Eur. J. Med. Chem. 35 (2000) 949–955

PII: S0223-5234(00)01170-3/SCO

Short communication

Triazolyl–benzimidazolones and triazolyl–benzotriazoles: new potential potassium channel activators. II

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Received 3 January 2000; revised 25 April 2000; accepted 26 April 2000

Abstract – This paper reports the synthesis and pharmacological evaluation of a series of 5-substituted-triazolyl–benzotriazoles (**2a–f**) and the corresponding series of 5-substituted-triazolyl–benzimidazolones (**6a–f**), as potential activators of the big-conductance calcium-activated potassium channels (BK_{Ca}). The synthesis and structure demonstration of the stock compounds of the two series have been described in our previous works, as well as the common starting compounds 4-carboxamido-5-(4-substituted-2-amino-anilino)-1,2,3-triazoles (**1a–f**). The triazolyl–benzotriazoles were obtained by diazotization, while the triazolyl–benzimidazolones were obtained by thermal intramolecular cyclization of ethoxycarbonylamino derivatives or directly with phosgene. Benzimidazolone compounds generally showed little effect whilst the compounds with a benzotriazole ring showed full efficacy, with vasorelaxing properties and potency parameters a little lower than that of the reference compound NS 1619. These effects were significantly reduced by an increased membrane depolarization. This depolarization-sensitive response is in agreement with the pharmacodynamic hypothesis of activation of potassium channels. © 2000 Éditions scientifiques et médicales Elsevier SAS

1,2,3-triazoles / benzotriazoles / benzimidazolones / potassium channel activators

1. Introduction

Potassium channel activators have been indicated as emerging drugs for the therapy of several cardiovascular, respiratory or CNS diseases [1]. The benzimidazolone derivative NS 1619 [1-(2-hydroxy-5-trifluoromethylphenyl)-5-trifluoromethyl-benzimidazolone] [2] is one of the best known activators of the big-conductance calcium-activated potassium channel subtype (BK_{Ca}), which is a potentially important pharmacological target for the above therapeutic approaches. Therefore, this molecule has been viewed as a pilot structure to project and develop original heterocyclic moieties, with a possible pharmacodynamic profile of BK_{Ca}-openers.

In a previous paper [3] we reported synthesis and pharmacological activity toward potassium channels, of some 5-(2-nitroanilino)-1,2,3-triazole derivatives (A), whose structures might be correlated to that of NS 1619 (*figure 1*).

Such open derivatives showed a high vasodilator activity as a probable consequence of their action on the K-channels.

As a continuation of this work, because the 5-(2-nitroanilino)-1,2,3-triazole derivatives represent common key intermediates, we report the synthesis and pharmacological evaluation, towards the BK_{Ca} channels, of the corresponding 5-substituted triazolyl benzimidazolones (B) and 5-substituted triazolyl benzotriazoles (C), whose structures appear more closely correlated to that of NS 1619 (*figure 1*).

2. Chemistry

The preparation of the new triazolyl–benzotriazole derivatives **2c–f** and triazolyl–benzimidazolone derivatives **6b–f** (*table 1*) is reported in *figure 2*.

* Correspondence and reprints.

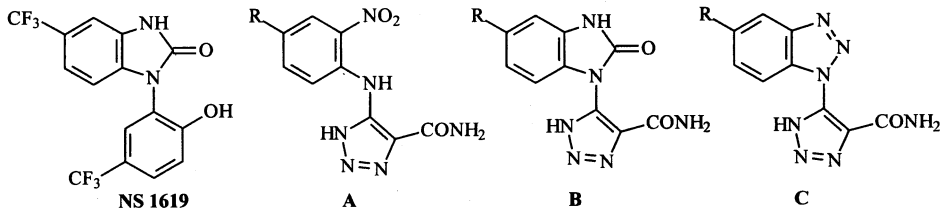


Figure 1. General reference structures.

The starting compounds 4-carboxamido-5-anilino-1,2,3-triazoles **1a–f**, useful for the synthesis of the two series of derivatives **2** and **6**, were described in the previously mentioned work [3].

