Carboxylic Acid Replacement Structure–Activity Relationships in Suosan Type Sweeteners. A Sweet Taste Antagonist.[1] 1

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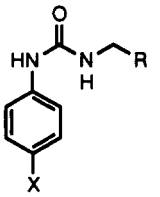
N-(4-Cyanophenyl)-*N'*-(2-carboxyethyl)urea (2), an analogue of suosan [1, *N*-(4-nitrophenyl)-*N'*-(2-carboxyethyl)urea], is a known high-potency sweetener derived from β-alanine. Sulfonic and phosphonic acid analogues of 2 were prepared to develop structure–activity relationships through modification of the carboxylic acid region of this family of sweeteners. Neither of the carboxylic acid replacements resulted in sweet analogues. However, we found that *N*-(4-cyanophenyl)-*N'*-[(sodiosulfo)methyl]urea (7) is an antagonist of the sweet taste response. The bitter taste response to caffeine, quinine, and naringin was also antagonized. Antagonist 7 was found to inhibit the sweet taste perception of a variety of sweeteners. Antagonist 7 had no effect on the sour or salty taste response.

The suosan family of high-potency sweeteners was discovered by Müller and Petersen.[2] This series of sweeteners consists of *N*-aryl-*N'*-(carboxymethyl)- or -(2-carboxyethyl)ureas. The carboxylic acid group contained in these compounds is commonly found in high potency sweeteners.[3] We explored modification of the carboxylic acid moiety to extend the structure–activity relationships (SAR) in the suosan class of sweeteners.

The use of carboxylic acid replacements is well documented in pharmaceutical research and has led to the discovery of compounds with agonist as well as antagonist activity.[4] Direct analogues of suosan, *N*-(4-nitrophenyl)-*N'*-(2-carboxyethyl)urea (1), were not prepared because of the safety concerns associated with nitroaryl compounds. Instead, *N*-(4-cyanophenyl)-*N'*-(2-carboxyethyl)urea (2)[5] was used as the parent compound. The



	R	X
1	CH_2CO_2H	NO_2 (suosan)
2	CH_2CO_2H	CN
3	CO_2H	CN

suosan family of sweeteners are not commercially viable because of their low water solubility, particularly at the pH used in carbonated soft drinks. The carboxylic acid surrogates chosen for synthesis were the sulfonic acid and the phosphonic acid group. The tetrazolyl group (with a pK_a approximately equivalent to the carboxylic acid) was previously explored, and the analogues were found to be weakly sweet.[6] We were interested in preparing the sulfonic acid and phosphonic acid analogues because of the differing acidity provided by these moieties and the expected increased water solubility of these analogues. We were also interested in screening these compounds as antagonists because in The NutraSweet Co. sweet taste agonist model, the carboxylic acid moiety occupies an important binding site.[7] Thus, while isosteric replacements often generate potent agonists, they may also yield antagonists.

A major class of taste modifying substances are sweet taste antagonists. Sweet taste antagonists can be divided functionally into two groups: antagonists which are active after a pretreatment phase and antagonists which are competitively active. The first group of antagonists work only if the tongue is treated with antagonist prior to tasting the sweet substance itself. Examples of this type of antagonist are gymnemic acid,[8] the ziziphins,[9] and hodulcin.[10] Whether these pretreatment sweet taste antagonists are competitive antagonists or not is still subject to debate.[11]

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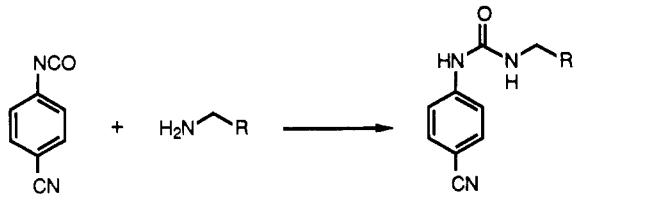

Compound	R
4	$CH_2PO_3Na_2$
5	PO_3Na_2
6	CH_2SO_3Na
7	SO_3Na

Figure 1.

