deviation of 0.04, were made at two different concentrations and volumes of phases. Following shaking for 30 min, the layers were separated, the aqueous portion was centrifuged at 9900g and then filtered, and absorbances were measured at 301, 261, and 290 nm for **4**, **5**, and **7**, respectively. In addition, the log *P* value of **5** was determined to be −0.76 using an octanol–water system, with the concentration of **5** determined as the inorganic phosphate by treating the sample with a sulfuric acid–perchloric acid solution[16] and quantitating phosphate concentrations using the method of Berenblum and Chain.[17]

Syntheses. The hydrazones (starting hydrazides' references cited) **2**,[18] **4**–**7**, **10**, and **11**,[19] **9**,[4] and **3** and **12**[20] (Table II) were prepared by refluxing the appropriate hydrazide with pyridine-2-carboxaldehyde or pyridine-2-carboxaldehyde 1-oxide[21] in absolute EtOH for 1 h in the presence of HOAc (1 mL). Where no recrystallization solvent is indicated, the products were isolated from the reaction mixture, washed with water, and dried in vacuo. No acetic acid was used in the case of **2**, **4**, **7**, or **9**. Diphenyl phosphorochloridothionate, required for the preparation of the hydrazide leading to **3** and **12**, was synthesized according to the procedure of Autenrieth and Hildebrand.[22] The MnO_2 required for the synthesis of pyridine-2-carboxaldehyde 1-oxide is best prepared by the method of Sondheimer et al.[23] Cupric chelates **13** and **14** were prepared by adding a saturated solution of cupric chloride dihydrate, in absolute EtOH, dropwise to **10** (1.5 g) or **12** (1.0 g) in 20 mL of absolute EtOH until no additional precipitate formed. The reaction mixtures were stirred 15 min. The precipitates were collected, washed several times with triply distilled H_2O, and dried in vacuo.

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(7) 2[methylamine (−0.57) + double bond (−0.30) + pyridine (+0.65)] + diethyl phenylphosphorothioate (+3.46) − 2Et (+1.00 each) + intramolecular hydrogen bonding (+0.65), given N and O are equivalent.
(8) **7** − 2(pyridine) (+0.65) + 2(pyridine *N*-oxide) (−1.69).
(9) Methylamine (−0.57) + double bond (−0.30) + pyridine *N*-oxide (−1.69) + diethyl phenylphosphorate (+1.64) − 2Et (+1.00 each) + intramolecular hydrogen bonding (+0.65), given N and O are equivalent.
(10) L. A. Cates, M. B. Cramer, and L. Williams, *J. Med. Chem.*, **21**, 143 (1978).
(11) Hexamethylphosphoric triamide (+0.28) − 2(branching of functional group) (−0.20 each) + Me (+0.50) + 2Cl (+0.39 each) + ring closure (−0.09) + intramolecular hydrogen bonding (+0.65).
(12) Tripropylphosphine oxide (+2.53) − 2Me (+0.50 each) + 2Cl (+0.39 each) + ring closure (−0.09) + intramolecular hydrogen bonding (+0.65).
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Synthesis of Spiro[isobenzofuran-1(3*H*),4'-piperidines] as Potential Central Nervous System Agents. 4. Central Nervous System Depressants

Richard C. Allen, Victor J. Bauer, Raymond W. Kosley, Jr., Arthur R. McFadden, Gregory M. Shutske,*

Chemical Research Department

Michael L. Cornfeldt, Stuart Fielding, Harry M. Geyer, III, and Jeffrey C. Wilker

Department of Pharmacology, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, New Jersey 08876. *Received May 26, 1978*

The synthesis of 1'-[3-(4-fluorobenzoyl)propyl]-3-phenylspiro[isobenzofuran-1(3*H*),4'-piperidine] (**2a**) and eight halo and methoxy analogues is described. The compounds were generally more potent per os than chlorpromazine in the Sidman avoidance paradigm in rats and less potent than haloperidol. 1'-[3-(4-Fluorobenzoyl)propyl]-3-(4-fluorophenyl)spiro[isobenzofuran-1(3*H*),4'-piperidine] (**2e**) approached the per os potency of haloperidol in this test and was shown to be active in inhibiting monkey avoidance also. Compound **2e** was much less active than haloperidol in antagonizing apomorphine-induced emesis in dogs, apomorphine-induced stereotypy in rats, and amphetamine-induced circling in lesioned rats. This lack of nonselective, dopamine-receptor blocking effects makes **2e** attractive as a potential neuroleptic.

