Pergamon

Bioorganic & Medicinal Chemistry, Vol. 5, No. 7, pp 1275–1283, 1997

PII: S0968-0896(97)00077-1
0968-0896/97 $17.00 + 0.00

Arylimino-1,2,4-Thiadiazolidinones: A New Family of Potassium Channel Openers

Ana Martínez,[a,*] Ana Castro,[a] Ignacio Cardelús,[b] Jesús Llenas[b] and José M. Palacios[b]
[a]*Instituto de Química Médica (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain*
[b]*Laboratorios Almirall, SA, Cardener, 68–74, 08024 Barcelona, Spain*

Abstract—A series of arylimino-1,2,4-thiadiazolidines were prepared using an efficient synthesis starting from thiadiazolopyridinium chlorides. All the compounds showed smooth muscular relaxant properties in rat portal veins. The different behaviour under highly depolarized conditions and the reduction of the biological effect by glyburide suggests that the arylimino-1,2,4-thiadiazolidin-3-ones may act, at least in part, via K^+-induced hyperpolarization of vascular smooth cells. © 1997 Elsevier Science Ltd.

Introduction

Recently, markedly increased attention has been focused on potassium channel openers[1] because of their potential value in the treatment of diseases[2] involving smooth muscle contraction, such as hypertension,[3] angina pectoris,[4] asthma,[5] and urinary incontinence.[6] The active research in this field has stimulated the synthesis of a chemically diverse group of compounds with the aim of developing drugs for smooth muscle relaxation.[7]

Although the exact nature of the K_{ATP}-channel opened by these compounds remains unclear,[8] and consequently it is unknown whether or not all of the structurally heterogenous potassium channel openers bind to the same active site,[9] a pharmacophore model in this class of compounds possessing four distinct areas (two lipophilic and two hydrogen bonding regions) was recently described.[10] In addition, in vitro structure–activity studies on pinacidil-type compounds led to the definition of a receptor binding model[11] in which three elements are present: a hydrogen bond-donating site flanked by a hydrogen bond-accepting interaction and a lipophilic pocket. However, the discovery of aryl cyanoguanidines[12] and hetereoaromatic cyanoamidines[13] (Fig. 1) as potent antihypertensive agents has shown that neither the pyridine nitrogen nor the hydrogen bonding region are necessary for the biological activity of pinacidil-type compounds. Only the lipophilic receptor binding site remains a common feature in all the active drugs and a recent report confirms its importance.[14]

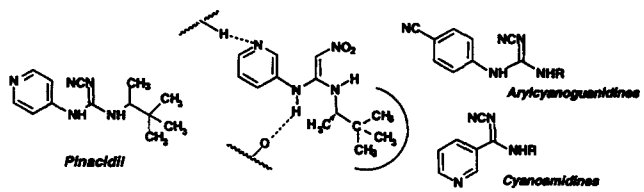


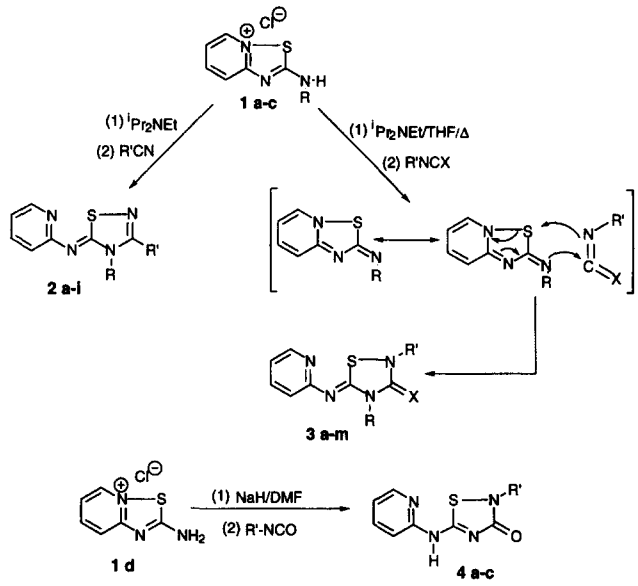
Figure 1.

Considering this background and continuing with our work on smooth muscle relaxants,[15] we report here the synthesis and the rat portal vein vasorelaxant activity of 1,2,4-thiadiazolidines.[16] This series of compounds is structurally different to the PCOs described but, in a very general sense, could be considered as conformationally restricted pinacidil related analogues. The new compounds have a central planar portion consisting of a heterocyclic framework capable of bearing pyridylimino or pyridylamino substituents and different alkyl groups to probe the position of the lipophilic binding site

Results and Discussion

Chemistry

The 1,2,4-thiadiazolidines prepared for this study were initially synthesized following a new and versatile method which involves the use of 1,2,4-thiadiazolo-[2,3-*a*]pyridinium chlorides as intermediates[17] (Scheme 1). These salts, that can be easily obtained by sulfuryl chloride oxidation of thioureas, present a high reactivity towards electrophiles which opens an easy and efficient synthetic pathway to many heterocycles bearing pyridylimino or pyridylamino substituents. Thus, reactions of thiadiazolopyridinium salts in a basic medium (diisopropylethylamine) with alkyl and aryl nitriles afforded 5-pyridylimino-3-substituted-1,2,4-thiadiazolines **2a–i**.[18] On the other hand, the nucleophilic addition of the thiadiazolopyridinium salts to alkyl and aryl isocyanates or isothiocyanates afforded *N*-2 substituted 1,2,4-thiadiazolidin-3-ones and 3-thiones **3a–m** in good yields after 2 h at tetrahydrofurane reflux temperature. When 2-amino-1,2,4-thiadiazolo[2,3-*a*]-pyridinium chloride **1d** is used, a stronger base such



Scheme 1.

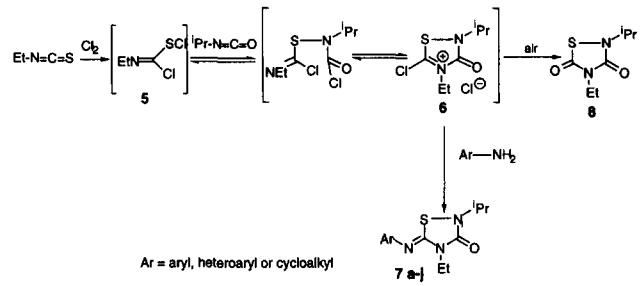
as sodium hydride is necessary to deprotonate the exocyclic nitrogen, which is the first step in the mechanism of these reactions, yielding pyridylamino derivatives **4a–c**.

In order to introduce different arylimino moieties in the 1,2,4-thiadiazolidin-3-one framework a second synthetic pathway, which is based on the reactivity of *N*-alkyl-*S*-[*N'*-(chlorocarbonyl)amino]isothio-carbamoyl chlorides,[19] has been employed. Thus, chlorination of ethylisothiocyanate in an inert atmosphere and subsequent reaction with *N*-isopropylisocyanate produces sparingly soluble 3-oxothiadiazolium salt **6** via the intermediate iminochloromethylsulfenyl chloride **5**. Salt **6** is an exceptionally reactive, white solid, which fumes heavily in moist air yielding after evolution of HCl, the 1,2,4-thiadiazolidine-3,5-dione **8**. Only when compound **6** is not isolated and manipulated under a dry nitrogen atmosphere, can it be easily converted in thiadiazolidinones **7a–h** by reaction with primary amines (aryl, heteroaryl, and cycloakyl) in a 1:3 salt:amine ratio respectively (Scheme 2).

