Table II. Hypotensive Activity of PAF and 1(S)-Me-PAF in Spontaneously Hypertensive Rats (SHR)[13]

drug	dose, mg/kg (po)	n^a	decrease in systolic blood pressure,[b] mmHg
C_{16}-PAF	0.3	5	4 ± 1.9
	3	5	5 ± 7.7
	25[c]	4	53 ± 3.9
1(S)-Me-PAF	0.1	5	35 ± 11.4
	1	4	69 ± 11.9
	10	5	*d*

[a] Number of SHRs in the experiments. [b] Values were given in mmHg 3 h after oral administration (po). [c] One of four animals was dead before measurements of blood pressure. [d] All SHRs used in the experiment were dead within 3 h after oral administration.

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Articles

Synthesis and Inhibitory Activity on Carbonic Anhydrase of Some New Sulpiride Analogues Studied by Means of a New Method

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The pharmacological activity of several new sulpiride analogues was studied by means of a new approach, based on a potentiometric technique with a pCO_2 sensor, capable of detecting carbonic anhydrase inhibition at equilibrium conditions. This procedure gives results stated as percent of inhibition of enzymatic activity (IP, inhibitory power). To prove the reliability of the proposed approach and to study structure–activity relationships, several new molecules were synthesized and tested in comparison with the two sulpiride enantiomers. A possible inhibition mechanism is discussed in terms of experimental evidence obtained from the interactions between the molecular structures of the new synthesized compounds and carbonic anhydrase.

Carbonic anhydrase (CA) is a well-known metalloenzyme[1,2] prevalent in plant and animal tissues as well as in bacteria; it is stable in solution under a wide range of conditions, for example from pH 4 to pH 11. Carbonic anhydrase in man is mainly located in the blood in a concentration of 1–2 g/L, but it is also present in many organs and tissues like the pancreas, kidneys, lungs, eyes, arterial walls, gastric mucosa, and the central nervous system. In mammalian red cells, the enzyme is constituted by a polypeptide chain with a molecular weight of about 30 000, and it contains one zinc atom per molecule.[2] Its role is to reversibly catalyze the so-called "hydration reaction" of carbon dioxide to carbonic acid (eq 1) at very

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^- \quad (1)$$

high rates. In particular, an "acid form" of the enzyme is required for the "dehydration", and a "basic form", for the "hydration" reaction. This implies a proton binding of the enzyme at some step of the catalytic pathway (eq 2), where

$$CO_2 + H_2O + E \rightleftharpoons HCO_3^- + EH^+ \rightleftharpoons HCO_3^- + E + H^+ \quad (2)$$

EH^+ and E represent respectively the acid and the basic forms of the enzyme.[2] Another possible aspect of these equilibrium reactions has recently been proposed[3] where the net classic reaction (1) is now the result of the addition of reactions 3 and 4.

$$EH_2O \rightleftharpoons EOH^- + H^+ \quad (3)$$

$$EOH^- + CO_2 + H_2O \rightleftharpoons HCO_3^- + EH_2O \quad (4)$$

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^- \quad (1)$$

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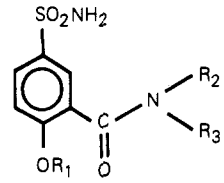
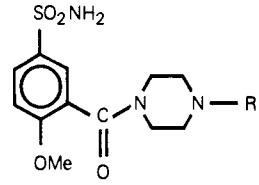
Table I. 2-Alkoxysulfamoylbenzamides



compd	R_1	R_2	R_3	method	mp, °C	recryst solvent	yield,[a] %	formula	anal.
2	Me	H	1-ethyl-3-piperidinyl	A	195–196	EtOH	30	$C_{15}H_{23}N_3O_4S$	C, H, N
3	Me	H	1-ethyl-4-piperidinyl	A	216–218	EtOH	35	$C_{15}H_{23}N_3O_4S$	C, H, N
4	Me	H	Et_2NCH_2 CH_2	B, C	191–192	EtOH	30	$C_{14}H_{23}N_3O_4S$	C, H, N
5	Me	H	$Me_2NCH_2CH(Me)$	A–C	202–203	*i*-PrOH	45	$C_{13}H_{21}N_3O_4S$	C, H, N
6	Me	H	$CH_2(CH_2CH_2)_2NCH_2CH_2$	B, C	230–231	DMF	43	$C_{15}H_{23}N_3O_4S$	C, H, N
7	Me	H	$O(CH_2CH_2)_2NCH_2CH_2CH_2$	B, C	162	EtOH	38	$C_{15}H_{23}N_3O_5S$	C, H, N
8	Me	Me	(*S*)-(1-ethyl-2-pyrrolidinyl)methyl	D	103–105	EtOH/C_6H_6	66	$C_{16}H_{25}N_3O_4S$	C, H, N
9	(*S*)-Et(Me)$CHCH_2$	H	(*S*)-(1-ethyl-2-pyrrolidinyl)methyl	A	158–159	*i*-PrOH	40	$C_{19}H_{31}N_3O_4S$	C, H, N
10	(*S*)-Et(Me)$CHCH_2$	H	(*R*)-(1-ethyl-2-pyrrolidinyl)methyl	A	157–158	*i*-PrOH	45	$C_{19}H_{31}N_3O_4S$	C, H, N

[a] Yield based on the last step.

