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Short communication

Synthesis and pharmacological evaluation of 2,3-dihydro-3-oxo-4*H*-thieno[3,4-*e*][1,2,4]thiadiazine 1,1-dioxides as voltage-dependent calcium channel blockers

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Abstract – The synthesis of a novel series of 2,3-dihydro-3-oxo-4*H*-thieno[3,4-*e*][1,2,4]thiadiazine 1,1-dioxides and their pharmacological evaluation as drugs with effects on the rat cardiovascular system are described. The compounds under study were synthesized via Curtius rearrangement of appropriate sulfamoylacylazides which, in turn, were prepared from known starting materials. In isolated rat portal vein, these thienothiadiazines, like verapamil and diazoxide, inhibited the spontaneous motility produced by KCl (20 mM). In addition, the new compounds, like verapamil and unlike diazoxide, also exhibited inhibitory effects in the same preparation when the cell membrane was depolarized by an increased extracellular KCl concentration (80 mM) and, consequently, the membrane potential approached a level close to the K^+ equilibrium potential. Further characterization of this inhibitory activity led to the identification of a selective inhibitory effect of the new compounds on KCl (80 mM)-induced $^{45}Ca^{2+}$ uptake in the same vascular tissue. When tested in vivo (anaesthetized normotensive rats), acute administration of verapamil, diazoxide and some of the most in vitro potent compounds in $^{45}Ca^{2+}$ uptake experiments produced a gradual, dose-dependent and sustained decrease in diastolic arterial blood pressure, devoid of cardiac effects. These results suggest that, like verapamil, the cardiovascular effects produced by the new thienothiadiazines seem to be due, at least in part, to a blockade of transmembrane voltage-dependent calcium channels present in vascular smooth muscle cells and not to an activation of ATP-sensitive K^+ channels. Compounds **5b**, **5e** and **5i** have been selected for further studies as antihypertensive agents. © 2000 Éditions scientifiques et médicales Elsevier SAS

2,3-dihydro-3-oxo-4*H*-thieno[3,4-*e*][1,2,4]thiadiazine 1,1-dioxides / calcium channel blockers / cardiovascular activity

1. Introduction

Modulation of ion transmembrane channels is the basis of therapy for a variety of illnesses [1, 2]. Despite the great number of different cation channels described, there is a high degree of structural homology among them, which makes it easy to find relatively close compounds with a differential pattern of cation modulation activity. This is the case for some cromakalim [3] and pinacidil [4] analogues. During an investigation focused on the discovery of novel potassium channel openers [5], we observed that several compounds belonging to the 1,2,6- and 2,1,3-thiadiazine ring systems did not fit with the diazoxide pattern in different pharmacological assays. These results and our interest in the search of cyclic sulfonamides with promising pharmacological activities [6–8] led us to prepare new compounds in which the benzene ring of known benzothiadiazines was substituted by thiophene. The substitution of the benzene ring of many therapeutic agents by an aromatic heterocycle was a useful recourse frequently employed to retain or enhance their pharmacological properties; thus, for example, several pyrido-1,2,4-thiadiazine 1,1-dioxides were recently prepared and demonstrated to be more potent and selective than diazoxide as potassium channel openers [9, 10]. In this context, we have synthesized a series

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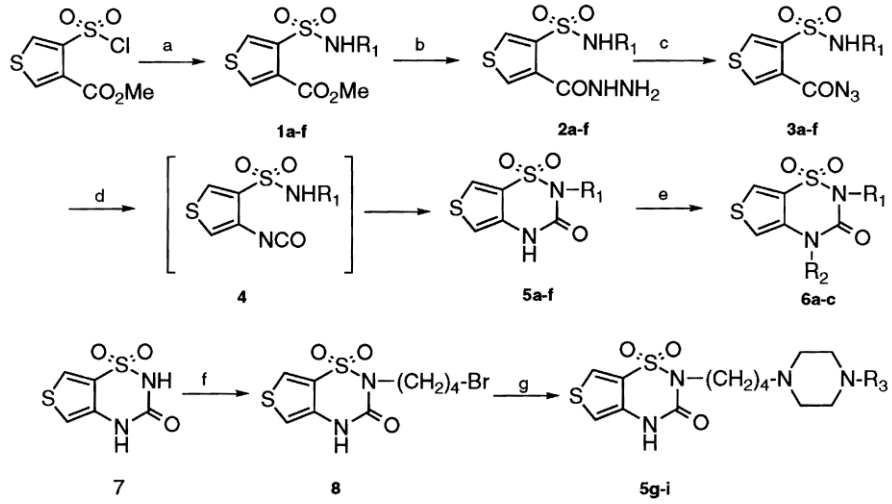


Figure 1. (a) R_1NH_2/THF; (b) N_2H_4 H_2O/EtOH; (c) 2N HCL or 2N HNO_3, $NaNO_2$, H_2O; (d) Δ/benzene (5a, b), xylene (5f) or $CHCl_3$ (5c, d, e); (e) NaH/DMF, R_2X; (f) NaH/DMF, $Br(CH_2)_4Br$; (g) NaH/DMF, $C_4H_9N_2–R_3$.

of 2,3-dihydro-3-oxo-4*H*-thieno[3,4-*e*][1,2,4]thiadiazine 1,1-dioxides **5** and **6**, which show a partial structural analogy with diazoxide, whose antihypertensive and smooth muscle relaxant activities have been described [1]. The introduction of phenyl, benzyl and dialkylaminoalkyl moieties in this new heterocyclic ring was based on the results obtained in our initial studies with these derivatives [5] and the presence of such substituents in the structures of several drugs of clinical use in the cardiovascular system [11, 12]. In the present paper we describe the synthesis of these new thienothiadiazines and their effects on spontaneous motility, on tension responses to increased extracellular KCl concentrations and on $^{45}Ca^{2+}$ uptake (basal and K^+-induced) in isolated rat portal vein, a vascular tissue widely used in the evaluation of the pharmacological effects of K^+ channel openers. Some of the most effective compounds in $^{45}Ca^{2+}$ uptake experiments were also evaluated as potential hypotensive agents in anaesthetized normotensive rats.

