Cerebrovasodilatation through Selective Inhibition of the Enzyme Carbonic Anhydrase. 3. 5-(Arylthio)-, 5-(Arylsulfinyl)-, and 5-(Arylsulfonyl)thiophene-2-sulfonamides

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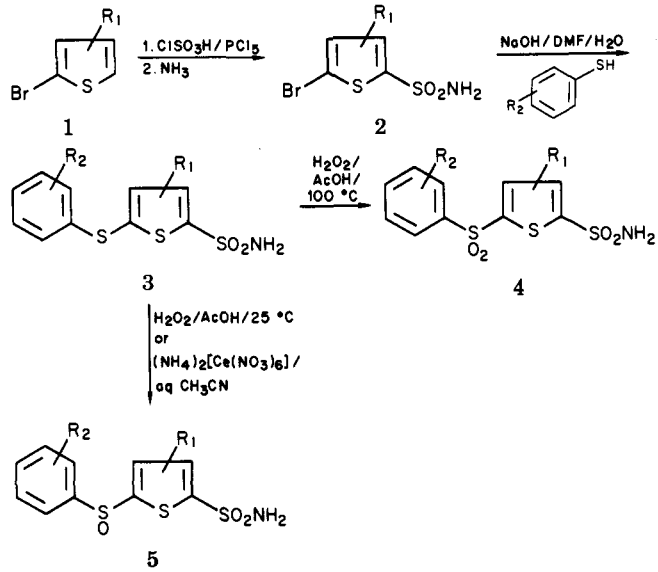
A series of 5-(arylthio)-, 5-(arylsulfinyl)-, and 5-(arylsulfonyl)thiophene-2-sulfonamides is described and anticonvulsant activities are listed for the compounds. In most cases, the sulfones had the highest activity and the sulfides the least. Sulfones with 3- or 4-halo substituents generally had the highest activity, and one analogue, 5-[(4-fluorophenyl)sulfonyl]thiophene-2-sulfonamide (**51**, UK-17022), had an anticonvulsant ED_{50} of 2 mg/kg when administered orally to mice. Compound **51** selectively increased cerebral blood flow in animals without an unacceptable level of diuresis.

Previous publications[1,2] have described how certain sulfonamide carbonic anhydrase inhibitors can increase carbon dioxide levels locally by inhibition of brain and/or erythrocyte carbonic anhydrase, thereby selectively increasing cerebral blood flow. This method of increasing cerebral blood flow has the advantage of being able to overcome autoregulation[3,4] without a decrease in blood pressure,[5] the only limiting factor being the responsiveness of the cerebral vasculature. The carbonic anhydrase inhibitor acetazolamide has been reported to increase cerebral blood flow in patients with cerebrovascular disease[5-7] but, since the compound is a potent inhibitor of kidney carbonic anhydrase, increases in cerebral blood flow can only be achieved at doses which cause marked diuresis[8] and, eventually, metabolic acidosis.

Many primary sulfonamides also exhibit anticonvulsant activity by virtue of their ability to cause enzyme inhibition in brain and/or erythrocytes.[9,10] Sulfonamides only inhibit carbonic anhydrase in their ionized form[11] but, since compounds pass through lipid barriers mostly in their un-ionized form, several potent carbonic anhydrase inhibitors do not cross the blood–brain barrier readily. However, an increase in lipophilicity can help to increase the ability of a compound to cross membranes. Thus, methazolamide (log P = 0.2) enters the brain more readily than acetazolamide (log P = –0.25) and shows considerably greater anticonvulsant activity, although the pK_a values of the two compounds are similar. In a previous publication[2] we showed that 6-*tert*-butyl-2-sulfamoylimidazo[2,1-*b*]-1,3,4-thiadiazole (UK-15454), which had a pK_a comparable with methazolamide but a much higher lipophilicity (log P = 2.15), was some 4 to 5 times more potent as an anticonvulsant.

In addition, we have reported[1] a series of substituted 1,4-benzenedisulfonamides, several of which are potent anticonvulsants. One of these compounds, 4-[(4-methoxypiperidino)sulfonyl]-2-chlorobenzenesulfonamide (UK-12130), also caused a selective increase in cerebral blood flow in man and animals.

Scheme I



Anticonvulsant activity is also associated with benzenesulfonamides containing a 4-(alkylsulfonyl) or 4-(arylsulfonyl) substituent.[12] 2-Amino-4-(benzenesulfonyl)benzenesulfonamide is reported to be a more potent anticonvulsant but a weaker diuretic than acetazolamide.[13]

Thiophene-2-sulfonamide is known to be at least as potent as benzenesulfonamide as a carbonic anhydrase inhibitor[8] and several simple derivatives have been reported to have diuretic activity.[14] As part of our program of preparing novel sulfonamides as anticonvulsants and cerebral vasodilators, we decided to prepare a series of 5-(arylsulfonyl)thiophene-2-sulfonamides. It was hoped that the increase in lipophilicity on introduction of the arylsulfonyl substituent would favorably influence the anticonvulsant activity relative to the diuretic activity.