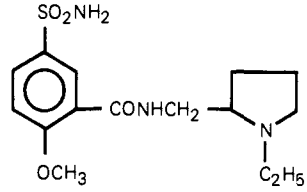
Table II. Piperazine Monoamides of 2-Methoxy-5-sulfamoylbenzoic Acid



compd	R	method	mp, °C	recryst solvent	yield,[a] %	formula	anal.
11	H	E	175 (HCl)	*i*-PrOH	81	$C_{12}H_{17}N_3O_4$·HCl	C, H, N
12	CH_2=$CHCH_2$	A	104–105	EtOAc	35	$C_{15}H_{21}N_3O_4S$	C, H, N
13	Me_2CHCH_2	A	147–149	*i*-PrOH	60	$C_{16}H_{25}N_3O_4S$	C, H, N
14	$CH_2(CH_2CH_2)_2CHCH_2$	A	110–112	EtOAc/C_6H_{12}	40	$C_{19}H_{29}N_3O_4S$	C, H, N
15	Et(Me)$CHCH_2$	A	184–186 (HCl)	*n*-BuOH	65	$C_{17}H_{27}N_3O_4S$·HCl	C, H, N
16	$EtOOCCH_2$	A	160–162	EtOH	40	$C_{16}H_{23}N_3O_6S$	C, H, N
17	(1-pyrrolidinylcarboxy)methyl	A	205–206	EtOH	60	$C_{18}H_{26}N_4O_5S$	C, H, N
18	$Me_2CHNHC(=O)CH_2$	A	225–228	EtOH	48	$C_{17}H_{26}N_4O_5S$	C, H, N
19	$PhCH_2$	A	186–187	EtOH	45	$C_{19}H_{23}N_3O_4S$	C, H, N
20	*p*-methoxybenzyl	A	160–162	*i*-PrOH	65	$C_{20}H_{25}N_3O_5S$	C, H, N
21	*m*-methoxybenzyl	A	178–179	*i*-PrOH	67	$C_{20}H_{25}N_3O_5S$	C, H, N
22	3,4,5-trimethoxybenzyl	F	182–184	EtOH	45	$C_{22}H_{29}N_3O_7S$	C, H, N
23	*p*-sulfamoylbenzil	F	184–185	MeOH	69	$C_{19}H_{24}N_4O_6S_2$	C, H, N

[a] Yield based on the last step.

Sulpiride[4] (1), a 2-methoxy-5-sulfamoylbenzamide, is a drug belonging to the class of orthopramides; among them,



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methochlopramide[5] was the first to be used therapeutically. It exhibits antidepressant[6] and antipsychotic[7] activities with no extrapyramidal side effects; moreover, it possesses antiemetic properties[8] and stimulates gastrointestinal motility.[4] From several points of view,[9] the pharmacological activity of sulpiride is so peculiar that it is considered an atypical neuroleptic,[10] mainly because it does not block the dopamine effects on the dopamine-sensitive adenilcyclase in the rat brain.[11]

It has recently been shown that sulpiride displays inhibitory power on different types of carbonic anhydrases[12,13] (particularly the *S*-(−) enantiomer displays a higher inhibitory activity than the *R*-(+) one[14]). This was easily

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predictable since it has been shown that aromatic or heterocyclic sulfonamides are very specific inhibitors of such enzymes[15] if the sulfamoyl group is not substituted.[16]

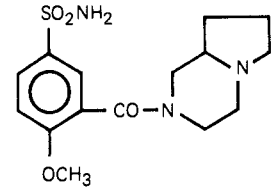
The purpose of this work is to show how both steric and structural changes of the sulpiride molecule are responsible for modification of the inhibitory activities of the new synthesized compounds related to sulpiride. This project was undertaken by using a recently developed method[12] that is based on a potentiometric technique with a pCO_2 electrode and proposes results stated as percent of inhibition of enzymatic activity (IP, inhibitory power). The results obtained with this method are not easily comparable to those obtained from previous techniques[17,18] since throughout our new procedure all thermodynamic parameters (pH, ionic strength, temperature, pressure, etc.) were fixed and kept constant, thus enabling us to draw conclusions derived exclusively from kinetic data. Despite the difficulties inherent in comparison with results obtained from other methods, a few key compounds were nonetheless tested according to Maren's procedure.[18a] The outcome of such comparisons revealed that results were in agreement within the limits of methodological differences.

Chemistry

The syntheses of the benzamides listed in Tables I and II were accomplished by usual methods, primary among them being the condensation of the 2-alkoxy-5-sulfamoylbenzoic acid with the suitable amine in pyridine solution in the presence of PCl_3[19] (method A). In some cases the formation of the amidic bond was achieved by heating at 120 °C for several hours an equimolar mixture of ethyl ester of 2-methoxy-5-sulfamoylbenzoic acid and the suitable amine[20] (method B) or by heating the mixture in diethylene glycol at 80 °C for a couple of days (method C).

Compound **8** was prepared by reacting the corresponding amine with the mixed anhydride obtained from 2-methoxy-5-sulfamoylbenzoic acid and diphenylphosphinyl chloride[21] in the presence of triethylamine (method D). By hydrogenolysis in the presence of Pd/C, compound **19** was transformed into compound **11** (method E) and the latter into compounds **22** and **23** by alkylation with 3,4,5-trimethoxybenzyl chloride and with 4-sulfamoylbenzyl chloride, respectively (method F).