The preparation and the chemical and spectroscopic structure demonstration of the stock compounds belonging to the two series are reported in our previous papers: triazolyl–benzotriazoles (**2a**: R=H;

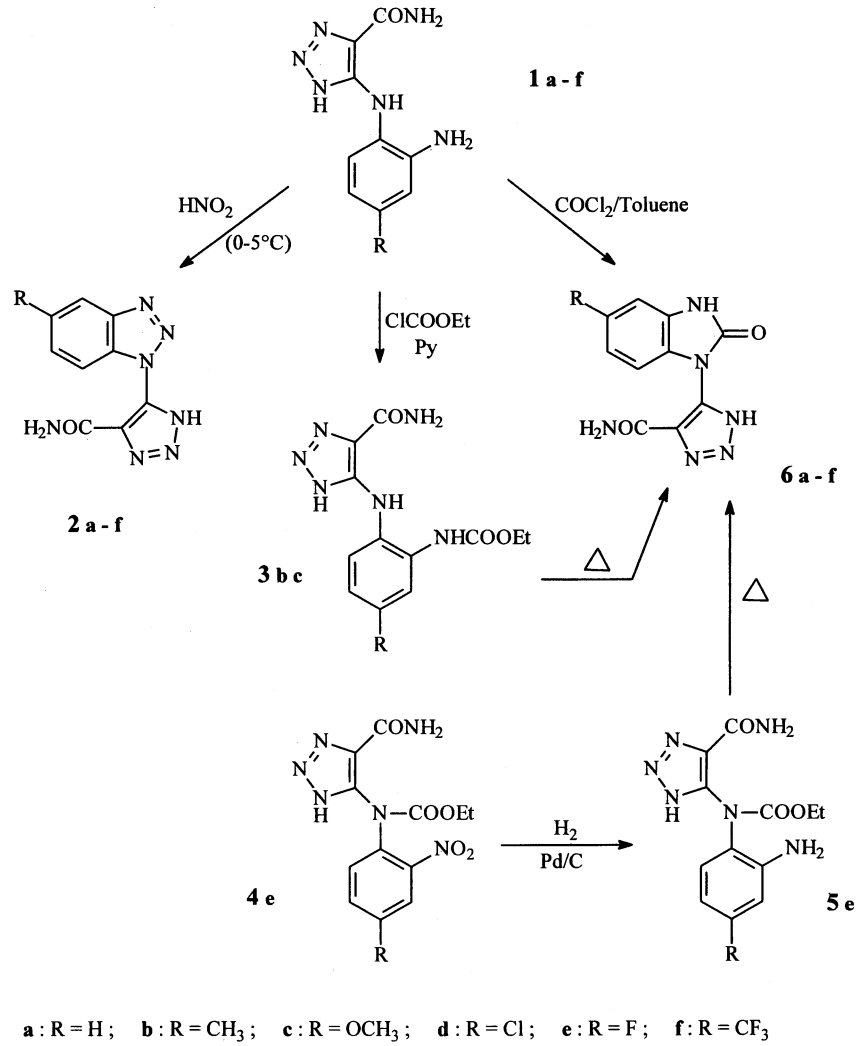


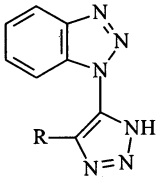
Figure 2. Synthetic routes for the preparation of compounds **2a–f** and **6a–f**.

2b: R=CH_3) [4] and triazolyl–benzimidazolone **6a** (R=H) [5].

The new 5-substituted-1-(4-carboxamido-1,2,3-triazol-5-yl)-benzotriazole derivatives **2c–f** were obtained by diazotization of the corresponding triazole derivatives **1c–f**, bearing the *ortho*-phenylendiamino moiety appropriate for conversion into benzotriazole.

