o-Chlorobenzenesulfonamidic Derivatives of (Aryloxy)propanolamines as β-Blocking/Diuretic Agents[1]

Violetta Cecchetti,[†] Arnaldo Fravolini,*[,†] Fausto Schiaffella,[†] Oriana Tabarrini,[†] Giancarlo Bruni,[‡] and Giorgio Segre[‡]

Istituto di Chimica Farmaceutica e Tecnica Farmaceutica, Università di Perugia, 06123 Perugia, Italy, and Istituto di Farmacologia, Università di Siena, 53100 Siena, Italy

Received April 28, 1992

A series of compounds **1b–f**, **2b–f**, and **3b–f** having an o-chlorobenzenesulfonamidic diuretic moiety variously linked to the nitrogen side chain of the β-blocking (aryloxy)propanolamine pharmacophore were prepared and tested for their β_1-adrenoceptor affinity. For all the active compounds, β-blocking and diuretic activities were investigated in rats; the structure–activity relationships are discussed. Some of the compounds displayed varying levels of both properties and among these, compounds **1c** and **2c** have been chosen for further development.

Introduction

In the long-term treatment of essential hypertension, no single drug has yet proved entirely satisfactory. Combined treatment is necessary to evoke an optimal result and a β-blocker/diuretic combination is widely used as first line therapy for hypertension management.

For the future, an antihypertensive drug having both β-blocker and moderate diuretic properties[2] in the same molecule would be of great interest. The advantage of such a compound over a simple combination lies in the fact that the single entity would be adsorbed, metabolized, and excreted at one rate in a given subject, thus increasing the likelihood that the two main biological activities remain in balance during the course of drug action.

To date, only a few attempts to synthesize hybrid molecules by combining the structures of a diuretic and β-adrenoceptor antagonist, have been described.[3] The majority of the reported attempts have failed, probably due to the intrinsic difficulty of accommodating the individual sets of structure–activity requirements for the two activities in a single molecule.

Our previously reported[4] approach to this symbiotic drug design was achieved by replacing the conventional alkyl substituent at the side chain nitrogen atom of β-blockers with a 2-(4-chloro-3-sulfamoylbenzamido)ethyl group. This type of substitution retained the structural requirements for the interaction with the β-adrenoceptor, due to the presence of a 2-amidoethyl group known to impart high β-blocking potency[5] and, at the same time, allowed the diuretic o-chlorobenzenesulfonamidic moiety[6] to be incorporated into the molecule. This replacement was made on oxypropanolamine and (iminoxy)propanolamine derivatives of 1,4-benzothiazine, previously reported by us as β-adrenoceptor antagonists,[7] on carteolol, selected for isosterism of the carbostyril nucleus with the 1,4-benzothiazine one, and on well-known propranolol. The pharmacological results obtained indicated that the incorporation of o-chlorobenzenesulfonamidic moiety into the molecule by an amidoethylamino group, in some cases, allowed the desired diuretic activity and also maintained the β-blocking one. Indeed, some of the resulting compounds (such as **1a**, **2a**, **3a**) (Figure 1) were found to exhibit differently modulated β-adrenoceptor blocking and diuretic activites.[4]

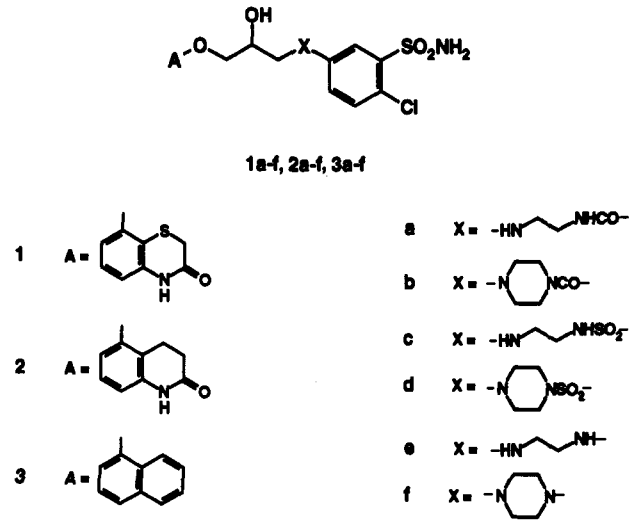


Figure 1. o-Chlorobenzenesulfonamidic derivatives of (aryloxy)-propanolamines.

As an extension of this work, we now report the synthesis and pharmacological evaluation of some new (1,4-benzothiazinyl-, (carbostyril-, and (naphthyloxy)propanolamine derivatives **1b–f**, **2b–f**, and **3b–f**, variously linked to the same diuretic o-chlorobenzenesulfonamidic moiety. In order to evaluate the role of the bridge linking the two pharmacophore moieties, the former amidoethylamino bridge ($NHCH_2CH_2NHCO$) was modified by (1) inclusion of the ethylenediaminic group in a rigid piperazinic moiety (**b**, **d**, **f**); (2) isosteric substitution of the amidic group with a sulfonamidic one (**c**, **d**); and (3) elimination of the amidic group (**e**, **f**) (Figure 1).

Chemistry

The (aryloxy)propanolamine derivatives **1b–f**, **2b–f**, and **3b–f**, reported in Figure 1 and Table I were prepared by the following standard chemical procedure: selected epoxide, either purchased or prepared by standard methods, was reacted with an equimolar amount of the appropriate amine derivative **3**, **5**, **7**, **9**, or **10** in refluxed EtOH (Scheme I).

