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Articles

Cerebrovasodilatation through Selective Inhibition of the Enzyme Carbonic Anhydrase. 1. Substituted Benzenedisulfonamides

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A series of substituted benzenedisulfonamide carbonic anhydrase inhibitors is described and their anticonvulsant activities are listed. One compound, 4-(4-methoxypiperidinosulfonyl)-2-chlorobenzenesulfonamide (**19**, UK-12130), was found to have anticonvulsant activity in animals at a dose level that gave only a minimal diuretic effect. **19** selectively increased cerebral blood flow in animals and man without producing an unacceptable level of metabolic acidosis.

As a consequence of the overall aging of the population, cerebrovascular disease is becoming a major medical and social problem. There is, therefore, an urgent need to discover an effective therapy to improve the function of the aging brain. It is well established that the degree and extent of the neurological deficit correlate with decreases in both cerebral blood flow and oxygen utilization,[1-3] but it is not certain which constitutes the primary deficit. Current therapy is mainly confined to vasodilator drugs,[4] even though these are usually unselective in their vasodilator activity and frequently lower blood pressure.

The physiological and pharmacological behavior of the cerebral vasculature is unique, inasmuch as drug actions exerted on most other vascular beds can only rarely be assumed to be operating similarly in the brain.[5] The most important mechanism in the autoregulation of cerebral blood flow is metabolic, involving a local feedback process in which carbon dioxide is the principal factor.[6] It is not surprising, therefore, that no agent has achieved cerebral vasodilatation of the potency and specificity produced by inhalation of 5-7% CO_2.[7,8] By this means, autoregulation is surmounted[9,10] and, since blood pressure is not decreased,[11] CO_2 achieves a dilation limited only by the responsiveness of the cerebral vasculature.[1,12] However, the gas is inconvenient to use in clinical practice, and, owing to the respiratory discomfort and anxiety it produces, is limited to intermittent usage.[13,14]

The cerebrovasodilator effect of CO_2 can be mimicked by inhibition of the enzyme carbonic anhydrase which catalyses the reversible hydration of carbon dioxide to carbonic acid.[15] This enzyme is present in erythrocytes, the brain, and renal tubules. It has been shown that the carbonic anhydrase inhibitor, acetazolamide, selectively increases cerebral blood flow both in patients with cerebrovascular disease[11,16,17] and also in normal experimental animals[17,18] when given iv at doses between 5 and 50 mg/kg. At these doses, however, acetazolamide also produces physiological changes in the other tissues which contain carbonic anhydrase, especially the kidney, where it elicits a marked, sometimes maximal, diuresis[15] which usually leads to metabolic acidosis.

The aim of our synthetic program was to obtain carbonic anhydrase inhibitors that had an increased in vivo selectivity for the enzyme in brain (and erythrocytes) and a decreased selectivity for the enzyme in kidney. We wish to report a series of substituted benzenedisulfonamide carbonic anhydrase inhibitors, one of which shows good cerebrovasodilator activity at a dose which elicits only a mild diuretic response.

Chemistry. Meerwein et al. have described a method for the preparation of benzenesulfonyl chlorides from anilines.[19] This involved diazotization of the aniline, followed by treatment of the resultant diazonium complex with an acetic acid solution containing sulfur dioxide and cupric chloride. Holland et al. subsequently extended this reaction to sulfanilamide and obtained 4-sulfamoylbenzenesulfonyl chloride in good yield.[20]

In a similar manner the anilines **1-6** (Scheme I) were diazotized to yield the appropriately substituted sulfonyl chlorides **7a-f** from which the requisite disulfonamides (Table I) were obtained after reaction with various amines. The aniline **4** is novel and was prepared from 3,5-dimethylacetanilide by straightforward chlorosulfonation and amination followed by hydrolysis. The 2- and 3-(trifluoromethyl)disulfonamides **21** and **24** were prepared by treatment of the benzylthio compound **8** with chlorine in aqueous acetic acid, followed by reaction of the sulfonyl chlorides with either ammonium hydroxide or 4-methoxypiperidine and reduction of the nitro group to afford **9** and **11**. The usual diazotization procedure yielded the