The new 5-substituted-1-(4-carboxamido-1,2,3-triazol-5-yl)-benzimidazolone derivatives **6b–f** were obtained by different synthetic routes. Thus, according to the previously employed procedure [5], the 5-anilino-triazole intermediates **1b** and **1c** were reacted with 1.2 equiv of ethyl chloroformate in pyridine solution, to give the corresponding ethoxycarbonylamino derivatives **3b** and **3c** in moderate yield. When heating **3b** and **3c** in boiling DMF, the expected benzimidazolone derivatives **6b** and **6c** were obtained in good yield, by intramolecular cyclization.

The 5-fluoro-benzimidazolone derivative **6e** was prepared by an alternative procedure, analogous to the previous one [5]: the 4-carboxamido-5-(4-fluoro-2-nitroanilino)-1,2,3-triazole [3] was heated in an excess of ethyl chloroformate to give the ethoxycarbonylamino derivative **4e** in high yield, which was converted to the corresponding amino derivative **5e** by catalytic hydrogenation at room temperature and pressure. Heating **5e** in boiling DMF, the expected benzimidazolone derivative **6e** was obtained.



7a : R = COOH
7b : R = H

Figure 3. Previously prepared compounds.

The other benzimidazolone derivatives **6d,f** together with the previously described **6a** [5], were obtained by a new direct procedure, starting from the suitable 4-carboxamido-5-(4-substituted-2-aminoanilino)-1,2,3-triazoles **1a,d,f** in pyridine solution at 0 °C, by reaction with a solution of 20% phosgene in toluene.

For pharmacological purposes, the triazolyl–benzotriazole derivatives **7a** and **7b** (*figure 3*) were also prepared as previously described [4], starting from **2a**, by alkaline hydrolysis and decarboxylation.

The structures of all the new compounds were assigned on the basis of known reaction mechanisms [6, 7] and our previous evidence [3–5] and were confirmed by analytical and spectroscopic methods.

^{1}H-NMR data for compounds **2** and **6** are reported in *table II*.

Table I. Physico-chemical properties.

Compound	R	Yield (%)	Crystall. solvent	M.p. (° C)	Analysis C, H, N	Mass *m/z*	
						M^+	Base
2a	H			[4]			
2b	CH_3			[4]			
2c	OCH_3	72	MeOH–H_2O	273–275	$C_{10}H_9N_7O_2$	259	44
2d	Cl	74	MeOH–H_2O	278–282	$C_9H_6N_7OCl$	263	137
2e	F	78	EtOH	283–285	$C_9H_6N_7OF$	247	121
2f	CF_3	59	EtOH	273–277	$C_{10}H_6N_7OF_3$	297	44
3b	CH_3	60	AcOEt	165–167	$C_{13}H_{16}N_6O_3$		
3c	OCH_3	33	AcOEt	208–210	$C_{13}H_{16}N_6O_4$	320	44
4e	F	83	AcOEt	201–203	$C_{12}H_{11}N_6O_5F$	338	44
5e	F	95	MeOH	142–144	$C_{12}H_{13}N_6O_3F$	308	136
6a	H			[5]			
6b	CH_3	76	AcOEt	295–300	$C_{11}H_{10}N_6O_2$	258	44
6c	OCH_3	75	AcOEt	253–256	$C_{11}H_{10}N_6O_3$		
6d	Cl	61	AcOEt/Pet. Eth.	318–322	$C_{10}H_7N_6O_2Cl$	278	44
6e	F	56	AcOEt/Pet. Eth.	>350	$C_{10}H_7N_6O_2F$	262	246
6f	CF_3	58	DMF/H_2O	287–290	$C_{11}H_7N_6OF_3$	312	44

Table II. ^{1}H-NMR data (δ, ppm) for compounds **2** and **6**.