The key step in this synthesis was the preparation of amine intermediates **3**, **5**, **7**, **9**, and **10** obtained as in Scheme II. (Arylamido)piperazine **3** and (arylsulfonamido)piperazine **7** were synthesized by acylation of a monoprotected piperazine derivative with 4-chloro-3-sulfamoylbenzoyl

† Università di Perugia.
‡ Università di Siena.

0022-2623/93/1836-0157$04.00/0

Scheme I

chloride and 4-chloro-3-sulfamoylbenzenesulfonyl chloride, respectively, and successive hydrolytic deprotection. In a similar fashion, using *N*-acetylethylenediamine and 4-chloro-3-sulfamoylbenzenesulfonyl chloride, (arylsulfonamido)ethylamine **5** was obtained. Starting from 4-chloro-3-sulfamoylaniline, *N*-arylethylenediamine **9** was prepared by reaction with 1-chloro-2-nitroethane followed by reductive hydrogenation over Raney nickel, while arylpiperazine **10** was synthesized by reaction with bis-(2-chloroethyl)amine, according to the general procedure of Brewster et al.[8]

Pharmacology

All target compounds **1b–f**, **2b–f**, and **3b–f** were first screened at the receptor level to determine their ability to displace the binding of [^{3}H]dihydroalprenolol ([^{3}H]-DHA) from turkey erythrocyte membranes (β_1-adrenoceptors) (Table I).

Subsequently, all active compounds **1c–3c**, **1e**, **3e**, **1f**, and **3f**, were evaluated for β-blocking potency in rats by the inhibition of isoprenaline-induced tachycardia by both intravenous and oral dosing (Table I).

Only the resulting active compounds were finally assayed for their diuretic activity in rats after oral administration (Table II).

In all of the tests, in addition to appropriate control drugs, 8-[(*tert*-butylamino)-2-hydroxypropoxy]-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine (**4a**)[7a] and/or *N*-[(4-chloro-3-sulfamoylbenzamido)ethyl]propanolamine derivatives **1a–3a**,[4] previously reported by us, were included for comparison.

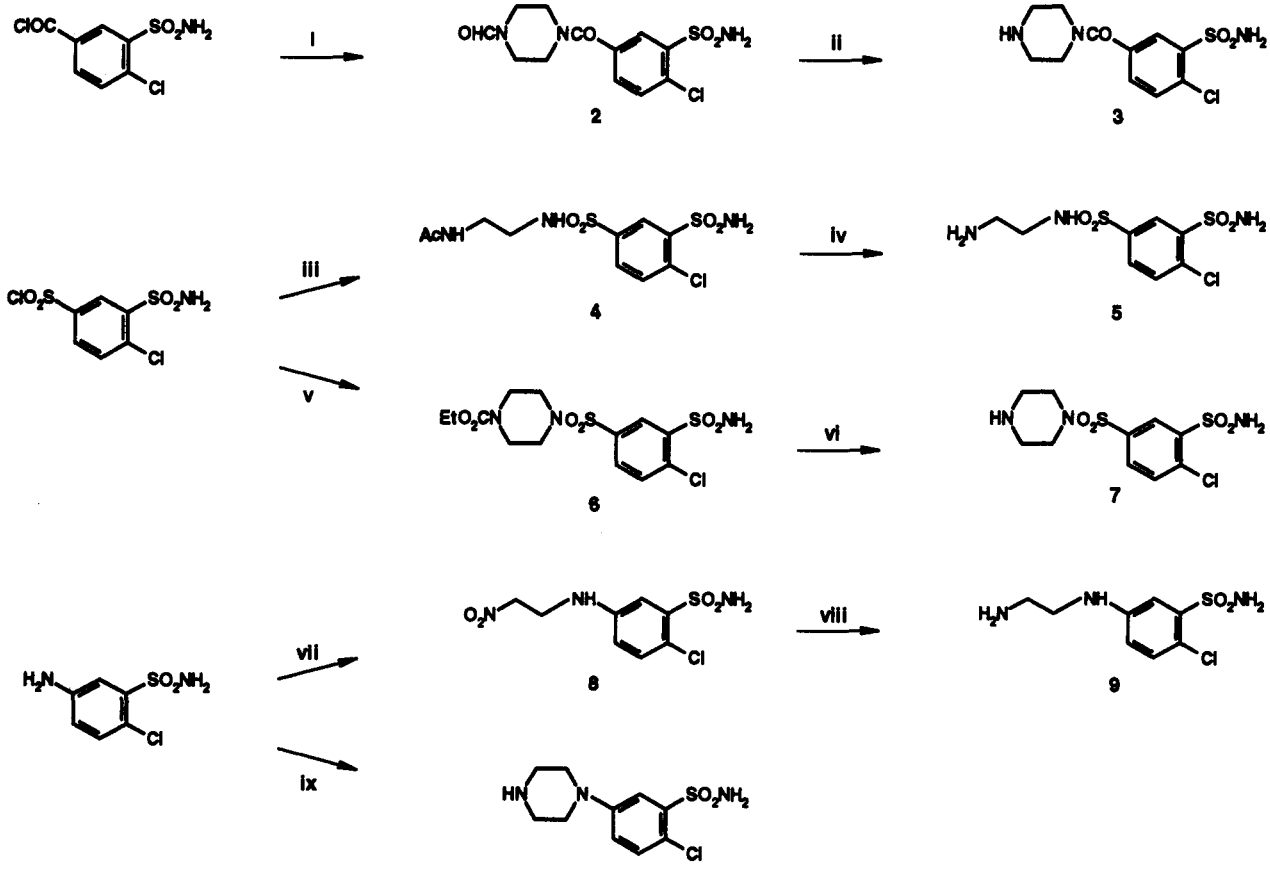
Results and Discussion

The object of this study was to evaluate the effects, on both β-blocking and diuretic activities, caused by variations induced in the bridge (X) connecting the (aryloxy)propanol β-blocking pharmacophore and the diuretic *o*-chlorobenzenesulfonamidic moiety.