Results and Discussion

Chemistry. (Arylthio)thiophenesulfonamides (**3**; Scheme I) were generally prepared by treatment of a 5-bromothiophene-2-sulfonamide (**2**) with an appropriate

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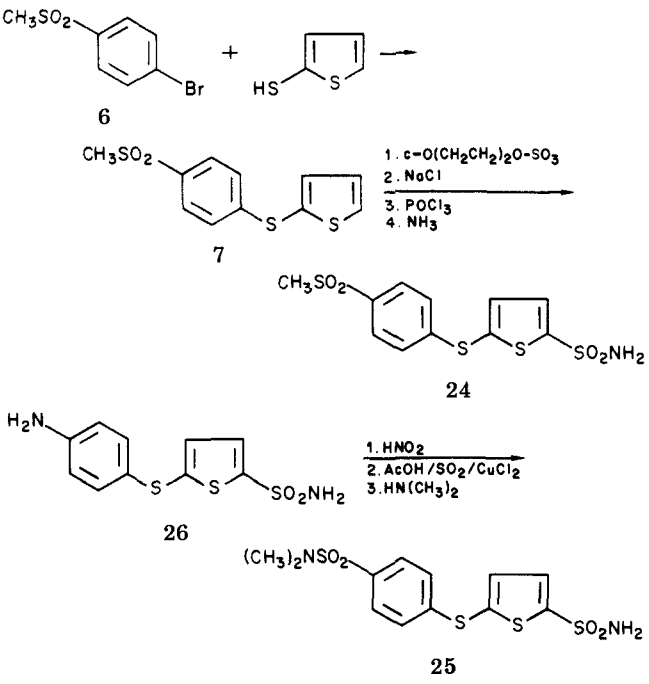
Scheme II



aryl thiol in aqueous dimethylformamide in the presence of sodium hydroxide.

The bromothiophenesulfonamides **2** were prepared from the corresponding bromothiophene **1** by treatment with a mixture of chlorosulfonic acid and phosphorus pentachloride, followed by ammonia. The substituted bromothiophenes are all known with the exception of 2-bromo-3-chlorothiophene, which was prepared by bromination of 3-chlorothiophene with *N*-bromosuccinimide in a mixture of chloroform and acetic acid. All the aryl thiols are known compounds with the exception of 4-isobutyrylaminothiophenol, which was prepared by treatment of 4,4′-diaminodiphenyl disulfide with isobutyric anhydride, followed by reduction of the product with zinc dust in acetic acid.

For the preparation of 5-[[(4-(methanesulfonyl)-phenyl]thio]thiophene-2-sulfonamide (**24**), 4-bromophenyl methyl sulfone was treated with thiophene-2-thiol in aqueous dimethylformamide in the presence of potassium hydroxide, and the product **7** was then treated successively with sulfur trioxide/dioxan and NaCl, followed by phosphorus oxychloride and ammonia.

For the preparation of the 4-(dimethylsulfamoyl) analogue **25**, the amine **26** was converted to the corresponding sulfonyl chloride by the method of Meerwein et al.,[15] and this was then treated with dimethylamine (Scheme II). Sulfoxides **4** were prepared from the sulfides either by oxidation with hydrogen peroxide in acetic acid at room temperature or with ceric ammonium nitrate according to the method of Ho and Wong.[16] Sulfones **5** were prepared by oxidation of the sulfides with hydrogen peroxide in hot acetic acid. The only exception was the 4-amino compound **61**, which was prepared by acid hydrolysis of the corresponding isobutyrylamino analogue **65**.

4-(Phenylsulfonyl)benzenesulfonamide (**68**),[12] required for comparison purposes, was prepared by oxidation of 4-(phenylthio)benzenesulfonamide.

Biology. The anticonvulsant activity of primary sulfonamides in mice depends on inhibition of the enzyme in brain or erythrocytes, or both, the factors determining the importance of the enzyme at each site being the potency of the drug and its comparative ease of entry into both brain and erythrocytes.[10] In order to identify compounds that might increase cerebral blood flow by raising carbon dioxide levels via carbonic anhydrase inhibition, anticonvulsant activity was measured by means of the mouse electroshock test. The anticonvulsant ED_{50} values of the (arylsulfonyl)thiophene-2-sulfonamides and the related sulfides and sulfoxides are listed in Table I, together with the ED_{50} values obtained for the standard compound **69**.

The in vitro inhibition of carbonic anhydrase was determined using enzymes prepared from mouse erythrocytes, and the results are shown in Table II. The assay was based on the method of Philpot and Philpot.[17]

As may be seen, an increase in activity results from replacement of the phenyl ring of **68** by thiophene (**43**). The sulfides were weakly active, the most notable exceptions being the 4-Cl (**18**) and 4-SCH_3 (**22**) analogues. However, oxidation of the sulfide linkage to sulfoxide improved potency in almost all cases. For example, the sulfoxide **32** was some 12 times more potent than its corresponding sulfide **8**. In general, the 3- or 4-halo compounds such as **34–38** were the most potent. Replacing a 4-halo group by 4-OCH_3 (**39**) or 4-CH_3 (**42**) was unfavorable.

