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ScienceDirect
European Journal of Medicinal Chemistry 43 (2008) 2618–2626
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
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Laboratory note

1,2,3-Triazol-carboxanilides and 1,2,3-triazol-(*N*-benzyl)-carboxamides as BK-potassium channel activators. XII

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Received 27 July 2007; received in revised form 28 February 2008; accepted 29 February 2008
Available online 7 March 2008

Abstract

The chemical structures of many synthetic activators of large-conductance calcium-activated potassium channels (BK channels) satisfy a simple pharmacophore model, consisting of two appropriately substituted phenyl rings connected by a linker of a heterogeneous nature. In this paper, a series of new compounds with modifications of the linker portion of the above pharmacophore are described. In particular, in these new derivatives, the linker portion is represented by a 1,2,3-triazole-carboxamide group, which can be viewed as a combination of two different kinds of linker, independently used in previous series of BK-openers: the amide function and the 1,2,3-triazole ring. The overall finding of this study indicated that the triazole-carboxamide derivatives were generally poorly effective and that this structural modification of the linker is deleterious for activity on BK channels. Therefore, it can be hypothesized that the increase of the steric hindrance of the linker and/or the increase of the distance between the two aromatic portions are negative for the interaction with the biological target.

Keywords: BK-potassium channel; Triazole-carboxamide; Triazole-carboxanilide; BK-activator

1. Introduction

Large-conductance, calcium-activated potassium channels (BKCa or BK channels) are distributed in both excitable and non-excitable cells and are involved in many cellular functions such as action potential repolarization; neuronal excitability; neurotransmitter release; hormone secretion; tuning of cochlear hair cells; innate immunity; and modulation of the tone of vascular, airway, uterine, gastrointestinal, and urinary bladder smooth muscle tissues [1–3].

The physiological activation of BK channels, induced mainly by two triggering signals, such as the rise of the intracellular free calcium ions and membrane depolarization, ensures massive flow of potassium ions (with a single channel conductance of 150–300 pS) to the extracellular side of the plasmalemma, membrane hyperpolarization and reduction of cellular excitability. Conversely, the availability of exogenous BK-activators can represent a pharmacological tool for the clinical management of pathological states, related to a cell hyperexcitability, such as asthma, urge incontinence and bladder spasm, gastric hypermotility, neurological and psychiatric disorders [1–3].

With regard to the cardiovascular system, BK channels ensure the predominant component of the outward K^+ current in vascular smooth muscle cells, playing fundamental roles in the modulation of the muscular tone of vessels [4,5]. Consequently, the vasorelaxing effects of exogenous BK-openers can be a rational basis for treatment of hypertension and/or other diseases related to an impaired contractility of vessels (for example, coronary vasospasm) [1,2].

Our research program about potential activators of BK channels considered heterocyclic compounds [6–9] which referred to the benzimidazolone derivatives NS 004 and NS1619

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doi:10.1016/j.ejmech.2008.02.032

(Fig. 1A) reported in the literature [10] as pioneer BK-openers. Thereafter, based on an hypothetical simple pharmacophore (Fig. 1B) consisting of two appropriately substituted phenyl rings connected by a linker, 1,2,3-triazole derivatives [11–13] and benzanilides [14–16] were synthesized and tested. In these compounds, the heterocyclic ring and the amide function, respectively, represented the linker.

In particular, after a first paper [14] which had shown a BK channel-induced vasorelaxing activity of *N*-(2-hydroxy-5-chlorophenyl)-2-methoxy-5-chlorobenzamide higher than NS1619, research on new compounds bearing the amide linker was extended, changing the benzanilide structure [15] by the introduction of a 5- or 6-membered aromatic heterocyclic substituent in the acid moiety of the amide, keeping unaltered the basic moiety as 2-hydroxy-5-chloro-anilide (Fig. 2A). In addition, an aromatic or aliphatic 6- or 5-membered heterocyclic substituent was introduced in the basic moiety of the amide, keeping unaltered the acid moiety as 2-hydroxy-5-chlorobenzoyl (Fig. 2B). The pharmacological results indicated that the presence of nitrogen heterocycles on the acid side of the amide linker seems to be a negative requirement, while furan and thiophene rings were tolerated. On the contrary, the introduction of unsaturated heterocyclic rings (pyridine and thiazole) on the basic side of the amide linker, led to biological activity, but the presence of aliphatic heterocycles lowered the pharmacological effect. The importance of the presence of a phenol function as an H-bond donor was confirmed.

