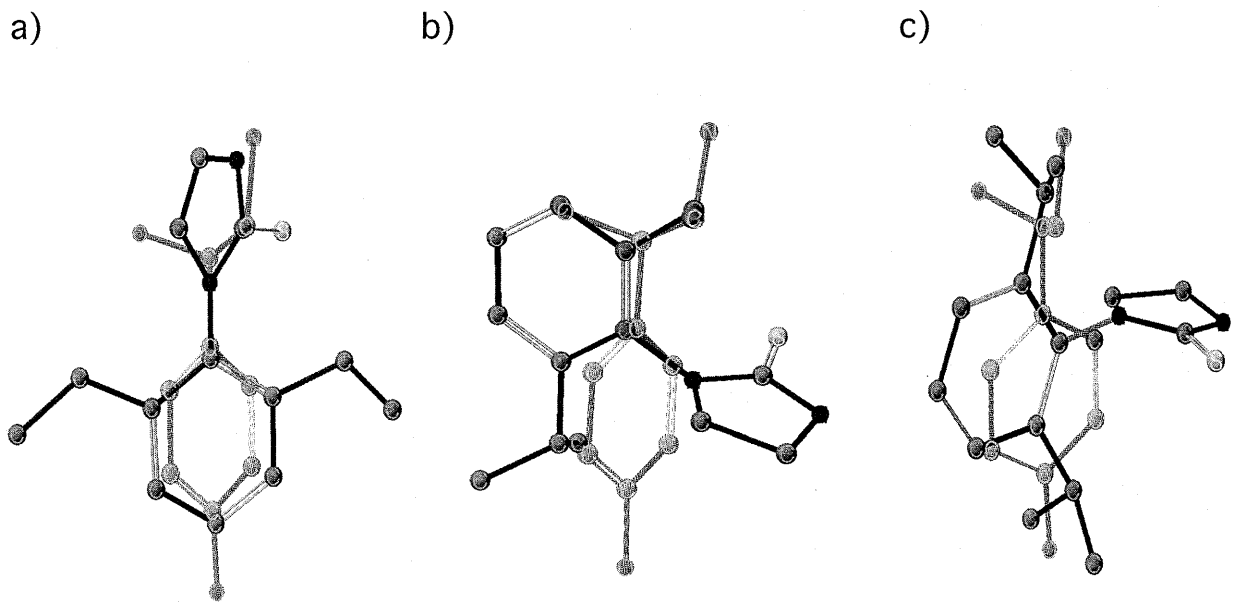


Fig. 2. Superimposed Computer-generated Structure of a) OA (light) and **48** (dark), b) OA (light) and **50** (dark), and c) OA (light) and **51** (dark). Hydrogen atoms are omitted for clarity. A similarity comparison of molecules was made by using the atom-based rigid-fit method offered by PowerFit 1.0 from MicroSimulations (Mahwah, NJ, U.S.A.). PowerFit uses the well-validated SEAL fitting potential as the objective fitting criteria, and it utilizes the "global search of best fit" from simulated annealing.[19,20] With this potential, fitting is scored by the volume-based steric overlay, Columbus-type electrostatic match, atom-type matching, distance constraints, and conformational energy.

final X-ray model of **50** is shown in Fig. 1. The bond distance, bond angles, and dihedral angles for **50** obtained by X-ray crystallography show good similarity with those obtained by energy minimization, suggesting that the process of energy minimization was reasonable for superimposition.[9] Compound **48** could be superimposed well on OA (Fig. 2a). The aromatic group of **48** can occupy space similar to that of OA, and the imidazolidine ring of **48** can occupy space similar to the ethylamine group of OA. Superimposition of energy-minimized OA and **48** revealed structural and conformational similarities that might account for the higher Vmax value of **48** compared to **50** and **51**, in which the phenyl and imidazolidine rings are held in a nonplanar conformation and would not superimpose well on OA (see Figs. 2b and 2c). SPIT **48** stimulated OA-sensitive adenylate cyclase with K_a of 8.5 μM, which is slightly larger than that (6.0 μM) generated by OA of K_a(OA)/K_a(**48**)=0.71. SPITs **50** and **51** had lower Vmax values than **48**, although **51** had higher potency [K_a(OA)/K_a(**51**)=2.1] than **48**. When energy-minimized **50** and OA are superimposed, the aromatic ring of OA is not apparently overlaid with that of **50**. Meanwhile, when energy-minimized **51** and OA are superimposed, the ethylamine group of OA is overlaid with the 2-isopropyl group of **51**, and *p*-OH of OA is overlaid with the 6-isopropyl group of **51**. Thus, superimposition of energy-minimized OA and **50** or **51** revealed structural and conformational dissimilarities that might account for the lower Vmax values of **50** and **51** compared to **48**.

There was a marked decrease in enzyme activation by alkylating at C_4 of the imidazolidine ring of **28** and **48** with high Vmax values and potency, leading to **29** and **49** with lower Vmax values, whose K_a values could not

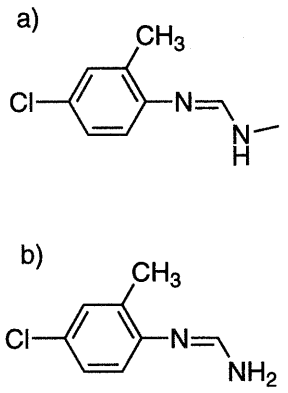


Fig. 3. Structures of a) *N*-Demethyl Chlordimeform and b) *N,N*-Didemethyl Chlordimeform.

be measured due to their low potency. Similarly, alkylating at C_5 of the imidazolidine ring of **12** caused a lower Vmax value, leading to **13**. Among the phenylethanolamines and formamidines, the addition of an *N*-methyl group to the terminal nitrogen increased the potency in terms of adenylate-cyclase activation at the OA-2 receptors: *N*-methyl OA (synephrine) was almost twice as potent as OA,[21] and the corresponding *N*-demethyl chlordimeform[13] was about twice as potent as *N,N*-didemethyl chlordimeform (see Fig. 3 for their structures). The *N*-terminal ring structure seemed to be the key feature distinguishing the enhancing effect on potency after *N*-methylation of the phenylethanolamines and formamidines, judging from the negative effects observed after alkylation at C_4 or C_5 of the imidazolidine rings of SPITs. This indicates that a certain degree of steric bulkiness and hydrophobicity (created by either

an imidazolidine ring structure or by alkylation of the ring) was favorable for binding and activating the adenylate cyclase, but that excessive bulkiness and hydrophobicity (created by the combination of an imidazolidine ring structure and alkylation of the ring) was not.

Taken together, the foregoing biochemical studies of adenylate-cyclase activation show that SPITs and related compounds with certain substituents could be potent agonists of OA receptors associated with the activation of adenylate cyclase. Furthermore, based on the comparison of OA and known OA agonists, it is proposed that new compounds could be synthesized which would possess similar structural features to OA and the OA agonists. These may help to point the way towards developing extremely potent and relatively specific octopaminergic agonists. However, the number of AITs and SPITs so far examined is still limited, therefore, in order to fully understand QSAR of these compounds, more detailed experiments are in progress.

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