In the first three papers in this series[1] we described a number of 3-phenylspiro[isobenzofuran-1(3*H*),4'-piperidines] of general formula 1. Compounds **1a** and **1b** exhibited potent antitetrabenazine activity and **1c** was active in lowering blood pressure in the spontaneously hypertensive rat. Since several compounds in the 3-aryl-

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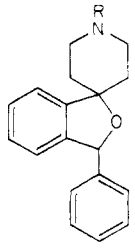
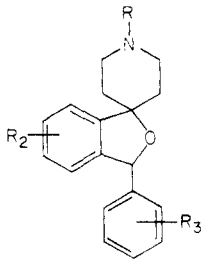
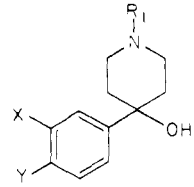


1a, R = H
b, R = OH
c, R = SC_6H_5

spiro[isobenzofuran-1(3*H*),4′-piperidine] series were candidates for development as antidepressants,[1a,b] efforts were made to capitalize on the ability of the unique parent heterocyclic ring system to form the basis for new central nervous system (CNS) drugs. Numerous alkyl, aralkyl, and heteroalkyl substituents were attached to the nitrogen of the spiro[isobenzofuran-1(3*H*),4′-piperidine] ring system with the aim of achieving compounds with CNS depressant activity. Of the compounds that were synthesized, the ones containing a 3-(4-fluorobenzoyl)propyl moiety, **2**, showed





3a, X = H; Y = Cl
b, X = CF_3; Y = H
c, X = H; Y = Br

2, R_2, R_3 = H, Cl, F, or OCH_3

$R_1 = (CH_2)_3COC_6H_4$-*p*-F

the most potential as CNS depressants. It was recognized that these compounds are structurally similar to some known neuroleptics—haloperidol (**3a**), trifluperidol (**3b**), and bromoperidol (**3c**). This paper describes the synthesis and pharmacology of nine such 3-arylspiro[isobenzofuran-1(3*H*),4′-piperidine] derivatives, **2**.

Chemistry. The synthesis of compounds **2** is described in Scheme I and their properties are given in Table I. The properties of the novel intermediates are given in Table II. The *N*-methyl compounds **4a–d,h**[1a] and **4e–g**[2] are described in previous publications. Compound **4i** was synthesized by the addition of phenyllithium to 6-chloro-1′-methylspiro[isobenzofuran-1(3*H*),4′-piperidin]-3-one (**7**)[3] and subsequent reduction of the 6-chloro-1′-methyl-3-phenylspiro[isobenzofuran-1(3*H*),4′-piperidin]-3-ol (**8**) with formic acid.[2] Treatment of **4a–i** with phenyl chloroformate in dichloromethane gave phenyl carbamates **5a–i** which were hydrolyzed with potassium hydroxide in ethylene glycol to give the secondary amines **6a–i**. Alkylation of the secondary amines with 2-(3-chloropropyl)-2-(4-fluorophenyl)-1,3-dioxolane in refluxing 1-butanol containing potassium carbonate, followed by hydrolysis of the ketals, gave the 3-arylspiro[isobenzofuran-1(3*H*),4′-piperidines] **2a–i**.

Pharmacology. The selective inhibition of a nondiscriminated avoidance response is a property common to neuroleptics, anxiolytics, and sedatives. The compounds in this series were tested in rats in the operant paradigm originally described by Sidman[4] and elegantly used by the Janssen group[5] to develop compounds, including **3a–c**. In this test, neuroleptics such as chlorpromazine or haloperidol will suppress a learned response that an animal has been trained to employ in order to avoid an unsignaled, unpleasant stimulus. Spiro[isobenzofuran-1(3*H*),4′-piperidines] **2a–i** (Table III) were generally more potent po than chlorpromazine and less potent than haloperidol in this test. They exhibited a delayed onset, with peak activity occurring 4–6 h after dosing. They also were often more potent po than ip. In these cases po ED_{50} values were calculated at the time of maximum effect. In general, the substituted analogues were more potent than the unsubstituted parent, **2a**. Within the methoxy analogues, 4′-substitution gave the most active compound, **2d**, with an ED_{50} of 3.0 mg/kg po, while the 5-substituted analogue **2b** and the 6-substituted analogue **2c** were less than half as potent. The fluoro analogues **2e–g** were approximately equipotent, with the 4′,6-difluoro compound **2g** being the most active compound in inhibiting Sidman avoidance in rats (ED_{50} = 2.2 mg/kg po). The chloro analogues presented some striking differences. Although the 6-chloro and 6-fluoro analogues **2i** and **2f** were approximately equipotent, the 4′-chloro analogue **2h**, in sharp contrast to the 4′-fluoro analogue **2e**, was only weakly active.