Further structural changes of the benzanilide pharmacophore concerned a deepening of the structure–activity relationships of benzanilide derivatives previously studied as BK channel activators [16] (Fig. 2C). In that paper, several substitutions on the phenyl rings of the reference benzanilides were reported, which possessed particular and specific properties from a mesomeric and/or steric point of view. The pharmacological results indicated that several compounds exhibited vasorelaxing effects which could not be attributed to the activation of BK channels, while two derivatives showed a clear profile of BK-activators with a vasodilator activity comparable to or slightly lower than that recorded for the reference benzimidazolone NS1619.

A third structural modification led to the introduction of methylenic spacers between the amide linker and the two aromatic rings, to evaluate the biological effect caused by this lengthening (Fig. 2D). The pharmacological results suggested that these structural modifications were generally deleterious [17].

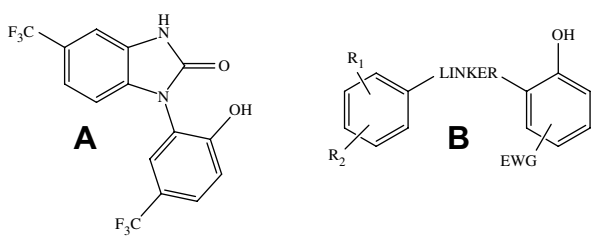


Fig. 1. Chemical structure of NS1619 (A). Pharmacophore model of BK-activators (B). R_1 and R_2 represent substituents of various nature. EWG is an electron withdrawing group.

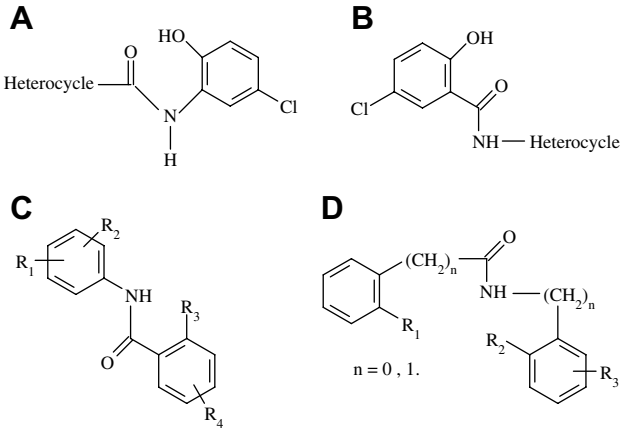


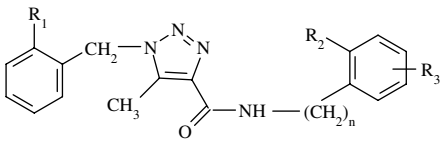
Fig. 2. Chemical structures of previously synthesized compounds (A–D).

In this new paper, our research program on potential activators of BK channels has been developed with further changes on the linker portion of the usual pharmacophore. For this purpose, on the basis of our previous results [13,14], the 1,2,3-triazole ring [13] and the amide function [14] were combined to give a triazole-carboxamide linker, by the synthesis and pharmacological evaluation of a new series of 1,2,3-triazol-carboxanilides and 1,2,3-triazol-(*N*-benzyl)-carboxamides corresponding to the general formula reported in Fig. 3.