2. Chemistry

The synthesis of the 2,3-dihydro-3-oxo-4*H*-thieno[3,4-*e*][1,2,4]thiadiazine 1,1-dioxides used in this study was achieved by two different methods (*figure 1*). In the first one [13], the known methyl sulfamoylthiophene-3-carboxylates **1a–f** [14] reacted with hydrazine hydrate in ethanol to give the hydrazides **2a–f**, which were readily converted to the 3-carboxy azides **3a–f** by the action of nitrous acid. The thiadiazines **5a–f** were prepared by ring closure of the intermediate isocyanates **4** produced in the Curtius rearrangement [15, 16] of carboxy azides **3a–f** in benzene, xylene or chloroform (*figure 1*). In the second method, compounds **5g–i** were better obtained by N-alkylation of the 2,3-dihydro-3-oxo-4*H*-thieno[3,4-*e*][1,2,4]thiadiazine 1,1-dioxide **7** [13] with 1,4-dibromobutane and subsequent reaction of the 4-bromobutyl derivative **8** with the corresponding 4-substituted piperazine in the presence of sodium hydride and *N*,*N*-dimethylformamide (DMF). Alkylation of **5b** under similar conditions (NaH/DMF/alkyl halide) gave the 2,4-disubstituted thiadiazines **6a–c** in good yields. The structures of all the newly synthesized compounds were established on the basis of analytical and NMR data (*table I*).

3. Biological results and discussion

Increased extracellular KCl concentrations (20 and 80 mM) have been reported to cause spontaneous myogenic activity or contractions in phasic vascular smooth muscle (e.g. rat portal vein) by depolarizing cell membranes and by increasing the influx of Ca^{2+} through L and T voltage-dependent calcium channels which, in turn, may induce CICR (Ca^{2+}-induced Ca^{2+} release) from intracellular stores [17].

The fact that these thiadiazines, like diazoxide and verapamil, antagonized the spontaneous mechanical activity induced by KCl (20 mM) in rat portal vein (*table II*) suggests the existence of a non-selective vasodilator

Table I. Yields and physico-chemical data of synthesized thiadiazines **5** and **6**.

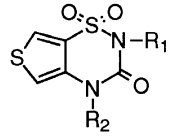
Compound	R_1	R_2	R_3	Yield (%)	M.p. (°C)	Recryst. solvent	Formula	Anal.
5a	*n*-Pr	H	–	69	132–134	EtOH-H_2O	$C_8H_{10}N_2O_3S_2$	C, H, N, S
5b	Bn	H	–	82	160–162	EtOH-H_2O	$C_{12}H_{10}N_2O_3S_2$	C, H, N, S
5c	3-Cl-Ph	H	–	67	245–247	MeOH	$C_{11}H_7ClN_2O_3S_2$	C, H, N, S
5d	4-F-Ph	H	–	54	260–262	EtOH	$C_{11}H_7FN_2O_3S_2$	C, H, N, S
5e	4-Cl-Ph	H	–	54	252–254 (d)	MeOH-H_2O	$C_{11}H_7ClN_2O_3S_2$	C, H, N, S
5f	4-MeO-Ph	H	–	82	182–183	toluene	$C_{12}H_{10}N_2O_4S_2$	C, H, N, S
5g	R_3-N⌒N-$(CH_2)_4$	H	Ph	72	120–122	EtOH	$C_{19}H_{24}N_4O_3S_2$	C, H, N, S
5h	R_3-N⌒N-$(CH_2)_4$	H	4-F-Ph	62	136–138	MeOH	$C_{19}H_{23}FN_4O_3S_2$	C, H, N, S
5i	R_3-N⌒N-$(CH_2)_4$	H	2-Pyrimidyl	70	132–134	EtOH	$C_{17}H_{22}N_6O_3S_2$	C, H, N, S
6a	Bn	Me	–	90	158–160	EtOH	$C_{13}H_{12}N_2O_3S_2$	C, H, N, S
6b	Bn	Et	–	91	130–142	EtOH	$C_{14}H_{14}N_2O_3S_2$	C, H, N, S
6c	Bn	*n*-Pr	–	74	134–136	MeOH	$C_{15}H_{16}N_2O_3S_2$	C, H, N, S

action (possibly related to either an intracellular activity or an inhibitory effect of the new compounds on the cell membrane) which involves, among other mechanisms, the blockade of Ca^{2+} influx through voltage-dependent Ca^{2+} channels and/or the opening of K^+ channels. Despite their chemical similarity to diazoxide, the predominant effect of the studied compounds on the smooth muscle cell membrane of rat portal vein does not seem to be due to the opening of ATP-sensitive K^+ channels (K_{ATP}), since diazoxide (present work), cromakalim and other K_{ATP} agonists antagonize the spontaneous motility but do not inhibit contractions induced by KCl at concentrations greater than about 30 mM in vascular smooth muscle. This latter effect is due to the fact that when the cell is depolarized by an increased extracellular KCl concentration (>30 mM), the membrane potential approaches the Nernst equilibrium potential for K^+ and, consequently, the opening of K_{ATP} by cromakalim, or any other K^+ channel opener, will then have very little influence on membrane potential [18]. In this context, it is interesting to note that the new compounds inhibited, unlike diazoxide, the contractions induced by KCl concentrations (80 mM) in rat portal vein (*table II*).