The concentration vs response curves of competitive sweet taste antagonists show a concentration dependence. Sweet taste antagonists which are thought to act competitively include methyl 4,6-dichloro-4,6-dideoxy-α-galactopyranoside,[12] *p*-nitrophenyl α-glucopyranoside,[13] chloramphenicol,[12] and 2-(4-methoxyphenoxy)propanoic acid.[14]

Results and Discussion

Phosphonic acid and sulfonic acid analogues of **2** and *N*-(4-cyanophenyl)-*N*-(carboxymethyl)urea (**3**) were prepared as shown in Figure 1. In the suosan series of sweeteners, the glycine derivative is much less potent than the β-alanine derivative.[2] However, both the phosphonic acid and sulfonic acid moieties are slightly larger than the carboxylic acid group.[15] Therefore, both β-alanine analogues and glycine analogues were prepared. The compounds were first screened for sweetness activity greater than or equal to 20 times a 2% sucrose solution [$P_w(2) = 20$]. If the compounds were found not to be sweet and have little or no other taste responses associated with them, they were screened for sweetness inhibition activity in aqueous sucrose solutions.

The phosphonic acid group has found utility as a carboxylic acid bioisostere in pharmaceutical research.[16] The phosphonic acid group was of interest because it affords two acidic protons with pK_as of 2.4 and 7.1, respectively, for methylphosphonic acid.[17] The phosphonic acid group would therefore be ionized at the pH of carbonated soft drinks (pH 3) which would lead to increased water solubility as compared to the corresponding carboxylic acid. Neither phosphonic acid **4** nor **5** was found to be sweet. When evaluated as antagonists against sucrose, neither

Table I. Evaluation of Sweet Taste Intensities of Sucrose Solutions Containing from 0 to 4 mg/mL of Antagonist **7**[a]

antag 7, mg/mL	% sucrose inten obsd on 0–15 scale[b] ± LSD[c] in aq sucrose solns at given %				
	3.5	5.6	7.5	10	12.5
0.0	4.0 ± 0.7	6.1 ± 1.1	8.5 ± 0.8	10.1 ± 0.8	12.3 ± 1.1
2.0	2.3 ± 0.6	3.6 ± 0.8	4.9 ± 1.4	7.3 ± 0.7	9.3 ± 1.5
3.0	1.6 ± 0.5	2.2 ± 1.1	4.2 ± 0.7	6.5 ± 1.2	8.6 ± 1.0
4.0	1.2 ± 0.8	1.3 ± 0.8	3.3 ± 1.1	4.5 ± 1.1	6.4 ± 1.0

[a] These data were generated using four trained panelists. Four samples were prepared for each inhibitor panel session. The solutions were made using a known concentration of sucrose and either 0.0, 2.0, 3.0, or 4.0 mg of inhibitor per mL of final solution. The solution containing no inhibitor provided an internal standard for that day's panel session. The samples were presented in a random order. Each panelist rated the sweetness of each sample against a series of standard sucrose solutions. Each panel session was replicated three times. The experiment outlined above was repeated several times using different sucrose concentrations in order to generate a set of concentration–response curves. [b] Sweetness intensity scale from 0 to 15 based on 0–15% sucrose solutions. [c] Error bars are least significant difference (LSD), $(2SD\sqrt{2/n})$, where n is equal to the number of panelists.

Table II. Evaluation of Sweet Taste Intensities of Aspartame Solutions Containing from 0 to 4 mg/mL of Antagonist **7**[a]

aspartame concn, ppm	% sucrose inten obsd on 0–15 scale ± LSD at given concns of 7			
	0 ppm	1000 ppm	2000 ppm	4000 ppm
250	5.34 ± 1.10	3.30 ± 1.20	2.11 ± 1.37	1.37 ± 1.20
500	7.55 ± 1.35	5.98 ± 0.95	4.48 ± 1.00	2.57 ± 1.26
750	9.78 ± 1.63	7.43 ± 1.43	6.24 ± 2.27	4.46 ± 1.44
1000	10.1 ± 0.69	8.83 ± 1.46	7.34 ± 1.65	5.85 ± 1.93
1500	11.4 ± 1.86	9.62 ± 1.62	8.35 ± 0.96	6.44 ± 1.30
2000	12.0 ± 1.47	10.5 ± 1.60	9.35 ± 1.51	6.91 ± 2.01
2500	12.9 ± 1.85	10.2 ± 1.93	8.95 ± 2.13	8.86 ± 2.22
3000	11.4 ± 2.60	10.7 ± 2.37	9.11 ± 2.02	7.82 ± 2.03

[a] See Table I, footnotes *b* and *c*.