Among the changes made, the inclusion of ethylenediaminic group in a rigid piperazine moiety proved to be very detrimental to binding at β_1-adrenoceptors (Table I), both in amidic (series **b**) and sulfonamidic (series **d**) derivatives (compare vs series **a** and **c**, respectively). On the other hand, this loss of affinity was not observed in series **f** where the piperazine group was directly linked to the *o*-chlorobenzenesulfonamidic moiety (compare vs series **e**). No correlation was found regarding the aryl ring (A); in series **c**, the carbostyril derivative **2c** displayed the highest affinity with a K_i value of 1.4 nM, similar to that of the control drugs, whereas in series **e** and **f** only the carbostyril derivatives **2e** and **2f** showed no affinity.

Only those compounds (**1c–3c**, **1e**, **3e**, **1f**, and **3f**) showing affinity in the binding study were tested in vivo by inhibiting isoprenaline-induced tachycardia in rats to

Scheme II [a]



[a] Reagents: (i) 1-formylpiperazine, benzene, 100 °C; (ii) MeOH, 36% HCl; (iii) $AcNHCH_2CH_2NH_2$, acetone, Et_3N; (iv) EtOH, 6 N HCl, reflux; (v) *N*-(ethoxycarbonyl)piperazine, acetone, Et_3N; (vi) 10% Na_2CO_3, reflux; (vii) $NO_2CH_2CH_2Cl$, THF; (viii) H_2/Raney Ni, EtOH; (ix) $NH(CH_2CH_2Cl)_2$·HCl, 1-butanol, reflux.

Table I. Physical and Biological Properties of (Aryloxy)propanolamines

compd[a]	reaction time (h)	% yield	mp, °C[b]	purification method[c]	formula[d]	inhibition of [^{3}H]DHA binding in turkey erythrocytes (β_1)[e] K_i (nM)	inhibition of INA-induced tachycardia in rats[f] ED_{50} (mg/kg, iv)[g]	ED_{50} (mg/kg, po)[h]
1a						7.4	0.67 ± 0.031	36.24 ± 3.465
2a						300	0.14 ± 0.039	17.92 ± 1.387
3a						820	3.17 ± 0.385	23.13 ± 1.157
1b	30	53	145–165	A	$C_{22}H_{25}ClN_4O_6S_2$	NA[i]	NT[j]	NT
2b	30	71	136–148	A	$C_{23}H_{27}ClN_4O_6S$	NA	NT	NT
3b	30	65	88–120	A	$C_{24}H_{26}ClN_3O_5S$	NA	NT	NT
1c	30	81	100–130	B	$C_{19}H_{23}ClN_4O_7S_3$	5.2	0.45 ± 0.016	34.70 ± 2.093
2c	30	37	103–115	B	$C_{20}H_{25}ClN_4O_7S_2$	1.4	1.80 ± 0.041	53.78 ± 3.185
3c	30	35	87–118	B	$C_{21}H_{24}ClN_3O_6S_2$	160	NA	NA
1d	15	50	218–223	C	$C_{21}H_{25}ClN_4O_7S_3$	NA	NT	NT
2d	20	39	225–230	B	$C_{22}H_{27}ClN_4O_7S_2$	NA	NT	NT
3d	12	55	208–212	C	$C_{23}H_{26}ClN_3O_6S_2$	NA	NT	NT
1e	30	12	176–186	B	$C_{19}H_{23}ClN_4O_5S_2$	7.8	0.96 ± 0.046	56.37 ± 3.498
2e	23	22	211–214	B	$C_{20}H_{25}ClN_4O_5S$	NA	NT	NT
3e	30	36	70–91	B	$C_{21}H_{24}ClN_3O_4S$	15	NA	40.69 ± 2.641
1f	14	63	218–222	D	$C_{21}H_{25}ClN_4O_5S_2$	140	NA	56.30 ± 22.050
2f	12	62	227–231	D	$C_{22}H_{27}ClN_4O_5S$	NA	NT	NT
3f	8	73	166–170	D	$C_{23}H_{26}ClN_3O_4S$	31	2.91 ± 0.083	62.52 ± 3.886
4a[k]						24	0.05 ± 0.003	1.53 ± 0.026
carteolol						1.9	0.06 ± 0.008	4.81 ± 0.255
propranolol						2.3	0.18 ± 0.023	22.33 ± 1.306

[a] See Figure 1. [b] Amorphous solids: melting point of these compounds is the temperature at which the white solid became a colorless glass with decomposition. [c] Solvent used with silica gel column or for recrystallization were as follows: (A) elution with gradient of $CHCl_3$ to 10% EtOH/$CHCl_3$, (B) gradient of $CHCl_3$ to 5% MeOH/$CHCl_3$, (C) isocratic elution with $CHCl_3$, (D) recrystallization from EtOH. [d] All compounds had elemental analyses within ±0.4% of theoretical value. [e] K_i values determined with seven dose levels of each inhibitor in the assay system described in the Experimental Section. [f] Mean ± SE for three separate observations per dosage. [g] Two minutes after INA (0.12 μg/kg) administration. [h] One hour after INA (0.12 μg/kg) administration. [i] NA = no active compound. [j] NT = not tested. [k] 4a = 8-[(*tert*-butylamino)-2-hydroxypropoxy]-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine (see literature[7a]).