The action of the novel thienothiadiazines on transmembrane voltage-dependent Ca^{2+} channels was demonstrated by experiments carried out with radiolabelled calcium. Basal $^{45}Ca^{2+}$ uptake, i.e. the amount of Ca^{2+} entering by means of leak channels [17], did not change by the addition of diazoxide (100 μM), verapamil (1 μM) and the thienothiadiazines (100 μM), whereas the same concentration of some of our compounds, like verapamil (1 μM) and unlike diazoxide (100 μM), strongly inhibited the uptake of $^{45}Ca^{2+}$ induced by KCl (80 mM) (*table III*). These results suggest that the thienothiadiazines may inhibit, at least in part, the contractions induced by KCl (80 mM) in rat portal vein by blocking transmembrane Ca^{2+} influx through voltage-dependent Ca^{2+} channels. The fact that these compounds exhibit IC_{50} values of K^+-induced $^{45}Ca^{2+}$ uptake approximately 100-fold higher than the IC_{50} value of verapamil suggests a lower potency for them as calcium channel blockers. To evaluate, however, the therapeutic potential and the future of our compounds as Ca^{2+} antagonists, it would be necessary to know their toxicity in order to calculate the corresponding LD_{50}/ED_{50} or LD_{50}/IC_{50} ratios and compare them with those of the reference drugs (e.g. verapamil). On the other hand, the potential effects of compounds **5**–**6** on the uptake of $^{45}Ca^{2+}$ induced by a little membrane depolarization could not be studied because KCl (20 mM) did not significantly increase basal $^{45}Ca^{2+}$ uptake in rat portal vein.

These results also permit us to conclude that the most effective compounds in antagonizing KCl (80 mM)-induced $^{45}Ca^{2+}$ uptake were the *N*-2 monosubstituted derivatives **5b** and **5e**. In contrast, the *N*-2, *N*-4 disubstituted compounds **6a** and **6c**, with IC_{50} values clearly higher ($P < 0.01$), showed the lowest inhibitory activity. The other *N*-2, *N*-4 disubstituted compound **6b**, like diazoxide, did not significantly alter the KCl (80 mM)-induced $^{45}Ca^{2+}$ uptake (*table III*). Substitution of the chlorine by a fluorine atom in the *para* position of the *N*-2 phenyl ring of **5e** or its change from *para* to *meta* position of the phenyl moiety led to compounds **5d** and **5c** which were less potent ($P < 0.05$ and $P < 0.01$ versus **5e**, respectively). Replacement of the *p*-chloro substituent in **5e** by a methoxyl group clearly impaired the activity. Introduc-

Table II. Inhibitory effects of thiadiazines **5** and **6** on KCl (20 mM)-induced spontaneous mechanical activity/KCl (80 mM)-induced contractions in isolated rat portal vein.



Compound	R_1	R_2	R_3	Concentration (μM)	KCl (20 mM)-induced spontaneous motility[a]	IC_{50} (μM)	KCl (80 mM)-induced contraction[b]
DMSO	–	–	–	14 (mM)	nt		nt
PEG	–	–	–	6.5 (mM)	5 ± 0.7		6 ± 0.9
Diazoxide	–	–	–	100	66 ± 4.1*	59 ± 3.4	8 ± 1.0
(±)-Verapamil	–	–	–	0.3	78 ± 5.3*	0.15 ± 0.012	95 ± 4.1*
5a	*n*-Pr	H	–	100	65 ± 4.0*	64 ± 4.3	75 ± 6.2*
5b	Bn	H	–	100	78 ± 5.3*	40 ± 4.1	94 ± 4.3*
5c	3-Cl-Ph	H	–	100	67 ± 3.8*	66 ± 4.2	79 ± 5.5*
5d	4-F-Ph	H	–	100	70 ± 5.4*	52 ± 4.6	90 ± 6.2*
5e	4-Cl-Ph	H	–	100	77 ± 4.7*	41 ± 3.8	94 ± 4.8*
5f	4-MeO-Ph	H	–	100	63 ± 4.5*	60 ± 4.0	72 ± 5.9*
5g	R_3-N N-$(CH_2)_4$	H	Ph	100	67 ± 4.6*	62 ± 4.3	77 ± 5.3*
5h	R_3-N N-$(CH_2)_4$	H	4-F-Ph	100	68 ± 5.2*	56 ± 3.9	81 ± 6.2*
5i	R_3-N N-$(CH_2)_4$	H	2-pyrimidyl	100	75 ± 5.1*	45 ± 3.7	93 ± 5.3*
6a	Bn	Me	–	100	69 ± 5.8*	63 ± 4.2	75 ± 4.6*
6b	Bn	Et	–	100	37 ± 4.9*	118 ± 6.6	45 ± 4.4*
6c	Bn	*n*-Pr	–	100	65 ± 5.7*	65 ± 4.4	71 ± 6.0*

[a] % of inhibition produced by the concentration indicated of each compound on KCl (20 mM)-induced spontaneous motility; [b] KCl (80 mM)-induced contractions. IC_{50} values represent the 50% inhibitory concentrations obtained for the compounds tested against spontaneous myogenic activity produced by KCl (20 mM). These values were calculated, as indicated in the methods, from the cumulative concentration–response curves of each compound. Data are presented as means ± SEM of six experiments. Level of statistical significance: nt: not tested. * $P < 0.01$ (Student *t*-test) with respect to the % of inhibition produced by the vehicle (PEG).