Neuroleptics can be distinguished from other CNS depressants in a conditioned avoidance–escape procedure such as the pole-climb avoidance paradigm.[6] In this test an animal is conditioned to respond (pole climb) to a stimulus (light and tone) in order to avoid an unpleasant foot shock. Should it fail to give the proper response in

Scheme I

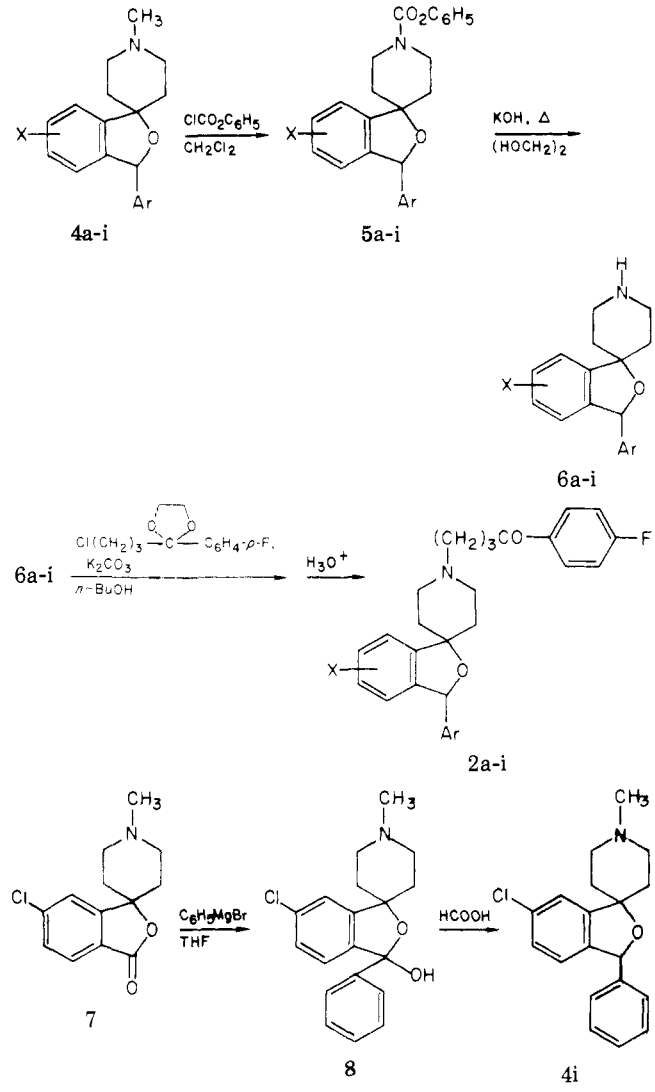
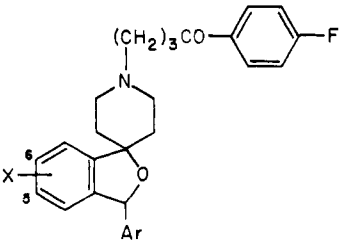


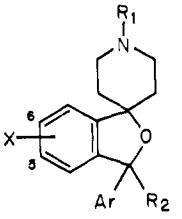
Table I. 1'-[3-(4-Fluorobenzoyl)propyl]-3-arylspiro[isobenzofuran-1(3*H*),4'-piperidines][a]



compd	X	Ar	mp,[b,e] °C	yield,[c] %	formula[d]
2a	H	C_6H_5	137-138	88	$C_{28}H_{28}FNO_2$
2b	5-CH_3O	C_6H_5	62-64	53	$C_{29}H_{30}FNO_3$
2c	6-CH_3O	C_6H_5	98-100	55	$C_{29}H_{30}FNO_3$
2d	H	4-$CH_3OC_6H_4$	113-114	66	$C_{29}H_{30}FNO_3$
2e	H	4-FC_6H_4	132-134	58	$C_{28}H_{27}F_2NO_2$
2f	6-F	C_6H_5	116-118	88	$C_{28}H_{27}F_2NO_2$
2g	6-F	4-FC_6H_4	113-115	65	$C_{28}H_{26}F_3NO_2$
2h	H	4-ClC_6H_4	131-133	64	$C_{28}H_{27}ClFNO_2$
2i	6-Cl	C_6H_5	90-92	43	$C_{28}H_{27}ClFNO_2$

[a] All compounds exhibited IR and ^1H NMR spectra consistent with the assigned structures. [b] Melting points are uncorrected. [c] Yield of analytically pure material; yields were not optimized. [d] All compounds analyzed correctly for C, H, and N within ±0.4% of theoretical values. [e] All compounds were recrystallized from acetonitrile.