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Table I

SO_2NH_2 / R, R_1 (positions 2, 3, 5, 6) / SO_2NR_2

no.	R	R_1	NR_2	mp, °C	recrystn solvent[e]	mol formula[a]	% yield	ED_{50}, mg/kg po, protection against max electroshock in mice	ED_{50}, mg/kg po, protection against max electroshock in mice of standard compd
13	H	H	c-$N(CH_2CH_2)_2N$-4-CH_3	214–215	H_2O-EtOH	$C_{11}H_{17}N_3O_4S_2$	30	40	24[b]
14	H	H	c-$N(CH_2CH_2)_2N$-4-Ph	218–220	H_2O-EtOH	$C_{16}H_{19}N_3O_4S_2$	25	16	24[b]
15	H	H	c-$N(CH_2CH_2)_2C(-OC_2H_2O-)$	217	H_2O-EtOH	$C_{13}H_{18}N_2O_6S_2$	40	38	29[b]
16	H	H	c-$N(CH_2CH_2)_2C(=O)$	194–195	H_2O-EtOH	$C_{11}H_{14}N_2O_5S_2$	90	47	21[b]
17	H	H	c-NC_5H_9-4-OC_2H_5	176–179	acetone–hexane	$C_{13}H_{20}N_2O_5S_2$	29	40	29[b]
18	H	H	c-NC_5H_9-4-OCH_3	188–190	acetone–hexane	$C_{12}H_{18}N_2O_5S_2$	69	25.63 ± 0.50 ($n = 49$)	
19	H	2-Cl	c-NC_5H_9-4-OCH_3	165–167	IPA	$C_{12}H_{17}ClN_2O_5S_2$	75	8.40 ± 0.19 ($n = 70$)	
20	H	3-Cl	c-NC_5H_9-4-OCH_3	122–123	IPA	$C_{12}H_{17}ClN_2O_5S_2$	57	21	25[b]
21	H	2-CF_3	c-NC_5H_9-4-OCH_3	177–179	IPA	$C_{13}H_{17}F_3N_2O_5S_2$	55	I[d]	
22	5-Cl	3-Cl	c-NC_5H_9-4-OCH_3	>300	IPA–MeOH	$C_{12}H_{16}Cl_2N_2O_5S_2$	10	I	
23	5-CH_3	2-Cl	c-NH_5H_9-4-OCH_3	156–157	H_2O-EtOH	$C_{13}H_{19}ClN_2O_5S_2$	52	I	
24	H	3-CF_3	c-NC_5H_9-4-OCH_3	136–136	IPA	$C_{13}H_{17}F_3N_2O_5S_2$	40	26	10[c]
25	6-CH_3	2-CH_3	c-NC_5H_9-4-OCH_3	148–149	IPA	$C_{14}H_{22}N_2O_5S_2$	60	I	
26	H	H	c-NC_5H_9-3-OCH_3	144–145	acetone–hexane	$C_{12}H_{18}N_2O_5S_2$	24	30	29[b]
27	H	H	c-NC_5H_9-4-$COCH_3$	174–175	IPA	$C_{13}H_{18}N_2O_5S_2$	51	I	
28	H	H	c-NC_5H_8-4(OH)-C_4H_9	166	acetone–hexane	$C_{15}H_{24}N_2O_5S_2$	12	100	29[b]
29	H	H	c-NC_5H_8-4(OH)-CH_3	183–185	acetone–hexane	$C_{12}H_{18}N_2O_5S_2$	48	31	26[b]
30	H	H	c-NC_5H_8-4(2-pyr)-OH	210–211	EtOH	$C_{16}H_{19}N_3O_5S_2$	32	40	29[b]
31	H	H	c-NC_5H_8-4(OCH_3)-CH_3	159	acetone–hexane	$C_{13}H_{20}N_2O_5S_2$	35	I	
32	H	H	$N(C_2H_5)C_3H_6OCH_3$	117–118	H_2O-EtOH	$C_{12}H_{20}N_2O_5S_2$	15	>40	26[b]
33	H	H	N, O, NCH_3, N, Ph	272–275	acetone–MeOH	$C_{20}H_{24}N_4O_5S_2$	35	>40	
34	H	H	c-NC_5H_9-4-CH_3	198–199	acetone–hexane	$C_{12}H_{18}N_2O_4S_2$	75	40	21[b]
35	H	2-Cl	N, H, N, O, O, N, H	335	DMA–H_2O	$C_{13}H_{15}ClN_4O_6S_2$	90	I	
36	H	2-Cl	c-$N(CH_2CH_2)_2O$-3-CH_3	159–162	IPA	$C_{11}H_{15}ClN_2O_5S_2$	41	11	10[c]
37	H	2-Cl	c-$N(CH_2CH_2)_2O$	187–188	H_2O-EtOH	$C_{10}H_{13}ClN_2O_5S_2$	70	10	26[b]
38	H	2-CF_3	c-$N(CH_2CH_2)_2O$	192–194	IPA–MEK	$C_{11}H_{13}F_3N_2O_5S_2$	47	I	
39	5-Cl	3-Cl	c-$N(CH_2CH_2)_2O$	215	IPA	$C_{10}H_{12}Cl_2N_2O_5S_2$	51	I	
40	H	2-Cl	N, O	130–132	H_2O–IPA	$C_{11}H_{15}ClN_2O_5S_2$	16	18	10[c]
41	H	2-Cl	c-$N(CH_2CH_2)_2S$	179–180	EtOH	$C_{10}H_{13}ClN_2O_4S_3$	57	15	9[c]
42	H	2-Cl	c-$N(CH_2CH_2)_2SO_2$	217–220	H_2O–MeOH	$C_{10}H_{13}ClN_2O_6S_3$	18	>40	9[c]
43	H	2-Cl	c-NC_5H_9-4-OH	220–223	acetone–hexane	$C_{11}H_{15}ClN_2O_5S_2$	12	100	27[b]
CH_3CONH … SO_2NH_2			(acetazolamide)					36	25[b]

[a] All compounds were analyzed for C, H, and N. [b] Compound **18** used as standard; standard deviation ±3.5. [c] Compound **19** used as standard; standard deviation ±1.6. [d] I signifies that the compound was inactive at doses up to 100 mg/kg. [e] IPA, 2-propanol; DMA, dimethylacetamide; MEK methyl ethyl ketone.

