dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to afford a 3.7 g of semisolid **38** that crystallized upon trituration with $CHCl_3$:[22] NMR ($CDCl_3$) δ 6.3 (br s, 1 H, OH), 7.00–8.15 (m, 3 H, Ar-H), 10.35 (br s, 1 H, COOH). This crude material (2 g, 9.2 mmol) in acetone (50 mL) was heated at reflux with MeI (5.6 g, 39 mmol) and K_2CO_3 (4.4 g, 31 mmol) for 48 h and stirred at room temperature for 64 h. The solid material was removed by filtration, and the solvent was evaporated under reduced pressure. The residual oil was distilled (Kugelrohr bath temperature 52 °C; 0.01 mmHg) to afford 2.3 g (100%) of **39**. Finally, a solution of the ester (2.25 g, 9.18 mmol) in anhydrous Et_2O (15 mL) was reduced by addition to a solution of AlH_3 (generated by the addition of 0.6 g of $AlCl_3$ to a suspension of 0.5 g of $LiAlH_4$ in 15 mL of Et_2O at 0 °C under a nitrogen atmosphere) and allowing the reaction mixture to heat at reflux for 18 h. Excess reducing agent was decomposed by the addition of 15% NaOH at 0 °C. The mixture was filtered, and the filtrate was dried ($MgSO_4$) and evaporated to dryness to yield 0.45 g (23%) of **40** as a colorless oil after distillation (Kugelrohr bath temperature 52 °C; 0.04 mmHg): NMR ($CDCl_3$) δ 3.8–4.0 (m, 5 H, OCH_3, CH_2), 4.6 (br s, 1 H, OH), 7.01–7.11 (m, 3 H, Ar-H). The product[34] was used without further characterization for the preparation of **36a**.

Radioligand Binding. The radioligand binding studies were performed as previously described in detail.[35] Briefly, frontal cortical regions of male Sprague–Dawley rats (200–250 g; Charles River and Harlan–Sprague) were dissected on ice and homogenized (1:10 w/v) in ice-cold 50 mM Tris HCl, 0.5 mM EDTA, and 10 mM $MgCl_2$ at pH 7.4 and centrifuged at 3000*g* for 15 min. The pellet was resuspended in buffer (1:30 w/v) incubated at 37 °C for 15 min and then centrifuged twice at 30000*g* for 10 min (with a resuspension between centrifugations). The final pellet was resuspended in 50 mM Tris HCl, 0.5 mM EDTA, 10 mM $MgCl_2$, 0.1% ascorbate, and 10^{-5} M pargyline.

Assays were performed in triplicate in a 2.0-mL volume containing 5 mg (wet weight) of tissue and 0.4 nM [^{3}H]ketanserin (76 Ci/mmol; New England Nuclear) for 5-HT2 receptor assays, and 10 mg (wet weight) of tissue and 1 nM [^{3}H]mesulergine (75.8 Ci/mmol; Amersham) for 5-HT1C receptor assays. Cinanserin (1.0 μM) was used to define nonspecific binding in the 5-HT2 assay. In the 5-HT1C assays, mianserin (1 μM) was used to define nonspecific binding, and 100 nM spiperone was added to all tubes to block the binding of [^{3}H]mesulergine to 5-HT2 receptors. Tubes were incubated at 37 °C for 15 min, filtered on Schliecher and Schuell (Keene, NH) glass fiber filters (presoaked in 0.1% polyethyleneimine), and washed with 10 mL of ice-cold buffer. The filters were counted at an efficiency of 50%.

Saturation and competition experiments were analyzed using an updated version of the program EBDA[36] to obtain equilibrium dissociation constants (K_D), B_{max}, Hill coefficients, and IC_{50} values. K_i values for competition experiments were obtained using the equation $K_i = IC_{50}/1 + (D^*/K_{D^*})$ where IC_{50} is the experimentally observed concentration of competing drug that inhibits 50% of specific binding, K_{D^*} is the equilibrium dissociation constant determined in saturation studies, and D^* is the concentration of radioactive ligand used in the competition assays.[37] 5-HT hydrogen oxalate and spiperone were obtained from Sigma (St. Louis, MO).