Table II. Novel Spiro[isobenzofuran-1(3*H*),4'-piperidine] Intermediates[a]



compd	R_1	X	Ar	R_2	mp,[b] °C	yield,[c] %	recrystn solvent[e]	formula[d]
4i	CH_3	6-Cl	C_6H_5	H	117-118	85	F	$C_{19}H_{20}ClNO$
5c	$CO_2C_6H_5$	6-CH_3O	C_6H_5	H	161-162	72	B	$C_{26}H_{25}NO_4$
5e	$CO_2C_6H_5$	H	4-FC_6H_4	H	181-183	79	B	$C_{25}H_{22}FNO_3$
5f	$CO_2C_6H_5$	6-F	C_6H_5	H	147-149	70	C-E	$C_{25}H_{22}FNO_3$
5g	$CO_2C_6H_5$	6-F	4-FC_6H_4	H	137-139	76	A-B	$C_{25}H_{21}F_2NO_3$
5i	$CO_2C_6H_5$	6-Cl	C_6H_5	H	159-160	84	C	$C_{25}H_{22}ClNO_3$
6f	H	6-F	C_6H_5	H	129-131	80	F	$C_{18}H_{18}FNO$
6i	H	6-Cl	C_6H_5	H	117-117.5	92	F	$C_{18}H_{18}ClNO$
8	CH_3	6-Cl	C_6H_5	OH	208-210	75	B-D	$C_{19}H_{20}ClNO_2$

[a-d] See corresponding footnotes to Table I. [e] A = water; B = acetonitrile; C = methanol; D = tetrahydrofuran; E = ethyl acetate; F = hexane.

order to avoid the shock, however, the pole is still available so that it can escape the shock once it is delivered. Typical neuroleptics show a wide separation between the dose that will suppress conditioned avoidance responding and the dose that will suppress the escape response. Sedatives and anxiolytics, on the other hand, show much less separation (see Table III). The compounds of this series which were active in inhibiting Sidman avoidance all showed large differences between the ED_{50} values for suppression of avoidance responding and for escape failures (Table III). Compounds **2a,b,e** were roughly as potent as chlorpromazine in suppressing avoidance in this test but caused escape failures at higher doses. Compound **2f** approached the potency of haloperidol in suppressing conditioned avoidance, again requiring a higher dose for escape failure. The weak activity of the 4'-chloro analogue **2h** was confirmed in this test. Compounds **2a,b,e,f** showed consistent activity in inhibiting Sidman and pole-climb avoidance in rats.

Table IV shows that the activity of **2b** and **2e** was maintained in suppressing Sidman avoidance in monkeys. The antiavoidance effect of **2e** was partially reversed by the anticholinergic agent, benztropine. This provided another indication of true neuroleptic activity.[7]

It is thought that antipsychotic agents exert their effects by dopamine-receptor blockade in the CNS,[8] particularly in the ventral limbic areas.[9] Furthermore, it is postulated that the troublesome Parkinsonian-like extrapyramidal (EPS) side effects of the clinically useful antipsychotics are due to nonselective, dopamine-receptor blockade in the corpus striatum.[9] Pharmacologically, it is observed that typical antipsychotics like chlorpromazine and haloperidol block the effects of dopamine agonists in a nonspecific manner. For example, they inhibit apomorphine-induced emesis in dogs and apomorphine-stereotyped behavior in rats.[10]

In view of this, it is interesting to note (Table III) that compounds **2b,e,g** were at least ten times less potent than haloperidol in blocking apomorphine emesis in dogs. It should be particularly noted that compound **2e** approached the po potency of haloperidol in Sidman avoidance and yet was at least two orders of magnitude less potent in blocking apomorphine emesis. This lack of antiapomorphine effects was seen in rat stereotypy also.

Table III. CNS Activity of 1'-[3-(4-Fluorobenzoyl)propyl]-3-arylspiro[isobenzofuran-1(3*H*),4'-piperidines][a,b]