Compound	4-H	6-H	7-H	5-subst.	NH and NH_2
2a	8.19	7.63	7.65	7.53	8.1, 7.8
2b	7.95	7.46	7.54	2.50	8.1, 7.7
2c	7.62	7.28	7.58	3.88	8.1, 7.8
2d	8.38	7.69	7.69	–	8.1, 7.8
2e	8.07	7.56	7.68	–	8.2, 7.7
2f	8.74	7.87	7.96	–	8.2, 7.8
6a	7.04	7.04	6.79	7.04	11.0, 7.7, 7.4
6b	6.90	6.89	7.64	2.30	11.2, 7.6, 7.4
6c	6.83	6.94	8.08	3.65	11.5, 8.4, 7.7
6d	8.07	7.37	8.32	–	10.7, 8.8, 7.8, 7.6
6e	7.57	6.95	7.10	–	11.0, 8.8, 8.1, 7.7
6f	7.62	7.50	8.35	–	10.8, 8.5, 7.6

3. Pharmacology

Pharmacological studies, performed in different circulatory districts, such as the rabbit pulmonary artery [8], the rat portal vein [9] and the rat aorta [10] demonstrated that high conductance calcium-activated potassium channels (BKCa) play an important role in modulating the vascular smooth muscle tone. Indeed, potassium-channel activation determines a membrane hyperpolarisation, followed by a lowering of the concentration of free intracellular calcium and consequently by miorelaxation. Thus, the functional evaluation of a vasorelaxing activity of the compounds tested was chosen as a preliminary screening method, to unmask a possible potassium channel opening effect.

4. Results and discussion

All the compounds tested belonging to the triazolyl–benzotriazole heterocyclic series (**2a–f**) showed vasorelaxing effects, consisting of a complete abolition of the contractile tone induced by the administration of KCl 20 mM. The potency orders of magnitude were slightly lower than those recorded for the reference compound NS1619 (*table III*). Preliminary observations of the structure-activity relationships suggested that the absence of any substituent in the 5 position of the benzene ring (**2a**) led to a decrease of potency (but not of efficacy). The experimental data seemed to suggest a possible correlation between the recorded potencies and the steric hindrance in this position. The methoxy group showed a negative influence on potency and efficacy. The benzotriazoles **7a,b** did not show any vasorelaxing properties, therefore the carboxamido group should appear as a useful structural requirement.

The triazolyl–benzimidazolone compounds (**6a–f**), albeit more similar to the reference molecule than the benzotriazoles, did not reach the same vasoactive properties. They showed partial vasorelaxing efficacy, as they were unable to cause a complete reversion of the KCl 20 mM-induced contractile tone. This partial efficacy (often about 50%) did not permit a satisfactory calculation of the potency parameter (IC_{50}) (*table III*).

Finally, a further investigation of the possible potassium channel opening mechanism of action was also performed in the isolated vessels. It is widely reported that drugs, acting through potassium channel activation, possess the highest degree of 'depolarisation-sensitive' inhibition of the functional responses, among the several classes of vasodilators [11]. Therefore, the compounds were also tested on isolated aortae, whose contractile tone was induced by a higher level of membrane depolarisation (due to the administration of KCl 80 mM). In such an experimental condition, significant decreases of potency and efficacy values were observed, fitting the profile of response expected for a potassium channel opener. The reference compound NS1619 did not show any significant decrease of activity, when administered to preparations pre-contracted by KCl 80 mM. This anomalous behaviour could be due to different ancillary mechanisms of action, whose existence has already been suggested by the literature [10]. Furthermore, the vasorelaxing effect of compound **2b** (con-

trol efficacy % = 100; control $pIC_{50} = 4.86 \pm 0.21$) resulted strongly inhibited by the non-selective potassium channel antagonists 1 mM tetraethylammonium chloride (efficacy % = 34 ± 5; pIC_{50} not calculable) and 3 mM 4-aminopyridine (efficacy % = 24 ± 1; pIC_{50} not calculable), whereas the K_{ATP}-selective blocking sulphanylurea glybenclamide did not produce any significant inhibition (control efficacy % = 100; $pIC_{50} = 4.63 \pm 0.17$). The above experimental data suggested a possible involvement of the calcium-activated potassium channel in the vasodilator properties of the tested compounds.