(34) Elliott, M.; Janes, N. F.; Pearson, B. C. Pyrethrins and related compounds. IX. Alkenylbenzyl and benzylbenzyl chrysanthemates. *J. Sci. Food Agr.* **1967**, *18*, 325–331; *Chem. Abstr.* **1968**, *68*, 49265.
(35) Titeler, M.; Lyon, R. A.; Davis, K. H.; Glennon, R. A. Selectivity of serotonergic drugs for multiple brain serotonin receptors. *Biochem. Pharmacol.* **1987**, *36*, 3265–3271.
(36) MacPherson, G. A. A practical computer-based approach to the analysis of radioligand binding experiments. *Comput. Programs Biomed.* **1983**, *17*, 107–114.
(37) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

N,N′-Disubstituted Guanidine High-Potency Sweeteners

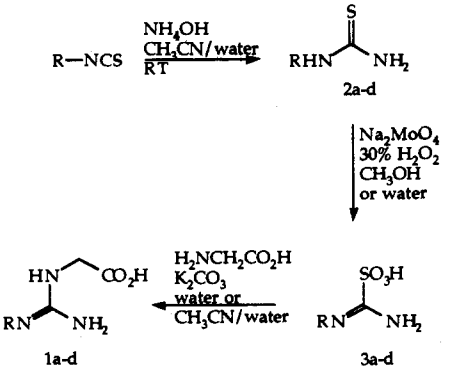
George W. Muller,* D. Eric Walters,*,† and Grant E. DuBois

Research & Development, NutraSweet Company, 601 E. Kensington Road, Mt. Prospect, Illinois 60056.
Received September 20, 1990

The role and function of the aryl group in the highly potent trisubstituted guanidine sweeteners **7a–d** was investigated. Four disubstituted guanidines, lacking the aryl group, were prepared. These guanidines contain a hydrophobic substituent on one nitrogen and a carboxymethyl group substituted on one of the other nitrogens. They were found to be tasteless or to have a significantly lower sweetness potency than the corresponding trisubstituted compounds. Possible rationales for the effects of structure on the sweet taste activity are discussed.

In 1986, Nofre, Tinti, and their co-workers reported a new series of *N*-(carboxymethyl)guanidines that are high-potency sweeteners.[1] A disubstituted guanidine, *N*-(carboxymethyl)-*N′*-(4-cyanophenyl)guanidine, was reported with a sweetness potency 2400 times that of sucrose relative to a 2% sucrose reference solution ($P_w(2) = 2400$). The same group later reported on a series of *N*-aryl-*N′*-(aryl/alkyl)-*N″*-(carboxymethyl)-trisubstituted guanidines, some of which have substantially increased sweetness potency relative to the earlier described disubstituted analogues. The most potent analogues exhibit sweetness potencies in excess of 100 000 times that of sucrose.[2] In the trisubstituted guanidines, two preferred aryl moieties are 4-cyanophenyl and 4-nitrophenyl. The structure–activity relationships (SAR) in the trisubstituted guanidine

Scheme I. Synthesis of N,N′-Disubstituted Guanidines



†Current address: Department of Biological Chemistry, The Chicago Medical School, 3333 Green Bay Road, North Chicago, IL 60064.

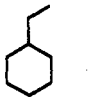
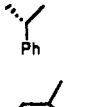
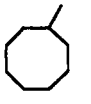
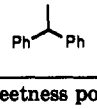
0022-2623/92/1835-0740$03.00/0

Table I. Reaction Yields for the Synthesis of N,N'-Disubstituted Guanidines

R	compd 2	yield, %	mp, °C	compd 3	yield, %	mp, °C	compd 1	yield, %	mp, °C
cyclohexylmethyl	**a**	74	151–153	**a**	75	190–191.5	**a**	30	226–227
(*S*)-α-methylbenzyl	**b**	76[a]	104–107	**b**	56	188–189	**b**[d]	76	230.5–231.5
cyclooctyl	**c**	78[a]	93–95	**c**	90	141–143	**c**[e]	40	>240
benzhydryl	**d**	83[b]	-	**d**	76[c]	198–202	**d**[d]	22	>240

[a] Crude product was soluble in reaction mixture and was purified by flash chromatography on silica gel [3/1 EtOAc/hexane (**2b**), 7/3 EtOAc/hexane (**2c**)]. [b] The crude product from the reaction slurry was recrystallized from EtOAc/hexane and the material from concentration of the reaction mixture and mother liquor was purified by flash chromatography (EtOAc). [c] After 16 h at room temperature, an additional 1.76 equiv of 30% H_2O_2 was added to the reaction mixture which was then heated to 40 °C for 4 h to complete the oxidation. [d] The product was obtained in pure form by filtration of the reaction mixture. [e] The crude product was purified by sonication in methylene chloride, followed by filtration to isolate the guanidine.