compd	Sidman avoidance, ED_{50}, mg/kg ip (rat)	pole-climb avoidance, ED_{50}, mg/kg ip (rat)		apomorphine emesis, ED_{50}, mg/kg po (dog)	amphetamine-induced circling, ED_{50}, mg/kg ip (rat)
		AR[k]	EF[l]		
2a	6.2 (5.5-7.0) 4.8 (4.5-5.1)[c]	8.6 (6.8-10.1)	>40	0.15 (0.13-0.18)	2.6 (1.9-3.3)
2b	9.5 (8.8-10.4)	9.8 (7.9-11.3)	>40	>1.0	9.9 (8.9-10.9)
2c	>10[c]	nt[d]	*e*	nt	nt
2d	3.0 (2.7-3.3)[c]	17.2 (15.7-18.8)	>40	0.16 (0.13-0.22)	10.2 (9.2-11.5)
2e	3.7 (3.4-4.3)[c]	4.3 (3.7-4.8)[c]	16.2 (13.6-20.6)[c]	>5.0	11.9 (10.2-14.4)
2f	2.7 (2.3-3.2)	0.95 (0.66-1.25)	31.1 (20.1-63.9)	0.047 (0.033-0.098)	4.6 (3.9-5.5)
2g	2.2 (1.9-2.5)[c]	18.6 (15.8-22.4)	>40	0.5[f]	9.7 (8.1-11.9)
2h	>10	>20	*e*	>10	*g*
2i	2.3 (1.9-3.0)	*h*	*e*	0.05 (0.04-0.14)	3.5 (3.0-4.3)
haloperidol	0.35 (0.30-0.40)[i]	0.53 (0.44-0.64)	7.3 (6.1-9.7)	0.06 (0.03-0.14)	0.10 (0.07-0.12)
chlorpromazine	2.3 (2.0-2.7) 7.5 (7.2-7.8)[c]	5.2 (4.7-5.7)	9.0 (8.2-10.1)	12.8 (7.8-16.6)	2.2 (2.0-2.5)
thioridazine	*j*	13.8 (10.9-16.5)	>60		13.3 (11.3-16.1)
pentobarbital		23.7 (21.5-27.0)	24.1 (23.0-25.4)		
diazepam		13.4 (11.0-15.8)	24.5 (21.9-30.0)		

[a] Figures in parentheses are 95% confidence limits. [b] Test procedures are described in the Experimental Section. [c] Administered po. [d] Not tested. [e] Not calculated. [f] A dose of 0.5 mg/kg produced a 50% decrease in emesis, but no ED_{50} was calculated. [g] A 47% increase in circling occurred at 5 mg/kg. [h] A dose of 5 mg/kg produced a 47% decrease in avoidance responding. [i] A po dose of 1.25 mg/kg produced a 50% reduction in responding, but no ED_{50} was calculated. [j] A dose of 20 mg/kg produced 15% inhibition. [k] AR = avoidance response. [l] EF = escape failure.

Table IV. Inhibition of Sidman Avoidance in Monkeys[a]

compd	decrease in responses, %				dose, mg/kg po
	1[b]	2	3	4	
2b	39	42	29	76	2.5
2c	84	77	–	–	10
2e	90	92	90	–	20[c]
	75	17	–	–	10
	32	21	–	–	5
chlorpromazine	54	83	70	68	5.0
	19	29	48	11	2.5
haloperidol	91	97	30	97	1.0
	9	68	2	26	0.75

[a] The test procedure is described in the Experimental Section. [b] A maximum of four monkeys was tested at a given dose. A "–" indicates that an animal was not tested at a given dose. [c] This effect was partially reversed by benztropine (1.25 mg/kg po).

Table V. Inhibition of Apomorphine and Amphetamine Stereotypies[a,b]

compd	inhibn of apomorphine stereotypy, ED_{50}, mg/kg sc (rat)	inhibn of amphetamine stereotypy, ED_{50}, mg/kg ip (rat)
2b	*c*	6.97 (5.11-9.52)
2e	*c*	11.8 (3.4-14.0)
haloperidol	0.20 (0.19-0.21)	0.21 (0.11-0.34)
chlorpromazine	5.4 (1.8-5.7)	1.7 (1.1-3.2)
thioridazine	nt[d]	15.8 (6.1-32.7)

[a] Figures in parentheses are 95% confidence limits. [b] The test procedure is described in the Experimental Section. [c] Inactive at 25 mg/kg. [d] Not tested.

Stereotyped behavior (compulsive licking, gnawing, and sniffing) in rats depends on a direct stimulation of receptors for dopamine in the CNS.[11] Antagonism of this behavior is a useful, if nonspecific, in vivo test for dopamine-receptor blocking activity. Apomorphine initiates stereotypy by directly stimulating dopamine receptors. Amphetamine, however, acts in an indirect manner by stimulating dopamine release, leading to stereotyped behavior.[11] Chlorpromazine and haloperidol antagonize both apomorphine- and amphetamine-induced stereotypy. Compounds **2b,e**, however, besides being much less active than haloperidol in antagonizing apomorphine-induced emesis in dogs, were inactive in blocking apomorphine-induced stereotypy in rats at the doses tested (Table V). They were, however, moderately active in antagonizing amphetamine-induced behavior.