5. Experimental protocols

5.1. Chemistry

Melting points were determined on a Kofler hot-stage and are uncorrected. IR spectra in Nujol mulls were recorded on a Mattson Genesis series FTIR spectrometer. ^{1}H-NMR spectra were recorded with a spectrometer Varian Gemini 2000 in DMSO-d_6 in δ units, using TMS as the internal standard. Mass spectra were performed with a Hewlett Packard MS/system 5988. Elemental analyses (C, H, N) were within ± 0.4% of the theoretical values and were performed on a Carlo Erba elemental analyzer Mod. 1106 apparatus. TLC data were obtained with Riedel de Haen, 37360 DC-Karten F_{254}, 0.2 mm, eluting with a 1:3 AcOEt/petroleum ether mixture. Petroleum ether corresponds to fraction boiling at 40–60 °C.

*5.2. 4-Carboxamido-5-(4-substituted-2-aminoanilino)-1,2,3-triazoles (**1a–f**)*

The preparation of the title compounds is reported in the literature [3].

*5.3. 5-Substituted-1-(4-carboxamido-1,2,3-triazol-5-yl)-benzotriazoles (**2c–f**)*

To an ice-cooled (0–5 °C) and stirred solution of 2.0 mmol (0.500 g) of the appropriate 4-carboxamido-5-(4-substituted-2-aminoanilino)-1,2,3-triazole (**1c**, **1d**, **1e** or **1f**) in 15 mL of 18% HCl, a solution of $NaNO_2$ (0.165 g, 2.4 mmol) in 10 mL of H_2O was added drop by drop. After 20 min, the ice-bath was removed and the stirring was continued at room temperature for 1 h. The precipitate obtained, consisting of the title compounds, was collected by filtration and washed with H_2O. The compounds were purified by crystallization (*table I*). ^{1}H-NMR data are reported in *table II*.

*5.4. 4-Carboxamido-5-(2-ethoxycarbonylamino-4-substituted-anilino)-1,2,3-triazoles (**3b,c**)*

To an ice-cooled and stirred solution of 0.500 g of **1b** [3] (2.15 mmol) or **1c** [3] (2.0 mmol) in 10 mL of anhydrous pyridine, 1.2 equiv of ethyl chloroformate were added. The solution was heated at 50 °C for 4 h and, after 20 h at room temperature, it was acidified with 18% HCl and extracted with $CHCl_3$. The organic layer was washed with 10% HCl, H_2O, dried ($MgSO_4$) and evaporated to give the title compounds, which were purified by crystallization (*table I*). ^{1}H-NMR data (δ): **3b**: 1.34 (t, 3H, CH_3); 2.24 (s, 3H, CH_3); 4.37 (q, 2H, CH_2); 6.92 (m, 1H, 5′-H); 7.19 (m, 1H, 3′-H); 7.44 (m, 1H, 6′-H); 5.7, 6.0, 7.1, 7.5 (5H, NH and NH_2). **3c**: 1.36 (t, 3H, CH_3); 3.65 (s, 3H, OCH_3); 4.42 (q, 2H, CH_2); 6.60 (m, 1H, 5′-H); 6.78 (m, 1H, 3′-H); 8.02 (m, 1H, 6′-H); 5.5, 6.3, 7.4 (5H, NH and NH_2).

*5.5. 4-Carboxamido-5-[N-(ethoxycarbonyl), N-(4-fluoro-2-nitrophenyl)]-amino-1,2,3-triazole (**4e**)*

A suspension of 4-carboxamido-5-(4-fluoro-2-nitroanilino)-1,2,3-triazole [3] (0.750 g, 2.82 mmol) in 10 mL of ethyl chloroformate was heated under reflux for 24 h. After cooling, the solution was poured into water-crushed ice, then neutralised (pH 6–7) with portions of solid $NaHCO_3$. After 3 h of stirring, the precipitate consisting of **4e** was collected by filtration, washed with H_2O and purified by crystallization (*table I*). ^{1}H-NMR data (δ): 1.38 (t, 3H, CH_3); 4.55 (q, 2H, CH_2); 7.85 (m, 1H, 6′-H); 8.09 (m, 1H, 5′-H); 8.64 (m, 1H, 3′-H); 8.1, 8.5, 11.6 (3H, NH_2 and NH).