The structures of all the new synthesized compounds were established according to their analytical and spectroscopic ^1H and ^{13}C NMR data (Tables 1–4), complemented with NOE, COSY, and HETCOR experiments.

Pharmacology

The vasodilating activity of arylimino-1,2,4-thiadiazolidines was first examined by measuring the inhibitory effect on the 20 mM KCl-induced contraction of rat portal vein at two different concentrations, following the procedure described by Jetley and Weston,[20] as shown in Table 5. Pinacidil was considered the standard reference for all the experiments. All the compounds



Scheme 2.

show moderate vasorelaxant properties. More interesting is the different biological behaviour of these compounds under highly depolarized conditions (using 80 mM KCl). The 1,2,4-thiadiazolidine series, in which the *N*-2 position is unsubstituted, shows a great inhibitory effect whilst the relaxation activity of the 1,2,4-thiadiazolidinone family under the same conditions is very weak. The differential ability to inhibit low versus high potassium concentration-induced contractions suggests that this new family of aryl-1,2,4-thiadiazolidin-3-ones may act, at least in part, via K^+-induced hyperpolarization of vascular smooth cells. To further characterize the putative mechanism of action, the best thiadiazolidinones (**3j**, **7f** and **7h**) were tested in the presence of glyburide (10^{-5} M) and compared with their respective relaxant potency when given alone in the isolated rat portal vein. The results obtained were as follows (change of IC_{50} in μM): compound **3j**: from 12.3 to 24.6, compound **7f**: from 17.9 to 36.0, and compound **7h**: from 22.4 to 42.3, when given alone or with glyburide, respectively. The fact that this classical potassium channel blocker reduces the thiadiazolidinones smooth muscle relaxant effect shows the intermediacy of K_{ATP} channels in the mechanism of action of this new family of arylimino-1,2,4-thiadiazolidinones.

Molecular modelling

In order to shed some light on the differential pharmacological profile of these compounds, a computer molecular modelling study was carried out by 3-D comparison of one selected member of each family (compounds **2f** and **3j**) and the standard reference pinacidil.

Previously, a full geometrical optimization at the restricted Hartree–Fock level (RHF) using the AM1 method[22] (MOPAC 5.0 program,[23] Chem-QM interface[24]) was performed on pinacidil to determine the preferred conformation. It is well known that cyanoguanidines can present conformational isomerism due to restricted rotation about the sp^2-carbon and nitrogen bonds and *E,Z* isomerism of the *N*-cyanoimino bond.[25,26] In the case of pinacidil, the calculated values of ΔH_f for the eight possible conformations (Fig. 2) show that it should exist predominantly in the staggered conformation Ih, the same independently described by Koga et al.[10] and Quast[11] using different theoretical methods.

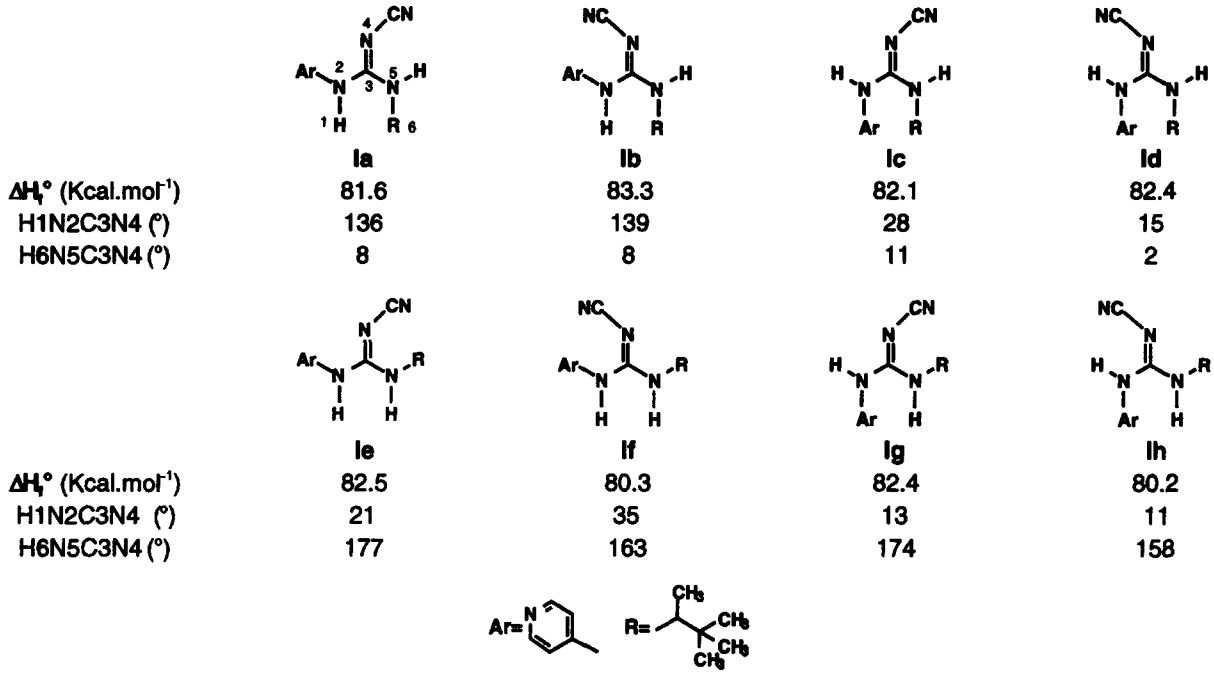


Figure 2.

Optimized geometries of the selected compounds **2f** and **3j** were obtained with AM1 calculations starting from conformations close to that determined by X-ray diffraction of 1,2,4-thiadiazolines[18] and considering the standard input geometries within Chem-X. A 3-D comparison of all these optimized structures and the low energy conformer Ih of pinacidil was performed by least-squares molecular rigid fitting.[27] The chosen atoms for molecular orientation were the three guanidine nitrogen atoms for Ih, and the two nitrogen and sulfur atoms linked to the thiadiazolidine carbon for the rest of the molecules. The best fittings are depicted in Figure 3 showing that in thiadiazolidine **2f** the alkyl group attached to the heterocycle moiety has a different orientation in the rigid derivative with regard to pinacidil, whilst in thiadiazolidinone **3j** the *N*-2 substituent overlay in the same area of the trimethylpropyl pinacidil moiety (Fig. 3). Considering the postulated lipophilic interaction in the receptor binding site,[11] this spatial difference that was found may explain the