tion of alkyl substituents in the *N*-2 or *N*-4 positions of the parent thieno[3,4-*e*][1,2,4]thiadiazine structure, as in **5a** and **6a–6c**, resulted in a significant decrease of the inhibitory activity (IC_{50} values significantly higher; $P < 0.01$ with respect to **5e**). Compound **5i**, bearing at *N*-2 a 4-[1-[4-(2-pyrimidyl)piperazinyl]]butyl] group, showed an inhibitory activity close to that of **5e**; however, other derivatives with similar piperazinyl substituents (**5g**, **5h**) exhibited lower IC_{50} values ($P < 0.01$ versus IC_{50} value of **5e** and **5i**). In this context, it must be pointed out that a number of intracellular mechanisms (different from the calcium antagonist activity (inhibition of $^{45}Ca^{2+}$ uptake) on the cell membrane) may be involved in the inhibitory effects of our compounds on the contractions induced by KCl (20/80 mM) in rat portal vein (see above). For this reason the SAR studies were carried out bearing only in mind the IC_{50} values of KCl-induced $^{45}Ca^{2+}$ uptake but not the IC_{50} values against KCl (20 mM)-induced contractions.

Taking into account the above results, the potential hypotensive activity of **5b**, **5e** and **5i** in anaesthetized normotensive rats was determined in order to show evidence for their correlation with the inhibitory effects produced by these in vitro potent compounds on KCl (80 mM)-induced $^{45}Ca^{2+}$ uptake in isolated rat portal vein. Thus, in vivo (anaesthetized normotensive rats), acute administration of verapamil (0.02, 0.05 and 0.1 mg/kg i.v.), diazoxide (1.5, 3 and 4.5 mg/kg i.v.) and compounds **5b**, **5e** and **5i** (0.3, 1 and 3 mg/kg i.v.) produced a rapid and dose-dependent fall in diastolic arterial pressure (DAP) without any significant change in heart rate (*table IV*). The maximal effect caused by each dose was reached approximately 0.5–1 min after treatment and persisted for over 10 min. Furthermore, the effective doses of verapamil and the studied compounds required to reduce basal DAP by 30% (ED_{30}) in anaesthetized normotensive rats correlated well (in order of potency) with the IC_{50} values needed for inducing an in vitro

Table III. Inhibitory effects of thiadiazines **5** and **6** on basal and KCl (80 mM)-induced $^{45}Ca^{2+}$ uptake (nmol/kg wet tissue) in isolated rat portal vein.

Compound	Concentration (μM)	Basal $^{45}Ca^{2+}$ uptake[a]	KCl (80 mM)-induced $^{45}Ca^{2+}$ uptake[b]	IC_{50} (μM)
DMSO	14 (mM)	54.36 ± 6.2	163.1 ± 15^{++}	
PEG	6.5 (mM)	nt	nt	
Diazoxide	100	45.4 ± 7.9	176.2 ± 14.0^{++}	
(±)-Verapamil	1	51.8 ± 7.2	81.4 ± 9.6**	0.32 ± 0.4
5a	100	50.6 ± 9.0	121.7 ± 9.7**	125.6 ± 7.8
5b	100	47.2 ± 8.1	98.3 ± 12.3**	83.2 ± 6.8
5c	100	49.4 ± 8.8	115.6 ± 9.0**	115.4 ± 6.2
5d	100	38.8 ± 7.0	104.4 ± 11**	94.6 ± 8.0
5e	100	56.0 ± 6.5	96.2 ± 9.13**	80.7 ± 6.2
5f	100	56.5 ± 7.6	132.5 ± 9.3*	133.5 ± 8.0
5g	100	42.3 ± 8.8	117.2 ± 9.7**	118.5 ± 7.2
5h	100	54.3 ± 7.3	108.6 ± 10**	101.7 ± 7.1
5i	100	45.7 ± 8.7	100.7 ± 11**	83.5 ± 5.8
6a	100	39.8 ± 7.0	123.1 ± 10**	127.1 ± 9.1
6b	100	49.9 ± 6.4	144.5 ± 9.5	
6c	100	52.1 ± 8.2	132.7 ± 9.1*	129.8±7.8

nt: not tested. Effects produced by the concentration indicated of each compound on basal[a] and KCl (80 mM)-induced $^{45}Ca^{2+}$ uptake[b]. IC_{50} values represent the concentration of the new compounds required to reduce KCl (80 mM)-induced $^{45}Ca^{2+}$ uptake by 50%. These values were calculated by the least squares linear regression, as indicated in methods, using at least three different concentrations of each compound which produced a KCl (80 mM)-induced $^{45}Ca^{2+}$ uptake inhibition between 20% and 80% of the maximal pharmacological response (100% of inhibition). Results are expressed as means ± SEM of at least six experiments. Level of statistical significance: * $P < 0.05$ and ** $P < 0.01$ (Student *t*-test) versus KCl (80 mM)-induced $^{45}Ca^{2+}$ uptake in absence of the vehicle (DMSO; $^{45}Ca^{2+}$ tissue content = 158 + 12.4 nmol/kg wet tissue; $P < 0.01$ with respect to basal $^{45}Ca^{2+}$ uptake, $n = 6$). $^{++}$ $P < 0.01$ versus basal $^{45}Ca^{2+}$ uptake in the absence of DMSO ($^{45}Ca^{2+}$ tissue content = 50.1 ± 8.6 nmol/kg wet tissue).

inhibitory effect on KCl (80 mM)-induced $^{45}Ca^{2+}$ uptake in isolated rat portal vein. This suggests that the calcium antagonist activity showed by the novel thienothiadiazines in rat portal vein smooth muscle cells may be responsible, at least in part, for the decrease in blood pressure observed in vivo (anaesthetized normotensive rat).