Table II. Sweetness Potencies [$P_w(X)$][a] of Di- and Trisubstituted Guanidine Sweeteners

R		
	**1a** weakly bitter at 1.0 mg/mL	**7a** $P_w(2)$ = 35 000 ref 2
	**1b** weakly bitter at 1.0 mg/mL	**7b** $P_w(2)$ = 28 000 ref 2
	**1c** $P_w(2)$ = 200	**7c** $P_w(2)$ = 170 000 ref 2
	**1d** $P_w(4)$ = 400	**7d** $P_w(2)$ = 200 000 ref 5

[a] Sweetness potencies were calculated on a weight basis relative to *X*% sucrose solution (weight/volume) as a reference.

sweetener series is suggestive of a relatively nonspecific hydrophobic function with regard to the *N'*-(aryl/alkyl) moiety. Preferred hydrophobic substituents were reported to be cycloaliphatic, substituted cycloaliphatic, benzyl, and substituted benzyl. To better understand the SAR of the guanidine sweeteners, a series of *N*-(aryl/alkyl)-*N'*-(carboxymethyl)-disubstituted guanidines was prepared. It was expected that evaluation of such compounds would allow a determination of the relative importance of the aryl and hydrophobic moieties in the highly potent trisubstituted guanidine sweeteners.

Chemistry

The four disubstituted guanidines (**1a–d**) which are the subject of this report were prepared by a combination of the methods of Maryanoff and co-workers[3] and Miller and Bischoff[4] (Scheme I). The yields of **1a–d** and of the intermediates **2a–d** and **3a–d** are given in Table I. The preparation of **1a–d** proceeded as expected from the reported methods except for the benzhydryl analogue, **1d**. In this case, heating was required in the Na_2MoO_4/H_2O_2 oxidation reaction. Otherwise, the *N*-benzhydrylthiourea was oxidized only to the intermediate sulfinate oxidation level.

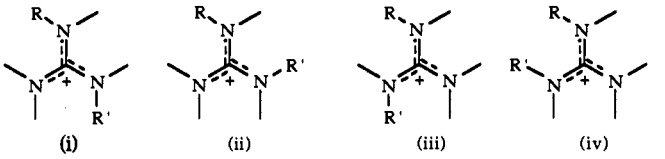
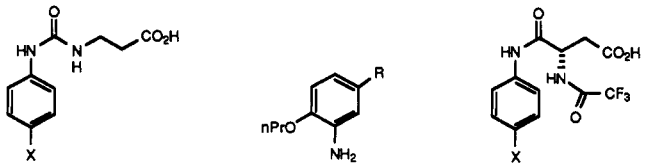


Figure 1. The four possible conformers of N,N'-disubstituted guanidines.

Results and Discussion

Nearly 100 sweet trisubstituted guanidines were reported by Nofre, Tinti, and co-workers.[2] Additional examples were recently described by Nagarajan and coworkers.[5] The compounds of highest sweetness potency in the series share the common feature of a 4-cyanophenyl or 4-nitrophenyl aryl substituent. Nitro and cyano aromatic functionalities are well-known in other structural classes of sweeteners. Specific examples are the aryl ureas **4a**[6] and **4b**,[7] the anilines **5a** and **5b**,[8] and the aspartyl anilides **6a** and **6b**.[9]



4a : X = NO_2 5a : R = NO_2 6a : X = NO_2

4b : X = CN 5b : R = CN 6b : X = CN

The four hydrophobic substituents embodied in **1a–d** were selected for their marked potentiating effects on the sweet taste activity in the trisubstituted guanidine series.[2,5] The sweetness potencies of the disubstituted guanidines **1a–d** which we determined and of the corresponding trisubstituted guanidines **7a–d** which have been reported are given in Table II. Interestingly, while disubstituted guanidines **1c** and **1d** are still sweet, they are nearly 3 orders of magnitudes less potent than the corresponding aryl-containing trisubstituted guanidines **7c** and **7d**. The