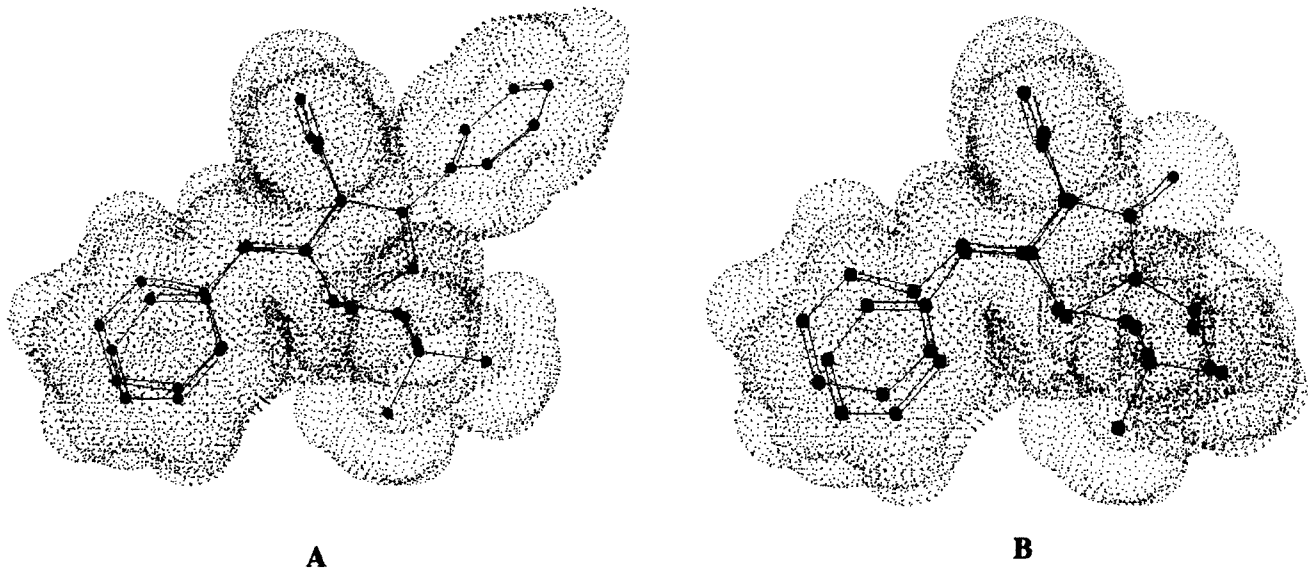
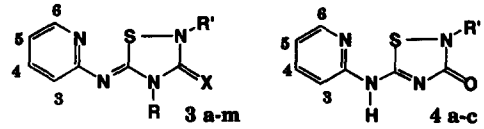


Figure 3. (A) Superimposition of thiadiazolidine **2f** (blue) and pinacidil (red). (B) Superimposition of thiadiazolidinone **3j** (blue) and pinacidil (red).

Table 1. ^{1}H NMR chemical shifts (ppm) and coupling constants (Hz) of compounds **3a–m** and **4a–c** ($CDCl_3$)



No	R	R′	H-3	H-4	H-5	H-6	R	R′	$J_{H_6H_5}$	$J_{H_6H_4}$	$J_{H_5H_4}$	$J_{H_5H_3}$	$J_{H_4H_3}$	$J_{H_6H_3}$
3a[a]	Et	Et	7.20 (dd)	7.60 (td)	6.84 (td)	8.24 (dd)	1.30 (t) 4.05 (c)	1.22 (t) 3.54 (c)	6.2	1.8	8.3	0.9	8.2	—
3b[b]	Et	Pr	7.32 (d)	7.70 (td)	6.95 (td)	8.33 (dd)	1.37 (t) 4.12 (c)	0.97 (t) 1.73 (m); 3.52 (m)	4.4	1.8	6.1	—	8.3	—
3c[c]	Et	chex.	7.21 (dd)	7.61 (td)	6.85 (td)	8.28 (dd)	1.29 (t) 4.06 (c)	3.97 (m) 1.96–1.55 (m)	5.3	1.8	8.3	1.0	8.3	0.9
3d[d]	Et	Ph	7.06 (m)	7.60 (m)	6.84 (t)	8.20 (dd)	1.32 (t) 4.14 (c)	7.06–7.60 (m) 4.50 (m)	5.4	—	—	—	—	—
3e[e]	Et	Adam.	7.20 (dd)	7.61 (td)	6.84 (td)	8.28 (dd)	1.29 (t) 4.03 (c)	1.68 (m); 2.27 (m) 2.29 (m)	4.4	0.9	5.2	1.4	6.9	—
3f[f]	Et	Me	7.26 (d)	7.64 (t)	6.88 (t)	8.26 (d)	1.32 (t) 4.07 (c)	3.06 (s)	4.6	1.7	6.9	—	8.1	—
3g[g]	Et	Me	7.39 (dd)	7.74 (td)	6.96 (td)	8.32 (dd)	1.38 (t) 4.56 (c)	3.39 (s)	5.3	1.6	5.6	1.2	8.3	—
3h[h]	Et	Allyl	7.36 (d)	7.73 (t)	6.96 (m)	8.32 (d)	1.38 (t) 4.57 (c)	5.93 (m) 5.28 (m)	4.4	—	—	—	8.3	—
3i[i]	Me	^{i}Pr	7.28 (dd)	7.68 (td)	6.94 (td)	8.34 (dd)	3.50 (s)	1.37 (d) 4.45 (m)	4.4	1.8	8.8	1.0	7.3	—
3j[j]	Et	^{i}Pr	7.22 (dt)	7.61 (td)	6.85 (td)	8.26 (dd)	1.29 (t) 4.07 (c)	1.31 (d) 4.37 (m)	5.2	1.7	6.4	1.1	8.2	0.9
3k[k]	Bn	^{i}Pr	7.58 (dd)	7.69 (td)	6.94 (td)	8.35 (dd)	5.23(s) 7.25–7.36 (m)	1.40 (d) 4.50 (m)	4.4	0.9	7.0	1.0	8.9	—
3l	Me	Me	7.27 (dd)	7.66 (td)	6.91 (td)	8.26 (ddd)	3.06 (s)	3.47 (s)	4.4	1.8	8.3	1.9	8.2	—
3m	Bn	Me	7.52 (dd)	7.60 (td)	6.85 (td)	8.22 (dd)	5.16 (s) 7.18–7.28 (m)	3.03 (s)	6.2	1.7	6.4	1.8	8.2	0.9
4a[l]		Et	8.27 (m)	7.78 (td)	6.99 (td)	8.27 (m)		1.37 (t) 3.75 (m)	5.2	1.5	5.2	0.7	7.3	—
4b[m]		iPr	8.17 (d)	7.72 (td)	6.93 (td)	8.23 (ddd)		1.38(d) 4.54 (m)	4.3	1.7	7.2	1.2	8.3	0.8
4c		chex.	8.29 (m)	7.78 (td)	6.99 (td)	8.29 (m)		1.22–2.16(m) 4.22 (m)	4.8	—	4.8	—	8.3	1.9

[a] $J_{CH_2CH_3} = 7.1$, $J_{CH_2CH_3} = 7.2$.
[b] $J_{CH_2CH_3} = 7.0$, $J_{CH_2CH_2} = 7.4$.
[c] $J_{CH_2CH_3} = 7.1$.
[d] $J_{CH_2CH_3} = 7.0$.
[e] $J_{CH_2CH_3} = 7.0$.
[f] $J_{CH_2CH_3} = 7.0$.
[g] $J_{CH_2CH_3} = 7.0$.
[h] $J_{CH_2CH_3} = 7.0$.
[i] $J_{CHCH_3} = 6.7$.
[j] $J_{CH_2CH_3} = 7.0$, $J_{CHCH_3} = 6.7$.
[k] $J_{CHCH_3} = 6.7$.
[l] $J_{CH_2CH_3} = 7.2$.
[m] $J_{CHCH_3} = 6.7$.

different biological behaviour of 1,2,4-thiadiazolidines and 1,2,4-thiadiazolidinones.