Oxidation of the sulfide linkage to sulfone resulted in potent compounds in almost every case. Once again the 3- or 4-halo substituents were preferred and the 4-F analogue **51** was 4 times more potent as an anticonvulsant agent than the standard compound **69**. Halo substitution in the 4 position of the thienyl ring, e.g., 4-Cl (**45**) and 4-Br (**46**), led to potent compounds, but the 3-Cl analogue (**63**) was very weak; this was in marked contrast to its corresponding sulfide **28**. A bulky lipophilic group at the 4 position of the phenyl ring was unfavorable for activity, e.g., **49** (4-*tert*-butyl).

Consideration of all the compounds in Table I led to the selection of **51** for further evaluation. **51** (UK-17 022) is moderately ionized at physiological pH (11.2%; pK_a = 8.29) and has a log *P* value of 1.81 (octanol–water). Table II shows that these thienylsulfonamides are more potent than the standard compound **69** as in vitro inhibitors of the carbonic anhydrase enzyme and most are slightly more potent than acetazolamide. Acute intravenous diuretic experiments in dogs showed that a dose of 2.5 mg/kg of **51** was 2.2 times more diuretic than the same dose of **69**, with respect to increases in urine volume and pH and in K^+ and HCO_3^- output.

In three conscious beagle dogs, intravenous administration of 1.0 and 2.5 mg/kg of **69** produced no increase in vertebral blood flow at the lower dose and a maximum 23.5 ± 3.5% increase at the higher dose. In four dogs, intravenous doses of 1.0, 2.5, and 5 mg/kg of **51** increased vertebral blood flow by 27 ± 3.2, 36 ± 4.0, and 46 ± 6.4%. The extent and duration of the increase in flow were ascertained by determining the area under the flow curves at the three iv doses. By this method, **51** was found to be 4.6 times superior to **69** in increasing vertebral blood flow. Oral administration of 10 mg/kg of **51** to three dogs produced a 58 ± 5.3% maximum increase in flow, the effect lasting for just over 4 h. In two anesthetized cats, iv administration of 2.5 mg/kg of **51** increased vertebral blood flow by a maximum of 50 ± 7%, the effect lasting for 2 h. At this dose there were no appreciable changes in

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femoral blood flow, blood pressure, or heart rate.

Conclusions

Replacement of the benzene ring of **68** by thiophene (**43**) leads to an increase in anticonvulsant activity. 5-(Arylsulfonyl)thiophene-2-sulfonamides, in general, are potent anticonvulsants and, with few exceptions, the corresponding arylthio analogues are markedly less active. As with previously reported primary sulfonamides, a high level of anticonvulsant activity is associated with cerebral vasodilator activity. Thus, **51** causes an increase in cerebral blood flow without producing an unacceptable level of diuresis.

Experimental Section

Pharmacology. (b) Electroshock Test.[17] 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 (2.5, 6.25, and 16 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 milligrams per kilogram) for each compound.

(b) Blood Flow and Diuresis. 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 central midline incision in the neck, and the probe was 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 flows were 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.

The diuretic effects of **51** and 4-[(4-methoxypiperidino)-sulfonyl]-2-chlorobenzenesulfonamide (**69**) were also assessed in dogs during vertebral flow measurements. Urine was collected vis a urethral catheter 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) Carbonic Anhydrase Inhibition Methodology. In vitro carbonic anhydrase activity was determined by a modification of the colorimetric method of Philpot and Philpot.[17] 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 an ice bath. The inhibitors were preincubated with the enzyme for 5 min prior to the addition of substrate. This procedure allowed for enzyme–inhibitor equilibrium to take place. The concentration of compound required to inhibit 50% of the enzyme activity was determined graphically. Approximately 2 units of enzyme activity was 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 a solution in Me_2SO-d_6 unless otherwise stated. The IR spectra were obtained with a Perkin-Elmer 237 spectrophotometer and the ^1H NMR spectra were obtained with a Varian Associates spectrometer Model A-60A.

2-Bromo-3-chlorothiophene (1, R_1 = 3-Cl). A solution of 3-chlorothiophene (26.5 g, 0.22 mol) and *N*-bromosuccinimide (40.0 g, 0.22 mol) in $CHCl_3$ (125 mL) and AcOH (125 mL) was heated under reflux for 1 h. After cooling, the solution was poured into H_2O, and the organic phase was separated, washed successively with dilute KOH solution and H_2O, and then dried over Na_2SO_4. After filtration, the solvent was evaporated off to give an oil, which was fractionally distilled to give pure 2-bromo-3-chlorothiophene: yield 24.0 g (55%); bp 73–75 °C (12 mm); NMR (neat liquid) δ 7.00 (d, 1, H-5, $J_{4,5}$ = 5.7 Hz), 6.65 (d, 1, H-4). Anal. (C_4H_2BrClS) C, H.