Scheme I

SO_2NH_2 / NH_2 —(1. HNO_2; 2. $AcOH$-SO_2-$CuCl_2$)→ SO_2NH_2 / SO_2Cl —(HNR_2R_3)→ SO_2NH_2 / $SO_2NR_2R_3$

1, R_1 = H
2, R_1 = 2-Cl
3, R_1 = 3,5-Cl_2
4, R_1 = 2,6-$(CH_3)_2$
5, R_1 = 2-Cl, 5-CH_3
6, R_1 = 3-Cl

7a, R_1 = H
b, R_1 = 2-Cl
c, R_1 = 3,5-Cl_2
d, R_1 = 2,6-$(CH_3)_2$
e, R_1 = 2-Cl, 5-CH_3
f, R_1 = 3-Cl
10, R_1 = 2-CF_3

I (see Table I)

SCH_2Ph / CF_3 / NO_2 (8) —(1. Cl_2-aq AcOH; 2. NH_4OH; 3. H_2-Pd)→ SO_2NH_2 / CF_3 / NH_2 (9) —(1. HNO_2; 2. $AcOH$-SO_2-$CuCl_2$)→ 10

8 —(1. Cl_2-aq AcOH; 2. HN(4-piperidinyl)-OCH_3; 3. H_2-Pd)→ 11

OCH_3 / N / SO_2 / CF_3 / NH_2 (11) —(1. HNO_2; 2. $AcOH$-SO_2-$CuCl_2$)→ OCH_3 / N / SO_2 / CF_3 / SO_2Cl (12) —(NH_4OH)→ 24

sulfonyl chlorides **10** and **12** and amination gave the required disulfonamides.

Biology. The anticonvulsant effect of the primary sulfonamides is mediated through carbonic anhydrase inhibition.[21] Direct correlation between in vitro carbonic anhydrase activity and a specific in vivo effect is unfortunately not possible, since the latter depends on an adequate concentration of drug reaching the desired site of action. Carbonic anhydrase is an extremely active enzyme and at least 99% inhibition is required to produce physiological effects.[15] In rats, carbonic anhydrase inhibitors as a class do not pass the blood–brain barrier, and in this species anticonvulsant activity depends solely on inhibition of the enzyme in erythrocytes.[22] In mice, the enzyme in brain, in erythrocytes, or at both sites is involved, the determining factors being the potency of the drug and its comparative ease of entry into both brain and erythrocytes.[22]

Harper[23] has suggested that control of cerebral blood flow is mediated by a predominantly metabolic mechanism via changes in intraparenchymal resistance (precapillary arterioles and small pial vessels). Alterations in pH, with consequent changes in intraparenchymal resistance, occur either generally as a consequence of changes in blood gas composition or locally from CO_2 and, to a small extent, from lactate, generated from cerebral glucose oxidation. In theory, inhibition of carbonic anhydrase in brain and/or erythrocyte should induce local increases in CO_2 in the vicinity of the intraparenchymal vessels. In order to identify compounds that might increase cerebral blood flow by raising CO_2 levels via carbonic anhydrase inhibition, the mouse electroshock test was used as the primary screen. The results are presented in Table I.

The in vitro inhibition of carbonic anhydrase was determined using enzymes prepared from mouse brain, kidney, and erythrocytes. The assay was based on the method of Philpot and Philpot.[24] None of the compounds showed any selectivity for enzyme from a particular tissue, the differences in ID_{50} values for each compound being within limits of variation. Table II shows the results.

Table II. In Vitro Carbonic Anhydrase Activity Activity and pK_a Values

compd	concn, M, producing 50% inhibn	pK_a
18	$2.7 \times 10^{-7} \pm 0.13^a$	9.42
19	$1.9 \times 10^{-7} \pm 0.12$	8.76
21	$2.0 \times 10^{-7} \pm 0.12$	8.12
acetazolamide	$2.0 \times 10^{-8} \pm 0.10$	7.4

[a] Standard deviation.

Results and Discussion

The diuretic and natriuretic activities of the sulfonamides are enhanced by an increase in their dissociation constants, and it has been concluded that the ionized form is the active species for inhibition of the enzyme.[25] However, high ionization at physiological pH, coupled with low lipid solubility, prevents penetration of drug through the blood–brain barrier since drugs pass through lipid barriers mostly in their un-ionized form. For example, benzolamide (2-benzenesulfonamide-1,3,4-thiadiazole-5-sulfonamide, pK_a = 3.2) is restricted from entering the brain as it is 99.99% ionized, is strongly (92%) bound to plasma protein, and has a low lipid solubility.[26] Despite this, benzolamide does have some anticonvulsant activity in rats and mice[22] because erythrocytes are permeable to anions and the small amount of unbound benzolamide is able to penetrate and eventually exert its effect. However, a maximum diuretic effect due to preferential inhibition of kidney carbonic anhydrase is encountered long before the anticonvulsant dose level is reached.