5. Experimental protocols

5.1. Chemistry

Melting points were determined on a Gallenkamp capillary apparatus and are uncorrected. Elemental analyses were performed with a Heraeus CHN-RAPID instrument. Analytical results which are only indicated by symbols were found to be within ± 0.4% of the theoretical values. ^{1}H-NMR spectra (300 MHz) and ^{13}C-NMR spectra (75 MHz) were recorded on a Varian XL-300 spectrometer in the indicated solvent. Chemical shifts are expressed in δ units from tetramethylsilane (TMS) as an internal standard. IR spectra were measured with a Shimadzu IR-435 spectrometer. Mass spectra were recorded on a Hewlett-Packard 5973 MSD instrument. Silica gel/TLC cards (Fluka, silica gel-precoated aluminium cards with fluorescent indicator 254 nm) were used for thin layer chromatography (TLC). Developed plates were visualized by UV light. Flash column chromatography was performed on columns packed with silica gel 60 (230–400 mesh) (Merck).

5.1.1. General procedure for the preparation of compounds **1a–f**. *Example: methyl 4-[N-(1-propyl)sulfamoyl]thiophene-3-carboxylate* **1a**

To a mixture of methyl 4-chlorosulfonylthiophene-3-carboxylate [14] (2.40 g, 10 mmol), potassium carbonate (0.14 g, 1 mmol) and THF (10 mL) was added dropwise a solution of 1-propylamine (0.8 mL, 10 mmol) in the same solvent, maintaining the temperature below 10 °C. The reaction mixture was refluxed for 6 h. The solvent was evaporated and the residue was treated with water, filtered off and dried ($MgSO_4$) to give **1a** as a white solid (2.0 g, 73%); m.p. 50–52 °C (ligroin); IR (KBr, cm^{-1}) 3 230 (NH), 1 705 (C=O), 1 325, 1 160 (SO_2), 1 250 (C–O); ^{1}H-NMR (DMSO-d_6) δ 8.40 (d, 1H, J = 3.0 Hz, thiophene), 8.20 (d, 1H, J = 3.0 Hz, thiophene), 6.80 (bs, 1H, exchanged with deuterium by D_2O addition, NH),

Table IV. Cardiovascular effects of **5b**, **5e** and **5i** in anaesthetized normotensive rats.

Compound	Dose (mg/kg, i.v.)	Effects on DAP[a]	ED_{30} (mg/kg, i.v.)	Effects on heart rate[b]
(±)-Verapamil	0.02	–13.4 ± 1.04*	0.046 ± 0.0035	1.4 ± 0.15
	0.05	–30.2 ± 2.78*		–4.1 ± 0.56
	0.1	–47.9 ± 3.75*		–6.2 ± 0.88
Diazoxide	1.5	–12.5 ± 0.87*	2.61 ± 0.19	0.7 ± 0.077
	3	–33.4 ± 2.39*		–2.3 ± 0.31
	4.5	–48.2 ± 3.44*		–3.4 ± 0.42
5b	0.3	–10.02 ± 0.65*	1.15 ± 0.077	–0.6 ± 0.065
	1	–30.6 ± 2.12*		–1.8 ± 0.24
	3	–42.1 ± 2.98*		–3.9 ± 0.53
5e	0.3	–12.4 ± 0.91*	0.94 ± 0.069	0.5 ± 0.055
	1	–32.2 ± 2.4*		–2.3 ± 0.29
	3	–46.5 ± 3.49*		–4.6 ± 0.67
5i	0.3	–8.2 ± 0.55*	1.39 ± 0.098	0.9 ± 0.11
	1	–24.3 ± 1.74*		–4.1 ± 0.58
	3	–41.6 ± 2.93*		–5.7 ± 0.84

[a] % of decrease (–) in DAP. [b] % of increase (+) or decrease (–) in heart rate. ED_{30} values represent the dosage of the new compounds required to reduce normal DAP by 30%. These values were calculated by the least squares linear regression, as indicated in the methods, using three different doses of each compound which produced a DAP decrease between 5 and 50% (this latter percentage is usually the maximal pharmacological response obtained in anaesthetized normotensive rats). The basal values of MAP and heart rate (measured in control and treated rats before treatment) were 81 ± 4 mm Hg and 372 ± 9 beats/min, respectively (n = 20). In control animals, the vehicle (saline/PEG at 20%) did not significantly modify these values (% of increase in DAP and decrease in heart rate = 1.4 ± 0.12 and –2.1 ± 0.28, respectively; n = 5). Data are expressed as means ± SEM of five experiments. Level of statistical significance: * $P < 0.01$ (Student t-test) versus control values.

2.80 (t, 2H, J = 7.3 Hz, CH_2N), 1.35 (m, 2H, CH_2), 0.75 (t, 3H, J = 7.3 Hz, CH_3). Anal. $C_9H_{13}NO_4S_2$ (C, H, N, S).