4,5-Dibromothiophene-2-sulfonamide (2, R_1 = 4-Br). Phosphorus pentachloride (41.6 g, 0.2 mol) was added portionwise with stirring to chlorosulfonic acid (58.2 g, 0.5 mol), and the resultant solution was cooled to 0 °C. 2,3-Dibromothiophene (48.4 g, 0.2 mol) was added with stirring over 15 min, and the resultant mixture was heated on a steam bath for 30 min. The mixture was then poured onto ice, and the solid was filtered off, washed with a small amount of H_2O, and dissolved in the minimum volume of acetone. The solution was cooled in ice and an excess of concentrated aqueous ammonia solution was added. The mixture was then diluted with water (1500 mL), and the precipitate was filtered off and recrystallized from MeOH/H_2O to give the pure product **2** (R_1 = 4-Br): yield 40.0 g (62%); mp 181–184 °C. Anal. ($C_4H_3Br_2NO_2S_2$) C, H, N.

The following dihalothiophene-2-sulfonamides were prepared in the same manner: **2** (R_1 = 3-Br), mp 135–138 °C. Anal. ($C_4H_3Br_2NO_2S_2$) C, H, N. **2** (R_1 = 3-Cl), mp 134–136 °C. Anal. ($C_4H_3ClBrNO_2S_2$) C, H, N. **2** (R_1 = 4-Cl), mp 159.5–161.5 °C. Anal. ($C_4H_3ClBrNO_2S_2$) C, H, N.

4,4′-Bis(isobutyrylamino)diphenyl Disulfide (71). 4,4′-Diaminodiphenyl disulfide (25 g, 0.1 mol) was added portionwise to isobutyric anhydride (25 mL) to give a clear solution which afterwards precipitated a solid. The mixture was heated with stirring on the steam bath for 10 min, cooled, and diluted with toluene. The solid was filtered off, washed with petroleum ether (40–60 °C), dried, and recrystallized from MeOH to give 4,4′-bis(isobutyrylamino)diphenyl disulfide: yield 15.6 g (40%); mp 192–193 °C. Anal. ($C_{20}H_{24}N_2O_2S_2$) C, H, N.

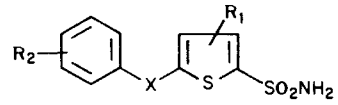
4-(Isobutyrylamino)thiophenol (72). A mixture of 4,4′-bis(isobutyrylamino)diphenyl disulfide (14.5 g, 0.037 mol), zinc dust (7.0 g), and AcOH (100 mL) was heated on a steam bath for 4 h. The hot solution was decanted from unreacted zinc and evaporated. The glassy residue was then triturated with cold dilute KOH solution and the mixture was filtered. The filtrate was acidified by the dropwise addition of concentrated HCl, and the resultant solid was filtered off, washed with H_2O, and dried to give **72**: yield 10.5 g (72%); mp 142–144 °C. Anal. ($C_{10}H_{13}NOS$) C, H, N.

5-(Phenylthio)thiophene-2-sulfonamide (8). To a solution of thiophenol (11.0 g, 0.1 mol) in DMF (250 mL) was added a solution of NaOH (4.0 g) in H_2O (20 mL), followed by 5-bromothiophene-2-sulfonamide (24.2 g, 0.1 mol), and the mixture was heated under reflux for 4 h. The solvent was then evaporated off under reduced pressure and the residue was poured into H_2O. The mixture was extracted with ether (4 × 100 mL), and the combined ethereal extracts were washed with H_2O, dried, and evaporated to give an oil which solidified on standing. Recrystallization of the solid from EtOAc/petroleum ether (40–60 °C) gave **8**: yield 13.7 g (50.7%); mp 98–100 °C. Anal. ($C_{10}H_9NO_2S_3$) C, H, N.

This method was used for the preparation of compounds **9–23** and **26–29** (Table I), starting from the appropriately substituted bromothiophenesulfonamides and -thiophenols.

5-[[4-(Dimethylsulfamoyl)phenyl]thio]thiophene-2-sulfonamide (25). A solution of sodium nitrite (0.78 g) in the minimum volume of H_2O was added to a stirred solution of 5-[(4-aminophenyl)thio]thiophene-2-sulfonamide (**26**) (12.86 g, 0.045 mol) in a mixture of THF (50 mL) and concentrated HCl (20 mL) at 0 °C, followed by magnesium chloride (1.6 g). The