Table II. Diuretic and Saluretic Activity in the Rat (0–5 h)

compd	dosage (mg/kg, po)	urinary output[a] (mL)	saluretic activity[b] (mequiv/kg) Na^+	K^+	Na^+/K^+
1a	8	1.2 ± 0.31	0.9	0.8	1.1
	15	1.7 ± 0.63	1.1	0.9	1.2
	30	2.0 ± 0.46	1.7	1.1	1.5
	50	4.3 ± 0.63	3.1	1.5	2.1
2a	8	2.8 ± 0.31	2.6	1.8	1.4
	15	2.6 ± 0.22	2.0	1.5	1.3
	30	3.3 ± 0.66	2.9	1.8	1.6
3a	8	5.2 ± 0.26	2.5	1.2	2.1
	15	4.8 ± 0.36	3.1	1.7	1.8
	30	2.9 ± 0.46	1.8	1.4	1.2
1c	8	3.5 ± 0.35	3.2	1.3	2.5
	15	3.7 ± 0.33	3.0	1.3	2.3
	30	5.7 ± 0.93	4.5	2.4	1.9
2c	8	2.2 ± 0.48	1.8	0.9	2.0
	15	3.1 ± 0.41	2.3	1.2	1.9
	30	4.6 ± 0.35	3.4	1.5	2.3
1e	8	2.6 ± 0.18	2.1	1.1	1.9
	15	3.3 ± 0.27	2.6	1.3	2.0
	30	3.1 ± 0.07	2.2	1.2	1.8
3e	8	2.4 ± 0.58	2.8	1.6	1.7
	15	3.2 ± 0.31	3.1	2.0	1.5
	30	3.5 ± 0.77	3.2	2.6	1.2
1f	8	2.6 ± 0.10	2.2	1.7	1.3
	15	3.1 ± 0.13	2.9	1.8	1.6
	30	3.5 ± 0.17	3.0	2.0	1.5
3f	8	1.9 ± 0.07	2.4	1.3	1.8
	15	2.2 ± 0.12	1.7	1.4	1.2
	30	2.9 ± 0.54	3.2	2.0	1.4
HCT[c]	8	4.0 ± 0.29	3.1	1.8	1.7
	15	5.9 ± 0.66	3.9	1.9	2.0
	30	7.3 ± 0.13	5.1	2.3	2.2
saline		2.0 ± 0.27	1.4	1.1	1.3

[a] Mean ± SE for five separate observations per dosage. [b] SE for the saluretic data were calculated and are less than 10% of the mean values. [c] HCT = hydrochlorothiazide.

evaluate their β-blocking potency (Table I). In general, when compared with the former derivatives **1a–3a**, the new (aryloxy)propanolamine derivatives did not display an improved β-blocking potency; they were less potent than the reference compounds **4a**, carteolol, and propanolol. After intravenous dosing, benzothiazinyl derivative **1c**, characterized by an sulfonamidoethylamino bridge (X), displayed the best activity (ED_{50} = 0.45 mg/kg), superior to the other carbostyril analogue **2c** (ED_{50} = 1.80 mg/kg) as well as to the all other benzothiazinyl derivatives **1a–f**. The naphthyl derivatives **3** were found to be inactive with the exception of **3f** (ED_{50} = 2.91 mg/kg). In general, these results were confirmed after oral dosing. However, **3e** and **1f**, usually inactive when administered intravenously, showed an unexpected activity.

Although the new derivatives did not show an improved β-blocking activity, they had a better diuretic one when compared with the former derivatives **1a–3a** (Table II). The diuretic activity was especially significant for the series **c** derivatives which showed an approximately 2-fold urinary output at 30 mg/kg compared to the control. In particular **1c**, displaying the best in vivo β-blocking activity after both intravenous and oral dosing, at 30 mg/kg po showed a urinary output equal to 96% of the effect produced by an equimolar hydrochlorothiazide dose of 15 mg/kg and a saluretic effect similar to hydrochlorothiazide at the same dose.

In short, the modifications induced in the bridge connecting β-blocking and diuretic pharmacophores confirmed the validity of the proposed symbiotic approach. Specifically, compounds **1c** and **2c** showed the desired pharmacologic profile.

It remains to be seen if this dual behavior lowers blood pressure. Nothing can be said, at the present time, about the antihypertensive activity of these compounds because no simple animal model of hypertension is available which responds satisfactory to both β-blocking and diuretic agents. Only after extensive studies, currently in progress

on compounds 1c and 2c, can their presumed efficacy as antihypertensive agents be confirmed.

Experimental Section

Melting points were determined in capillary tubes (Buchi melting point apparatus) and are uncorrected. Elemental analyses were performed on a Carlo Erba Model 1106 elemental analyzer, and the data for C, H, and N are within ±0.4% of the theoretical values. ^{1}H NMR spectra were recorded at 90 MHz (Varian EM 390) or 200 MHz (Bruker AC-200) with Me_4Si as internal standard and Me_2SO-d_6 as solvent. Chemical shifts are given in ppm (δ), and the spectral data are consistent with the assigned structures. Mass spectra were recorded on Varian MAT 311 A. Reagents and solvents were purchased from common commercial suppliers and were used as received. Column chromatography separations were carried out on Merck silica gel 40 (mesh 70–230) and flash chromatography on Merck silica gel 60 (mesh 230–400). Organic solutions were dried over anhydrous Na_2SO_4 and concentrated with a Buchi rotary evaporator at low pressure. Yields are of purified product and were not optimized. The physical properties of the target compounds **1b–f**, **2b–f**, and **3b–f** are summarized in Table I.