5.1.2. General procedure for the preparation of compounds **2a–f**. *Example: 4-[N-(1-propyl)sulfamoyl]thiophene-3-carbohydrazide* **2a**

A mixture of compound **1a** (0.58 g, 2 mmol) and hydrazine hydrate (98%) (0.30 mL, 6 mmol) in ethanol (5 mL) was refluxed for 1 h. Upon cooling, the precipitated solid was filtered off, washed with ice cold ethanol and recrystallized to yield **2a** as a white solid (0.44 g, 76%); m.p. 146–148°C (EtOH); IR (KBr, cm^{-1}) 3 200 (NH); 1 610 (C=O); 1 320, 1 160 (SO_2); ^{1}H-NMR (DMSO-d_6) δ 8.17 (d, 1H, J = 3.0 Hz, thiophene), 8.06 (d, 1H, J = 3.0 Hz, thiophene), 7.00–3.00 (bs, 4H, exchanged with deuterium by D_2O addition, NH), 2.76 (t, 2H, J = 7.3 Hz, CH_2N), 1.40 (m, 2H, CH_2), 0.76 (t, 3H, J = 7.3 Hz, CH_3); Anal. $C_8H_{13}N_3O_3S_2$ (C, H, N, S).

5.1.3. General procedure for the preparation of compounds **3a–f**. *Example: 4-[N-(1-propyl)sulfamoyl]thiophene-3-carboxy azide* **3a**

To a solution of compound **2a** (0.50 g, 2 mmol) in 2 N nitric acid (1.40 mL) was added dropwise a solution of sodium nitrite (0.15 g, 2.1 mmol) in water (0.50 mL), maintaining the reaction temperature below 10 °C. The mixture was stirred at this temperature for 2 h and the insoluble product was filtered, washed with water and dried ($MgSO_4$) to yield **3a**, (0.44 g, 84%). The compound was pure enough to be used as such in the following step. IR (KBr, cm^{-1}) 3 350 (NH), 2 160, 1 210 (N_3), 1 680 (C=O); 1 330, 1 165 (SO_2).

5.1.4. General procedure for the preparation of compounds **5a–f**. *Example: 2-(1-propyl)-2,3-dihydro-3-oxo-4H-thieno[3,4-e][1,2,4]thiadiazine 1,1-dioxide* **5a**

A suspension of the carboxy azide **3a** (0.50 g, 2 mmol) in dry benzene (20 mL) was refluxed for 4 h. The precipitate was filtered off and recrystallized to give **5a** as a white solid; IR (KBr, cm^{-1}) 3 260 (NH), 1 675 (C=O), 1 330, 1 160 (SO_2); ^{1}H-NMR (DMSO-d_6) δ 11.21 (s, 1H, exchanged with deuterium by D_2O addition, NH), 8.55 (d, 1H, J = 3.2 Hz, thiophene), 6.99 (d, 1H, J = 3.2 Hz, thiophene), 3.66 (t, 2H, J = 7.3 Hz, CH_2), 1.60 (m, 2H, CH_2), 0.84 (t, 3H, J = 7.3 Hz, CH_3); ^{13}C-NMR (DMSO-d_6) δ 149.2 (C-3), 132.3 (C-4a), 126.0 (C-7), 124.9 (C-7a), 106.6 (C-5), 42.0 (CH_2), 22.2 (CH_2), 10.8 (CH_3); MS (EI) m/z 246 (M^+).

The synthesis of thiadiazines **5b–f** from their respective intermediates **2b–f** and **3b–f** following this same procedure was recently reported [19].

5.1.5. 2-(4-Bromobutyl)-2,3-dihydro-3-oxo-4H-thieno[3,4-e][1,2,4]thiadiazine 1,1-dioxide **8**

To a solution of the thiadiazine **7** (1.73 g, 8.5 mmol) in dry DMF (20 mL), under an inert atmosphere, was added slowly sodium hydride (60% dispersion in mineral oil, 0.34 g, 8.5 mmol) maintaining the temperature below 10 °C. After 15 min, 1,4-dibromobutane (1.8 g, 1.0 mL, 8.5 mmol) was added and the reaction mixture was stirred at 70 °C for 7 h. The solvent was evaporated to dryness and the crude solid was treated with water and extracted with ethyl acetate. The organic layer was washed with water, dried and concentrated under reduced pressure. The residue was purified by silica gel flash chromatography using ethyl acetate/hexane 1:1 as eluent. Compound **8** was isolated as white crystals (0.97 g, 34%); m.p. 111–113 °C (EtOH); IR (KBr, cm^{-1}) 1 680 (C=O), 1 340, 1 160 (SO_2); ^{1}H-NMR (DMSO-d_6) δ 11.22 (s, 1H, exchanged with deuterium by D_2O addition, NH), 8.55 (d, 1H, J = 3.2 Hz, thiophene), 7.00 (d, 1H, J = 3.2 Hz, thiophene), 3.75 (t, 2H, J = 6.8 Hz, CH_2), 3.53 (t, 2H, J = 6.8 Hz, CH_2Br), 1.78 (m, 4H, $2CH_2$); MS (EI) *m/z* 338.7 (M^+); Anal. $C_9H_{11}BrN_2O_3S_2$ (C, H, N, S).