Thus, the operant avoidance blocking behavior of compounds **2b,e** indicated their potential as neuroleptics, while their inability to antagonize apomorphine in vivo showed a lack of nonspecific, dopamine-receptor blocking effects. These data suggested that these compounds could be of potential antipsychotic utility in man but without the undesirable EPS reactions resulting from nonselective, dopamine-receptor blockade.

This view was further strengthened by the ED_{50} values of 9.9 and 11.9 mg/kg ip that **2b** and **2e** displayed, respectively, in inhibiting amphetamine-induced circling in lesioned rats (Table III). This test gives an indication of direct, dopamine-receptor blockade in the basal ganglia and, as such, may predict the EPS side effect liability of potential antipsychotics.[12] Haloperidol, for example, is particularly effective (ED_{50} = 0.10 mg/kg ip) in inhibiting this circling behavior. Thioridazine, however, a compound with less EPS liability,[13] has an ED_{50} of 13.3 mg/kg ip. Compounds **2b,e** thus fall in the range of thioridazine.

By balancing conditioned avoidance behavior against undesirable dopamine-receptor blocking effects, the 4'-fluoro analogue **2e** was chosen for further development as a potential neuroleptic with a unique pharmacological profile.

Experimental Section

The structures of all compounds are supported by their IR (Perkin-Elmer 457) and ^1H NMR (Jeolco C60HL, tetramethylsilane) spectra. Melting points were taken on a Thomas-Hoover capillary melting point apparatus. All melting points are uncorrected. Elemental analyses were performed by Micro-Tech Labs, Skokie, Ill.

The synthesis of starting materials **4a–d,h**[1a] and **4e–g**[2] is described elsewhere. The properties of the novel compounds are described in Tables I and II.

6-Chloro-1'-methylspiro[isobenzofuran-1(3*H*),4'-piperidin]-3-one (7). Compound 7 was prepared as described by Rodriguez et al. in 40% yield: mp 214–216 °C (lit.[3] mp 209–213 °C).

6-Chloro-1'-methyl-3-phenylspiro[isobenzofuran-1(3*H*),4'-piperidin]-3-ol (8). To a stirred solution of 260 mL of

2.3 N phenyllithium (0.6 mol) in 70:30 benzene/ether was added a warm solution of **7** (50.3 g, 0.2 mol) in 1 L of THF and 400 mL of toluene. An ice bath was applied occasionally to keep the reaction temperature below 40 °C. After the addition was complete, the reaction was stirred for 3 h and then quenched with 200 mL of H_2O. The organic phase was separated, washed with additional H_2O, dried, and concentrated in vacuo to give crystalline **8**. This was filtered and washed with Et_2O to give 49.5 g (75%) of **8**.

6-Chloro-1'-methyl-3-phenylspiro[isobenzofuran-1(3*H*),4'-piperidine] (4i). As described for compounds **4e–g**,[2] **8** (47.5 g, 0.144 mol) was treated with 400 mL of refluxing 98% formic acid to give 38.6 g (85%) of **4i**.

6-Chloro-1'-(phenoxycarbonyl)-3-phenylspiro[isobenzofuran-1(3*H*),4'-piperidine] (5i). To a solution of **4i** (34.8 g, 0.11 mol) in 200 mL of CH_2Cl_2 was added 20 g (0.13 mol) of phenyl chloroformate in 200 mL of CH_2Cl_2. After 16 h the solvent was removed in vacuo and the resulting solid triturated with Et_2O to give 38.8 g (84%) of **5i**. Compounds **5a–h** were prepared analogously.

6-Chloro-3-phenylspiro[isobenzofuran-1(3*H*),4'-piperidine] (6i). Ethylene glycol (800 mL) was added to a freshly prepared solution of 74 g of potassium hydroxide pellets in 35 mL of H_2O. Compound **5i** (32.1 g, 0.076 mol) was then added and the resulting suspension brought to reflux with vigorous stirring. After refluxing for 30 min the solution (now homogeneous) was poured into 2.5 L of ice and H_2O, and the precipitated **6i** was filtered off. This material was precipitated once from MeOH and H_2O to give 21 g (91%) of **6i**. Compounds **6a–h** were prepared analogously.

6-Chloro-1'-[3-(4-fluorobenzoyl)propyl]-3-phenylspiro[isobenzofuran-1(3*H*),4'-piperidine] (2i). Compound **6i** (8.0 g, 0.027 mol) was dissolved in 50 mL of 1-butanol and 15 g of anhydrous K_2CO_3 was added. This suspension was brought to reflux and 2-(3-chloropropyl)-2-(4-fluorophenyl)-1,3-dioxolane (9.0 g, 0.037 mol) was added dropwise. After refluxing for 20 h, the inorganic salts were filtered off and the solvent was removed in vacuo. The crude ketal thus obtained was then hydrolyzed by boiling briefly in 300 mL of MeOH and 150 mL of 3 N HCl. This solution was chilled, washed with hexane, made basic, and extracted with $CHCl_3$. The dried organic extracts were concentrated in vacuo to give an oil that crystallized from CH_3CN to give 5.34 g (43%) of **2i**. Compounds **2a–h** were prepared in a similar manner.