The starting point for our work was a series of benzenedisulfonamides prepared by Holland et al. as potential anticonvulsant agents.[20,27] Table I lists the compounds to be discussed. As may be seen, compounds **14** and **18** proved superior to acetazolamide in the mouse electroshock test. Moving the methoxy group of **18** to the 3 position of the piperidine ring (**26**) or going from 4-methoxy (**18**) to 4-ethoxy (**17**) resulted in lower anticonvulsant activity. Activity was lost, or substantially diminished, when the piperidine ring was opened as in **32** or when the 4-methoxy group of **18** was replaced either by acetyl (**27**) or by an alkyl group (**34**). Spiropiperidines **33** and **35** also proved ineffective.

Insertion of a chloro substituent at position 2 of the phenyl ring proved highly beneficial. Comparison of compound **19** with **18** shows that anticonvulsant activity was more than doubled. However, moving the chlorine to position 3 (**20**) was not advantageous, and disubstitution as in **22**, **23**, **25**, and **39** abolished all anticonvulsant activity. Replacement of 2-Cl by 2-CF_3, **21** and **38**, also led to loss of anticonvulsant activity even at doses of 100 mg/kg. This was somewhat unexpected, particularly as both compounds have comparable effects on carbonic anhydrase in vitro (Table II).

Consideration of all the compounds in Table I led to the

selection of **19** for further evaluation. **19** (UK-12130) is weakly ionized at physiological pH (3.8%; pK_a = 8.76) but is more lipophilic (log P = 0.62, octanol–water) than is acetazolamide (50% ionized, pK_a = 7.4; log P = –0.25). Thus, **19** should possess a lower affinity for the anion transport system in the renal cortex and should also have a greater ability to cross membranes, e.g., the blood–brain barrier. Although not as potent as inhibitor of carbonic anhydrase as acetazolamide (Table II), drug concentrations of **19** in the brain of mice were seven to eight times higher than those produced by an equivalent (molar) dose of acetazolamide.[28] Also, acute diuretic experiments in rats showed that **19** was much less potent than acetazolamide with respect to increases in urine volume and pH and in K^+ and HCO_3^- output.[28] ED_{50} values for urine output after oral administration to rats were **19**, 13 mg/kg; **18**, 22 mg/kg; and acetazolamide, 5 mg/kg.

A Toribara dialysis experiment showed that at 20 μg/mL **19** was 65% bound to plasma proteins. Blood level data indicated that the half-life of elimination from dog plasma was 3–4 h following iv administration. The major metabolite was the 4-hydroxypiperidino compound **43**.[29] Administration of 2.5, 5, and 10 mg/kg iv of **19** to dogs increased vertebral blood flow by maxima of 22, 28, and 52%, respectively. There was no significant effect on blood pressure, heart rate, total peripheral resistance, or stroke volume.

In anesthetized cats, increased vertebral blood flow (31%, 5 mg/kg iv) produced by **19** was not accompanied by any significant changes in femoral blood flow, blood pressure, or heart rate.[28]

19 has also been shown to have a selective cerebrovasodilator action in baboons[30] and man[31,32] and has undergone clinical evaluation in patients suffering from cerebrovascular disease. The results of this work will be published elsewhere.

Experimental Section

Pharmacology. (a) Electroshock Test.[24] Male mice weighing between 18 and 28 g were used, ensuring that in any one assay the weight range about the mean was within ±2 g. The electroshock stimulus was applied via electrodes placed on the corneal surface of the eyes for a duration of 200 ms. The current necessary to ensure the production of a maximal (tonic/tonic extensor) convulsion in all untreated mice was 20 (where the mean weight was 20 g or less) or 25 mA (where the mean weight exceeded 20 g).

The compounds to be tested were ball-milled with glass beads for up to 24 h in 0.1% (v/v) Tween-80 in saline and the suspension was administered by gavage. Three dose levels, 6.25, 16, and 40 mg/kg, were administered to groups of ten mice 2 h prior to electroshock. A control group was similarly given 0.1% (v/v) Tween-80 in saline (0.1 mL/10 g of body weight). The convulsions were graded as no effect, clonic/clonic, clonic/tonic, or tonic/tonic (maximal). Anticonvulsant activity was expressed as the percentage protection from the maximal seizure effect at each dose level. Table I shows the protective ED_{50} values (in mg/kg) for each compound.

(b) Diuretic Activity. The diuretic effect of selected compounds in rats was investigated. Male rats, 150–200 g, were deprived of food overnight, water being withdrawn 2 h prior to drug administration. A fluid load of isotonic saline (2.5 mL/100 g of body weight po) was given immediately before administration of drug. The animals were divided into groups of eight and the compounds to be tested were administered at three dose levels of 12.5, 25, and 50 mg/kg in separate experiments (acetazolamide was also tested at 5 mg/kg). Vehicle was administered to control groups (0.5 mL/100 g of body weight of 0.1% Tween-80 in saline). The animals were placed individually in metabolism cages and 4 h later urine volume and electrolyte outputs were determined.

The diuretic effects of **19** and acetazolamide were also assessed in dogs during vertebral flow measurements. Urine was collected via catheters at 30-min intervals for 2 h prior to and 3 h after the administration of test compound or vehicle, and volume and electrolyte outputs were determined.