Method A was also followed in preparing (i) compound **26**, starting from 1,4-diazabicyclo[4.3.0]nonane (**29**) (obtained by a new procedure) and (ii) compound **28**, by utilizing 1-benzylpiperazine[22] and 2-sulfamoyl-4-imidazolecarboxylic acid.[23] The synthesis of compound **27** was performed by alkylation of 1-benzylpiperazine with 2-methoxy-5-sulfamoylbenzyl chloride (**30**).



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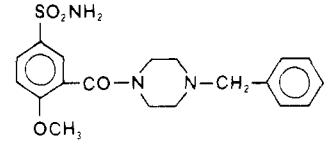

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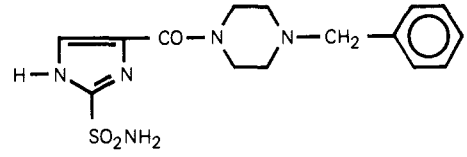

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Several amines used for the preparation of the compounds mentioned in this work were commercially available; others we prepared. The (*S*)-1-ethyl-2-(aminomethyl)pyrrolidine was obtained by the reduction with $LiAlH_4$ of (*S*)-1-acetylproline, treatment of the primary alcohol with $SOCl_2$, and then reaction of the chloro intermediate with ammonia.[24]

(*R*)-1-Ethyl-2-(aminomethyl)pyrrolidine was obtained by separation of racemic 1-ethyl-2-(aminomethyl)-pyrrolidine with (*R*,*R*)-tartaric acid.[25]

The piperazine acylation, unlike its alkylation, generally does not yield monoacyl derivatives in good amounts[26,27] except in the case of reactions with chlorocarbonates.[27] The amines we prepared in order to synthesize the compounds reported in Table II were therefore obtained partly by monoalkylation of the piperazine[22] and partly by alkylation of 1-carbethoxypiperazine[27] and subsequent hydrolysis.

(*S*)-1,4-Diazabicyclo[4.3.0]nonane (**29**) was prepared by reducing the diketopiperazine obtained by intramolecular cyclization of 1-glycylproline with $LiAlH_4$.

The benzoic acid required for the preparation of compounds **9** and **10** was obtained by nucleophilic displacement of chloride in 2-chloro-5-sulfamoylbenzoic acid[28] with sodium (*S*)-isoamyl alcoholate in dimethylformamide.

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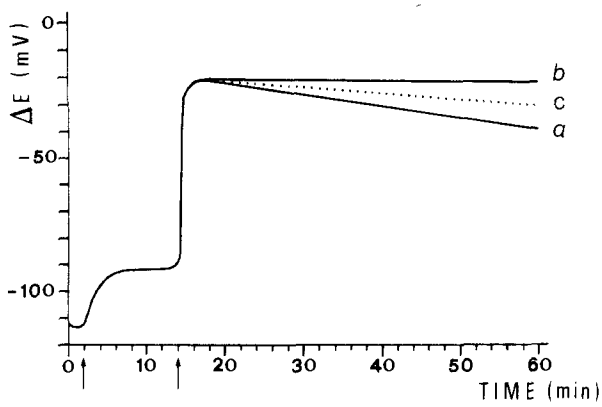

Figure 1. Potentiometric trends of our measurements. Ordinates: recorded electromotive force (in millivolts). Abscissae: time (in minutes). The arrows (2nd and 14th minute) correspond to the subsequent additions of the aliquots of the following: (a), (c) 0.1 mL of $NaHCO_3$ and 1.0 mL of CA and 5.0 mL of $NaHCO_3$; (b) 0.1 mL of $NaHCO_3$ and 1.0 mL of H_2O and 5.0 mL of $NaHCO_3$.

Results and Discussion

Experimental evidence regarding mechanisms of carbonic anhydrase inhibition was obtained in the present research program on the basis of the interactions occurring between the enzyme and previously unknown specific molecular species endowed with peculiar properties.

Past investigation on sulfonamides was mainly concerned with the synthesis of molecules primarily displaying increased inhibition of CA (e.g., acetazolamide and its derivatives[29]), even if studies of a wide number of benzamides whose structures were correlated to that of the sulpiride molecule were also performed.[30]

We are no longer primarily concerned with inhibitory activity per se but with a combined potential for inhibition and selective organ-targeted transport. Thus, we faced the problem at hand by considering the sulpiride molecular structure as the starting matrix for designing new drugs. Consequently, the carbonic anhydrase activity was measured both in the presence and in the absence of different molecular structures, by determining the rate of carbon dioxide evolution from a bicarbonate solution to which the enzyme was added using a pCO_2 electrode as a probe. It is worthwhile to point out that the proposed method assures equilibrium conditions in this system, given that the most important thermodynamic parameters (e.g., temperature, pressure, pH, ionic strength, CO_2 concentration) were fixed and their influence controlled, allowing us to monitor enzymatic activity mainly on the basis of kinetic parameters. Under such conditions, we have obtained a physicochemical model suitable for the phenomenological study of the differential inhibitory potentials promoted by chemical changes in the structures of the new synthesized compounds.