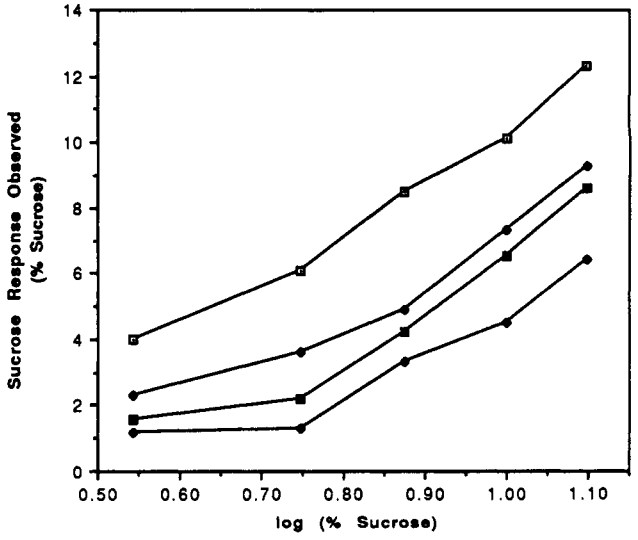

Figure 2. Inhibition of sucrose taste response by compound 7. Inhibitor concentrations: 0 ppm (□), 2000 ppm (◆), 3000 ppm (■), 4000 ppm (◆).

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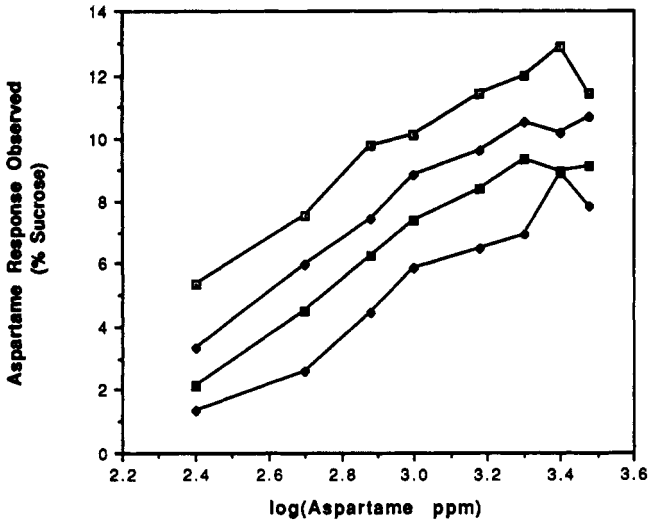

Figure 3. Inhibition of aspartame taste response by compound 7. Inhibitor concentrations: 0 ppm (□), 1000 ppm (◆), 2000 ppm (■), 4000 ppm (◆).

compound was found to be active.

The sulfonic acid moiety is found in high-potency sweeteners such as cyclamate and its analogues,[18] dihydrochalcone derivatives,[19] and stevioside derivatives.[20] Sodium lauryl sulfate, a surfactant, has been reported to be a taste modifier which may act by disrupting the taste cell membrane.[14] Heptyl and octyl sulfonate[21] and hexyl and heptyl sulfate[22] have been claimed in European patent applications as sweet taste inhibitors. The sulfonic acid group contains one acidic proton with a $pK_a < 1$. Thus, ureas containing a sulfonic acid group should have increased water solubility as compared to the carboxylic acid derivatives. *N*-(4-Nitrophenyl)-*N'*-[(sodiosulfo)ethyl]urea (8), derived from 4-nitrophenyl isocyanate and taurine (2-aminoethanesulfonic acid) has been reported to be not sweet.[2,23] Ureas 6 and 7 were found to be tasteless at concentrations up to 4 mg/mL (4000 ppm) in water. Both were evaluated as antagonists against sucrose, and 7 was found to be active. Antagonist 7 was evaluated at concentrations of 2000, 3000, and 4000 ppm in 3.5%, 5.6%, 7.5%, 10.0%, and 12.5% aqueous sucrose solutions. The results are shown in Table I and Figure 2. The concentration–response curves shown in Figure 2 are roughly parallel as is observed for a competitive antagonist.[24] A similar series of concentration–response curves were generated for aspartame (Table II and Figure 3). In the case of aspartame, the concentration–response curve with the antagonist appear to be leveling out below the maximal response of aspartame. This type of behavior is indicative of a noncompetitive inhibitor.