Conclusion

The use of thiadiazolopyridinium chlorides as intermediates in heterocyclic synthesis has led to a new class of vasorelaxant agents: the arylimino-1,2,4-thiadiazolidin-3-ones.

The K^+-induced hyper-polarization of vascular smooth muscle cells is involved in their biological behaviour. Further efforts, including the application of neuronal networks, are being carried out in order to optimize the vasorelaxant properties of these new lead compounds.

Experimental

Chemistry

General procedures. Reagents were purchased from commercial suppliers and used without further purification. Reaction solvents were distilled from an appro-

Table 2. ^{1}H NMR chemical shifts (ppm) and coupling constants (Hz) of compounds **7a–h** ($CDCl_3$)

No	Ar	iPr			Et			Aryl
		CH	CH_3	J_{CHCH_3}	CH_2	CH_3	$J_{CH_2CH_3}$	
7a	3-pyridyl	4.57 (q)	1.15 (d)	6.6	3.38 (c)	1.29 (t)	7.1	8.31 (H-6, H-2); 7.25 (H-5, H-4)
7b	4-pyridyl	4.57 (q)	1.17 (d)	6.6	3.37 (c)	1.28 (t)	7.0	8.46 (H-6, H-2, J = 4.7); 6.86 (H-5, H-3, J = 4.7)
7c	4-CN–C_6H_5	4.55 (q)	1.15 (d)	6.6	3.35 (c)	1.27 (t)	7.1	7.56 (H*o*CN, J = 8.7); 7.01 (H*m*CN, J = 8.7)
7d	C_6H_5	4.56 (q)	1.17 (d)	6.6	3.89 (c)	1.25 (t)	7.1	7.32 (H*m*, J = 7.9); 7.11 (H*p*, J = 7.3); 6.95 (H*o*, J = 7.2)
7e	C_6H_{11}	4.56 (q)	1.13 (m)	6.6	3.72 (c)	1.13 (m)	7.0	2.53 (CHcyclo.); 1.13–1.72 ($(CH_2)_n$)
7f	4-OMe–C_6H_5	4.55 (q)	1.13 (d)	6.6	3.82 (c)	1.27 (t)	7.0	6.79–6.89 (Ar-H); 3.73 (OCH_3)
7g	3-OMe–C_6H_5	4.55 (q)	1.13 (d)	6.6	3.80 (c)	1.23 (t)	7.1	7.12 (H*m*OCH_3, J = 8.2); 6.59–6.44 (Ar-H; 3.67 (OCH_3)
7h	3,4-diOMe–C_6H_5	4.57 (q)	1.15 (d)	6.7	3.85 (c)	1.30 (t)	7.1	6.75 (Ar-H, J = 8.1); 6.51 (Ar-H, J = 8.1); 3.82 (OCH_3)

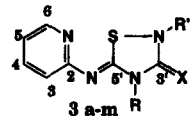
priate drying agent before use. Flash column chromatography was carried out at medium pressure using silica gel (E. Merck, Grade 60, particle size 0.040–0.063 mm, 230–240 mesh ASTM) with the indicated solvent as eluent. Melting points were measured on a Reichert–Jung Thermovar and are uncorrected.

^{1}H NMR spectra were recorded at room temperature using a Brucker AM-200 (200 MHz) and a Varian XL-300 (300 MHz) spectrometers. ^{13}C NMR spectra were recorded at room temperature using the same spectrometers operating at 50 and 75 MHz, respectively. Chemical shifts are reported in δ values (ppm) relative to internal Me_4Si and J values are reported in hertz.

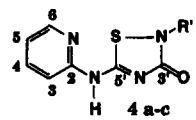
Elemental analyses were performed by the analytical department at CNQO (CSIC) and the results obtained were within ±0.4% of the theoretical values.

General method for the synthesis of 5-(2-pyridylimino)-1,2,4-thiadiazolidin-3-ones. A suspension of the corresponding 2-alkylamino-1,2,4-thiadiazolo[2,3-*a*]-pyridinium chloride[18] (1 mmol), alkyl- or arylisocyanate or isothiocyanate (1 mmol) and *N*,*N*′-diisopropyl-ethyl-

Table 3. ^{13}C NMR chemical shifts of compounds **3a–m** and **4a–c** ($CDCl_3$)



3 a-m



4 a-c

No.	R	R′	C-2	C-3	C-4	C-5	C-6	C-5′	C-3′	R	R′
3a	Et	Et	153.17	117.17	138.34	119.92	140.97	157.80	158.70	13.52; 38.11	15.06; 39.28
3b	Et	Pr	153.03	116.66	137.86	119.49	140.55	157.42	158.41	12.97; 38.86	11.12; 22.58; 44.47
3c[a]	Et	chex	152.45	116.69	137.83	119.52	141.15	157.53	158.33	13.00; 38.72	53.33
3d[b]	Et	Ph	151.17	116.65	138.11	119.33	138.63	156.81	159.21	12.89; 38.80	—
3e[c]	Et	Adam.	151.84	116.37	137.70	119.22	141.18	157.66	158.33	12.88; 38.23	—
3f	Et	Me	153.21	116.60	137.82	119.35	140.03	157.18	158.40	12.94; 38.86	28.59
3g	Et	Me	155.74	117.15	138.48	120.01	139.27	158.74	173.08	12.45; 43.82	33.44
3h[d]	Et	Allyl	155.59	117.18	138.45	119.95	139.47	158.69	172.56	12.41; 43.66	43.50
3i	Me	^{i}Pr	152.64	116.91	137.97	119.41	141.08	157.25	158.77	30.01	45.97; 21.70
3j	Et	^{i}Pr	152.46	116.77	137.87	119.54	141.13	157.51	158.19	12.99; 38.71	21.73; 45.89
3k[e]	Bn	^{i}Pr	152.55	116.93	137.89	119.73	141.16	157.31	158.23	46.65	21.75; 46.03
3l	Me	Me	153.88	116.92	138.10	119.47	140.25	157.25	159.24	30.37	28.89
3m[f]	Bn	Me	153.36	116.80	137.88	119.64	140.16	157.17	158.53	46.87	28.78
4a	—	Et	151.61	114.48	139.04	117.74	143.06	166.15	162.12	—	15.08; 37.53
4b	—	^{i}Pr	150.76	113.75	139.07	117.78	143.47	166.73	162.16	—	22.56; 45.92
4c[g]	—	chex	151.03	113.92	139.05	117.75	143.47	166.67	161.93	—	53.30

[a]C-cyclohexyl: 32.31; 25.71; 25.29.
[b]C-phenyl: 137.47; 128.86; 125.13; 123.26.
[c]C-adamantyl: 29.68; 36.30; 40.59; 57.46.
[d]C-allyl: 118.38; 131.91.
[e]C-arom: 127.54; 128.79; 128.79; 136.71.
[f]C-arom: 127.53; 128.26; 128.74; 136.56.
[g]C-cyclohexyl: 25.36; 25.83; 33.29.