In Figure 1 we report a typical example of our experiments. The ordinates represent the difference of electromotive force (in millivolts), the abscissae the time (in minutes). The CO_2 evolution, occurring at 25 ± 0.1 °C, under constant stirring and at pH 7.0 (phosphate buffer 0.1 M) was measured in different experimental conditions:

(a) To 30 mL of phosphate buffer were added two aliquots of a $NaHCO_3$ solution (0.1 and 5.0 mL, respectively) and one aliquot of CA solution (1.0 mL). The two aliquots of $NaHCO_3$ were diluted in solution at the 2nd and 14th minute of the assay, and the CA was diluted just before the second addition of sodium bicarbonate. The $NaHCO_3$ was from a stock solution 0.1 M resulting in a final concentration of 1.4×10^{-2} M; the CA was from a stock solution of 0.7 g/L resulting in a final concentration of 2.0×10^{-2} g/L. The slope of the final part of the curve is $\Delta E/\Delta t = 0.38$ mV/min.

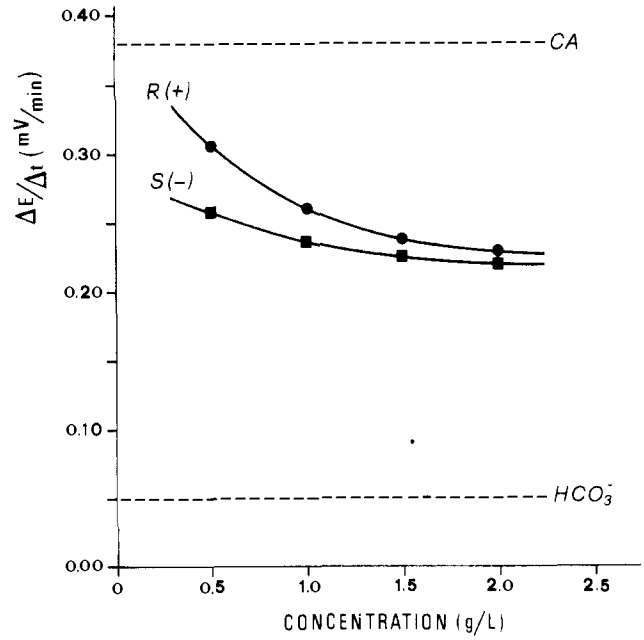

Figure 2. Different behavior of the two sulpiride enantiomers (*S* and *R*) at different concentrations.

(b) The same conditions as described in (a) were used, but without CA. To obtain the identical total volume as in (a), 1.0 mL of distilled water was added instead of 1.0 mL of CA. As can be seen from the flat portion at the end of the curve the potential is now almost stable; i.e., $\Delta E/\Delta t$ is very low (5 mV/min).

(c) The same conditions as described in (a) were used, but with the presence of the inhibitor, diluted in the buffer solution from the beginning of the assay.

All three experiments were carried out for 60 min in a 50-mL beaker. As can be seen, the (c) curve (dotted line) is obviously between (a) and (b). Moreover, the higher the inhibitory power of compound added, the higher the upward shift of the plot. This behavior can be easily expressed as $\Delta E/\Delta t$, which represents the degree of inhibition reached by that particular compound as a function of its concentration in solution, the concentration of CA in solution being constant.

In Figure 2 ($\Delta E/\Delta t$ vs. concentration) the data referring to two sulpiride enantiomers (*S* and *R*) are compared to CA alone (upper dotted line) and $NaHCO_3$ alone (lower dotted line). It is evident that (*S*)-sulpiride displays an inhibitory activity higher than the (*R*)-sulpiride,[14] as also confirmed by pharmacological tests carried out in vivo.[31]

All of these graphical data can be expressed in a more suitable way, as listed in Table III (IP, inhibitory power) that gives the values of percent inhibition for different concentrations of the inhibitors considered, calculated according to eq 5, where $(\Delta E/\Delta t)_{In}$ is the slope (mV/min) of the portion at the end of the curve related to the com-

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$$IP = 1 - \frac{(\Delta E/\Delta t)_{In} - (\Delta E/\Delta t)_{HCO_3^-}}{(\Delta E/\Delta t)_{CA} - (\Delta E/\Delta t)_{HCO_3^-}} \quad (5)$$

pound under investigation at that particular concentration (see line c of Figure 1), $(\Delta E/\Delta t)_{HCO_3^-}$ is the slope related to the $NaHCO_3$ solution (see line b of Figure 1), and $(\Delta E/\Delta t)_{CA}$ is the slope related to the $NaHCO_3$ + CA solution (see line a of Figure 1). In our case $(\Delta E/\Delta t)_{HCO_3^-}$ = 0.05 mV/min and $(\Delta E/\Delta t)_{CA}$ = 0.38 mV/min so that eq 5 becomes eq 6.

$$IP = 1 - \frac{(\Delta E/\Delta t)_{In} - 0.05}{0.33} \quad (6)$$

As one can see, the IP value tends toward 0 (minimal inhibition) for $(\Delta E/\Delta t)_{In} \rightarrow 0.38$ and toward 1 (maximal inhibition) for $(\Delta E/\Delta t)_{In} \rightarrow 0.05$. Hence, the efficacy of this approach lies not only in the thermodynamic equilibrium conditions under which the analyses were performed but also in the maximal reproducibility of the results obtained. For indeed, the simple $(\Delta E/\Delta t)_{In}$ values, which necessarily depend in part on electrode characteristics (e.g., response time, filling solution, and membrane conditions) are no longer considered as absolute values themselves but instead are compared with $(\Delta E/\Delta t)_{HCO_3^-}$ and $(\Delta E/\Delta t)_{CA}$. In other words it is now possible to eliminate any distortion in our perception of inhibitory activity.