Pharmacological Methods. Compounds were administered using distilled water and a suitable surfactant except where otherwise indicated. ED_{50} values were calculated during a representative time of peak activity using computerized linear regression analysis.

Sidman Avoidance in Rats. In an apparatus similar to that described by Janssen,[5] male Sprague–Dawley rats (Charles River) were trained to avoid an unsignaled shock by repetitive lever-pressing responses. A shock–shock interval of 15 s and a response–shock interval of 30 s were used. A drug's effect on the performance of each animal was compared to the performance data generated in the previous nondrug session. Each animal thereby served as its own control. Responses constituted the basic measure of performance during a specific time interval and were reported as percent of control responses. The ED_{50} of a test compound was that dose which caused a 50% reduction in responding at the time of maximum effect.

Sidman Avoidance in Monkeys. Male squirrel monkeys (*Saimiri sciureus*) were trained as described above for rats. A shock–shock interval of 20 s and a response–shock interval of 20 s were used. The basic data were accumulated as with rats over a 4-h test session. The results were reported as a percent decrease in responses.

Pole-Climb Avoidance in Rats. Male Long–Evans rats (Blue Spruce Farms) were trained and tested in experimental chambers similar to those described by Cook,[6] with the following modifications. On the top of the experimental chamber a speaker and a light were situated. A smooth stainless steel pole, 1 in. in diameter, was suspended by a spring through a hole in the center of the chamber top. The pole could be pulled down approximately $^1/_8$ in. by a weight greater than 200 g to activate a microswitch. When a rat jumped on the pole and activated the microswitch, a response was recorded. Due to the spring tension, a rat could not hold the pole down while standing on the grid floor and because of the smooth surface it could not remain on the pole any length of time.

The activation of both light and speaker together was used as the conditioning stimulus (CS). The CS was presented alone for 5 s and for the following 30 s the CS was coincident with a scrambled shock from the grid floor, the unconditioned stimulus (UCS).

A response (pole jump) during the CS period terminated the CS and the following UCS + CS. This was considered an avoidance response (AR). Response when both CS and UCS were present terminated both stimuli and was an escape response (ER). Although all stimuli were terminated by AR and ER, their respective clocks maintained the prescribed durations and in effect the intertrial interval (ITI) was expanded. A response during this ITI time was not recorded and had no effect on the system.

The rats which consistently reached or exceeded 80% avoidance without any escape failures were used with experimental compounds. The animals were then compared with their solvent control data in avoidance responses and escape failures (EF). Two ED_{50} values were calculated, representing, respectively, the dose that caused a 50% decrease in AR and the dose that caused a 50% increase in EF at the time of maximum effect.

Apomorphine-Induced Emesis in Dogs. Adult beagle dogs of either sex were given the test compound po as a mixture with lactose in a gelatin capsule; they were then dosed with a standard 0.15 mg/kg dose of apomorphine hydrochloride subcutaneously at various intervals after administration of the test compound. The initial screen was usually a dose of 1 mg/kg po of the test compound. The dogs were first observed for overt behavioral effects, e.g., pupillary response to light, changes in salivation, sedation, tremors, etc. After the administration of apomorphine the dogs were observed for stereotypic sniffing and gnawing and for the emetic response. Emesis was defined as wretching movements followed by an opening of the mouth and either attempted or successful ejection of stomach content. The ED_{50} was defined as the dose that produced 50% inhibition in this emetic response at the time of maximum effect.

Stereotypy in Rats. Groups of male Wistar rats were given the drug 1 h prior to apomorphine or amphetamine challenge and were placed in individual stainless steel cages. A control group received the vehicle. The rats were challenged with apomorphine hydrochloride (Merck; 1.25 mg/kg iv) or *d*-amphetamine sulfate (Smith Kline & French Labs; 10 mg/kg iv) and then observed at 5, 10, and 20 min after apomorphine challenge or 55 and 65 min after amphetamine challenge. Stereotypic activity was defined as sniffing, licking, or chewing behavior that occurred in a repetitive manner and was noted as follows: 0 = no unusual activity; 1 = occasional sniffing, licking, or chewing but with periods of normal behavior longer than periods of stereotypy; 2 = frequent sniffing, licking, or chewing but with occasional brief periods of normal behavior; 3 = constant sniffing, licking, or chewing without interruption, compulsive gnawing possibly present.