8-(2,3-Epoxypropoxy)-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine[7a] and 5-(2,3-epoxypropoxy)-3,4-carbostyril[9] were prepared by the cited literature methods while 1-(2,3-epoxypropoxy)-naphthalene was obtained from Aldrich Chimica.

1-(4-Chloro-3-sulfamoylbenzoyl)-4-formylpiperazine (2). A suspension of 4-chloro-3-sulfamoylbenzoyl chloride[10] (1.8 g, 7.08 mmol) in benzene (150 mL) was added portionwise to a stirred solution of 1-formylpiperazine (2.2 g, 19.30 mmol) in benzene (50 mL) at room temperature. The mixture was heated at 100 °C for 20 h and then allowed to cool to room temperature. The resulting precipitate was filtered off and washed with saturated aqueous $NaHCO_3$ and H_2O. Recrystallization from EtOH gave **2** (1 g, 43%) as a white solid: mp 220–222 °C. Anal. ($C_{12}H_{14}ClN_3O_4S$) C, H, N.

1-(4-Chloro-3-sulfamoylbenzoyl)piperazine (3). The suspension of compound **2** (0.7 g, 2 mmol) in MeOH (30 mL) and 36% HCl (2.75 mL) was stirred at room temperature for 18 h. The resulting solution was evaporated to dryness to give a white solid (mp 265–267 °C) which was dissolved in H_2O (2 mL) and alkalinized (ca. pH 9) by the addition of saturated aqueous $NaHCO_3$. The precipitated solid was collected and recrystallized from EtOH to yield **3** (0.34 g, 53%) as a white solid: mp 234–236 °C; ^{1}H NMR δ 2.35–2.65 (4 H, m, CH_2NHCH_2 piperazine), 2.30–3.40 (5 H, m, $CH_2N(CO)CH_2$ piperazine and NH), 7.45–7.70 (2 H, m, H-5 and H-6), 7.92 (1 H, d, J = 2.5 Hz, H-2); MS m/z 303.5 (M^+). Anal. ($C_{11}H_{14}ClN_3O_3S$) C, H, N.

***N*-[(4-Chloro-3-sulfamoylphenyl)sulfonyl]-*N*'-acetylethylenediamine (4).** A solution of 4-chloro-3-sulfamoylbenzenesulfonyl chloride[11] (2 g, 6.89 mmol) in acetone (30 mL) was added dropwise at room temperature to a stirred solution of *N*-acetylethylenediamine (0.84 g, 8.22 mmol) and triethylamine (0.96 mL, 6.89 mmol) in acetone (2 mL). When the addition was complete, the reaction mixture was evaporated to dryness to give a semisolid residue which was dissolved in warm H_2O (30 mL) and then extracted several times with EtOAc. The organic phases were combined, dried, and evaporated to dryness. The resulting residue was purified by flash chromatography eluting with gradient of $CHCl_3$ to 10% MeOH/$CHCl_3$ yielding **4** (1.47 g, 60%) as a white solid: mp 155–158 °C. Anal. ($C_{10}H_{14}ClN_3O_5S_2$) C, H, N.

***N*-[(4-Chloro-3-sulfamoylphenyl)sulfonyl]ethylenediamine (5).** A suspension of acetyl derivative **4** (2.3 g, 6.47 mmol) in EtOH (100 mL) and 6 N HCl (23 mL) was refluxed for 32 h. After cooling, the separated white solid was filtered off (mp 251–253 °C dec) and then dissolved in H_2O (10 mL). The resulting solution was made basic (ca. pH 8) by the addition of a saturated aqueous Na_2CO_3 solution and the precipitated solid was collected, dried, and recrystallized from EtOAc/EtOH to yield **5** (1.3 g, 64%) as an off-white solid: mp 178–180 °C; ^{1}H NMR δ 2.45–2.65 and 2.68–2.90 (each 2 H, m, $NHCH_2CH_2NHSO_2$), 5.20 (5 H, br s, SO_2NH_2, SO_2NH and NH_2), 7.70–7.95 (2 H, m, H-5 and H-6), 8.25 (1 H, d, J = 2.5 Hz, H-2). Anal. ($C_9H_{12}ClN_3O_4S_2$) C, H, N.

1-[(4-Chloro-3-sulfamoylphenyl)sulfonyl]-4-(ethoxycarbonyl)piperazine (6). A solution of 4-chloro-3-sulfamoylbenzenesulfonyl chloride[11] (2 g, 6.89 mmol) in acetone (25 mL) was added dropwise at room temperature to a stirred solution of *N*-(ethoxycarbonyl)piperazine (1.09 g, 6.89 mmol) and triethylamine (0.96 mL, 6.89 mmol) in acetone (15 mL). After the addition was complete, the reaction mixture was stirred at room temperature for 20 min. The precipitated solid was collected and recrystallized from EtOH to give **6** (1.5 g, 53%) as a white solid: mp 212–214 °C. Anal. ($C_{13}H_{18}ClN_3O_6S_2$) C, H, N.