Table I. Chemical Data and Anticonvulsant Activity



no.	X	R_1	R_2	mp, °C	recrystn solvent[a]	mol formula[b]	% yield	ED_{50}, mg/kg po, protection against max electroshock in mice[c]	ED_{50} (69)/ED_{50} (compd) anticonvulsant potency ratio relative to standard compound[d]
8	S	H	H	98–100	EtOAc–PE	$C_{10}H_9NO_2S_3$	50	19	0.3
9	S	3-Cl	H	95.5–97.5	EtOAc–PE	$C_{10}H_8ClNO_2S_3$	46	I	
10	S	4-Cl	H	128–130	*i*-PrOH–PE	$C_{10}H_8ClNO_2S_3$	60	I	
11	S	4-Br	H	124–126	benzene–PE	$C_{10}H_8BrNO_2S_3$	52	I	
12	S	H	2-CH_3	72–73	benzene–PE	$C_{11}H_{11}NO_2S_3$	77	>16	<0.5
13	S	H	4-CH_3	120.5–122.5	EtOAc–PE	$C_{11}H_{11}NO_2S_3$	56	>40	<0.2
14	S	H	4-$C(CH_3)_3$	73–74	benzene–PE	$C_{14}H_{17}NO_2S_3$	56	I	
15	S	H	3-F	70–72	CCl_4	$C_{10}H_8FNO_2S_3$	56	>6	<1.3
16	S	H	4-F	106–107	EtOAc–PE	$C_{10}H_8FNO_2S_3$	59	7	1.0
17	S	H	3-Cl	107–108	EtOAc–PE	$C_{10}H_8ClNO_2S_3$	77	>6	<1.2
18	S	H	4-Cl	137–139	EtOAc–PE[e]	$C_{10}H_8ClNO_2S_3$	41	3	2.0
19	S	H	4-Br	143–144	EtOAc–PE[e]	$C_{10}H_8BrNO_2S_3$	49	>6	<1.2
20	S	H	3-CF_3	95–96	MeOH–H_2O	$C_{11}H_8F_3NO_2S_3$	42	6	1.3
21	S	H	4-OCH_3	98–99	EtOAc–PE	$C_{11}H_{11}NO_3S_3$	56	>40	<0.2
22	S	H	4-SCH_3	99–100	EtOAc–PE	$C_{11}H_{11}NO_2S_4$	46	4	1.75
23	S	H	4-OH	124–126	H_2O	$C_{10}H_9NO_3S_3$	51	I	
24	S	H	4-SO_2CH_3	123–125	EtOH–H_2O	$C_{11}H_{11}NO_4S_4$	10	5	1.4
25	S	H	4-$SO_2N(CH_3)_2$	104–106	EtOAc–PE	$C_{12}H_{14}N_2O_4S_4$	14	>6	<1.3
26	S	H	4-NH_2	134–135	MeOH–H_2O	$C_{10}H_{10}N_2O_2S_3$	64	7	1.0
27	S	H	4-$NHCOCH_3$	154–155	EtOAc	$C_{12}H_{12}N_2O_3S_3$	51	9	1.1
28	S	3-Cl	4-$NHCOCH_3$	190–193	EtOH–PE	$C_{12}H_{11}ClN_2O_3S_3$	34	5	1.4
29	S	4-Cl	4-$NHCOCH_3$	167–168	EtOAc–PE	$C_{12}H_{11}ClN_2O_3S_3$	57	I	
30	S	H	4-$NHCOCH(CH_3)_2$	184–185	EtOAc–PE	$C_{14}H_{16}N_2O_3S_3$	67	I	
31	S	H	3,4-CH=CHCH=CH	156	EtOAc	$C_{14}H_{11}NO_2S_3$	37	I	
32	SO	H	H	130–131	*i*-PrOH–PE	$C_{10}H_9NO_3S_3$	47[h]	2	3.5
33	SO	4-Cl	H	117–119	*i*-PrOH–PE	$C_{10}H_8ClNO_3S_3$	66[h]	>6	<1.3
34	SO	H	3-F	110–111	$CHCl_3$–PE	$C_{10}H_8FNO_3S_3$	65[h]	2.5	3.2
35	SO	H	4-F	123–124	$CHCl_3$	$C_{10}H_8FNO_3S_3$	79[i]	3	2.0
36	SO	H	3-Cl	164–165	$CHCl_3$	$C_{10}H_8ClNO_3S_3$	85[i]	4	1.75
37	SO	H	4-Cl	140–141	$CHCl_3$	$C_{10}H_8ClNO_3S_3$	62[i]	2	3.5
38	SO	H	4-Br	134–135	$CHCl_3$	$C_{10}H_8BrNO_3S_3$	68[i]	5	1.4
39	SO	H	4-OMe	116–118	*i*-PrOH–PE[f]	$C_{11}H_{11}NO_4S_3$	62[h]	>6	<1.3
40	SO	H	4-SO_2CH_3	153–156	EtOH	$C_{11}H_{11}NO_3S_3$	48[i]	>16	<0.4
41	SO	H	4-$NHCOCH_3$	195–197	MeOH–H_2O	$C_{12}H_{12}N_2O_4S_3$	38[h]	4	1.5
42	SO	H	4-CH_3	118–120	*i*-PrOH–PE	$C_{11}H_{11}NO_3S_3$	63[h]	>6	<1.3
43	SO_2	H	H	137.5–139	EtOAc–PE	$C_{10}H_9NO_4S_3$	75	2	3.5
44	SO_2	3-Cl	H	151–152	EtOAc–PE	$C_{10}H_8ClNO_4S_3$	72	33	0.2
45	SO_2	4-Cl	H	147–148	EtOAc–PE	$C_{10}H_8ClNO_4S_3$	72	3	2.3
46	SO_2	4-Br	H	186–188	EtOAc–PE	$C_{10}H_8BrNO_4S_3$	81	3	2.3
47	SO_2	H	2-CH_3	161–162	EtOH–H_2O	$C_{11}H_{11}NO_4S_3$	85	6	1.2
48	SO_2	H	4-CH_3	163.5–164.5	EtOAc–PE	$C_{11}H_{11}NO_4S_3$	67	8	0.8
49	SO_2	H	4-$C(CH_3)_3$	161–162	EtOAc–PE	$C_{14}H_{17}NO_4S_3$	73	I	
50	SO_2	H	3-F	155–156	EtOAc–PE	$C_{10}H_8FNO_4S_3$	62	2	3.5
51	SO_2	H	4-F	153–154	EtOH–H_2O	$C_{10}H_8FNO_4S_3$	79	2	4.0
52	SO_2	H	4-Cl	159–161	AcOH–H_2O	$C_{10}H_8ClNO_4S_3$	78	3	2.3
53	SO_2	H	4-Br	179–180	AcOH–H_2O	$C_{10}H_8BrNO_4S_3$	93	4	1.75