There are many structural classes of sweeteners.[3,25] Although no sweet taste receptor has been isolated, the sweet taste phenomenon is thought to be receptor mediated. The question of single versus multiple sweet taste receptors has yet to be resolved.[26] Sensory experiments of synthetic sweetener mixtures where synergy is observed between different classes of sweeteners has led to the proposal of more than one type of sweet taste receptor.[27] Single taste nerve fiber experiments are indicative of multiple sweet taste receptors.[28] We were interested in determining if antagonist 7 showed selectivity between structurally distinct sweetener classes. A selective an-

Table III. Evaluation of Sweet Taste Intensities of Aqueous Solutions Containing 10 Different Sweeteners with and without Antagonist 7[a]

compound	concn, ppm	% sucrose equiv inten obsd on 0–15 scale ± LSD at given concns of 7	
		0 ppm	4000 ppm
sucrose	100 000	9.60 ± 1.45	3.23 ± 1.44
aspartame	750	9.88 ± 1.53	3.87 ± 0.84
sucralose	152	8.52 ± 1.30	1.43 ± 0.84
saccharin	330	8.15 ± 1.45	4.06 ± 1.41
acesulfame-K	1000	7.88 ± 2.65	4.59 ± 1.92
MAG	3000	6.97 ± 2.58	5.28 ± 2.42
Na cyclamate	5000	10.1 ± 1.73	6.05 ± 1.72
NHDHC	250	8.85 ± 2.47	5.98 ± 2.11
rebaudioside A	638	7.62 ± 1.00	3.11 ± 0.99
thaumatin	40	8.86 ± 1.77	6.23 ± 1.85

[a] See Table I, footnotes *b* and *c*.

Table IV. Evaluation of Bitter Taste Intensities of Aqueous Solutions of Selected Bitter Compounds with and without Antagonist 7[a,b]

compound	concn, ppm	bitterness rating obsd on 0–15 scale ± LSD at given concns of 7	
		0 ppm	4000 ppm
caffeine	1100	8.48 ± 1.27	4.77 ± 2.35
quinine	15	11.8 ± 4.23	5.73 ± 2.50
naringin	290	10.5 ± 2.65	7.80 ± 2.63

[a] Bitterness intensity scale from 0 to 15 based on 0–0.20% caffeine solutions. [b] See Table I, footnote *c*.

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tagonist could permit classification of receptors. Eight different classes of sweeteners were evaluated with antagonist 7.

The sweeteners were tested at concentrations previously determined to be isosweet to a 10% sucrose solution.[29] The high-potency sweeteners evaluated were aspartame, sucralose, sodium saccharin, acesulfame-K, monoammonium glycyrrhyzinate (MAG), sodium cyclamate, neohesperidin dihydrochalcone (NHDHC), rebaudioside A, and thaumatin. The sweet taste responses to all nine of the above sweeteners were depressed in the presence of 7 (4 mg/mL). The antagonism data are tabulated in Table III. The high-potency sweeteners with slow onset and lingering sweet taste were the least depressed. This may be due to washing out of the antagonist before activation by the sweetener, or a short duration of action of the antagonist.

The activity of 7 against the three other basic tastes (sour, salt, and bitter) was also evaluated. Neither salt (NaCl and KCl) nor sour (citric acid) was antagonized by 7. However, the bitter taste response to caffeine, quinine, and naringin was reduced in the presence of 7 (Table IV), suggesting a relationship between the sweet and bitter taste responses.