(1) Nofre, C.; Tinti, J.-M.; Chatzopoulos-Ouar, F. European Patent Appl. 0 195 730 Sept. 24, 1986.
(2) Nofre, C.; Tinti, J.-M.; Chatzopoulos-Ouar, F. European Patent Appl. 0 241 395, Oct. 14, 1987.
(3) Maryanoff, C. A.; Stanzione, R. C.; Plampin, J. N.; Mills, J. E. A Convenient Synthesis of Guanidines from Thioureas. *J. Org. Chem.* **1986**, *51*, 1882–1884.
(4) Miller, A. E.; Bischoff, J. J. A Facile Conversion of Amino Acids to Guanidino Acids. *Synthesis* **1986**, 777–779.
(5) Nagarajan, S.; DuBois, G. E.; Kellogg, M.; Hellekant, G. Design and Synthesis of Super Potent Guanidines as Probes for the Sweet Taste Receptor. *Abstract of Papers*; 199th National Meeting of the American Chemical Society, Boston, MA; American Chemical Society: Washington, DC, 1990; AGFD 61.
(6) Petersen, S.; Müller, E. A New Group of Sweet Substances. *Chem. Ber.* **1948**, *81*, 31–38.
(7) (a) Tinti, J.-M.; Nofre, C.; Durozard, D. Studies on Sweeteners Requiring the Simultaneous Presence of Both the NO_2/CN and COO Groups. *Naturwissenschaften* **1981**, *68*, 143. (b) Tinti, J.-M.; Nofre, C.; Peytavi, A.-M. Interaction of Suosan with the Sweet Taste Receptor. *Z. Lebensm. Unters. Forsch.* **1982**, *175*, 266–268.
(8) Tinti, J.-M.; Durozard, D.; Nofre, C. Sweet Taste Receptor: Evidence of Separate Specific Sites for COO^- and NO_2/CN Groups in Sweeteners. *Naturwissenschaften* **1980**, *67*, 193–194.

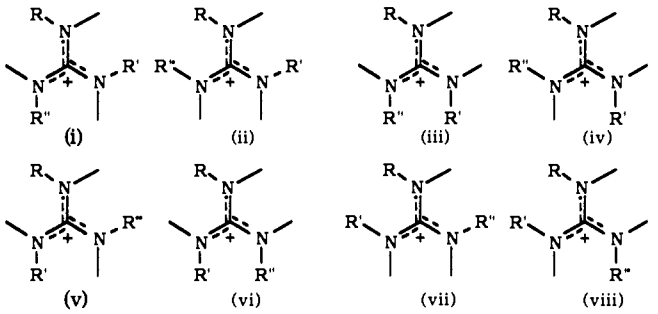


Figure 2. The eight possible conformers of N,N',N''-trisubstituted guanidines.

Table III. Relative Energies and Fractional Boltzmann Populations for Conformers for **1d** and **7d**

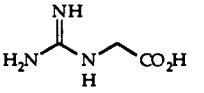
compd	conformer	relative energy (kcal/M)	Boltzmann population at 30 °C
1d	i	0.0	0.47
	ii	0.5	0.22
	iii	0.2	0.31
	iv	12.3	≪0.01
7d	i	0.0	0.51
	ii	1.5	0.04
	iii	7.7	≪0.01
	iv	2.9	<0.01
	v	0.2	0.34
	vi	5.6	≪0.01
	vii	1.1	0.08
	viii	1.9	0.02

disubstituted analogues **1a** and **1b** were not observed to be sweet at the concentrations evaluated (≤1 mg/mL).

There are several plausible rationales for the precipitous decreases in sweetness potencies which we have observed. We have considered two hypotheses: one based on conformational considerations and one based on molecular recognition units.