5.1.6. Preparation of thienothiadiazines **5g–i**

5.1.6.1. 2-[4-[1-(4-Phenylpiperazinyl)]butyl]-2,3-dihydro-3-oxo-4H-thieno[3,4-e][1,2,4]thiadiazine 1,1-dioxide **5g**

To a solution of compound **8** (1.5 g, 4.4 mmol) in dry THF (40 mL) was added dropwise 1-phenylpiperazine (0.72 g, 4.4 mmol). The reaction mixture was heated under reflux for 9 h. The THF was evaporated in vacuo and the oily residue was treated with 40% NaOH and extracted with ethyl acetate. The organic layer was separated, washed with water and brine, and dried ($MgSO_4$). Removal of the solvent furnished **5g** as a white solid which was recrystallized. IR (KBr, cm^{-1}): 3 270 (NH), 1 670 (C=O), 1 330, 1 145 (SO_2); ^{1}H-NMR (DMSO-d_6) δ 8.80 (bs, 1H, exchanged with deuterium by D_2O addition, NH), 7.86 (d, 1H, J = 3.2 Hz, thiophene), 7.34–7.30 (m, 2H, benzene), 6.93 (m, 3H, benzene), 3.67 (t, 4H, J = 4.9 Hz, $2CH_2N$), 3.27–3.22 (m, 8H, $4CH_2N$), 1.85–1.79 (m, 4H, $2CH_2$); ^{13}C-NMR (DMSO-d_6) δ 153.3 (C-3), 133.9 (C-4a), 131.5 (C-7), 126.0 (C-7a), 110.1 (C-5), 150.6, 128.9, 119.2, 115.7 (Ph), 47.9, 47.7, 43.2 (CH_2N), 24.6 (CH_2CH_2); MS (EI) *m/z* 420 (M^+).

5.1.6.2. 2-[4-[1-[4-(4-Fluorophenyl)-piperazinyl]]butyl]-2,3-dihydro-3-oxo-4H-thieno[3,4-e][1,2,4]thiadiazine 1,1-dioxide **5h**

This compound was prepared from **8** and 1-(4-fluorophenyl)piperazine by the above procedure. IR (KBr, cm^{-1}): 3 370 (NH), 1 670 (C=O), 1 330, 1 140 (SO_2); ^{1}H-NMR (DMSO-d_6) δ 8.63 (s, 1H, exchanged with deuterium by D_2O addition, NH), 8.31 (d, 1H, J = 3.5 Hz, thiophene), 7.80 (d, 1H, J = 3.5 Hz, thiophene), 7.07–6.99 (m, 4H, benzene), 3.54 (t, 4H, J = 5.0 Hz, $2CH_2N$), 3.21–3.12 (m, 8H, $4CH_2N$), 1.75–1.68 (m, 4H, $2CH_2$); ^{13}C-NMR (DMSO-d_6) δ 153.3 (C-3), 134.0 (C-4a), 131.6 (C-7), 126.0 (C-7a), 110.2 (C-5), 158.6, 153.9 (J_{C-F} = 235.8 Hz, C-4′), 147.6, 147.5 (J_{C-F} = 2.1 Hz, C-1′), 117.7, 117.6 (J_{C-F} = 7.7 Hz, C-2′), 115.6, 115.1 (J_{C-F} = 21.9 Hz, C-3′), 48.8, 43.3, (CH_2N), 24.7 (CH_2CH_2); MS (EI) *m/z* 438 (M^+).

5.1.6.3. 2-[4-[1-[4-(2-Pyrimidyl)-piperazinyl]]butyl]-2,3-dihydro-3-oxo-4H-thieno[3,4-e][1,2,4]thiadiazine 1,1-dioxide **5i**

This compound was prepared from **8** and 1-(2-pyrimidyl)piperazine by the above procedure. IR (KBr, cm^{-1}): 3 375 (NH), 1 660 (C=O), 1 345, 1 140 (SO_2); ^{1}H-NMR (DMSO-d_6) δ 8.60 (s, 1H, exchanged with deuterium by D_2O addition, NH), 8.39 (d, 2H, J = 4.7 Hz, pyrimidine), 8.30 (d, 1H, J = 3.5 Hz, thiophene), 7.80 (d, 1H, J = 3.5 Hz, thiophene), 6.66 (t, 1H, J = 4.7 Hz, pyrimidine)), 3.81 (m, 4H, $2CH_2N$), 3.49 (m, 4H, $2CH_2N$), 3.17 (t, 4H, J = 4.7 Hz, $2CH_2N$), 1.70 (m, 4H, $2CH_2$); ^{13}C-NMR (DMSO-d_6) δ 153.4 (C-3), 133.9 (C-4a), 131.5 (C-7), 126.0 (C-7a), 110.4 (C-5), 161.0, 157.9, 110.1, (pyrimidil), 47.7, 42.9, 42.8 (CH_2N), 24.6 (CH_2CH_2); MS (EI) *m/z* 422 (M^+).

The thiadiazines **6a–c** were prepared by a previously described procedure [19] consisting on the reaction of **5b** with the corresponding alkyl halides in the presence of NaH as the base and DMF as the solvent.

5.2. Biological methods

5.2.1. In vivo experiments. Anaesthetized normotensive rats

Normotensive male Wistar-Kyoto rats (WKY rats, 300–320 g, Iffa-Credo), purchased from Criffa (Barcelona, Spain), were anaesthetized by intraperitoneal injection of pentobarbital (80 mg/kg, i.p.) and cannulated following a method previously described [20]. After blood pressure and heart rate had stabilized, vehicle (saline solution of polyethylene glycol-300 (PEG) at 20%; 1 mL/kg, for control group) diazoxide, (±)-verapamil or the selected compound solutions (in saline/PEG at 20% for treated groups) were injected cumulatively as a bolus at 20 min intervals through the femoral vein, in order to observe the effects on blood pressure and heart rate. A maximum of three doses were given to each animal.