2. Chemistry

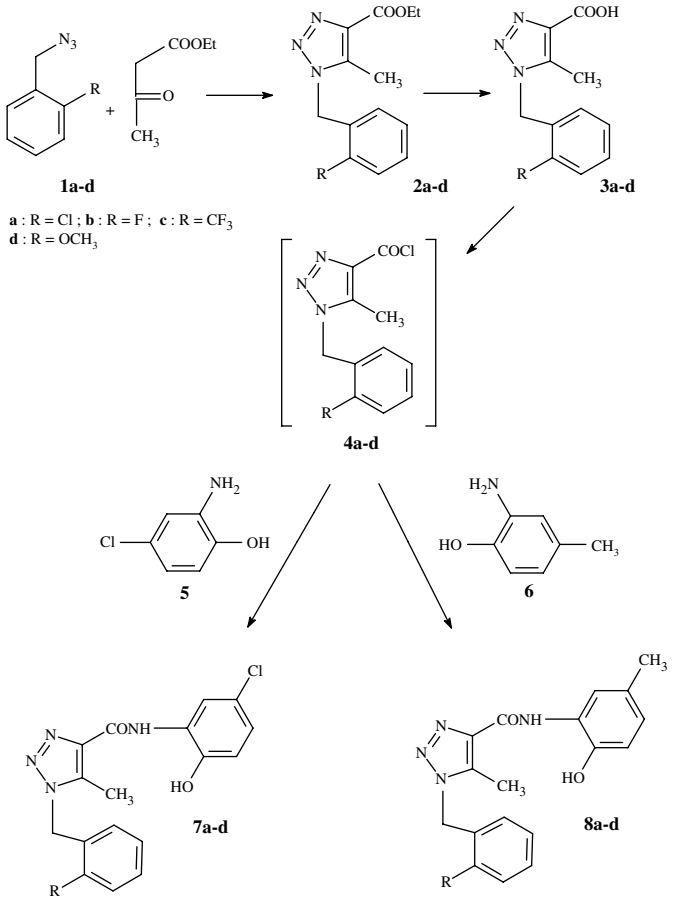
Scheme 1 reports the preparation of a series of 1,2,3-triazol-carboxanilides bearing in the 1 position of the triazole ring an ortho-substituted benzyl group to give the structure the flexibility useful for the biological activity [13]. The anilino substituent of the 1,2,3-triazol-4-carboxanilide bears a phenolic hydroxyl, a well-known requirement useful for the biological activity, in the ortho position of the phenyl ring, in its turn bearing a chlorine substituent (compounds **7a–d**) or a methyl substituent (compounds **8a–d**).

Thus, starting from the suitable azides (2-chlorobenzyl- [18], 2-fluorobenzyl- [19], 2-trifluoromethylbenzyl- [20] or 2-methoxybenzyl- [13]), the expected 1,2,3-triazolesters **2a–d** were obtained in satisfactory yields by 1,3-dipolar cycloaddition reaction to ethyl acetoacetate, carried out in dimethyl sulphoxide in the presence of anhydrous potassium carbonate. Alkaline hydrolysis provided the corresponding carboxylic acids **3a–d** which were converted to the respective acyl chlorides **4a–d**, by heating with thionyl chloride. These



n = 0 : Triazolcarbxanilides ; n = 1 : Triazol-N-benzylamide

Fig. 3. General formula of the new series of 1,2,3-triazol-carboxanilides and 1,2,3-triazol-(*N*-benzyl)-carboxamides.



Scheme 1. Synthetic route for compounds **7a–d** and **8a–d**.

last compounds were not isolated, but, after the evaporation of the reagent, were dissolved in anhydrous toluene and to the solution obtained an equimolar amount of the suitable aniline and triethylamine (TEA) in toluene solution were added, then the mixture was heated under reflux for several hours. The 2-hydroxy-5-chloro-aniline (**5**) provided derivatives **7a–d**, while the 2-hydroxy-5-methyl-aniline (**6**) gave the analogous derivatives **8a–d**.

Scheme 2 reported the preparation of another series of 1,2,3-triazol-anilides, obtained by a similar regiospecific 1,3-dipolar cycloaddition reaction of the suitable azide (**1a**, **b** or **d**) to the selected acetoacetanilides, *N*-(phenyl)-acetoacetamide (**9**), *N*-(4-chlorophenyl)-acetoacetamide (**10**) or *N*-(2-methoxyphenyl)-acetoacetamide (**11**).

By this single-step route, triazolamides bearing substituents with different mesomeric and steric properties, both on the acid component and on the basic one of the amide, were made available.