Examination of molecular models suggests that the disubstituted guanidines should have greater conformational flexibility than the trisubstituted compounds. A consequence of this flexibility might be that inactive conformers are favored energetically relative to the desired active conformer which is predominant in the trisubstituted series. To investigate the relative conformational energies of the di- and trisubstituted guanidines, we carried out molecular mechanics calculations using Still's MACROMODEL program[10] with additional parameters developed by the method of Hopfinger and Pearlstein.[11] Atomic charges were obtained using the INDO/S method.[12] Figure 1 illustrates the four conformers possible for a disubstituted guanidine (in the planar, charged form), and Figure 2 shows the eight conformers possible for the corresponding trisubstituted guanidine. Not surprisingly, conformers i, ii, and iii are relatively accessible for the disubstituted guanidines, while conformers i and v are strongly favored for the trisubstituted guanidines. Table III lists the relative energies and fractional Boltzmann populations (calculated at 30 °C) for compounds **1d** and **7d**. Based on our receptor modeling studies,[13] we have postulated the active conformation for trisubstituted guanidines to be i (Figure 2, R = aliphatic substituent, R' = CH_2COOH, R'' = Ar). This in turn corresponds to conformer ii for the disubstituted guanidines (R = aliphatic substituent, R' = CH_2COOH). Tinti and Nofre[14] propose that conformer v of Figure 2 is the active one. In any case, for both di- and trisubstituted compounds, the expected active conformer is readily accessible from an energetic point of view.

The second, and perhaps more likely, rationale which we considered for the diminished activities of the disubstituted compounds is that the aryl moieties of the trisubstituted compounds may be involved in important receptor binding interactions. The electron-deficient aromatic substitutents of **7a–d** may function as dipolar or π-stacking recognition units for complementary functionality at the receptor. Aryl–aryl interactions are well-known as key contributors to molecular recognition events.[15-18] Our results suggest that the 4-cyanophenyl moiety in the sweet guanidines, ureas, anilines, and aspartyl anilides may play an important role in the initiation of the sweet taste response, possibly via a π-stacking interaction. Clearly, however, the sweet taste activities of **1c** and **1d** show that the aryl moiety is *not* essential for activity. In addition, the earlier results of Nofre, Tinti, and co-workers demonstrate that the hydrophobic substituents of **7a–d** are *not* essential for receptor activation. Thus, the essential pharmacophore of the guanidine sweeteners may simply be guanidinoacetic acid (**11**). However, guanidinoacetic



11

acid appears not be sweet. At a concentration of 0.5% in water, no sweetness is detected. Thus, we conclude that guanidinoacetic acid requires the appropriate aryl or alkyl substituent for a detectable level of activity.

In summary, it has been found that *N*-alkyl-*N'*-(carboxymethyl)guanidines are of much lower sweetness potency than the corresponding *N*-alkyl-*N'*-(carboxymethyl)-*N''*-(4-cyanophenyl)guanidines. Thus, an appropriately sub-

(9) (a) Rodriguez, M.; Goodman, M. Peptide Sweeteners. 7. Taste Relationships of Trifluoroacetyl-L-aspartylanilides. *J. Med. Chem.* 1984, *27*, 1668–1672. (b) Lapidus, M.; Sweeney, M. L-4'-Cyano-3-(2,2,2-trifluoroacetamido)succinanilic Acid and Related Synthetic Sweetening Agents. *J. Med. Chem.* 1973, *16*(2), 163–166.

(10) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MacroModel—An Integrated Software system for Modeling Organic and Bioorganic Molecules Using Molecular Mechanics. *J. Comp. Chem.* 1990, *11*, 440–467.

(11) Hopfinger, A. J.; Pearlstein, R. A. Molecular Mechanics Force-Field Parameterization Procedures. *J. Comp. Chem.* 1984, *5*, 486–499.

(12) Ridley, J. E.; Zerner, M. C. An Intermediate Neglect of Differential Overlap Technique for Spectroscopy: Pyrrole and Azines. *Theor. Chim. Acta (Berlin)* 1973, *32*, 111–134.

(13) Culberson, J. C.; Walters, D. E. Three-Dimensional Model for the Sweet Taste Receptor: Development and Use. In *Sweeteners: Discovery, Molecular Design and Chemoreception*; Walters, D. E., Orthoefer, F. T., DuBois, G. E., Eds.; American Chemical Society: Washington, DC, 1991; pp 214–223.

(14) Tinti, J.-M.; Nofre, C. Why does a Sweetener Taste Sweet? A New Model. In *Sweeteners: Discovery, Molecular Design and Chemoreception*; Walters, D. E., Orthoefer, F. T., DuBois, G. E., Eds.; American Chemical Society: Washington, DC, 1991; pp 206–213.

(15) Nogrady, T. *Medicinal Chemistry: A Biochemical Approach*, 2nd ed.; Oxford University Press: New York, 1988; p 43.