54	SO_2	H	3-CF_3	148	EtOAc-PE	$C_{11}H_8F_3NO_4S_3$	86	5	1.6
55	SO_2	H	4-OH	164–166	H_2O	$C_{10}H_9NO_5S_3$	70	I	
56	SO_2	H	4-OCH_3	126–127	EtOAc-PE	$C_{11}H_{11}NO_5S_3$	63	6	1.0
57	SO_2	H	4-SO_2CH_3	238–240	MEK-PE	$C_{11}H_{11}NO_6S_4$	72	4	1.75
58	SO_2	H	4-$SO_2N(CH_3)_2$	226–228	MEK-PE	$C_{12}H_{14}N_2O_6S_4$	74	I	
59	SO_2	H	3-$COCH_3$	138.5–140.5	EtOAc-Pe	$C_{12}H_{11}NO_5S_3$	83	3	2.3
60	SO_2	H	4-$CONH_2$	240–242	AcOH-H_2O	$C_{11}H_{10}N_2O_5S_3$	58	4	2.0
61	SO_2	H	4-NH_2	195–196	MeOH-H_2O	$C_{10}H_{10}N_2O_4S_3$	61	2	3.5
62	SO_2	H	4-$NHCOCH_3$	261–263	MeOH	$C_{12}H_{12}N_2O_5S_3$	56	2	4.0
63	SO_2	3-Cl	4-$NHCOCH_3$	230–233	AcOH-H_2O	$C_{12}H_{11}ClN_2O_5S_3$	67	>6	<1.3
64	SO_2	4-Cl	4-$NHCOCH_3$	120–125	acetone-H_2O	$C_{12}H_{11}ClN_2O_5S_3$	62	6	1.3
65	SO_2	H	4-$NHCOCH(CH_3)_2$	218–220	i-PrOH-PE	$C_{14}H_{16}N_2O_5S_3$	92	4	2.0
66	SO_2	H	3,4-CH=CHCH=CH	228–230	MeOH	$C_{14}H_{11}NO_4S_3$	71	8	0.75
67	SO_2	4-CH_3		183–184	EtOH	$C_{11}H_{11}NO_4S_3$	63	3	2.7
68				190–192[g]	EtOH-H_2O	$C_{12}H_{11}NO_4S_2$	40	10	0.9
69				(UK-12130)				8.40 ± 0.19 (n = 70)	1.0
70				(acetazolamide)				36	0.2

[a] i-PrOH, isopropyl alcohol; MEK, methyl ethyl ketone; PE, petroleum ether (refers to the fraction boiling between 60 and 80 °C unless otherwise indicated). [b] All compounds were analyzed for C, H, and N. [c] Compound 69 used as the standard in each determination; standard deviation ±1.68. [d] The ratio ED_{50} (69)/ED_{50} (compd) is based upon the ED_{50} value obtained for 69 during the separate electroshock experiments on each compound. [e] 80–100 °C petroleum ether. [f] 40–60 °C petroleum ether. [g] Literature[12] mp 182 °C. [h] Method A. [i] Method B.

Table II. In vitro Carbonic Anhydrase Activity (Erythrocytes) and pK_a Values

compd	concn, M, producing 50% inhibn	pK_a [b]
18	$3 \times 10^{-9} \pm 0.10$[a]	9.19
37	$2.6 \times 10^{-8} \pm 0.12$	8.57
46	$4.2 \times 10^{-9} \pm 0.12$	7.98
51	$9.0 \times 10^{-9} \pm 0.13$	8.29
68	$6.4 \times 10^{-8} \pm 0.10$	9.10
69	$1.9 \times 10^{-7} \pm 0.12$	8.76
acetazolamide	$2.0 \times 10^{-8} \pm 0.10$	7.4

[a] Standard deviation. [b] In water, except for 18 which was determined in 30% aqueous methanol.