It is noteworthy that orthopramides displaying neuroleptic activities but missing the sulfamoyl group (like compound **24**[30a] or sultopride **25**[32]) do not show any inhibitory activity against CA. This finding stresses the close correlation existing between CA inhibition and the presence of sulfamoyl group. Nevertheless, the basic action of the sulfonamide group can be modulated by differing structures present in the different types of molecular species considered. So, for instance, the molecular structures of the four compounds considered in the plots of Figure 3, in which we also report their values according to Maren method,[18a] have a main part in common and differ only with respect to relatively small portion. However, in spite of these small differences occurring in a molecular region that is relatively far from the sulfamoyl group, e.g. in the side chain bound to one of piperazine nitrogen, the activities of the molecules are remarkably different, as shown also by the IP values related to these four compounds, as listed in Table III. Of course, these differences are more evident in low concentrations of the inhibitors and tend to disappear in higher ones. Furthermore, in general the molecules listed in Table I show an activity lower than those reported in Table II. This fact represents a rather new feature in the knowledge of the specific activities of benzamides toward CA, as compared, for example, with their activity as dopamine antagonists.[29c] In fact, in this last case, the studies performed on model systems[33] seem to support the hypothesis that hydrogen-bond formation between the proton bound to the amidic nitrogen and the oxygen of the methoxyl group plays a very important role. This event does not occur in the case of the compounds listed in Table II where a disubstituted nitrogen is always present. Moreover, these last compounds, if compared to the most common benzamides, show a remarkable conformational rigidity. Their structures are, indeed, almost planar, as evidenced by Dreiding's models, apart from the groups bound to the nitrogen atom of the amine group.

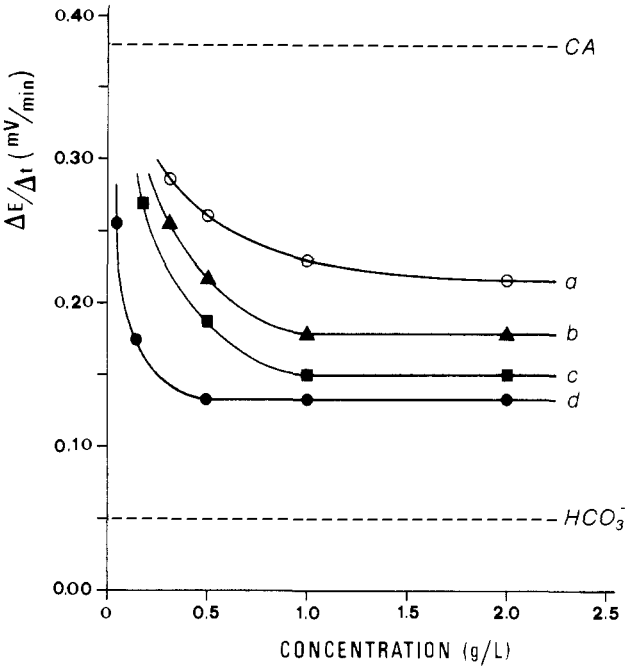

Figure 3. Comparison of inhibitory power among four strictly related molecules (see Table II) with their IC_{50} values derived according to Maren's procedure and presented here for purpose of comparison (IC_{50}): (a) RV 12348 (**11**), 1.7×10^{-3} M; (b) RV 12345 (**13**), 1.5×10^{-3} M; (c) RV 12346 (**20**), 4.8×10^{-4} M; (d) RV 12347 (**21**), 4.7×10^{-4} M.

A sharp improvement of the activity takes place for all the compounds obtained by substituting the methoxy group of the sulpiride molecule with a more hindered alkoxy group as in compounds **9** and **10**. Moreover, this improvement cofirms that the activity is higher for product (*S*) than for product (*R*).

Compound **8** is a sulpiride analogue having a methyl group on the amidic nitrogen and showing a remarkable increase of activity as further demonstration of the improvement, in the system under consideration, of an *N*-dialkylamide derivative when compared with the respective N-monoalkylated derivative.

At the first stage of our work we noticed that compound **7** showed activity higher than the one of sulpiride, and we considered particularly interesting the presence in the molecule of two nitrogen atoms connected by three carbon atoms. Therefore, we synthesized and tested first compound **3** and then the remaining compounds reported in Table II. The synthesis of these compounds followed the synthesis of compound **26**, obtained by transforming the sulpiride molecule into a more rigid structure by means of a bridge between the two nitrogen atoms, the aminic and the amidic ones. The piperazine ring formation was the most important result achieved and allowed the synthesis of the most active compounds,[34] namely the ones from **16** to **23**.