1-[(4-Chloro-3-sulfamoylphenyl)sulfonyl]piperazine (7). A suspension of **6** (2.4 g, 5.83 mmol) in an aqueous solution of 10% Na_2CO_3 (30 mL) was refluxed for 20 h. After cooling, the reaction mixture was adjusted to pH 8 by the addition of a solution of 6 N HCl. The precipitated solid was filtered off, washed with H_2O, dried, and recrystallized from EtOH giving **7** (1.9 g, 96%) as a yellowish-white solid: mp 274–276 °C dec; ^{1}H NMR δ 2.55–2.97 (8 H, m, piperazine CH_2), 7.80–7.92 (2 H, m, H-5 and H-6), 8.20 (1 H, d, J = 2.5 Hz, H-2). Anal. ($C_{10}H_{14}ClN_3O_4S_2 \cdot H_2O$) C, H, N.

***N*-(4-Chloro-3-sulfamoylphenyl)-2-nitroethyleneamine (8).** A mixture of 4-chloro-3-sulfamoylaniline[12] (11 g, 53 mmol) and 1-chloro-2-nitroethane[13] (24 g, 219 mmol) in dry THF (100 mL) was stirred at room temperature for 8 h. The mixture was then evaporated to dryness and the residue purified by flash chromatography eluting with 1% MeOH/CH_2Cl_2 to give **8** (4.3 g, 29%) as a white solid: mp 142–145 °C. Anal. ($C_8H_{10}ClN_3O_4S$) C, H, N.

***N*-(4-Chloro-3-sulfamoylphenyl)ethylenediamine (9).** A stirred solution of **8** (3.7 g, 13.24 mmol) in absolute EtOH (400 mL) was hydrogenated over Raney nickel (0.8 g) at room temperature and atmospheric pressure for 22 h. The mixture was filtered over Celite, and the filtrate was evaporated to dryness and purified by flash chromatography eluting with CH_2Cl_2/MeOH/$NH(C_2H_5)_2$ 73:25:2 to give **9** (2.0 g, 61%) as a white solid: mp 257–260 °C; ^{1}H NMR δ 2.60–2.72 and 2.90–3.15 (each 2 H, m, $NH_2CH_2CH_2NH$), 4.18 (4 H, br s, NH_2 and SO_2NH_2), 6.03–6.20 (1 H, m, NH), 6.60 (1 H, dd, J = 3 and 8.4 Hz, H-6), 7.08 (1 H, d, J = 3 Hz, H-2), 7.14 (1 H, d, J = 8.4 Hz, H-5); MS m/z 249.7 (M^+). Anal. ($C_8H_{12}ClN_3O_2S$) C, H, N.

1-(4-Chloro-3-sulfamoylphenyl)piperazine (10). A mixture of bis(chloroethyl)amine hydrochloride (1.73 g, 9.68 mmol) and 4-chloro-3-sulfamoylaniline (2 g, 9.68 mmol) in 1-butanol (60 mL) was refluxed for 48 h and then cooled. Anhydrous K_2CO_3 (1.33 g, 9.6 mmol) was then added and the mixture was refluxed for another 48 h. After cooling, the separated amorphous solid was filtered off and dissolved in H_2O (10 mL) and the resulting solution was made basic (ca. pH 8) by the addition of a saturated aqueous $NaHCO_3$. The white precipitate was collected, dried, and recrystallized from *i*-PrOH to give **10** (0.98 g, 37%) as a snow-white solid: mp 215–217 °C; ^{1}H NMR δ 2.70–2.95 and 2.98–3.20 (each 4 H, m, piperazine CH_2), 6.98 (1 H, dd, J = 3 and 9 Hz, H-6), 7.28 (1 H, d, J = 9 Hz, H-5), 7.37 (1 H, d, J = 3 Hz, H-2); MS m/z 275.7 (M^+). Anal. ($C_{10}H_{14}ClN_3O_2S$) C, H, N.

General Procedure for the Preparation of (Aryloxy)propanolamines 1b–f, 2b–f, 3b–f. This procedure is illustrated for the preparation of 1-[4-(4-chloro-3-sulfamoylbenzoyl)-1-piperazinyl]-3-[(3,4-dihydro-3-oxo-2*H*-1,4-benzothiazin-8-yl)oxy]-propan-2-ol (**1b**).

A mixture of piperazine derivative **3** (0.64 g, 2.11 mmol) and 8-(2,3-epoxypropoxy)-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine[7a] (0.5 g, 2.11 mmol) in absolute EtOH (300 mL) was refluxed for 30 h. The solvent was then evaporated to dryness, and the solid residue was purified by column chromatography eluting with gradient of $CHCl_3$ to 10% EtOH/$CHCl_3$ to give **1b** (0.6 g, 53%) as a white amorphous solid: mp 145–165 °C; ^{1}H NMR δ 2.40–2.60 (6 H, m, $CH_2N(CH_2)CH_2$), 3.30–3.70 (6 H, m, SCH_2 and $CH_2N(CO)CH_2$), 3.90–4.10 (3 H, m, $OCH_2CH(OH)$), 4.90 (1 H, br s, OH), 6.60 and 6.70 (each 1 H, d, J = 8.1 Hz, H-5 and H-7 benzothiazine), 7.10 (1 H, t, J = 8.1 Hz, H-6 benzothiazine), 7.60 (1 H, dd, J = 8.3 and 1.7 Hz, H-6), 7.70 (1 H, d, J = 8.0 Hz, H-5), 7.75 (2 H, br s, SO_2NH_2), 7.95 (1 H, d, J = 1.7 Hz, H-2), 10.50 (1 H, s, NH). Anal. ($C_{22}H_{25}ClN_4O_6S_2$) C, H, N.