*5.6. 4-Carboxamido-5-[N-(ethoxycarbonyl), N-(4-fluoro-2-aminophenyl)]-amino-1,2,3-triazole (**5e**)*

To a solution of **4e** (0.300 g, 1.0 mmol) in 150 mL of MeOH was added 0.030 g of 10% Pd/C and the mixture was hydrogenated at room temperature and pressure. The catalyst was filtered off, washed with hot MeOH and the filtrate was evaporated in vacuo, to give the title compound, which was purified by crystallization (*table I*). ^{1}H-NMR data (δ): 1.36 (t, 3H, CH_3); 4.47 (q, 2H, CH_2); 6.43 (m, 1H, 6′-H); 6.58 (m, 1H, 5′-H); 7.48 (m, 1H, 3′-H); 5.1, 7.9, 8.0, 8.3 (5H, NH_2 and NH).

*5.6.1. 5-Substituted-1-(4-carboxamido-1,2,3-triazol-5-yl)-benzimidazolones (**6b,c,e**)*

A solution of 0.500 g (1.60 mmol) of the appropriate ethoxycarbonyl derivative **3b**, **3c** or **5e** in 10–15 mL of DMF was heated under reflux for 4–5 h. The reaction mixture was concentrated in vacuo (1/2–1/3 of the original volume) and H_2O was added to precipitate the title compounds, which were collected by filtration and crystallised from the appropriate solvent (*table I*). ^{1}H-NMR data are reported in *table II*.

*5.6.2. 5-Substituted-1-(4-carboxamido-1,2,3-triazol-5-yl)-benzimidazolones (**6a,d,f**)*

To an ice-cooled (0–5 °C) and stirred solution of 1.0 mmol of the appropriate compound **1a** [4], **1d** [3] or **1f** [3] in 8 mL of anhydrous pyridine, a solution of 20% phosgene in toluene (0.8 mL, 1.6 mmol) was added. The reaction mixture was stirred for 2 h, then the ice-bath was removed and stirring continued for 20 h. Dilution with H_2O and acidification (pH 2–3) with 10% HCl caused precipitation of the title compounds, which were collected by filtration and crystallised from the appropriate solvent (*table I*). ^{1}H-NMR data are reported in *table II*.

Table III. Pharmacological effects of the compounds tested[a].

Compound	pIC_{50}	Efficacy %	Dep.
2a	3.95 ± 0.059	100	+
2b	4.86 ± 0.21	100	+
2c	3.81 ± 0.049	70	+
2d	4.26 ± 0.090	100	+
2e	4.03 ± 0.036	100	+
2f	4.43 ± 0.11	100	+
6a		ineffective	
6b	4.03 ± 0.17	71	+
6c		ineffective	
6d	[b]	53	+
6e	[b]	48	+
6f	[b]	55	+
NS 1619	5.30 ± 0.29	100	−

[a] The values of potency (pIC_{50}) are expressed as the mean ± SEM. The efficacy parameter indicates the maximal vasorelaxation, as a % of the contractile tone induced by KCl 20 mM. The potency parameters of compounds possessing a low efficacy (50% or lower) could not be calculated. The effectiveness (+) or the ineffectiveness (−) of the increased depolarisation (KCl 80 mM) to reduce the activity of the compounds, are also shown (Dep.).

[b] The pIC_{50} values could not be calculated because of the low efficacy %.

5.7. Pharmacology

All the experimental procedures were carried out following the guidelines of the European Community Council Directive 86-609.

To determine a possible vasodilator mechanism of action, the compounds were tested on isolated thoracic aortae of male normotensive Wistar rats (250–350 g).