Finally, we also tested the influence of the replacement of the aromatic ring supporting the sulfamoyl group with other isosteric molecular arrangements, e.g. heterocyclic ring as in compound **28**; the lack of the methoxy group does not affect the activity on CA, while the presence of the sulfamoyl group is essential. Moreover, from this last experiment it was found that a six-membered aromatic ring is not essential for activity. In fact, compound **19** was shown to be less active than its analogous **28** in which this ring was replaced by a five-membered heterocyclic ring.

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Table III. Percent of Inhibition Values (100IP) as a Function of Different Inhibitor Concentrations Calculated by Expression 6

compd	N	0.2 g/L	0.5 g/L	1.0 g/L	1.5 g/L	2.0 g/L
RV 12366	23	70.5	76.5	77.3	77.3	77.7
RV 12361	28	68.9	75.8	76.4	76.7	77.0
RV 12347	21	66.7	73.5	74.6	74.7	75.8
RV 12346	20	63.6	70.5	73.5	73.5	75.0
RV 12367	22	61.4	69.7	72.0	72.7	73.5
RV 12316	19	58.3	69.7	70.5	71.2	72.0
RV 12358	18	54.6		69.7		69.7
RV 12351	16		62.1		69.7	71.2
RV 12325	26	46.2	61.4	72.0	73.5	74.2
RV 12344	14	51.5	60.6	69.7	72.7	74.2
RV 12343	15	46.2	59.1	71.2	72.0	72.0
RV 12304	7	45.5	60.6	68.2	69.7	
RV 12350	17	45.5	57.6	69.7	69.7	
RV 12328	9	42.4	57.6	65.2	66.7	67.4
RV 12329	10	41.7	56.8	63.6	65.2	66.7
RV 12326	8	39.4	54.6	65.2	68.2	68.9
RV 12359	27	37.9	53.0	63.6	65.2	66.7
RV 12345	13	36.4	50.8	62.1	63.6	63.6
RV 12342	12	36.4	47.0	60.6	62.1	62.1
RV 12313	6	39.4	43.9	54.6	60.6	
RV 12348	11	30.3	40.9	50.0	53.0	56.1
RV 12324	3		37.1	47.0	51.5	53.0
RV 12309	(S)-1		37.9	43.9	47.0	50.0
RV 12318	5		34.1	47.0	51.5	53.0
RV 12315	4		24.2	39.4	49.2	51.5
RV 12308	(R)-1		20.5	37.9	43.9	47.0
RV 12310	2			25.8	37.1	42.4
RV 12333	24	a	a	a	a	a
RV 12306	25	a	a	a	a	a

[a] No inhibition detected.

Currently in progress are additional tests using this procedure in order to further clarify inhibitory mechanisms, as well as pharmacological assays to verify in vivo the present in vitro results.[35]

Experimental Section

Chemical Syntheses. The C, H, N analyses of all compounds reported in this paper were within ±0.4% of the theoretical values. Melting points were determined in a Büchi capillary melting point apparatus and have not been corrected. All purified compounds had IR and ^{1}H NMR spectra compatible with their structures and were homogeneous by TLC. Analytical thin-layer chromatography was conducted on precoated plates of silica gel 60 F254 Merck using the mixture *i*-PrOH–MeOH 30% NH_4OH (8:1:1) as eluent. IR spectra were obtained on a Perkin-Elmer 457 spectrophotometer. ^{1}H NMR spectra were recorded on a Hitachi Perkin-Elmer R-24 or on a Varian XL-200 spectrometer with Me_4Si as internal standard.

The appropriate amine used in the preparation of compounds **1**, **10**, and **25** was supplied by ICROM (Milano, Italy); those used in the preparation of compounds **2**, **4**, and **5** were supplied by Fluka (Buchs, Switzerland); those used in the preparation of compounds **6** and **7** were supplied by Aldrich-Europe (Beerse, Belgium); and those used in the preparation of compounds **16–18** were supplied by Finorga (Courbevoie, France). *N*-Benzylpiperazines used in the preparation of compounds **11**, **19–22**, and **28** were prepared according to known procedures.[22] Furthermore, known methods were adopted for the synthesis of amines utilized in the preparation of compound **3**,[36] of compound **8**[24] [(*S*)-1-ethyl-2[(*N*-methylamino)methyl]pyrrolidine; bp 81–83 °C (20 mmHg)], of compounds **12–14** [1-(cyclohexylmethyl)piperazine; bp 94–96 °C (1 mmHg)] and **15**.[26,27]

Sulpiride (**1**) is industrially produced by Ravizza S.p.A. and can be prepared in the laboratory by method C. (*S*)-2-[(3,5-Dibromo-2,6-dimethoxybenzamido)methyl]-1-ethylpyrrolidine (**24**) was synthesized following the known procedure[30a] as well as 2-[[2-methoxy-5-(ethylsulfonyl)benzamido]methyl)]-1-ethylpyrrolidine (**25**; sultopride).[32]

2-Sulfamoyl-4-imidazolecarboxylic acid was prepared by known procedure.[23]