Summary and Conclusions

In examining carboxylic acid replacements in the suosan series of urea sweeteners, we have found the carboxylic acid moiety optimal with respect to sweetness potency. Substitution of the phosphonic acid group for the carboxylic acid group of suosan leads to analogues which are not sweet or have a potency of less than 2.5 times that of 2% sucrose solution.[30] Direct substitution of the sulfonic acid group for the carboxylic acid group leads to an inactive analogue. However, substitution of a sulfomethyl group for the 2-carboxyethyl group of 2 results in an analogue which is a sweet taste antagonist. Antagonist 7 was found to be active against a variety of sweetener classes. This general activity lends support to a single sweet taste receptor; however, this lack of selectivity may be due to similarities in the transduction pathway for the sweet taste response. It should be noted that the antagonist potency ($pA_2 \approx 2$) is quite low relative to agonist activity, thus a nonspecific inactivation cannot be ruled out. It has been postulated that the sweet and bitter taste sensations are activated via a common receptor or transduction mechanism.[31] The activity of 7 against bitter taste responses to caffeine, quinine, and naringin is consistent with this hypothesis, though again a nonspecific antagonism cannot be ruled out. We do not think that the mechanism of 7 is analogous to the previously mentioned sulfonate and sulfate sweet taste inhibitors since no activity was observed for homologue 6. However, a simple membrane disruption cannot be ruled out.

Experimental Section

Tasting Protocol. Compounds were first evaluated at 0.01, 0.1, 1.0, and 4.0 mg/mL in water by two trained panelists. Taste evaluations were obtained using the method described previously by DuBois et al.[32] Six concentrations of sucrose (2%, 5%, 7.5%, 10%, 12%, and 16%) were used to standardize sweetness intensity ratings of a taste panel on a 15-cm line scale. The panel was trained to assign intensity values of 2, 5, 7.5, 10, 12, and 15 to these concentrations, respectively. Five concentrations of caffeine (0.02, 0.03, 0.08%, 0.15%, and 0.20%) were used to standardize bitterness intensity rating of a taste panel on a 15-cm line scale. The panel was trained to assign intensity values of 2.2, 4, 7.2, 11.5, and 15.7 to these concentrations, respectively. All standards were dissolved in deionized water.

A single concentration of compound 7 (4 mg/mL) was used to determine the inhibition efficacy on a range of taste qualities including sweet, sour, salty, and bitter. The sweeteners are given in Table III, and the bitter tastants are shown in Table IV. Two salty compounds (NaCl and KCl) and one sour compound (citric acid) were tasted as well. Two solutions were prepared for each taste quality, one containing the tastant and the other containing the tastant with compound 7. Each of the solutions was evaluated by the method outlined above unless otherwise noted (see Table I).

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 on a General Electric QE-300 spectrometer. Microanalyses were performed by Midwest Microanalytical.

***N*-(4-Cyanophenyl)-*N'*-[(disodiophosphono)ethyl]urea (4).** To a stirred suspension of 4-cyanophenyl isocyanate (2.30 g, 16.0 mmol) in 50 mL of CH_3CN was added a solution of (2-aminoethyl)phosphonic acid (2.00 g, 16.0 mmol) and NaOH (1.28 g, 32.0 mmol) in 10 mL of H_2O. After 16 h, the reaction slurry was filtered and the solid was dried to afford 4.37 g (87%) of crude product. The crude product was recrystallized from H_2O (15 mL) to afford 2.90 g (57%) of the product as white needles: mp >290 °C; ^1H NMR (D_2O/TSP) δ 7.65 (d, J = 8.7 Hz, 2 H), 7.44 (d, J = 8.7 Hz, 2 H), 3.5–3.34 (m, 2 H), 1.85–1.60 (m, 2 H); ^{13}C NMR (D_2O/TSP) δ 159.7, 146.5, 136.3, 123.1, 121.7, 106.4, 39.4, 33.3, 31.6;[33] IR (KBr) cm^{-1} 3380 (br), 2240, 1700, 1670, 1600, 1550, 1330, 1080, 1050, 970. Anal. Calcd for $C_{10}H_{10}N_3O_4Na_2P \cdot 1.83H_2O$: C, 34.70; H, 3.98; N, 12.14; P, 8.95. Found: C, 34.76; H, 3.91; N, 12.01; P, 9.24.