(16) Rebek, J. Model Studies in Molecular Recognition. *Science* 1987, *235*, 1478–1484.

(17) Kollman, P. A. In *Burger's Medicinal Chemistry Part I. The Basis of Medicinal Chemistry*; Wolff, M. E., Ed., John Wiley & Sons: New York, 1980; Chapter 7, pp 313–330.

(18) Pirkle, W. H.; Pochapsky, T. C. Chiral Molecular Recognition in Small Bimolecular Systems: A Spectroscopic Investigation into the Nature of Diastereomeric Complexes. *J. Am. Chem. Soc.* 1987, *109*, 5975–5982.

stituted aryl group augments sweetness potency in the guanidine sweetener class. The sweet-tasting guanidines which lack this aryl moiety which we have described clearly indicate that the aryl group is not a requisite for sweetness. However, these analogues are in general 3 orders of magnitude less potent than corresponding aryl-substituted derivatives. Thus, we conclude that the essential pharmacophore in the guanidinoacetic acid sweeteners is an *N*-substituted-*N'*-(carboxymethyl)guanidine. Important functionality for receptor binding is present in guanidinoacetic acid. However, it appears to be insufficient for receptor activation and this critical role may be provided by either, or both, the aryl- or alkylguanidine substituents.

Experimental Section

Sensory data was obtained by evaluation of compounds at 0.01, 0.1, and 1.0 mg/mL in distilled water by two trained tasters, and the resulting sensory data was averaged.

Melting points were obtained on a Thomas-Hoover Unimelt capillary apparatus and are not corrected. IR spectra were taken as KBr pellets using a Perkin-Elmer Model 283 or 681. NMR spectra were obtained either on a Varian FT-80 or on a General Electric QE-300 spectrometer. Microanalyses were performed by Midwest Microlab, 7212 N. Shadeland Ave, Indianapolis, IN. Chromatography was performed according to the method of Still[19] on silica gel. The isothiocyanates were purchased from Fairfield Chemical Company and Trans World Chemicals. Care should be taken in running the hydrogen peroxide oxidation reaction due to its exothermicity.

***N*-(Cyclohexylmethyl)thiourea (2a).** To a stirred solution of cyclohexylmethyl isothiocyanate (9.14 g, 58.9 mmol) in 90 mL of acetonitrile was added 11.3 mL of 15 N ammonium hydroxide (170 mmol). After 19 h, the reaction mixture was filtered, and the white solid was washed with copious amounts of ether. The solid was air-dried to afford 4.60 g (45%) of the desired thiourea. The filtrate was concentrated and the residue was slurried in ether. The slurry was filtered to afford an additional 2.96 g (29%) of thiourea: mp 151–153 °C; ^{1}H NMR (DMSO-d_6) δ 7.7–7.5 (m, 1 H, NH), 7.5–6.65 (2 m, 2 H, NH_2), 3.3–3.1, 3.0–2.75 (2 m, 2 H), 1.8–1.4 (m, 6 H), 1.3–1.0 (m, 3 H), 1.0–0.7 (m, 2 H); IR (KBr, cm^{-1}) 3400, 3280, 3180, 2990, 2850, 1629, 1595, 1564, 1480. Anal. ($C_8H_{16}N_2$) C, H, N.

[*N*-(Cyclohexylmethyl)imino]aminomethanesulfonic Acid (3a). To a stirred, ice bath cooled suspension of **2a** (7.11 g, 41.3 mmol) and sodium molybdate dihydrate (0.15 g, 0.62 mmol) in 40 mL of methanol was added 13.5 mL of 30% hydrogen peroxide at a rate such that the reaction temperature did not rise above 20 °C. After the addition was complete, the ice bath was removed. The reaction temperature rose. When the reaction exotherm was complete, the reaction mixture was cooled to 10 °C and filtered, and the solid was washed with water. The solid was dried in vacuo (40 °C, <1 mm) to yield 6.80 g (75%) of the sulfonic acid: mp 190–191.5 °C; ^{1}H NMR (DMSO-d_6) δ 9.65–9.50 (m, 1 H, NH), 9.25–9.0 (m, 2 H, NH_2), 3.06 (t, 2 H, J = 6.1 Hz, NCH_2), 1.65–1.45 (m, 6 H), 1.25–1.0 (m, 3 H), 1.0–0.75 (m, 2 H); ^{13}C NMR (DMSO-d_6) δ 165.6, 47.6, 36.0, 29.7, 25.9, 25.1.