(c) Blood Flow. Right vertebral artery blood flow was monitored in conscious dogs after the chronic implantation of a Doppler ultrasonic flow probe around the artery. The artery was approached via a ventral midline incision in the neck and the probe placed on the artery distal to its exit point from the thoracic cavity and proximal to its entry into the cervical vertebrae. The probe was exteriorized via a path under the skin to the dorsal surface of the neck. Flow and heart rate measurements were made for 2 h prior to and 3 h after administration of the test compound or vehicle.

Vertebral and femoral artery blood flow was monitored using electromagnetic flow probes in anesthetized cats (induction, halothane, nitrous oxide/oxygen 4:1 v/v; maintenance, chloralose, 70 mg/kg iv) and positive pressure ventilation. Blood flow, blood pressure, and heart rate were monitored continuously.

(d) Carbonic Anhydrase Inhibition Methodology. In vitro carbonic anhydrase activity was determined by a modification of the colorimetric method of Philpot and Philpot.[24] The activity was expressed in terms of enzyme units and calculated from the expression

$$EU = (T_0 - T)/T$$

where EU represents enzyme units, T_0 the time of the uncatalyzed reaction in seconds, and T the time of the catalyzed reaction in seconds. All reactions were carried out in a cold room maintained at 5 ± 1 °C. The inhibitors were preincubated with the enzyme for 10 min prior to the addition of substrate. This procedure allowed for enzyme–inhibitor equilibrium to take place. The concentration required to inhibit 50% of the enzyme activity was determined graphically. Approximately two units of enzyme activity were utilized in each experiment.

Chemistry. All melting points are uncorrected and were obtained using an Electrothermal capillary melting point apparatus. The structures of all compounds were confirmed by their IR and NMR spectra, the latter being determined as solution in Me_2SO-d_6. The IR spectra were obtained with a Perkin-Elmer 237 spectrophotometer and the NMR spectra with a Varian Associates spectrometer, Model A-60A.

4-Amino-2,6-dimethylbenzenesulfonamide (4). 3,5-Dimethylacetanilide (225 g, 1.38 mol) was added portionwise to chlorosulfonic acid (375 mL) with stirring. The solution was stirred for 3 h at 90 °C before cooling and pouring onto a vigorously stirred mixture of ice–benzene–ethyl acetate. The organic layer was separated, dried, and concentrated. Petroleum ether (1500 mL) was then added and the brown oily solid that precipitated was removed by decantation. More petroleum ether was added, whereupon the sulfonyl chloride crystallized as a white solid: 112 g (31%); mp 140–142 °C.

The sulfonyl chloride (17 g, 0.065 mol) was dissolved in dry CH_2Cl_2 (200 mL) and ammonia gas was passed into the solution until precipitation was complete. The solid was collected and recrystallized from MeOH to give pure 4-sulfamoyl-3,5-dimethylacetanilide (**44**): 11.0 g (70%); mp 227–228 °C. Anal. ($C_{10}H_{14}N_2O_3S$) C, H, N.

The acetanilide **44** (7.5 g) was hydrolyzed by means of boiling NaOH (8.0 g) in H_2O (100 mL). After heating at reflux for 1 h, the solution was adjusted to pH 6.0, whereupon the desired product **4** precipitated, was collected, and recrystallized from *i*-PrOH: yield, 469 g (80%); mp 185–186 °C. Anal. ($C_8H_{13}N_2O_2S$) C, H, N.

2-Chloro-4-(chlorosulfonyl)benzenesulfonamide (7b, R_1 = 2-Cl). 4-Amino-2-chlorobenzenesulfonamide (**2**)[33] (80.5 g, 0.39 mol) was added to a solution of concentrated HCl (200 mL) in H_2O (150 mL) and the mixture stirred for 1 h at 0 °C. To this was added a solution of sodium nitrate (26.9 g, 0.39 mol) in H_2O (45 mL) at such a rate of addition that the temperature did not rise above 0 °C. The mixture was stirred for 15 min and then quenched onto a solution of sulfur dioxide (100 g, 1.56 mol) and cupric chloride (4 g) in CH_3COOH (280 mL). The reactants were then stirred for 1 h, H_2O (500 mL) added, and stirring continued for a further 45 min. The product was collected by suction filtration, washed with H_2O, dried in vacuo at 60 °C, and re-

crystallized from petroleum ether–acetone to give 74 g (68%), mp 163–165 °C (lit.[33] mp 162–164 °C). All the sulfonyl chlorides were prepared by the above method from the appropriate anilines, **1**,[20] **3**,[34] **4**, **5**,[35] **6**,[36] **9**,[37] and **11**.

7a: R_1 = H; mp 155–156 °C (lit.[20] mp 155–156 °C). **7c**: R_1 = 3,5-dichloro; mp 144–146 °C (lit.[33] mp 144–146 °C). **7d**: R_1 = 2,6-dimethyl; mp 139–140 °C; yield 55%. Anal. ($C_8H_{10}ClNO_4S_2$) C, H, N. **7e**: R_1 = 2-chloro-5-methyl; mp 163–164 °C; yield 47%. Anal. ($C_7H_7Cl_2NO_4S_2$) C, H, N. **7f**: R_1 = 3-chloro; mp 124–126 °C; yield 42%. Anal. ($C_6H_5Cl_2NO_4S_2$) C, H, N. **10**: mp 148–154 °C.[38] **12**: mp 117–119 °C; yield 48%. Anal. ($C_{13}H_{15}ClF_3NO_5S_2$) C, H, N.