Table 4. ^{13}C NMR chemical shifts of compounds **7a–h** ($CDCl_3$)

No.	Ar	C-3	C-5	Et	^{i}Pr	Ar
7a	3-Pyridyl	153.43	152.25	12.36 38.53	20.81 46.89	145.52 (C2′); 144.94 (C3′); 142.96 (C6′); 128.11 (C4′); 151.18 (C2′)
7b	4-Pyridyl	155.66	153.40	12.54 38.79	21.03 47.14	151.95 (C4′); 151.18 (C2′); 116.29 (C3′)
7c	4-CN-C_6H_5	153.44	152.91	12.56 38.83	21.04 47.17	118.88 (CN), 151.90 (C4′); 133.73 (C2′); 121.95 (C3′); 107.66 (C1′)
7d	C_6H_5	153.77	150.70	12.44 38.41	20.76 46.72	148.94 (C1′); 129.39 (C4′); 124.42 (C3′);120.78 (C2′)
7e	C_6H_{11}	154.87	145.83	12.43 38.20	20.89 46.69	83.78; 33.59; 25.55; 24.83
7f	4-OMe–C_6H_5	154.03	151.06	12.64 38.56	20.99 46.79	55.32 (OCH_3), 156.65 (C1′); 142.32 (C4′); 121.93 (C3′); 114.73 (C2′)
7g	3-OMe–C_6H_5	153.45	150.58	12.23 38.17	20.54 46.87	54.72 (OCH_3), 160.31 (C5′); 150.01 (C3′); 129.93 (C1′); 112.40 (C2′); 110.05 (C4′); 106.25 (C6′)
7h	3,4-diOMe–C_6H_5	153.87	151.15	12.59 38.52	20.92 46.74	55.72 (OCH_3), 149.71 (C3′); 146.10 (C4′); 142.77 (C1′); 115.51 (C6′); 111.21 (C5′); 105.69 (C2′)

amine (1 mmol) in THF (20 mL) was refluxed for 2 h. The solvent was evaporated to dryness in vacuo and the residue was chromatographed on a silica gel column using the appropiate eluent.

2,4-Diethyl-5-(2-pyridylimino)-1,2,4-thiadiazolidin-3-one (3a). Reagents: 2-ethylamino-1,2,4-thiadiazolo[2,3-*a*]-pyridinium chloride and ethylisocyanate. Silica gel chromatography column eluent: ethyl acetate:hexane (1:3); yield 73%; mp 89–90 °C. Anal. calcd for $C_{11}H_{14}N_4OS$: C, 52.78; H, 5.64; N, 22.38; S, 12.81. Found: C, 52.75; H, 5.63; N, 22.02; S, 12.74.

4-Ethyl-2-propyl-5-(2-pyridylimino)-1,2,4-thiadiazolidin-3-one (3b). Reagents: 2-ethylamino-1,2,4-thiadiazolo-[2,3-*a*]pyridinium chloride and propylisocyanate. Recrystallization solvent: ethanol/water; yield 30%; mp 93–95 °C. Anal. calcd for $C_{12}H_{16}N_4OS$: C, 54.52; H, 6.10; N, 21.19; S, 12.12. Found: C, 54.69; H, 6.35; N, 21.21; S, 11.97.

4-Ethyl-2-cyclohexyl-5-(2-pyridylimino)-1,2,4-thiadiazolidin-3-one (3c). Reagents: 2-ethylamino-1,2,4-thiadiazolo[2,3-*a*]pyridinium chloride and cyclohexylisocyanate. Silica gel chromatography column eluent: ethyl acetate:hexane (1:3); yield 78%; mp 90–91 °C. Anal. calcd for $C_{15}H_{20}N_4OS$: C, 59.18; H, 6.62; N, 18.40; S, 10.53. Found: C, 59.25; H, 6.69; N, 18.64; S, 10.21.

4-Ethyl-2-phenyl-5-(2-pyridylimino)-1,2,4-thiadiazolidin-3-one (3d). Reagents: 2-ethylamino-1,2,4-thiadiazolo-[2,3-*a*]pyridinium chloride and phenylisocyanate. Silica gel chromatography column eluent: ethyl acetate: hexane (1:3); yield 45%; mp 90–92 °C. Anal. calcd for $C_{15}H_{14}N_4OS$: C, 60.38; H, 4.73; N, 18.78; S, 10.74. Found: C, 60.17; H, 5.05; N, 18.38; S, 10.53.

4-Ethyl-2-adamantyl-5-(2-pyridylimino)-1,2,4-thiadiazolidin-3-one (3e). Reagents: 2-ethylamino-1,2,4-thiadiazolo[2,3-*a*]pyridinium chloride and adamantylisocyanate. Recrystallization solvent: methanol; yield 57%; mp 163–164 °C. Anal. calcd for $C_{15}H_{20}N_4OS$: C, 63.83; H, 7.05; N, 15.67; S, 8.97. Found: C, 63.98; H, 6.92; N, 15.67; S, 8.80.

4-Ethyl-2-methyl-5-(2-pyridylimino)-1,2,4-thiadiazolidin-3-one (3f). Reagents: 2-ethylamino-1,2,4-thiadiazolo-[2,3-*a*]pyridinium chloride and methylisocyanate. Silica gel chromatography column eluent: ethyl acetate: hexane (3:1); yield 52%; mp 124–125 °C. Anal. calcd for $C_{10}H_{12}N_4OS$: C, 50.82; H, 5.12; N, 23.71; S, 13.56. Found: C, 50.89; H, 5.30; N, 24.00; S, 13.70.

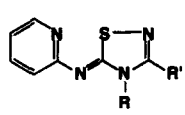
4-Ethyl-2-methyl-5-(2-pyridylimino)-1,2,4-thiadiazolidin-3-thi-one (3g). Reagents: 2-ethylamino-1,2,4-thiadiazolo-[2,3-*a*]pyridinium chloride and methilisothiocyanate. Silica gel chromatography column eluent: ethyl acetate:hexane (1:6); yield 41%; mp 117–119 °C. Anal. calcd for $C_{10}H_{12}N_4S_2$: C, 47.59; H, 4.79; N, 21.85; S, 25.41. Found: C, 47.82; H, 4.76; N, 22.10; S, 25.25.