Thus 2-chlorobenzylazide (**1a**) reacted with the acetoacetanilides **9–11** to give the corresponding triazol-carboxanilides **12a**, **13a** and **14a**, while 2-fluorobenzylazide (**1b**) reacted with the same acetoacetanilides to give the analogous derivatives **12b**, **13b** and **14b**. Similarly 2-methoxybenzylazide (**1d**) reacted to give the corresponding derivatives **12d**, **13d** and **14d**.

The presence of a methoxy group on the benzyl substituent of **12d**, **13d** and **14d** allowed to obtain a phenolic hydroxyl on the acid component of the 1,2,3-triazolamide (compounds **15**, **16**, **19**), via cleavage of the ether function with boron tribromide. In addition, the presence of a methoxyl on the anilino substituent of **14a**, **b** and **d** allowed the obtainment of a phenolic function on the basic component of the 1,2,3-triazolanilide (compounds **17–19**).

Clearly, two phenol functions are present on the derivative **19**.

Finally, Scheme 3 reports the preparation of a series of 1,2,3-triazolbenzylamides obtained according to the usual reaction sequence, starting from the acyl chlorides **4a**, **b** and **d**.

Each acyl chloride was reacted with benzylamine (**20**), 2-chlorobenzylamine (**21**) or 2-methoxybenzylamine (**22**) in toluene under reflux, in the presence of TEA, to give the expected 1-(2-substituted-benzyl)-4-carboxybenzylamido-5-methyl-1*H*-1,2,3-triazoles **23a**, **b**, **d**, **24a**, **b**, **d** and **25a**, **b**, **d**, respectively, in good yield. Also these derivatives have substituents which are different for mesomeric and steric properties; in addition they have a methylene bridge on the nitrogen atom of the amido linker, which lends more flexibility to the whole molecule.

Also in this case, the presence of a methoxyl in the *ortho* position of the benzyl substituent on the triazole nitrogen of the compounds **23d**, **24d** and **25d** allowed the introduction of a phenol function, generally useful for the biological activity.

The demethylation reaction, carried out in the usual manner with boron tribromide, gave the derivatives **26**, **27** and **30**.

Similarly, the cleavage of the ether function of the 2-methoxybenzylamides **25a**, **b** and **d** allowed the introduction of a hydroxyl function on the benzylamido component too, with obtainment of the compounds **28–30**. Clearly, two phenol functions are present on the derivative **30**.

The structures of all the newly prepared compounds were confirmed by analytical and spectroscopic data (Tables 1 and 2).

3. Pharmacology

As a preliminary indication about a possible BK-activating mechanism of action, the vasodilating effect of the new compounds was studied *in vitro* on isolated rat aortic rings precontracted with KCl 20 mM.

4. Results and discussion

In this work new potential BK-activators have been projected and synthesized on the basis of the general pharmacophoric model, reported in Fig. 1. In particular, in these new derivatives, the linker portion of the pharmacophore is represented by a 1,2,3-triazole-carboxamide group, which can be viewed as a combination of two different kinds of linker, independently used in other series of BK-openers.

(a) The amide function, which can be considered a simple and satisfactory linker portion, present in previous series of derivatives of ours [14–17].

(b) The 1,2,3-triazole ring, also used in previous work [11–13] of ours and belonging to a wider ''family'' of heterocyclic linkers [1].