2-Chloro-4-(4-methoxypiperidinosulfonyl)benzenesulfonamide (19). Triethylamine (4.2 mL) and 4-methoxypiperidine (3.15 g, 0.028 mol) were added to a solution of 2-chloro-4-(chlorosulfony)benzenesulfonamide (8.7 g, 0.03 mol) in acetone (100 mL). The mixture was stirred at room temperature for 1 h and then poured into 50 mL of 1 N HCl. The resulting product was removed by suction filtration, washed with H_2O, and recrystallized from *i*-PrOH to give pure **19**: 8.4 g (76%); mp 165–167 °C. Anal. C, H, N.

This method was used for the preparation of the majority of the compounds listed in Table I starting from the appropriate amine and the appropriately substituted benzenesulfonyl chlorides **7a–f**. Compound **16** was prepared by acid hydrolysis of **15**.

4-*N*-(4-Oxopiperidinosulfonyl)benzenesulfonamide (16). A solution of the ketal **15** (0.5 g) in 30% aqueous DMF and concentrated HCl (1 mL) was stirred under reflux for 1 h. The mixture was evaporated to a small volume and the resultant precipitate filtered off and recrystallized from aqueous EtOH to give 0.45 g (90%), mp 194–195 °C. Anal. C, H, N.

4-(Benzylthio)-3-(trifluoromethyl)nitrobenzene (8). 4-Chloro-3-(trifluoromethyl)nitrobenzene (44.8 g, 0.2 mol) and *S*-benzylthiouronium chloride (40.8 g, 0.2 mol) were dissolved in ethanol (150 mL). KOH (22.4 g, 0.4 mol) in EtOH (100 mL) was then added with stirring and the mixture heated at reflux for 3 h. After cooling, the precipitate was filtered off, washed with H_2O, and recrystallized from *i*-PrOH to give 45 g (72%), mp 91–93 °C. Anal. ($C_{14}H_{10}F_3NO_2S$) C, H, N.

4-Amino-2-(trifluoromethyl)benzenesulfonamide (9). Chlorine gas was bubbled through a suspension of **8** (50 g) in aqueous CH_3COOH (500 mL). The temperature was maintained below 30 °C by cooling in an ice bath. After 5.5 h the gas flow was halted and the solution poured into ice water (1 L). The sulfonyl chloride layer was separated, washed with bicarbonate and then with water, and finally warmed under reduced pressure to remove volatile products. Petroleum ether (200 mL) was added to the residue which was shaken vigorously, and the mixture was cooled. The solid 4-nitro-2-(trifluoromethyl)benzenesulfonyl chloride was then filtered off, washed with petroleum ether, and dried to yield 34.1 g (74%), mp 73–75 °C (lit.[38] mp 80–81 °C). The sulfonyl chloride (34.1 g) was added to a cooled well-stirred 0.88 N ammonia solution (140 mL) and stirring was continued for a further 3 h before evaporating to a reduced volume and acidifying with 2 N HCl. The mixture was warmed and sufficient aqueous EtOH added to effect solution. The mixture was then cooled and the resultant precipitate filtered off, washed with cold water, dried, and recrystallized from H_2O to give 4-nitro-2-(trifluoromethyl)benzenesulfonamide: 25.1 g (79%); mp 161–164 °C (lit.[37] mp 160–161 °C).

The above nitro compound (25 g, 0.092 mol) was dissolved in MeOH (100 mL) and 1 g of palladium on barium sulfate catalyst added. The mixture was hydrogenated at 50 psi at room temperature until uptake ceased. Working up in the usual manner gave a crude product that was recrystallized from aqueous EtOH to give 15.1 g (68%), mp 188–190 °C (lit.[37] 196–197 °C).

4-(4-Methoxypiperidinosulfonyl)-3-(trifluoromethyl)aniline (11). 4-Nitro-2-(trifluoromethyl)benzenesulfonyl chloride was prepared as described above from **8**. The solid sulfonyl chloride (2.89 g, 0.01 mol) was then stirred at room temperature with 4-methoxypiperidine (1.15 g, 0.01 mol) in CH_2Cl_2 (40 mL) containing triethylamine (1.0 g). After 2 h the solvent was removed in vacuo and the residue treated with a 50:50 mixture of H_2O–2-propanol. The required product, 4-(4-methoxypiperidinosulfonyl)-2-(trifluoromethyl)nitrobenzene (**45**), was then filtered in vacuo and recrystallized from *i*-PrOH: 1.8 g (49%); mp 141–143 °C. Anal. ($C_{13}H_{15}F_3N_2O_5S$) C, H, N.

45 (13.5 g) was dissolved in methanol (200 mL) and 1 g of 5% Pd/C catalyst added. The mixture was hydrogenated at 50 psi at a temperature of 50 °C. When uptake of hydrogen had ceased, the catalyst was filtered off and the filtrate evaporated to dryness in vacuo. The resultant solid was recrystallized from 2-propanol to give 8.9 g (72%), mp 145–147 °C. Anal. ($C_{13}H_{17}F_3N_2O_3S$) C, H, N.