resulting yellow suspension was warmed to 30 °C and added in one portion to a solution of sulfur dioxide (5 mL) and cupric chloride (0.6 g) in a mixture of AcOH (7.5 mL) and benzene (4 mL) at 30 °C. The mixture was stirred at room temperature for 30 min and poured into H_2O, and the resulting mixture was extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed well with H_2O, followed by Na_2CO_3 solution. Aqueous dimethylamine (25%, w/v, 25 mL) was added to the organic layer, and the mixture was stirred for 15 min, cooled in ice, and acidified with dilute HCl. The organic layer was separated and the aqueous layer was washed several times with EtOAc. The combined organic layer and extracts were washed with H_2O and dried over $MgSO_4$. Filtration and evaporation of the filtrate gave an oil which was chromatographed on silica gel. A small amount of impurity was eluted with toluene, and then elution with $CHCl_3$ gave an oil which solidified on scratching. Crystallization from EtOAc/petroleum ether (bp 40–60 °C) gave **25**: yield 2.0 g (14%); mp 104–106 °C. Anal. ($C_{12}H_{14}N_2O_4S_4$) C, H, N.

5-[[4-(Methylsulfonyl)phenyl]thio]thiophene-2-sulfonamide (24). KOH (2.8 g) in H_2O (10 mL) was added to a solution of thiophene-2-thiol (5.8 g, 0.05 mol) and 4-bromophenyl methyl sulfone (11.25 g, 0.048 mol) in DMF (50 mL), and the solution was heated under reflux for 7 h. After cooling, the solution was allowed to stand at room temperature overnight, poured into H_2O, and extracted with $CHCl_3$ (3 × 100 mL). The combined organic extracts were washed with H_2O and dried over $MgSO_4$, and after filtration the solvent was evaporated off to give a brown oil. The oil was dissolved in ether and the solution was decanted off from some tarry material. The ether solution was evaporated to give 2-[[4-(methylsulfonyl)phenyl]thio]thiophene (**7**) as an oil, which crystallized on standing.

The crude product **7** (7.5 g, 0.027 mol) was dissolved in $CHCl_3$ (100 mL) and added to a suspension of sulfur trioxide/dioxane complex (1 equiv) in 1,2-dichloroethane at 0 °C. The solution was stirred at room temperature for 1 h and then poured into H_2O. The aqueous layer was separated and saturated with NaCl. The solid was filtered off and dried to yield 5-[[4-(methylsulfonyl)-phenyl]thio]thiophene-2-sulfonic acid as the sodium salt. This was suspended in $POCl_3$ (10 mL), and the mixture was then heated to reflux for 1.5 h. The resulting mixture was cooled, poured into ice, and, when the ice had melted, was extracted with $CHCl_3$ (2 × 75 mL). The $CHCl_3$ extract was evaporated and the residue was dissolved in a small volume of acetone. An excess of concentrated ammonia solution was then added and the mixture was diluted with H_2O to give a gum, which solidified on standing. The solid was crystallized three times from aqueous EtOH to give **24**: yield 1.0 g (10% based on **7**); mp 123–125 °C. Anal. ($C_{11}H_{11}NO_4S_4$) C, H, N.

5-(Phenylsulfinyl)thiophene-2-sulfonamide (32). Method A. H_2O_2 (30%; 0.5 mL) was added to a solution of 5-(phenylthio)thiophene-2-sulfonamide **8** (1.0 g, 0.0037 mol) in AcOH (10 mL), and the solution was allowed to stand at room temperature for 3 days. The solution was poured into H_2O, and the solid was filtered off, washed with H_2O, dried, and crystallized from isopropyl alcohol/petroleum ether to give **32**: yield 0.50 g (47%); mp 130–131 °C. Anal. ($C_{10}H_9NO_3S_3$) C, H, N.

5-[(4-Chlorophenyl)sulfinyl]thiophene-2-sulfonamide (37). Method B. Ceric ammonium nitrate (11.1 g, 0.02 mol) was added in one portion to a solution of 5-[(4-chlorophenyl)thio]-thiophene-2-sulfonamide (**18**; 1.53 g, 0.005 mol) in 75% (v/v) aqueous acetonitrile (80 mL), and the solution was stirred at room

temperature for 20 min and then poured into H_2O (300 mL). The mixture was extracted with ether (3 × 100 mL), and the combined ethereal extracts were washed with H_2O and dried over $MgSO_4$. Filtration and evaporation of the solvent gave an oil. On trituration with petroleum ether (40–60 °C) the oil solidified, and crystallization from $CHCl_3$ gave the product **37**: yield 1.0 g (62%); mp 140–141 °C. Anal. ($C_{10}H_8ClNO_3S_3$) C, H, N.

Compounds **33–36** and **38–42** (Table I) were all prepared by either method A or B.

5-(Phenylsulfonyl)thiophene-2-sulfonamide (43). A solution of 5-(phenylthio)thiophene-2-sulfonamide (**8**; 5.0 g, 0.018 mol) and 30% H_2O_2 (5.0 mL) in AcOH (50 mL) was heated on a steam bath for 1 h. H_2O was added to the hot solution until crystallization commenced and the mixture was then allowed to cool. The product was filtered off, washed with H_2O, dried, and crystallized from EtOAc/petroleum ether (60–80 °C) to give **43**: yield 4.2 g (75%); mp 137.5–139 °C. Anal. ($C_{10}H_9NO_4S_3$) C, H, N. A polymorph of mp 170–172 °C was also obtained.