Binding Determinations. Pellets containing β_1 type adrenergic receptors were obtained from turkey erythrocyte mem-

branes as described in the literature.[14] [^{3}H]Dihydroalprenolol ([^{3}H]DHA) obtained from New England Nuclear (NEN), having a specific activity of 99.9 Ci/mmole and a radiochemical purity >98.5%, was used as ligand.

β-Adrenergic receptor binding was determined as follows: 100 μL of membranes (431 μg/mL of protein diluted 1:8 v/v) were incubated for 15 min at 37 °C with 6 nM [^{3}H]DHA and 100 μL of various concentrations of the test compound (dissolved in DMSO 5%) in 90 mM sodium chloride and 12 mM Tris, pH = 7.5 (total volume 1 mL). The incubations were stopped by adding 3 mL of cold buffer followed by rapid filtration through glass fiber filter disks (Whatman GF/B). The samples were subsequently washed with 4.5 mL of the same buffer and placed into scintillation vials; 10 mL of Filter-count (Packard) liquid scintillation cocktail was then added to each vial and counted using a scintillation spectrometer (Packard TRI-CARB 300C). Nonspecific binding was defined as nondisplaceable binding in the presence of 10 μM propranolol and specific binding as the difference between total and nonspecific binding. Blank experiments were carried out to determine the effect of the solvent (5%) on binding. The concentration of the test compounds that inhibited [^{3}H]DHA or binding by 50% (IC_{50}) were determined by log-probit analysis with seven concentrations of the displacers, each performed in duplicate. The IC_{50} values obtained were used to calculate apparent inhibition constants (K_i) by the method of Cheng and Prusoff.[15] The K_i values for the inhibition of [^{3}H]-DHA binding are reported in Table I and compared with *tert*-butyl-1,4-benzothiazine derivative **4a**, carteolol, and propranolol.

Anti-Isoprenaline Activity. The β-adrenoceptor blocking activity was studied in vivo by the inhibition of tachycardia induced by isoprenaline (INA) in rats.[16] For this purpose 0.12 μg/kg of isoprenaline were injected intravenously (jugular vein) into male Wistar rats, weighing 250–300 g, previously anaesthetized with sodium nembutal (55 mg/kg, ip). The increased in heart rate (HR) was evaluated by electrocardiograph. After several minutes, when the normal heart rate was restored, the test compound was administered intravenously, dissolved in DMSO, or orally, suspended in 1% gum arabic. Responses to isoprenaline were obtained 2 min after intravenous or 1 h after oral administration. Blank experiments were carried out to determine the effect of the solvent on the test. The comparison was made with two known β-blockers: carteolol and propranolol. Three rats were used per group and the results are reported in Table I as ED_{50} values obtained by log-probit analysis.

Diuretic Activity. Groups of five male CD rats, weighing 150–170 g, were used. All test compounds and hydrochlorothiazide (HCT), as control drug, were orally administered dissolved or suspended in 25 mL/kg of saline. Control animals received 25 mL/kg of saline only. The rats were starved and deprived of water for 18 h prior to the dosing and, after the administration of the test compounds, were immediately placed singly in metabolic cages. No food or water was supplied during the experimental period. Urine was collected during the 0–5-h interval in volumetric graduate cylinders and was analyzed for sodium and potassium by flame photometry. The results are reported in Table II.

Acknowledgment. We are grateful to Mr. Roberto Bianconi for skillful synthetic assistance. The research was assisted in part by a grant from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica.

Supplementary Material Available: ^{1}H NMR data of intermediates and target compounds not reported in the text and antihypertensive activity in SHR model of compound 1c (4 pages). Ordering information is given on any current masthead page.

References

(1) This work was previously presented in part: Cecchetti, V.; Fravolini, A.; Schiaffella, F.; Tabarrini, O.; Bruni, G.; Segre, G. An Approach to Diuretic-β-Adrenergic Blocking Agents. International Symposium on Medicinal Chemistry, Jerusalem, Israel, September 1990, p 41.

(2) Gifford, R. W.; Borazanian, R. A. Traditional First-Line Therapy: Overview of Medical Benefits and Side Effects. *Hypertension* **1989**, *13*(1), 119–124.

(3) (a) Willard, A. K.; Smith, R. L.; Cragoe, E. J. Potential Diuretic-β-Adrenergic Blocking Agents: Synthesis of 3-[2-[(1,1-Dimethylethyl)amino]-1-hydroxyethyl]-1,4-dioxino[2,3-*g*]quinolines. *J. Org. Chem.* **1981**, *46*, 3846–3852. (b) Bouley, E.; Teulon, J. M.; Cazes, M.; Cloarec, A.; Deghenghi, R. [*p*-[(Thienylcarbonyl)amino]phenoxy]propanolamine Derivatives as Diuretic and β-Adrenergic Receptor Blocking Agents. *J. Med. Chem.* **1986**, *29*, 100–103. (c) Kau, S. T.; Howe, B. B.; Li, J. H.-Y.; Smith, L. H.; Keddie, J. R.; Barlow, J. J.; Giles, R. E.; Goldberg, M. E. ICI 147,798: A Novel Diuretic Agent with Beta Adrenoceptor Blocking Activity. *J. Pharmacol. Exp. Ther.* **1987**, *242*, 818–826.

(4) Cecchetti, V.; Schiaffella, F.; Tabarrini, O.; Zhou, W.; Fravolini, A.; Goi, A.; Bruni, G.; Segre, G. Symbiotic Approach to Drug Design: *N*-[(4-Chloro-3-sulfamoylbenzamido)ethyl]propanolamine Derivatives as β-Adrenergic Blocking Agents with Diuretic Activity. *Eur. J. Med. Chem.* **1991**, *26*, 381–386.