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References and Notes

(1) D. Simard, J. Oleson, O. B. Paulson, N. A. Lassen, and E. Skinhoj, *Brain*, **94**, 273 (1971).
(2) C. P. Larson, W. K. Ehrenfel, J. G. Wade, and E. J. Wylie, *Surgery*, **62**, 31 (1967).
(3) S. A. Hegedus and R. T. Shackleford, *J. Am. Med. Assoc.*, **191**, 279 (1965).
(4) *Br. Med. J.*, **3**, 38 (1969).
(5) L. Sokoloff, *Pharmacol. Rev.*, **11**, 1 (1959).
(6) C. Fieschi and L. Bozzao in "International Encyclopaedia of Pharmacology and Therapeutics", A. Carpi, Ed., Pergamon Press, New York, N.Y., 1972, p 203.
(7) J. S. Meyer and A. D. Ericsson in ref 6, p 253.
(8) M. Nickerson in "Pharmacological Basis of Therapeutics", L. S. Goodman and A. Gilman, Ed., Macmillan, London, 1970, p 745.
(9) N. A. Lassen, *Circ. Res., Suppl.*, **15**, 201 (1964).
(10) A. M. Harper, *Acta Neurol. Scand., Suppl.*, 14, 94 (1965).
(11) D. L. Ehrenreich et al., *Arch. Neurol. (Chicago)*, **5**, 125 (1961).
(12) J. S. Meyer, F. Gotoh, M. Akiyama, and S. Yoshitake, *Circulation*, **36**, 197 (1967).
(13) L. C. McHenry and M. E. Jaffe, *Gen. Practitioner*, **37**, 98 (1968).
(14) N. A. Lassen, *Circ. Res.*, **34**, 749 (1974).
(15) T. H. Maren, *Physiol. Rev.*, **47**, 595 (1967).
(16) F. Gotoh, J. S. Meyer, and M. Tomita, *Arch. Int. Med.*, **117**, 39 (1966).
(17) J. B. Posner and F. Plum, *J. Clin. Invest.*, **39**, 1246 (1960).
(18) J. W. Severinghaus and N. A. Lassen, *Circ. Res.*, **20**, 272 (1967).
(19) H. Meerwein, G. Dihmar, R. Gollner, K. Hafner, and D. Steinfort, *Chem. Ber.*, **90**, 841 (1957).
(20) G. F. Holland, W. H. Funderburk, and K. F. Finger, *J. Med. Chem.*, **6**, 307 (1963).
(21) W. D. Gray, T. H. Maren, G. H. Simon, and F. H. Smith, *J. Pharmacol. Exp. Ther.*, **121**, 160 (1957).
(22) W. D. Gray and C. E. Rauh, *J. Pharmacol. Exp. Ther.*, **156**, 383 (1967).
(23) A. M. Harper, "9th Princeton Conference on Cerebral Vascular Diseases", J. P. Wishnant and B. A. Sandok, Ed., Grune and Stratton, New York, N.Y., 1974, p 27.
(24) F. J. Philpot and J. L. Philpot, *Biochem. J.*, **30**, 2191 (1936).
(25) F. Nakeya, N. Yata, A. Kamada, and M. Aoki, *Chem. Pharm. Bull.*, **17**, 2000 (1969).
(26) D. M. Travis, C. Wiley, and T. H. Maren, *J. Pharmacol. Exp. Ther.*, **151**, 464 (1966).
(27) G. F. Holland, W. M. McLamore, and G. D. Laubach, U.S. Patent 3 165 550 (1965).
(28) M. J. Parry and M. J. Randall, unpublished results.
(29) D. A. Stopher, *Proc. Anal. Div. Chem. Soc.*, **131**, 354 (1976).
(30) A. M. Harper, unpublished results.
(31) E. Skinhoj, "Proceedings of the 7th International Symposium on Cerebral Blood Flow and Metabolism", Harper, Jennett, Miller, and Rowan, Ed., Edinburgh, Nov 7, 1975.
(32) D. J. Wyper, C. J. McAlpine, K. Jawad, and B. Jennet, *J. Neurol., Neurosurg. Psychiatry*, **39**, 885 (1976).
(33) V. Petrow, O. Stevenson, and A. M. Wild, *J. Pharm. Pharmacol.*, **12**, 705 (1960).

(34) H. Heidrich, G. Faust, W. Fielder, D. Lehmann, and W. Poepel, *Chem. Abstr.*, **70**, 77557q (1969).
(35) E. Cohen, B. Klarberg, and J. R. Vaughan Jr., *J. Am. Chem. Soc.*, **82**, 2731 (1960).
(36) E. Cingolani, *Gazz. Chim. Ital.*, **78**, 275 (1948).
(37) W. T. Caldwell and A. N. Sayin, *J. Am. Chem. Soc.*, **73**, 5125 (1951).
(38) G. Ziegler and J. N. Sprague, Belgium Patent 756626 (1969).