Compounds **44–60** and **62–67** (Table I) were all prepared by this method.

5-[(4-Aminophenyl)sulfonyl]thiophene-2-sulfonamide (61). A mixture of 5-[[4-(isobutyrylamino)phenyl]sulfonyl]thiophene-2-sulfonamide (**65**; 1.8 g, 0.005 mol) and 15% aqueous HCl (20 mL) was heated under reflux until solution was complete (2 h). After cooling, the solution was neutralized by the addition of solid $NaHCO_3$, and the resultant precipitate was filtered off and crystallized from MeOH/H_2O to give **61**: yield 0.9 g (61%); mp 195–196 °C. Anal. ($C_{10}H_{10}N_2O_4S_3$) C, H, N.

4-(Phenylsulfonyl)benzenesulfonamide (68). Diphenyl sulfide (18.6 g, 0.1 mol) was dissolved in $CHCl_3$ (50 mL) and chlorosulfonic acid (11.7 g) was added dropwise with cooling to the solution over a period of 15 min. The solution turned deep violet and a vigorous evolution of HCl occurred. After the solution was stirred at room temperature for 30 min, the $CHCl_3$ was evaporated to give an oil. PCl_5 (21 g) was added in portions to this oil, and the mixture was warmed on the steam bath for 10 min. The resultant clear solution was then evaporated, the residual oil was dissolved in $CHCl_3$, and the solution was washed with H_2O and dried ($MgSO_4$). The residue obtained by filtration and evaporation of the filtrate was added in portions to a stirred solution of concentrated ammonia (150 mL), and the resultant solid was filtered off and dissolved in ether. The ether solution was dried ($MgSO_4$) and filtered, and the filtrate was evaporated. The residue was crystallized from EtOH/H_2O to give 4-(phenylthio)benzenesulfonamide: yield 12.0 g (45%); mp 138–140 °C (lit.[18] mp 129–130 °C). 4-(Phenylthio)benzenesulfonamide (5.0 g, 0.02 mol) was added to 30% H_2O_2 (10 mL) in AcOH (50 mL), and the solution was heated on a steam bath for 1 h. The solution was then cooled in ice, and the resultant solid was filtered off and crystallized from EtOH/H_2O to give **68**: yield 2.2 g (40%); mp 190–192 °C (lit.[11] mp 182 °C).

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Novel Synthesis of (*S*)-1-[5-(Benzoylamino)-1,4-dioxo-6-phenylhexyl]-L-proline and Analogues: Potent Angiotensin Converting Enzyme Inhibitors

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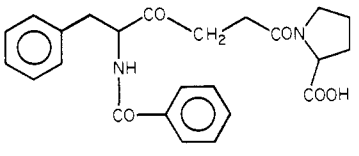
A new approach was developed for the synthesis of (*S*)-1-[5-(benzoylamino)-1,4-dioxo-6-phenylhexyl]-L-proline (**1**) and 23 analogues. The δ-(acylamino)-γ-keto acid intermediates were obtained by a modified Dakin–West reaction using 3-carbomethoxypropionyl chloride. Acylation of L-proline and recrystallization of the mixture of diastereomers gave the optically pure title compound in three reaction steps. The in vitro angiotensin converting enzyme (ACE) inhibitory activity of **1** was confirmed. Some of the novel analogues (**6**, **11**, **13**, and **17**) were also found to be potent inhibitors of ACE in vitro with an IC_{50} of 1.4–8.8 × 10^{-9} M (IC_{50} for captopril = 0.9 × 10^{-8} M). In vivo these compounds (**6**, **11**, **17**, and **18**) were much less active than captopril, especially by the oral route. Against angiotensin I (AI) challenge in normotensive conscious rats, **1** and **6** produced <50% inhibition at 30 mg/kg po but 57 to 82% inhibition at 3 mg/kg iv. Inhibition by both routes lasted <1 h. In renal hypertensive rats, **1** and **15** of its analogues failed to produce significant blood pressure lowering effects, in contrast to the marked effects of captopril. Near maximum inhibition of AI was achieved by continuous intravenous infusions of **1** and **20**, suggesting that limited oral activity may be due to degradation and/or clearance.

Angiotensin converting enzyme (ACE) inhibitors hold great promise in the treatment of hypertension.[1] Recently a very potent ACE inhibitor (**1**), an analogue of the tripeptide Bz-Phe-Gly-Pro, was disclosed[2] in which the NH of the amide portion of Phe-Gly was replaced by a methylene group.



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In the original synthesis[2] the key intermediate, (*S*)-δ-(benzoylamino)-γ-oxobenzenehexanoic acid, was obtained via a lengthy procedure in 11% yield. In order to explore the structure–activity relationsip of this series, a more

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