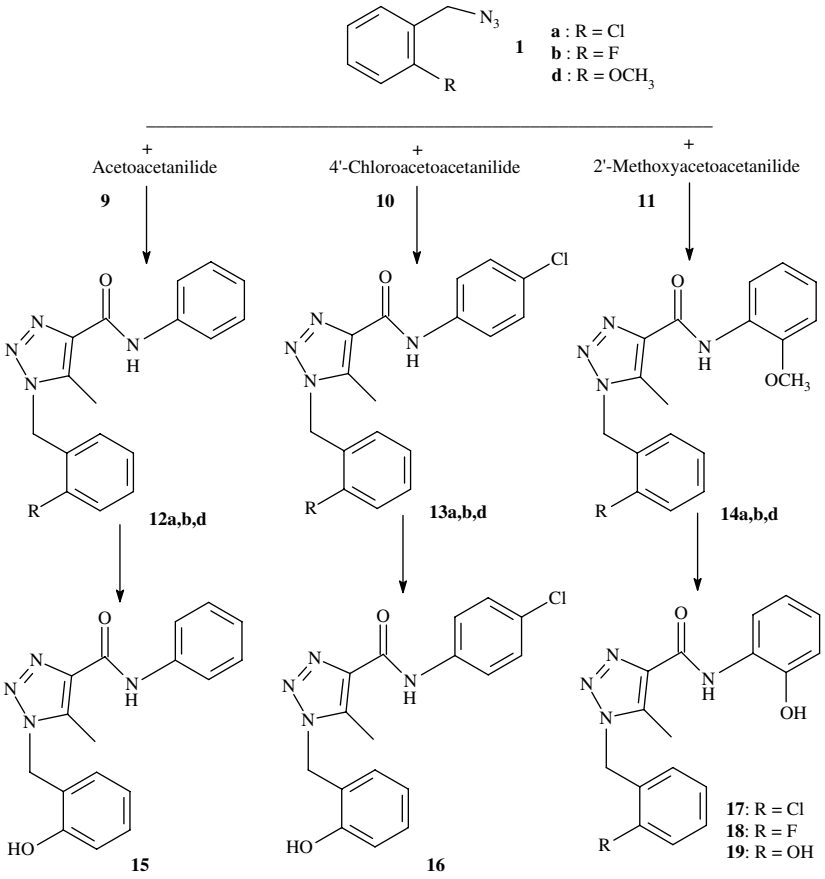
As a preliminary indication about a possible BK-activating property, all the target triazole-carboxamide derivatives of this series were tested by functional pharmacological tests, in order to evaluate a vasorelaxing effect. The results are summarized in Table 3. Compounds **7c**, and **8a–c** resulted almost ineffective and all these compounds are characterised by the presence of the phenyl hydroxyl group on the aromatic ring directly bound to the amidic nitrogen. This same structural characteristic is also present in **7a**, **b**, **d** and **8d**, as well as in compounds **17** and **18**, which globally exhibited modest levels of vasorelaxing efficacy (indeed, only compound **17** showed an efficacy slightly higher than 50%). Although many derivatives showing a phenol hydroxyl group on the benzyl bound to the triazole ring were also devoid of high levels of efficacy, it is noteworthy that this structural characteristic is shared by only the three derivatives (**15**, **19** and **30**) which exhibited high levels of vasorelaxing efficacy, although with potency parameters significantly lower than that of the reference compound NS1619. This remark seems to indicate that the insertion of the hydroxy group (widely recognized as a fundamental requirement for the interaction with BK channels) in the aromatic ring at the side of the triazole moiety of the linker is preferable. As regards the influence of halogen atoms, it is particularly evident that the presence of a fluorine in the structure exerted a negative impact, as emerged from the lowered efficacy recorded in compounds **18** and **29**. In order to obtain more detailed information about the mechanism of action, compound **15**, exhibiting the highest levels of potency and efficacy in this series, was also tested in the presence of tetraethylammonium chloride (10 mM). The presence of this potassium channel blocker antagonized the vasorelaxing responses evoked by **15**, indicating the probable involvement of BK-activation.

In conclusion, the overall finding of this study indicated that the triazole-carboxamide derivatives showed a poor activity and that this structural modification of the linker is deleterious for activity on BK channels. Therefore, in agreement with earlier results [16,17], it can be confirmed that the increase of the steric hindrance of the linker and/or the increase of the distance between the two aromatic portions is deleterious for the interaction with the biological target.