(5) (a) Large, M. S.; Smith, L. H. β-Adrenergic Blocking Agents. 19. 1-Phenyl-2-[[(substituted-amido)alkyl]amino]ethanols. *J. Med. Chem.* **1980**, *23*, 112–117. (b) Large, M. S.; Smith, L. H. β-Adrenergic Blocking Agents. 22. 1-Phenoxy-3-[[(substituted-amido)alkyl]amino]-2-propanols. *J. Med. Chem.* **1982**, *25*, 1286–1292. (c) Large, M. S.; Smith, L. H. β-Adrenergic Blocking Agents. 24. Heterocyclic Substituted 1-(Aryloxy)-3-[[(amido)alkyl]amino]propan-2-ols. *J. Med. Chem.* **1982**, *25*, 1417–1422. (d) Large, M. S.; Smith, L. H. β-Adrenergic Blocking Agents. 23. 1-[(Substituted-amido)phenoxy]-3-[[(substituted-amido)alkyl]amino]propan-2-ols. *J. Med. Chem.* **1983**, *26*, 352–357. (e) Mauleòn, D.; Dolors Pujol, M.; Rosell, G. β-Adrenergic Antagonists: *N*-Alkyl and *N*-Amidoethyl(aryl-alkoxy)propanolamines Related to Propranolol. *Eur. J. Med. Chem.* **1988**, *23*, 421–426.

(6) (a) Boissier, G. R.; Dumont, C.; Lesbros, J. Diuretic Activity of 3-Sulfamido-4-chlorobenzoic Acid (SD 141.08) and of 3-Sulfamido-4-chlorobenzamide (SD 141.12). *Therapie* **1966**, *21*, 331–340. (b) Jackman, G. B.; Petrow, V.; Stephenson, O.; Wild, A. M. Diuretic Agents. VI. Some Sulfamoylbenzoic Acids. *J. Pharm. Pharmacol.* **1962**, *14*, 679–686. (c) Hoefle, M. L.; Blouin, L. T.; DeWald, H. A.; Holmes, A.; Williams, D. Diuretics. 4-Substituted 3-Sulfamoylbenzoic Acids Hydrazides. *J. Med. Chem.* **1968**, *11*, 970–973.

(7) (a) Cecchetti, V.; Fravolini, A.; Fringuelli, R.; Schiaffella, F.; Mascellani, G.; Pagella, P. G.; Rugarli, P. L. Synthesis and β-Adrenergic Blocking Activity of Oxypropanolamines of 3,4-Dihydro-3-oxo-2H(1,4)benzothiazine *Farmaco Ed. Sci.* **1987**, *42*, 61–75. (b) Cecchetti, V.; Fringuelli, R.; Schiaffella, F.; Fravolini, A.; Bruni, G.; Fiaschi, A. I.; Segre, G. Synthesis and β-Adrenergic Blocking Activity of 1,4-Benzothiazine Oxime Ethers. *Eur. J. Med. Chem.* **1989**, *24*, 479–484.

(8) Brewster, K.; Coult, D. B.; Pinder, R. M. 1-Phenylpiperazines: Potential Antagonists of Lysergic Acids Diethylamide. *Chem. Therapeut.* **1972**, 87–91.

(9) Nakagawa, K.; Murakami, N.; Yoshizaki, S.; Tominaga, M.; Mori, H.; Yabuuchi, Y.; Shintani, S. Derivatives of 3,4-Dihydrocarbostyril as β-Adrenergic Blocking Agents. *J. Med. Chem.* **1974**, *17*, 529–533.

(10) Parke, Davis Co. 4-Halo-3-sulfamoylbenzoic Acids Esters. Brit. 915,259; *Chem. Abstr.* **1964**, *61*, 6962.

(11) Boehringer, C. F. & Soehne G.m.b.H. Asymmetrically Substituted Disulfonamides. Brit. 898,243; *Chem. Abstr.* **1962**, *57*, 12386.

(12) Jucker, E.; Lindenmann, A.; Schenker, E.; Flückiger, E.; Taeschler, M. Synthetic Drugs. XII. Constitution and Saluretic Effect of 3-Sulfamoyl-4-chlorobenzoic Acid Derivatives and Related Compounds. *Arzneim.-Forsch.* **1963**, *13*, 269–280.

(13) Zefirov, N. S.; Koz'min, A. S.; Zhdankin, V. V.; Nikulin, A. V.; Zyk, N. V. Lithium Perchlorate as a Reagent for Synthesis of Covalently Bonded Organic Perchlorates via Electrophilic Additions of Halogens and Nitronium Tetrafluoroborate to Olefins. *J. Org. Chem.* **1982**, *47*, 3679–3684.

(14) Minneman, K. P.; Weiland, G. A.; Molinoff, P. B. A Comparison of the Beta-Adrenergic Receptor of the Turkey Erythrocyte with Mammalian $Beta_1$ $Beta_2$ Receptors. *Mol. Pharmacol.* **1979**, *17*, 1–7.

(15) Cheng, Y. C.; Prusoff, W. H. Relationship between the Inhibition Constant (K_I) and the Concentration of Inhibitor which Causes 50% Inhibition (I_{50}) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

(16) Mylecharane, E. J.; Raper, C. 2-Nitrilophenoxypropanolamines: β-Adrenoceptor Antagonism in the Rat. *Arch. Int. Pharmacodyn.* **1973**, *202*, 163–170.