Method A. General Procedure. A stirred solution of amine (30 mmol) in 60 mL of anhydrous pyridine was cooled with an ice bath and treated with a solution of PCl_3 (10 mmol) in 10 mL of anhydrous pyridine. The mixture was allowed to come to room temperature, stirred for 1 h, treated with the appropriate carboxylic acid (25 mmol), and then heated at 100–120 °C for 4 h. The residue, obtained by evaporating pyridine in vacuo, was dissolved in 10% NaOH (50 mL) and the resulting solution washed with Et_2O (2 × 30 mL); the aqueous layer was acidified with HOAc to pH 6–7, treated with charcoal, alkalinized to pH 8–9 with 30% NaOH, and left in a refrigerator overnight. The precipitate was filtered off, washed with water, and crystallized from the appropriate solvent to give compounds **2**, **5**, **13**, **14**, **16–18**, and **28**. Obtained in similar experimental conditions, compound **3** was isolated by extraction from the ammonia solution and usual workup; compounds **9** and **10**, slightly soluble in NaOH solution, were filtered off, washed with water, and dissolved in 5% HOAc, the solution was treated with charcoal, and the products were precipitated with 30% NH_4OH; compounds **12** and **19** were precipitated from the NaOH solution and washed with ether by adding HOAc to pH 7.5; compound **15**, obtained as compounds **12** and **19**, was purified as the hydrochloride from *n*-BuOH; compounds **20** and **21** were purified as the hydrochlorides from EtOAc; compound **25** was isolated by extraction of the NaOH solution with $CHCl_3$ and purified as the hydrochloride; compound **26** was purified by flash chromatography on silica gel with CH_3OH as eluent.

Method B. General Procedure. A mixture of 2-methoxy-5-sulfamoylbenzoic acid ethyl ester (50 mmol) and the appropriate amine (50 mmol) was heated at 120 °C for 8 h. After cooling, the product was dissolved in 5% HOAc and the resulting solution treated with charcoal and then with 30% NH_4OH. After the solution was refrigerated overnight, the precipitate was filtered off and crystallized from the appropriate solvent to give compounds **4–7**.

Method C. General Procedure. A 16-mmol portion of the appropriate amine was added to a solution of 2-methoxy-5-sulfamoylbenzoic acid ethyl ester (3.8 g, 14 mmol) in 13 mL of diethylene glycol, and the mixture was heated at 80 °C for 48 h.

(35) Gessa, G. L. and colleagues, personal communication, unpublished results.

(36) (a) *Organic Syntheses*; Wiley: New York, 1955; Collect. Vol. III, p 258. (b) Fuson, R. C.; Parham, W. E.; Reed, L. J. *J. Am. Chem. Soc.* **1946**, *68*, 1239.

After cooling, the precipitate was filtered off and dissolved in 5% HOAc; the solution was heated while stirring, and treated with charcoal, and filtered, and the amide precipitated with 30% NH_4OH. The solid was crystallized from the appropriate solvent to give compounds 1 and 4–7.

Method D. A stirred solution of 2-methoxy-5-sulfamoylbenzoic acid (2.3 g, 10 mmol) in 50 mL of anhydrous THF was cooled to −10 °C and treated with diphenylphosphinyl chloride[21] (2.6 g, 11 mmol) and then dropwise with triethylamine (11 mmol). After stirring for 1 h, a solution of (*S*)-1-ethyl-2-[(*N*-methylamino)-methyl]pyrrolidine (1.42 g, 10 mmol) in triethylamine (1.5 mL) was added, and the resulting solution was allowed to come to room temperature by stirring for 12 h. After filtration, the solvent was removed under vacuum, the residue dissolved in 1 N HCl, and the resulting solution treated with charcoal; Na_2CO_3 was added and the product extracted with EtOAc. The organic layer, after being washed with water, was dried on Na_2SO_4, and the product was precipitated by adding petroleum ether. The precipitate was crystallized to give pure compound 8 (2.3 g, 66%).

Method E. Pd/C (5%; 1.0 g) was added to a solution of compound **19** (5.0 g, 12.8 mmol) in 100 mL of EtOH, and the mixture was hydrogenated while stirring under atmospheric pressure at 50 °C for 8 h. After removal of the catalyst, the solution was treated with ethanolic HCl; filtration of the precipitate and crystallization gave pure compound 11, 3.5 g (81%).

Method F. NaOH (20%; 3.0 mL) and 4-sulfamoylbenzyl chloride (3.5 g, 17 mmol) were subsequently added to a suspension of compound 11 (4.5 g, 15 mmol) in 20 mL of water. The mixture was refluxed for 3 h. After cooling, the precipitate was filtered off, washed with water, and crystallized to give pure compound **23**, 5.0 g (69%). Compound **22** was prepared analogously.

(*S*)-1,4-Diazabicyclo[4.3.0]nonane (29). A suspension of phtalimidoglycine (5.75 g, 27.5 mmol) in 50 mL of a solution of pyridine (0.25 mL) and $SOCl_2$ (2.25 mL) in $CHCl_3$ was refluxed for 2 h. Then, (*S*)-proline (3.05 g, 26.5 mmol) was added, and refluxing was continued for additional 4 h. After cooling, the solution was washed with water and dried over Na_2SO_4; after addition of $CHCl_3$ (25 mL), EtOH (32.5 mL), and 100% hydrazine hydrate (2.65 mL), the mixture was stirred at room temperature for 12 h and then refluxed for 24 h. After filtration of the precipitate, the solvent was evaporated under vacuum and the product was crystallized from $CHCl_3$–Et_2O to give 1,4-diazabicyclo[4.3.0]nonane-2,5-dione, 3.0 g (75%). This product was reduced in THF solution (30 mL) with a suspension of $LiAlH_4$ (1.52 g) in THF (100 mL), refluxing the mixture for 8 h. After the mixture was cooled at 0 °C, water (4 mL) in THF (20 mL) was added to the mixture and then 20% NaOH (10 mL) and water (35 mL) were added. After filtration of the precipitated salt, the solvent was removed and the product was distilled in vacuo to give compound **29**: 1.35 g (55%); bp 74–75 °C (10 mmHg).