***N*-(Cyclohexylmethyl)-*N'*-(carboxymethyl)guanidine (1a).** To a stirred solution of glycine (0.563 g, 7.50 mmol) and potassium carbonate (1.04 g, 7.50 mmol) in 17 mL of water was added **3a** (1.65 g, 7.50 mmol) in small portions over 5 min. The reaction mixture was stirred for 48 h at room temperature and for 1 h at reflux. The reaction mixture was filtered, and the solid was washed with water, followed by ether, and dried to afford 0.714 g (45%) of crude product. The crude product (604 mg) was purified by recrystallization to yield to 0.410 g (30% overall) of the desired guanidine: mp 226–227 °C; ^{1}H NMR (CD_3CO_2D) δ 4.04 (s, 2 H, CH_2CO), 3.04 (d, 2 H, J = 7.0 Hz, NCH_2), 1.8–1.45 (m, 6 H), 1.35–1.05 (m, 3 H), 1.01–0.80 (m, 2 H); IR (KBr, cm^{-1}) 3420, 3290, 2920, 2850, 1704, 1650, 1620, 1405, 1380, 1370, 1350. Anal. ($C_{10}H_{19}N_3O_2$) C, H, N.

Registry No. **1a**, 138460-19-2; (*S*)-**1b**, 138460-20-5; **1c**, 128169-40-4; **1d**, 138460-21-6; **2a**, 66892-28-2; (*S*)-**2b**, 25144-91-6; **2c**, 128169-48-2; **2d**, 92192-94-4; **3a**, 138460-22-7; (*S*)-**3b**, 138460-23-8; **3c**, 128194-24-1; **3d**, 138460-24-9; **7a**, 116869-63-7; (*S*)-**7b**, 116869-20-6; **7c**, 116869-52-4; **7d**, 138460-25-0; $(NH_2)_2$-C=NH, 113-00-8; (*S*)-PhCH(CH_3)NCS, 24277-43-8; $(Ph)_2$CHNCS, 3550-21-8; cyclohexylmethyl isothiocyanate, 52395-66-1; cyclooctyl isothiocyanate, 33522-04-2.

Supplementary Material Available: Experimental data for compounds **1b–d**, **2b–d**, and **3b–d** (5 pages). Ordering information is given on any current masthead page.

(19) Still, W. C.; Kahn, M.; Mitra, A. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* 1978, *43*, 2923.

Synthesis, Cardiac Electrophysiology, and β-Blocking Activity of Novel Arylpiperazines with Potential as Class II/III Antiarrhythmic Agents

Gary B. Phillips,* Thomas K. Morgan, Jr., William C. Lumma, Jr., Robert P. Gomez, Joan M. Lind, Randall Lis, Thomas Argentieri, and Mark E. Sullivan

Departments of Medicinal Chemistry and Pharmacology, Berlex Laboratories, Inc., 110 East Hanover Avenue, Cedar Knolls, New Jersey 07927. *Received June 25, 1991*

A series of novel arylpiperazines have been prepared in an attempt to incorporate both class II (β-receptor blocking) and class III antiarrhythmic properties in a single molecule. The key step in the preparation of the new compounds involves a regioselective heterocyclic ring formation. All but four compounds significantly prolonged action potential duration in canine cardiac Purkinje fibers (class III activity). All but one of the compounds demonstrated β-receptor affinity in a competitive binding assay and three had β_1-receptor selectivity. Compared to sotalol, a reference class II/III agent, arylpiperazine **7a** (4-[(methylsulfonyl)amino]-*N*-[(4-phenylpiperazin-2-yl)methyl]benzamide) demonstrated β_1-selectivity and was 1 order of magnitude more potent in the in vitro class III and the β_1-receptor screens. Compound **7a** was evaluated further and found to be effective in preventing programmed electrical stimulation-induced arrhythmias in conscious dogs (class III activity) and against epinephrine-induced arrhythmias in halothane anesthetized dogs (class II activity).

Due to the variety of pathophysiological causes that may contribute to the development of a life-threatening arrhythmia, no single agent is effective in all cases. Our approach has been to prospectively develop an agent with multiple focused activities within a single compound based on the assumption that the combined therapies will be

0022-2623/92/1835-0743$03.00/0