5.2.2. In vitro experiments.

Contractions studies in isolated rat portal vein

Hepatic portal veins were dissected and prepared from male WKY rats (290–350 g), according to the general procedure described elsewhere [5]. After an equilibration period of 30 min in normal Krebs bicarbonate solution (KBS), this KBS was changed to a modified medium containing KCl (20 mM) instead of the equivalent amount of NaCl in order to maintain the osmolarity constant. The spontaneous motility induced by KCl (20 mM) was measured by using a specific designed software (Letica) which calculates the AUC (area under the curve) every 3 min. When the spontaneous myogenic activity of the tissue in response to KCl (20 mM) had stabilized, the experimental procedure was started. Thus, increasing cumulative concentrations of diazoxide (10–200 μM), (±)-verapamil (0.03–1 μM) or the compounds (10–200 μM) were added to the bath at approximately 15–20 min intervals (time necessary to obtain a steady-state inhibitory effect in the presence of each concentration tested), in order to study the effects of these drugs on spontaneous mechanical activity induced by KCl (20 mM).

Other groups of preparations were used to assess the relaxant activity of the new compounds on KCl (80 mM)-induced contractions. In these preparations, isometric contractions induced by KCl (80 mM), instead of the equivalent amount of NaCl in order to maintain the isotonicity of the medium, were obtained. After achieving a stable contraction, solely the inhibitory effects produced by a single concentration of (±)-verapamil (0.3 μM), diazoxide (100 μM), or the new compounds (100 μM) were studied per portal vein segment, since the contractions induced by KCl (80 mM) were maintained without significant tension changes in control rings for only 2–3 min and, thereafter, they relaxed throughout the time. In all the contraction studies carried out in isolated rat portal vein, control tissues were simultaneously subjected to the above procedures, but omitting the drug and adding the vehicle (appropriate PEG dilutions).

5.2.3. $^{45}Ca^{2+}$ uptake

Portal vein segments were equilibrated for at least 60 min in KBS containing 1.5 mM, instead of 2.5 mM $CaCl_2 \cdot 2H_2O$. The general procedure used was similar to one previously described [20] in rat aorta. The vasoconstrictor agent used was KCl (80 mM). To investigate the actions of the vehicle [dimethyl sulfoxide (DMSO), control group] or diazoxide (50, 100 and 200 μM), verapamil (0.1, 0.3 and 1 μM) and the new compounds [in a range of 30–200 μM, depending on their potency (for treated groups)] on basal and induced $^{45}Ca^{2+}$ uptake, the tissues were exposed to them 20 min before and during the incubation period with $^{45}Ca^{2+}$.

5.2.4. Data presentation and statistical analysis

Unless otherwise specified, results shown in the tables are expressed as means ± SEM. Significant differences between two means ($P < 0.05$ or $P < 0.01$) were determined by Student's two-tailed *t*-test for unpaired data, where appropriate. In anaesthetized normotensive rats, hypotensive activity is expressed as ED_{30}, the dosage of the new compounds required to reduce normal DAP by 30%, which was calculated by the least squares linear regression using a linear fitting analysis program (Origin 5.0), of log dosage (in mg/kg i.v.) on pharmacological response (maximum % reduction in DAP achieved approximately 0.5–1 min after administration) obtained with each dose. KCl (20 mM)-induced spontaneous myogenic activity (in the presence or absence of the different concentrations of the studied compounds) was expressed as a percentage of the maximal spontaneous motility (E_{max} = 100%) produced by KCl (20 mM) before the addition of the tested compound. Concentration–response curves for the inhibitory effects of our molecules on KCl (20 mM)-induced spontaneous mechanical activity were analysed using a sigmoidal curve-fitting analysis program (Origin 5.0) and the 50% inhibitory concentrations (IC_{50}) of these drugs were calculated. The % of inhibition of the tested compounds (at 100 μM) against KCl (20 mM)-induced spontaneous motility/KCl (80 mM)-induced contractions was calculated in comparison with the maximal control spontaneous motility/contraction (E_{max} = 100%) previously obtained before the addition of each compound.

In the experiments with radioactive calcium, $^{45}Ca^{2+}$ vascular tissue (portal vein segments) uptake was calculated from the formula: $^{45}Ca^{2+}$ uptake [nmol/kg wet tissue] = [c.p.m. in tissue/kg wet tissue] × [nmol $^{45}Ca^{2+}$ in 1 L solution/c.p.m. in 1 L solution). In this expression, the numerator of the second factor is the concentration of $^{45}Ca^{2+}$, not the total Ca^{2+} concentration. In these experiments, Ca^{2+} antagonist activity is expressed as IC_{50}, the concentration of the new compounds required to reduce KCl (80 mM)-induced $^{45}Ca^{2+}$ uptake by 50%, which was calculated by the least squares linear regression, using a linear fitting analysis program (Origin 5.0), of log concentration (in μM) on pharmacological response [% reduction in KCl (80 mM)-induced $^{45}Ca^{2+}$ uptake] obtained with each concentration.

5.2.5. Drugs, chemicals and radioisotopes

The drugs and radioisotopes used in the experiments were: heparin sodium salt (Analema), pentobarbital so-

dium salt (Grindsted Products), diazoxide, (±)-verapamil hydrochloride (Sigma Chemical Co.), $^{45}Ca^{2+}$ (New England Nuclear). Diazoxide, an K_{ATP} agonist, and verapamil, a voltage-dependent calcium channel blocker, were used as standard references in all experiments. The new compounds, diazoxide and (±)-verapamil hydrochloride were dissolved in saline/PEG (Sigma) at 20% for i.v. administration, in KBS/PEG for contractions studies, or in DMSO (Sigma) for experiments with radiolabelled calcium, immediately before use. Pentobarbital and heparin were dissolved in saline.

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