Design of Potent Antagonists of the Vasopressor Response to Arginine-vasopressin

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As part of a program in which we are attempting to design and synthesize antagonists of the vasopressor response to arginine-vasopressin (AVP), [1-deaminopenicillamine]arginine-vasopressin (dPAVP), [2-(*O*-methyl)tyrosine]-arginine-vasopressin [Tyr(Me)AVP], and [1-deaminopenicillamine,2-(*O*-methyl)tyrosine]arginine-vasopressin [dPTyr(Me)AVP] were synthesized by the solid-phase method and assayed for vasopressor, antidiuretic, and oxytocic activities. Tyr(Me)AVP has a vasopressor potency of 9.7 ± 0.5 units/mg and an antidiuretic potency of 386 ± 36 units/mg. These values are 2.5 and 120%, respectively, of the corresponding potencies of AVP. The analogue is an antagonist of the in vitro response to oxytocin (pA_2 = 7.44 ± 0.12). dPAVP has an antivasopressor pA_2 of 7.45 ± 0.11. Its antidiuretic potency is 42.2 ± 2 units/mg, 2.5% that of its parent, 1-[deamino]arginine-vasopressin (dAVP). It is an antagonist of the in vitro response to oxytocin (pA_2 value = 6.93 ± 0.10). dPTyr(Me)AVP has an antivasopressor pA_2 of 7.96 ± 0.05 and an antidiuretic potency of 3.5 ± 0.5 units/mg. It is also an antagonist of the in vitro oxytocic response to oxytocin (pA_2 value = 7.61 ± 0.14). It is thus one of the most potent vasopressor antagonists reported to date.

We recently described the synthesis and properties of [1-deaminopenicillamine,4-valine,8-D-arginine]vasopressin (dPVDAVP).[1] This peptide was designed to explore the possibility that the substitution of two methyl groups for the two hydrogens on the β-carbon at position 1 of [1-deamino,4-valine,8-D-arginine]vasopressin (dVDAVP) might convert this potent antidiuretic agonist into an antagonist of the antidiuretic response. A similar substitution in oxytocin[2] and in deamino-oxytocin[2] had converted these oxytocic agonists into potent antagonists of the in vitro oxytocic response. Furthermore, [1-deaminopenicillamine]oxytocin was shown to be a weak antagonist of the vasopressor response to lysine-vasopressin (LVP).[3] Although not an antagonist of the antidiuretic response, dPVDAVP was found to be one of the most potent antagonists of the vasopressor response to arginine-vasopressin (AVP) yet reported.[1]

The high antivasopressor potency of dPVDAVP encouraged us to undertake a systematic investigation of those structural changes in AVP which would facilitate the design of analogues of AVP possessing potent and selective antivasopressor properties. The approach we have followed is based essentially on that utilized in the design of dVDAVP[4] and of [4-threonine,7-glycine]oxytocin,[5] peptides which exhibit highly potent and selective antidiuretic and oxytocic activities, respectively. These peptides were designed by combining in each one those structural changes which individually in analogues of AVP and oxytocin enhanced antidiuretic and oxytocic selectivity relative to AVP and oxytocin, respectively. Our objective then was (a) to determine which structural changes in AVP would individually produce analogues possessing reduced or antagonistic vasopressor properties and (b) to combine these in one molecule in the hope that their effects would be additive and thereby produce an antagonist with greater antivasopressor potency than the individual parent analogues. In this report we present the synthesis and some pharmacological properties of three analogues designed in this fashion: [1-deaminopenicillamine]arginine-vasopressin (dPAVP), [2-(*O*-methyl)tyrosine]arginine-vasopressin [Tyr(Me)AVP], and [1-deaminopenicillamine,2-(*O*-methyl)tyrosine]arginine-vasopressin [dPTyr(Me)AVP]. The rationale for selecting these three to start with was as follows.

(a) Penicillamine Substitution. The penicillamine residue has been substituted in oxytocin,[2] in deamino-oxytocin,[2] and in dVDAVP[1] but it has not been substituted in AVP, in LVP, or in their deamino derivatives. In dVDAVP, as we have seen, it led to a dramatic and selective increase in antivasopressor properties. As a starting point for these studies, therefore, it seemed highly appropriate to determine its effects on the properties of the highly active antidiuretic agonist, [1-deamino]arginine-vasopressin (dAVP).[6,7] dPAVP was chosen for synthesis on this basis.

(b) *O*-Methyltyrosine Substitution. Methylation of the phenolic hydroxyl group of the tyrosine residue at position 2 in oxytocin, besides leading to antioxytocic properties, also gave rise to weak antivasopressor activity in the resultant [2-(*O*-methyl)tyrosine]oxytocin.[8-11] This same substitution in LVP led to a drastic reduction in vasopressor potency [from 258 units/mg in LVP to 2.4 units/mg in [2-(*O*-methyl)tyrosine]lysine-vasopressin[12-14]] coupled with marked tachyphylaxis of the vasopressor response to the analogue and inhibition of the response to vasopressin.[15] We were thus curious to determine the effects of the *O*-methyltyrosine substitution on the vasopressor properties of AVP. Tyr(Me)AVP was selected for synthesis on this basis.

(c) Combination of Penicillamine Substitution and 2-*O*-Methyltyrosine Substitution. No analogue of oxytocin or of vasopressin containing the combination of these two changes has been reported. We were intrigued by the possibility that their effects might be additive. If so, we would produce a peptide with even greater antivasopressor potency than either dPAVP or Tyr(Me)AVP.

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