2-Methoxy-5-sulfamoylbenzyl Chloride (30) and 4-Sulfamoylbenzyl Chloride. They were prepared from 2-methoxy-5-sulfamoylbenzoic acid ethyl ester and 4-sulfamoylbenzoic acid ethyl ester respectively by reduction with $LiAlH_4$ in THF and treatment of the resulting benzylic alcohols with $SOCl_2$.

Enzymatic Experiments. The potentiometric measurements were carried out according to this new procedure already described and discussed.[12,14]

An Orion 901 ionalyzer connected to a Perkin-Elmer 56 recorder was used. The pCO_2 sensor used was an Orion combined electrode, Model 950200, filled with Orion 950202 filling solution.

All measurements were carried out at pH 7.0 (0.1 M phosphate buffer), at constant temperature (25 ± 0.1 °C) in a thermostated bath and with constant stirring.

All reagents were analytical grade. Carbonic anhydrase from bovine erythrocytes was supplied by Boehringer Mannheim (2000 U/mg) and by Sigma Catalog No. C2522 (2500 U/mg) and No. C7500 (2500 U/mg). The experiments performed employing different samples of carbonic anhydrase always gave the same results. Carbonic anhydrase from human erythrocytes was supplied by Calbiochem Behring Catalog No. 215830 (2300 U/mg) containing isoenzymes A and B. In this latter case tests were performed only on both sulpiride enantiomers; no differences were detected with respect to results obtained using carbonic anhydrase from bovine erythrocytes as far as IP value is concerned.

Tests to check for the eventual presence of acetylcholinesterase in the CA samples were also performed. The results were always negative.

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Registry No. **2**, 51218-14-5; **3**, 102535-19-3; **4**, 5607-65-8; **5**, 102535-20-6; **6**, 102535-21-7; **7**, 102535-22-8; **8**, 102535-23-9; **9**, 102535-24-0; **10**, 102535-25-1; **11**, 102535-26-2; **12**, 102535-27-3; **13**, 102535-28-4; **14**, 102535-29-5; **15**, 102535-30-8; **16**, 102535-31-9; **17**, 102535-32-0; **18**, 102535-33-1; **19**, 57479-93-3; **20**, 102535-34-2; **21**, 102535-35-3; **22**, 102535-36-4; **23**, 102535-37-5; **26**, 102535-38-6; **27**, 102535-39-7; **28**, 102573-55-7; **29**, 93643-24-4; $Et_2NCH_2CH_2NH_2$, 100-36-7; $Me_2NCH_2CH(Me)NH_2$, 108-15-6; $CH_2(CH_2CH_2)_2NCH_2CH_2NH_2$, 27578-60-5; $O(CH_2CH_2)_2NCH_2CH_2CH_2NH_2$, 123-00-2; $HN(CH_2CH_2)_2NCH_2CH{=}CH_2$, 13961-36-9; $HN(CH_2CH_2)_2NCH_2CHMe_2$, 5308-28-1; $HN(CH_2CH_2)_2NCH_2CH(CH_2CH_2)_2CH_2$, 57184-23-3; $HN(CH_2CH_2)_2NCH_2CH(Me)Et$, 82499-91-0; $HN(CH_2CH_2)_2NCH_2COOEt$, 40004-08-8; $HN(CH_2CH_2)_2NCH_2({=}O)NHCHMe_2$, 39890-42-1; $HN(CH_2CH_2)_2NCH_2Ph$, 2759-28-6; (1-ethyl-3-piperidinyl)amine, 6789-94-2; (1-ethyl-4-piperidinyl)amine, 50534-45-7; [(*S*)-(1-ethyl-2-pyrrolidinyl)methyl]methylamine, 102535-40-0; [(*S*)-(1-ethyl-2-pyrrolidinyl)methyl]amine, 22795-99-9; [(*R*)-(1-ethyl-2-pyrrolidinyl)methyl]amine, 22795-97-7; *N*-[(1-pyrrolidinylcarboxy)methyl]piperazine, 39890-45-4; *N*-(*p*-methoxybenzyl)-piperazine, 21867-69-6; *N*-(*m*-methoxybenzyl)piperazine, 2213-32-3; 2-methoxy-5-sulfamoylbenzoic acid, 22117-85-7; ethyl 2-methoxy-5-sulfamoylbenzoate, 33045-53-3; (*S*)-2-(2-methylbutoxy)-5-sulfamoylbenzoic acid, 102535-41-1; 1-(chloromethyl)-3,4,5-trimethoxybenzene, 3840-30-0; α-chloro-4-sulfamoyltoluene, 102153-43-5; phthalimidoglycine, 4702-13-0; (*S*)-proline, 147-85-3; 1,4-diazabicyclo[4.3.0]nonane-2,5-dione, 3705-27-9; carbonic anhydrase, 9001-03-0.