The rats were killed by cervical dislocation under light ether anaesthesia and bled. The aortae were immediately excised, freed of extraneous tissues and the endothelium was removed by gently rubbing the intimal surface of the vessels. Aortic rings were suspended, under a preload of 2 g, in 10 mL organ baths, containing Tyrode solution (composition of saline in mM: NaCl 136.8; KCl 2.95; $CaCl_2$ 1.80; $MgSO_4 \cdot 7H_2O$ 1.05; NaH_2PO_4 0.41; $NaHCO_3$ 11.9; Glucose 5.5), thermostated at 37 °C and continuously bubbled with a mixture of O_2 (95%) and CO_2 (5%). Changes in tension were recorded by means of an isometric transducer (Basile mod. 7005), connected with a unirecord microdynamometer (Basile mod. 7050).

After an equilibration period of 60 min, the endothelial integrity was confirmed by acetylcholine (ACh) (55 μM)-induced relaxation of norepinephrine (NE, 1 μM)-precontracted tissues. A relaxation $< 20\%$ of the NE-induced contraction was considered representative of an acceptable lack of the endothelial layer, while the organs, showing a relaxation $\geq 20\%$ (i.e. significant presence of the endothelium), were not used in the experimental procedures. Thirty to 40 min after confirmation of the endothelium removal, the aortic preparations were contracted by treatment with a single concentration of KCl (20 mM) and when the contraction reached a stable *plateau*, 3-fold increasing concentrations of the compounds (10 nM–1 mM) were added cumulatively. In parallel sets of experiments, to investigate the influence of a higher level of depolarisation on the responses evoked by the compound tested, the aortic preparations were contracted by KCl 80 mM. Then, 3-fold increasing concentrations of the compounds (10 nM–1 mM) were added cumulatively.

Preliminary experiments showed that both the KCl (20 and 80 mM)-induced contractions remained constant in a stable tonic state for at least 40 min.

In other sets of experiments, the non-selective potassium channel blocker tetraethylammonium chloride (1 mM), the non-selective potassium channel blocker 4-aminopyridine (3 mM) or the ATP-sensitive potassium channel selective blocker glybenclamide (1 μM) were added, before the KCl (20 mM)-induced contraction, followed by the administration of selected compounds.

Norepinephrine hydrochloride (Sigma), acetylcholine chloride (Sigma), tetraethylammonium chloride (Aldrich), 4-aminopyridine (Sigma) and KCl were dissolved in bi-distilled water. Glybenclamide (1 mM) was dissolved by sonication in an aqueous solution of NaOH (0.1 N) and further diluted in bi-distilled water. All the synthesised derivatives and the reference compound NS 1619 (RBI) were dissolved (10 mM) in aqueous NaOH (0.1 N). All further dilutions were performed in bi-distilled water. All solutions were prepared immediately before the pharmacological experimental procedures. Previous experiments showed a complete ineffectiveness of the administration of the vehicle.

5.7.1. Data analysis

The efficacy of the vasorelaxing responses was expressed as a maximal relaxant effect (E_{max}), calculated as a % of the contractile tone developed by the smooth muscle preparation, after the depolarising stimulus induced by KCl 20 mM. The above parameters were calculated by means of non-linear regression analysis of the sigmoidal concentration-response curves (computer program: GraphPad Prism), and were expressed as the mean of six experiments. Previous experiments demonstrated an almost complete quantitative equivalence between the contractile responses evoked by the two different concentrations of KCl, since the concentration 20 mM could substantially induce a maximal effect, in endothelium denuded aortic rings.

The parameter of potency of the vasorelaxing effects was expressed as pIC_{50}, representing the negative logarithm of the vasodilator molar concentration determining a half reduction of the contractile tone induced by the contractile agent. The above parameters were calculated by means of non-linear regression analysis of the sigmoidal concentration–response curves (computer program: GraphPad Prism), and were expressed as the mean $\pm$ SEM of six experiments.

The statistical comparison of experimental data was performed by the two-tailed Student t-test and Anova. A value of $P < 0.05$ was considered as representative of significant differences.

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