The scores for stereotypy were added and averaged for each animal. The percent inhibition shown by the drug was calculated and an ED_{50} was determined as the dose required to produce 50% inhibition of stereotypy.

Rat Circling. Male Wistar rats were anesthetized (sodium pentobarbital, 18 mg/kg ip) and the head was placed in a stereotaxic device and positioned in the plane of the Koenig and Klippel atlas. A radio frequency electrode was aimed at the zona compacta of the substantia nigra (coordinated anterior 2.18, lateral 2.00, and dorsal ventral 2.0 from instrument zero) through a hole drilled in the exposed skull. The lesion was made using a radio frequency lesion generator at a temperature of 50–55 °C for 45 s. The electrode was then withdrawn; the animal was sutured and allowed a 1-week recovery period. Specially constructed opaque plastic spheres attached to solid-state programming equipment served as test chambers. The number of full turns ipsilateral to the lesion was recorded on an automatic printout counter every 15 min for a 2-h test period. To determine the control values for turning, each subject was injected with 5 mg/kg ip of *d*-amphetamine and immediately placed in the chamber on two successive periods separated by a week.

Test compounds were typically administered 60 min prior to challenge by 5 mg/kg ip of amphetamine. The ED_{50} was calculated as the dose that produced a 50% decrease from control values in amphetamine-induced circlings.

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Three-Dimensional Mapping of the Sweet Taste Receptor Site

Piero A. Temussi,*

Istituto Chimico, University of Naples, 80134 Naples, Italy

Francesco Lelj,

Dipartimento di Chimica, University of Calabria, Cosenza, Italy

and Teodorico Tancredi

L.C.M.I.B. of C.N.R., Arco Felice, Naples, Italy. *Received February 6, 1978*

The active sites of the receptors for sweet and bitter tastants are shown to be related by a simple symmetry operation. This relationship, in turn, allows the identification of the critical geometrical features of both receptor sites. The model proposed for the sweet site is shown to be consistent with a large number of (conformationally rigid) sweet molecules.

Two of the so-called primary taste qualities,[1] i.e., sweet and bitter, can be elicited by a wide variety of apparently unrelated molecules.[1,2] It is the purpose of the present work to show that the active sites of sweet and bitter receptors are related by a simple symmetry operation and that it is possible to find the main geometrical features of both sites by using the shapes of conformationally rigid molecules as idealized molds.

Although the existence of common features among tastants had been suspected long ago, in relation with the theory of selective adsorption[3] of membrane proteins, it has been only in comparatively recent times that one of these features has been actually identified.

Shallenberger and Acree[4] have shown that a characteristic common to nearly all known sweet substances is an entity composed of a basic (B) and acidic (AH) group spaced of about 0.3 nm that should interact with a similar (complementary) entity of the receptor site, possibly through a pair of hydrogen bonds. Shallenberger has also shown[5] that the drastic differences in taste existing between amino acid enantiomers may be explained by the presence in the receptor site of a spatial barrier located at approximately 0.3 nm from the line joining AH and B. That is, sweet amino acids must have an *R* configuration since their side chain would otherwise "invade" the spatial barrier.

These facts (which henceforth shall be referred to as "Shallenberger's theory") do not account satisfactorily for all aspects of sweet taste. In particular, Shallenberger's theory gives no clues for the understanding of the large differences in relative sweetness among known tastants and does not explain why substances with the right AH–B entity (such as simple geometric isomers of known sweeteners) are completely tasteless. A dramatic example of this type is afforded by the two isomers of the oxime of anisaldehyde; the anti isomer is very sweet but the syn isomer is tasteless.[2]

A trivial way to overcome these difficulties may be to invoke the existence of multiple (specialized) sites for a given taste sensation. Evidence in favor of the existence of multiple sites both for the bitter and sweet taste has, in fact, been given by various authors.[6,7]

On the other hand, rather than attributing every peculiarity to an increasing number of distinct receptors it is certainly more fruitful to deepen the analysis of the properties of a given receptor site, trying to explain the taste of the greatest possible number of molecules in terms of a single site if it can be clearly identified.

In fact, the number of sweet molecules described by Shallenberger[4] in terms of the AH–B entity is large enough to justify the reference to a common receptor site. For the sake of clarity it may be convenient to classify the features of the active site as electronic or geometric although in some instances such a classification can appear somewhat artificial. The main "electronic" feature of this site is obviously represented by the AH–B entity itself, whereas the spatial barrier represents a simple geometric feature. It is the purpose of this paper to show how this view of

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