***N*-(4-Cyanophenyl)-*N'*-[(disodiophosphono)methyl]urea (5).** To a stirred solution of 4-cyanophenyl isocyanate (0.437 g, 3.03 mmol) in 7.5 mL of acetonitrile was rapidly added a solution of (2-aminomethyl)phosphonic acid (0.336 g, 3.03 mmol) and NaOH (0.242 g, 6.05 mmol) in 2 mL of water. The reaction mixture was stirred for 21 h and then concentrated. The residue was slurried in 20 mL of water, and the slurry was filtered to remove small amounts of yellow solid. The filtrate was concentrated to yield a white solid. This solid was recrystallized from ethanol/water to afford 0.112 g of white solid which was not the desired product. The mother liquor was concentrated, and the residue was recrystallized from ethanol/water to afford 0.576 g of impure urea. The impure urea was recrystallized twice more, once from ethanol/water and once from acetone/water to yield 0.310 g (34%) of the urea as white needles: ^1H NMR (D_2O) δ 7.67 (d, 2 H, J = 8.8 Hz), 7.47 (d, 2 H, J = 8.8 Hz), 3.20 (d, 2 H, J = 12.5 Hz); ^{13}C NMR (D_2O) δ 160.6, 145.3, 135.0, 121.7, 120.4, 105.1, 42.9, 41.329;[34] IR (KBr) cm^{-1} 3400, 2220, 1680, 1600, 1560, 1510, 1400, 1320, 1240, 1180. Anal. Calcd for $C_9H_8N_3O_4Na_2P \cdot 6.36\ H_2O$: C, 26.13; H 5.05; N, 10.16. Found: C, 26.12; H, 4.93; N, 10.26.

***N*-(4-Cyanophenyl)-*N'*-[(sodiosulfo)ethyl]urea (6).** To a stirred solution of 4-cyanophenyl isocyanate (4.07 g, 28.3 mmol) in 50 mL of acetonitrile was rapidly added a solution of taurine (3.54 g, 28.3 mmol) and NaOH (1.13 g, 28.3 mmol) in 15 mL of water. After 23 h, the reaction mixture was concentrated. The

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(33) Due to phosphorus–carbon coupling, nine carbon peaks are observed (not the expected eight).

(34) Due to phosphorus–carbon coupling, eight carbon peaks are observed (not the expected seven). The carbon peaks at 160.6, 42.9, and 41.3 ppm were very weak.

residue was diluted with water (50 mL), and the resulting slurry was filtered to remove small amounts of solid. The aqueous was concentrated to a thick oil and crystallized from ethanol/water to yield 4.55 g of slightly impure urea. Another 1.30 g of impure urea was isolated from the mother liquor. Both crops of the urea were combined and recrystallized from ethanol/water (cooled to 4 °C) to afford 2.42 g (29%) of the urea as a white powder: mp 259–265 °C; ^{1}H NMR (DMSO-d_6) δ 9.44 (s, 1 H), 7.8–7.5 (m, 4 H), 6.67 (t, 1 H, J = 5.3 Hz), 3.5–3.3 (m, 2 H, overlapping water peak), 2.65 (t, 2 H, J = 6.2 Hz); ^{13}C NMR (DMSO-d_6) δ 154.5, 145.3, 133.1, 119.6, 117.4, 102.1, 50.8, 36.0; IR (KBr) cm^{-1} 3500, 3360, 3180, 3100, 2240, 1700, 1600, 1540, 1520, 1420, 1320, 1240, 1200, 1180. Anal. Calcd for $C_{10}H_{10}N_3O_4NaS\cdot0.67H_2O$: C, 39.57; H, 3.73; N, 13.84. Found: C, 39.56; H, 3.53; N, 13.70.