4-Ethyl-2-allyl-5-(2-pyridylimino)-1,2,4-thiadiazolidin-3-thione (3h). Reagents: 2-ethylamino-1,2,4-thiadiazolo-[2,3-*a*]pyridinium chloride and allylisothiocyanate. Silica gel chromatography column eluent: ethyl acetate: hexane (1:3); yield 41%; mp 117–119 °C. Anal. calcd for $C_{12}H_{14}N_4S_2$: C, 51.77; H, 5.06; N, 20.12; S, 23.03. Found: C, 52.01; H, 4.93; N, 20.31; S, 23.20.

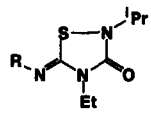
4-Methyl-2-isopropyl-5-(2-pyridylimino)-1,2,4-thiadiazolidin-3-one (3i). Reagents: 2-methylamino-1,2,4-thiadiazolo[2,3-*a*]pyridinium chloride and isopropylisocyanate. Silica gel chromatography column eluent: ethyl acetate:hexane (1:2); yield 35%; mp 97–98 °C. Anal. calcd for $C_{11}H_{14}N_4OS$: C, 52.77; H, 5.63; N, 22.38; S, 12.80. Found: C, 53.01; H, 5.64; N, 22.40; S, 12.71.

4-Ethyl-2-isopropyl-5-(2-pyridylimino)-1,2,4-thiadiazolidin-3-one (3j). Reagents: 2-ethylamino-1,2,4-thiadiazolo[2,3-*a*]pyridinium chloride and isopropylisocyanate. Recrystallization solvent: methanol/water; yield 72%; mp 103–105 °C. Anal. calcd for $C_{12}H_{16}N_4OS$: C, 54.52; H, 6.10; N, 21.19; S, 12.12. Found: C, 54.50; H, 6.30; N, 21.35; S, 12.40.

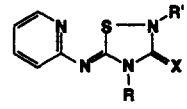
Table 5. Biological data for compounds **2a–i**, **3a–m**, **4a–c**, and **7a–h**



2a-i



7a-h



3a-m, 4a-c

Compd	R	R′	% Inhibition K^+ 20 mM[a] 10^{-5} M	% Inhibition K^+ 20 mM[a] 10^{-6} M	% Inhibition K^+ 80 mM[a] 10^{-5} M	Compd	R	R′	X	% Inhibition K^+ 20 mM[a] 10^{-5} M	% Inhibition K^+ 20 mM[a] 10^{-6} M	% Inhibition K^+ 80 mM[a] 10^{-5} M
2a	Me	Me	30±4	—	29±1	**3a**	Et	Et	O	31±2	9±2	12±3
2b	Me	Et	66±3	—	67±5	**3b**	Et	Pr	O	24±2	12±2	2±1
2c	Me	Ph	38±9	—	51±1	**3c**	Et	C_6H_{11}	O	30±6	13±8	9±1
2d	Et	Me	85±6	—	86±4	**3d**	Et	C_6H_5	O	16±6	7±4	2±1
2e	Et	Et	65±6	—	67±5	**3e**	Et	Adamantyl	O	9±3	2±8	2±1
2f	Et	Ph	67±3	—	88±2	**3f**	Et	Me	O	42±2	17±3	3±2
2g	Bn	Me	52±12	—	47±6	**3g**	Et	Me	S	35±7	6±4	12±2
2h	Bn	Et	17±3	—	26±2	**3h**	Et	Allyl	S	1±10	0±8	14±3
2i	Bn	Ph	32±3	—	41±1	**3i**	Me	^{i}Pr	O	27±5	7±7	11±5
						3j	Et	^{i}Pr	O	60±20	11±2	−9±11
7a	3-Pyridyl		24±3	13±1	4±1	**3k**	Bn	^{i}Pr	O	21±4	7±2	0±0
7b	4-Pyridyl		19±2	5±3	0±5	**3l**	Me	Me	O	19±6	4±6	0±4
7c	4-CN-C_6H_5		18±3	9±2	4±1	**3m**	Bn	Me	O	18±6	−5±5	3±2
7d	C_6H_5		30±1	20±3	2±2							
7e	C_6H_{11}		14±3	12±2	11±2	4a	—	Et	O	25±5	9±2	0±0
7f	4-OMe-C_6H_5		67±6	15±2	0±9	**4b**	—	^{i}Pr	O	27±3	10±2	−3±2
7g	3-OMe-C_6H_5		49±8	34±4	13±7	**4c**	—	C_6H_{11}	O	26±2	8±2	12±3
7h	3,4-OMe-C_6H_5		51±4	19±7	34±5							

[a]Effect over the rat portal vein spontaneous movement. The results are showed in % inhibition (mean±M.E.E., n = 3–5) respected the basal response. Pinacidil is used as standard (% inhibition K^+ 20 mM = 94±4 at 10^{-5} M, 74±5 at 10^{-6} M. % inhibition K^+ 80 mM = −3±5 at 10^{-5} M.)

4-Benzyl-2-isopropyl-5-(2-pyridylimino)-1,2,4-thiadiazolidin-3-one (3k). Reagents: 2-bencylamino-1,2,4-thiadiazolo[2,3-*a*]pyridinium chloride and isopropylisocyanate. Recrystallization solvent: ethanol/water; yield 61%; mp 120–121 °C. Anal. calcd for $C_{17}H_{18}N_4OS$: C, 62.55; H, 5.55; N, 17.16; S, 9.82. Found: C, 62.31; H, 5.60; N, 17.00; S, 9.62.

2,4-Dimethyl-5-(2-pyridylimino)-1,2,4-thiadiazolidin-3-one (3l). Reagents: 2-methylamino-1,2,4-thiadiazolo-[2,3-*a*]pyridinium chloride and methylisocyanate. Silica gel chromatography column eluent: ethyl acetate:hexane (1:1); yield 25%; mp 124–126 °C. Anal. calcd for $C_9H_{10}N_4OS$: C, 48.63; H, 4.53; N, 25.21; S, 14.42. Found: C, 48.29; H, 4.89; N, 25.04; S, 14.71.

4-Benzyl-2-methyl-5-(2-pyridylimino)-1,2,4-thiadiazolidin-3-one (3m). Reagents: 2-bencylamino-1,2,4-thiadiazolo[2,3-*a*]pyridinium chloride and methylisocyanate. Silica gel chromatography column eluent: ethyl acetate:hexane (1:2); yield 55%; mp 155–156 °C. Anal. calcd for $C_{15}H_{14}N_4OS$: C, 60.38; H, 4.73; N, 18.78; S, 10.74. Found: C, 60.41; H, 4.71; N, 18.61; S, 10.52.

General method for the preparation of 5-(2-pyridylamino)-1,2,4-thiadiazolidin-3-ones. Sodium hydride (0.5 mmol) and the corresponding alkylisocyanate (0.5 mmol) were added to a solution of 2-amino-1,2,4-thiadiazolo[2,3-*a*]pyridinium chloride[18] (0.5 mmol) in DMF (15 mL). The reaction mixture was stirred at 100 °C for 4 h. The solvent was evaporated under reduced pressure. Water was added to the resulting residue and the precipated solid formed was filtered off and purified by recrystallization.