***N*-(4-Cyanophenyl)-*N'*-[(sodiosulfo)methyl]urea (7).** To a stirred solution of 4-cyanophenyl isocyanate (42.3 g, 293 mmol) in 500 mL of acetonitrile was added a solution aminomethanesulfonic acid (33.3 g, 300 mmol) and NaOH (12.0 g, 300 mmol) in 100 mL of H_2O. The reaction slurry was stirred for 24 h and then filtered to yield 52 g of crude product. The crude product was slurried in 200 mL of hot H_2O (90 °C) and filtered to remove symmetrical urea impurities. The filtrate was concentrated to 100 mL, heated to reflux, filtered through cotton, and then allowed to cool. The resulting white crystals were isolated by filtration and dried in vacuo to afford 37.6 g (46%) of the desired urea: mp 270–280 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.37 (s, 1 H, NH), 7.57 (d, 2 H, J = 9.0 Hz, Ar), 7.52 (d, 2 H, J = 9.0 Hz, Ar), 7.23 (t, 1 H, J = 6.0 Hz, NH), 3.99 (d, 2 H, J = 6.0 Hz, CH_2); ^{13}C NMR (DMSO-d_6) δ 154.1, 145.0, 132.9, 119.5, 117.5, 102.3, 55.9; IR (KBr) cm^{-1} 3600 (br), 3580, 2240, 1720, 1600, 1560, 1520, 1420, 1180, 1060. Anal. Calcd for $C_9H_8N_3O_4NaS\cdot0.98H_2O$: C, 36.66; H, 3.40; N, 14.27. Found: C, 36.65; H, 3.02; N, 14.27.

Registry No. 1, 140-46-5; 4, 139583-43-0; 5, 139583-44-1; 6, 139583-45-2; 7, 134555-22-9; 4-cyanophenyl isocyanate, 40465-45-0; 2-aminoethanephosphonic acid, 2041-14-7; aminomethanephosphonic acid, 1066-51-9; taurine, 107-35-7; aminomethanesulfonic acid, 13881-91-9.

Selective β_3-Adrenergic Agonists of Brown Adipose Tissue and Thermogenesis. 1. [4-[2-[(2-Hydroxy-3-phenoxypropyl)amino]ethoxy]phenoxy]acetates

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The ester methyl [4-[2-[(2-hydroxy-3-phenoxypropyl)amino]ethoxy]phenoxy]acetate (8) has been identified as the most interesting member of a series of selective β_3-adrenergic agonists of brown adipose tissue and thermogenesis in the rat. In vivo it acts mainly via the related acid 10. Potency was generally markedly reduced by placing substituents on the phenyl ring of the phenoxypropanolamine unit of 8; only the 2-fluoro analogue 16 had comparable potency to 8. Other structure–activity relationships are discussed. Further testing of 8 (ICI 198157) has shown that in the rat it stimulates the β_3-adrenergic receptor in brown adipose tissue at doses lower than those at which it affects β_1 and β_2 adrenergic receptors in other tissues. It increases metabolic rate, as judged by an increase in oxygen consumption, and in the genetically obese Zucker rat it causes a reduced rate of weight gain. This class of compound may be useful in the treatment of obesity in man.

There is considerable evidence to suggest that the sympathetic nervous system (SNS) is important in the control of energy balance and weight regulation.[1] Agents which directly or indirectly enhance the SNS have been shown to be thermogenic and to cause weight reduction in obese animals[2] and man.[3] Although such compounds are useful pharmacological tools, their value as therapies for the treatment of obesity may be limited by lack of selectivity (at adrenergic receptors or in enhancing local concentrations of noradrenaline) and by lack of tissue specificity. The discovery that brown adipose tissue (BAT) is an important site of thermogenesis in the rat[4] indicated a tissue-specific means of modulating energy expenditure. The physiological effector of BAT is noradrenaline acting through β-adrenoceptors.[5] Work which suggested that β-receptors on BAT were different from conventional β_1 or β_2 subtypes on other tissues[6] pointed to a way of selectively stimulating BAT and thermogenesis without producing unwanted side effects on atria and trachea and other β_1- and β_2-receptor-mediated tissues. A human gene encoding for a third (β_3) β-adrenoceptor has been isolated recently and evidence presented for its presence in adipose tissue.[7]

We have synthesized various novel β_3-adrenergic agonists which show appropriate selectivity for BAT. Three tests were used in a screen to identify compounds of interest. The first was designed to determine whether compounds increased the core temperature of post-cold-adapted rats (see Pharmacology section). Core temperature can be increased by a variety of mechanisms and it was necessary to identify and eliminate those compounds which do not act by stimulting thermogenesis in BAT, i.e. which act by a nonspecific or toxic mechanism. This was achieved in the second test by measuring any increase in GDP binding

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