2-Ethyl-5-(2-pyridylamino)-1,2,4-thiadiazolidin-3-one (4a). Reagents: 2-amino-1,2,4-thiadiazolo[2,3-*a*]pyridinium chloride and ethylisocyanate. Recrystallization solvent: ethanol; yield 54%; mp 119–120 °C. Anal. calcd for $C_9H_{10}N_4OS$: C, 48.63; H, 4.53; N, 25.20; S, 14.42. Found: C, 48.60; H, 4.65; N, 25.74; S, 14.36.

2-Isopropyl-5-(2-pyridylamino)-1,2,4-thiadiazolidin-3-one (4b). Reagents: 2-amino-1,2,4-thiadiazolo[2,3-*a*]pyridinium chloride and isopropylisocyanate. Recrystallization solvent: acetonitrile; yield 56%; mp 218–220 °C. Anal. calcd for $C_{10}H_{12}N_4OS$: C, 50.82; H, 5.12; N, 23.71; S, 13.56. Found: C, 51.26; H, 5.38; N, 23.48; S, 13.76.

2-Cyclohexyl-5-(2-pyridylamino)-1,2,4-thiadiazolidin-3-one (4c). Reagents: 2-amino-1,2,4-thiadiazolo[2,3-*a*]pyridinium chloride and cyclohexylisocyanate. Recrystallization solvent: ethanol; yield 92%; mp 248–250 °C. Anal. calcd for $C_{13}H_{16}N_4OS$: C, 56.60; H, 5.57; N, 20.02; S, 11.32. Found: C, 56.49; H, 5.83; N, 20.27; S, 11.60.

4-Ethyl-2-isopropyl-1,2,4-thiadiazolidine-3,5-dione (8). Chlorine (0.28 g, 4 mmol) was added slowly to a solution of ethyl isothiocyanate (0.28 g, 3.3 mmol) in dry hexane (25 mL) at −15 °C to −10 °C. Chlorine was

generated by the addition of HCl 35% (1.55 mL) to MnO_4K (0.25 g). The temperature of the reaction mixture was carefully controlled during the addition step. At this point, *N*-ethyl-*S*-chloroisothiocarbamoyl[19] chloride was formed. Afterwards, isopropyl isocyanate (0.25 g, 3 mmol) was added. The mixture was stirred at room temperature for 12 h and the white solid, which fumes heavily in moist air, is separated in a dry nitrogen atmosphere by suction filtration, to provide **6** (0.50 g, 70%). This compound was used in the next synthetic step without further purification. A solution of **6** (0.48 g, 2 mmol) in EtOH (20 mL) was stirred at room temperature for 12 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using ethyl acetate: hexane (1:3) to give **8** (0.07 g, 19%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.63 (q, 1H, J_{CHCH_3} = 6.6 Hz, $CH(CH_3)_2$), 3.69 (c, 2H, $J_{CH_2CH_3}$ = 6.2 Hz, CH_2CH_3), 1.24 (d, 9H, CH_2CH_3 and $CH(CH_3)_2$). ^{13}C NMR (50 MHz, $CDCl_3$) δ 166.10 and 152.21 (C-5 and C-3 thiadiazolidine moiety), 46.90 (N-*C*H), 37.38 (N-*C*H_2), 21.16 (*C*H_3), 13.06 (*C*H_3). Anal. calcd for $C_7H_{112}N_2O_2S$: C, 44.65; H, 6.42; N, 14.88; S, 17.03 %. Found: C, 44.28; H, 6.18; N, 14.93; S, 16.92 %.

General method for the synthesis of 5-arylimino-1,2,4-thiadiazolidin-3-ones. To a solution of salt **6** (2 mmol) in THF (20 mL), the corresponding amine (6 mmol) was added. The resulting mixture was stirred at room temperature for 12 h, and then filtered. The filtrate was evaporated to dryness in vacuo and the residue purified by silica gel column chromatography using the appropiate solvent.

4-Ethyl-5-(3-pyridylimino)-2-isopropyl-1,2,4-thiadiazolidin-3-one (7a). Reagents: 3-pyridylamine. Silica gel chromatography column eluent: ethyl acetate:hexane (3:1); yield 64%; oil. Anal. calcd for $C_{12}H_{16}N_4OS$: C, 54.52; H, 6.10; N, 21.19; S, 12.12. Found: C, 54.81; H, 6.30; N, 21.35; S, 11.89.

4-Ethyl-5-(4-pyridylimino)-2-isopropyl-1,2,4-thiadiazolidin-3-one (7b). Reagents: 4-pyridylamine. Silica gel chromatography column eluent: ethyl acetate:hexane (3:1); yield 67%; mp 60–62 °C. Anal. calcd for $C_{12}H_{16}N_4OS$: C, 54.52; H, 6.10; N, 21.19; S, 12.12. Found: C, 54.30; H, 6.40; N, 20.92; S, 11.89.

4-Ethyl-5-(4-cyanophenylimino)-2-isopropyl-1,2,4-thiadiazolidin-3-one (7c). Reagents: 4-cyanoaniline. Silica gel chromatography column eluent: ethyl acetate:hexane (1:3); yield 22%; mp 84–85 °C. Anal. calcd for $C_{14}H_{16}N_4OS$: C, 58.31; H, 5.59; N, 19.43; S, 11.11. Found: C, 58.22; H, 5.68; N, 19.27; S, 10.76.

4-Ethyl-5-(phenylimino)-2-isopropyl-1,2,4-thiadiazolidin-3-one (7d). Reagents: aniline. Silica gel chromatography column eluent: ethyl acetate:hexane (1:6); yield 28%; mp 35–36 °C. Anal. calcd for $C_{13}H_{17}N_3OS$: C, 59.28; H, 6.50; N, 15.95; S, 12.17. Found: C, 58.99; H, 6.71; N, 15.69; S, 11.89.

4-Ethyl-5-(cyclohexylimino)-2-isopropyl-1,2,4-thiadiazolidin-3-one (7e). Reagents: cyclohexylamine. Silica gel chromatography column eluent: ethyl acetate:hexane (1:6); yield 11%; mp 32–33 °C. Anal. calcd for $C_{13}H_{23}N_3OS$: C, 57.95; H, 8.60; N, 15.59; S, 11.90. Found: C, 57.67; H, 8.51; N, 15.34; S, 11.70.

4-Ethyl-5-(4-methoxyphenylimino)-2-isopropyl-1,2,4-thiadiazolidin-3-one (7f). Reagents: 4-methoxyaniline. Silica gel chromatography column eluent: ethyl acetate:hexane (1:3); yield 24%; mp 58–60 °C. Anal. calcd for $C_{14}H_{19}N_3O_2S$: C, 57.31; H, 6.53; N, 14.32; S, 10.93. Found: C, 57.12; H, 6.25; N, 14.63; S, 10.87.

4-Ethyl-5-(3-methoxyphenylimino)-2-isopropyl-1,2,4-thiadiazolidin-3-one (7g). Reagents: 3-methoxyaniline. Silica gel chromatography column eluent: ethyl acetate:hexane (1:5); yield 54%; oil. Anal. calcd for $C_{14}H_{19}N_3O_2S$: C, 57.31; H, 6.53; N, 14.32; S, 10.93. Found: C, 57.02; H, 6.25; N, 14.56; S, 11.07.

4-Ethyl-5-(3,4-dimethoxyphenylimino)-2-isopropyl-1,2,4-thiadiazolidin-3-one (7h). Reagents: 4-methoxyaniline. Silica gel chromatography column eluent: ethyl acetate:hexane (1:5); yield 27%; mp 77–78 °C. Anal. calcd for $C_{15}H_{22}N_3O_2S$: C, 58.41; H, 7.19; N, 13.63; S, 10.40. Found: C, 58.28; H, 7.02; N, 13.52; S, 10.21.

Biological methods

Reagents and solutions. All the compounds tested were dissolved in polyethyleneglycol 300 (Merck) 20% in distilled water at 10^{-2} M. Further dilution, if necessary, was done with modified Krebs solution (see below). Pinacidil was synthetized in the Chemistry Department (Almirall).

Isolated rat portal vein. Male Wistar rats weighing 290–310 g were killed by cervical dislocation, and the hepatic portal vein was excised and placed in an oxygenated Krebs–Henseleit solution maintained at room temperature. Portal veins were dissected free of adhering connective tissue and cut longitudinally. One end of each portal vein was tied to a stainless-steel rod, placed in an organ bath containing the same solution at 37 °C, and continuously oxygenated with a mixture of 5% CO_2 in O_2. Contractions were recorded with a Letica polygraph (model 4000) and stored on a PC for further analysis. The muscle preparations were equilibrated in normal Krebs solution for at least 30 min while the resting tension was adjusted at 1 g. During the equilibration period, tissues were washed twice with fresh solution. Afterwards, the bath solution was changed to a modified one containing 20 mM K^+ and the experimental procedure was started when a stable spontaneous motility had been achieved. Compounds under study were added cumulatively and usually 20 min were enough to reach maximal inhibitory responses. The ability of glyburide to block the relaxant effect was also investigated with the best compounds by adding it at 10^{-5} M 20 min before the concentration–

relaxation curve was started. Another group of preparations was used to assess the relaxant activity of these compounds on K^+ 80 mM induced contractions. At the end of each experiment, pinacidil (10^{-6} M) was added to all baths. Spontaneous motility was measured by using specifically designed software (Letica) which calculates the AUC (area under the curve) every 3 min.

Acknowledgements

Financial support from CICYT (project no. SAF 93-710 and SAF 96-107) is gratefully acknowledged.

References

1. Empfield, J. R.; Russell, K. *Ann. Rep. Med. Chem.* **1995,** *39,* 81.

2. Robertson, D. W.; Steinberg, M. I. *J. Med. Chem.* **1990,** *33,* 1529.

3. Russell, K.; Brown, F. J.; Warwick, P.; Forst, J.; Grant, T.; Howe, B.; Kau, S. T.; Li, J. H.; McLaren, F. M.; Shapiro, H. S.; Trivedi, S. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2727.

4. Brown, T. J.; Chapman, R. F.; Mason, J. S.; Palfreyman, M. N.; Vicker, N.; Walsh, R. J. *J. Med. Chem.* **1993,** *36*, 1604.

5. Morley, J. *Trends Pharmacol. Sci.* **1994**, *15*, 463.

6. Grant, T. L.; Ohnmacht, C. J.; Howe, B. B. *Trends Pharmacol. Sci.* **1994**, *15*, 402.

7. Weston, A. H.; Hamilton, T. C. *Potassium Channel Modulators*; Blackwell Scientific: Oxford, 1992.

8. Ashford, M. L. J.; Bond, C. T.; Blair, T. A.; Adelman, J. L. *Nature (London)* **1994**, *370*, 456.

9. Edwards, G.; Weston, A. H. *Annu. Rev. Pharmacol. Toxicol.* **1993**, *33*, 597.

10. Koga, H; Ohta, M.; Sato, H.; Ishizawa, T.; Nabata, H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 625.

11. Manley, P. W.; Quast, U. *J. Med. Chem.* **1992**, *35*, 2327.

12. Atwal, K. S.; Moreland, S.; McCullough, J. R.; O'Reilly, B. C.; Ahmed, S. Z.; Normandin, D. E. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 83.

13. Nakajima, T.; Izawa, T.; Kashiwabara, T.; Nakajima, S.; Munezuka, Y. *Chem. Pharm. Bull.* **1994**, *42*, 2475.

14. Takemoto, T.; Eda, M.; Okada, T.; Sakashita, H.; Matzno, S.; Gohda, M.; Ebisu, H.; Nakamura, N.; Fukaya, C.; Hihara, M.; Eiraky, M.; Yamanouchi, K.; Yokoyama, K. *J. Med. Chem.* **1994**, *37*, 18.

15. Castro, A.; Martínez, A.; Cardelús, I.; Llenas, J. *Bioorg. Med. Chem.* **1995**, *2*, 179.

16. Castro, A.; Martínez, A.; Cardelús, I.; Llenas, J.; Palacios, J. M. Abstract P110, II Congreso Conjunto Italo-Español de Química Terapeútica, Ferrara, August 1995.

17. Castro, A.; Martinez, A. *Heterocycles* **1994**, *38*, 1737.

18. Martinez, A.; Castro, A.; Fonseca, I.; Cano, F.; Martinez-Ripoll, M.; Albert, A. *Heterocycles* **1996**, *48*, 2657.

19. Ottman, G.; Hooks, H. *J. Heterocycl. Chem.* **1967**, *4*, 366.

20. Jetley, M.; Weston, A. H. *Br. J. Pharmacol.* **1980**, *68*, 311.

21. Robertson, D. W.; Steinberg, M. I. *Ann. Rep. Med. Chem.* **1989**, *24*, 91.

22. Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985,** *107*, 3902.

23. Stewart, J. J. P. QCPE Bull. *9*, 10 (1989). MOPAC V5.0 QCPE program no. 455.

24. Chem-QM in Chem-X molecular modeling program Chemical Design Ltd, Oxford (1993).

25. Rao, C. N. R.; Rao, K.; Goel, A.; Balasubramanian, D. *J. Chem. Soc. (A)* **1971**, 3077.

26. Bassilev, G.; Koleva, V.; Ilieva, M.; Galabov, B. *J. Mol. Struct.* **1982**, *82*, 35.

27. Itai, A.; Tomioka, N.; Yamada, M.; Inoue, A.; Kato, Y. *3D QSAR in Drug Design*; Kubiny, H., Ed.; ESCOM Science: The Netherlands, 1993.

(*Received in U.S.A.* 29 *October* 1996; *accepted* 5 *February* 1997)