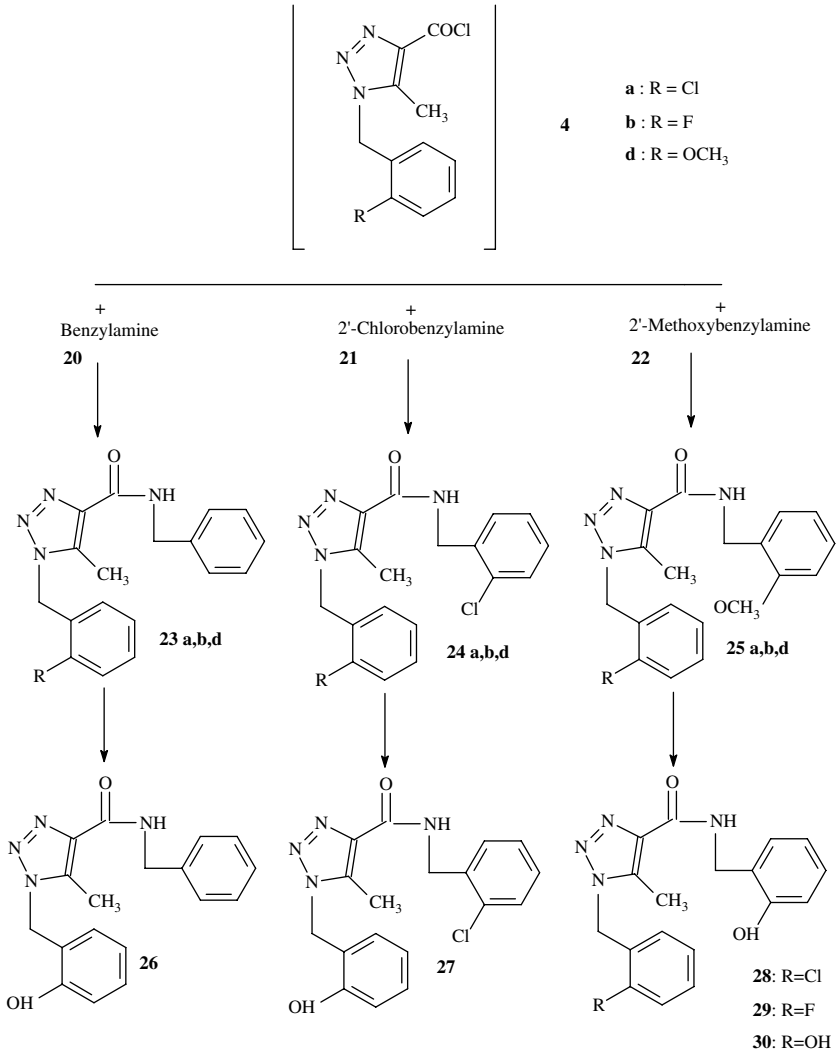
5. Experimental

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 Varian Gemini 200 spectrometer in DMSO-d_6, in δ units, using TMS as internal standard. TLC



Scheme 2. Synthetic route for compounds **15–19**.



Scheme 3. Synthetic route for compounds **26**–**30**.

was performed on precoated silica gel F_{254} plates (Merck). 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.

*5.1.1. 1-(2-Substituted-benzyl)-4-carbethoxy-5-methyl-1H-1,2,3-triazoles (**2a–d**)*

To a solution of 5.0 mmol of the appropriate azide (**1a**, **b**, **c** or **d**) and ethyl acetoacetate (0.715 g, 5.5 mmol) in 8–10 mL of anhydrous DMSO, 5–6 g of finely powdered K_2CO_3 was added and the suspension was stirred at 35–40 °C for 48 h. The reaction mixture was diluted with H_2O to precipitate the title compounds, stirring was continued for 1 h then the solid compounds were collected by filtration, washed with H_2O and purified by crystallization (Table 1).

*5.1.2. 1-(2-Substituted-benzyl)-4-carboxy-5-methyl-1H-1,2,3-triazoles (**3a–d**)*

A solution of 7.0 mmol of the appropriate triazolester (**2a**, **b**, **c** or **d**) in 10 mL of 10% NaOH and 10 mL of EtOH was heated under reflux for 3–4 h. The reaction mixture was concentrated, diluted with H_2O and acidified (pH ≅ 2) to precipitate the title compounds which were collected by filtration and purified by crystallization (Table 1).

*5.1.3. N-(2-Hydroxy-5-chlorophenyl)-1-(2-substituted-benzyl)-5-methyl-1H-1,2,3-triazol-4-carboxamides (**7a–d**)*

A solution of 3.0 mmol of the suitable carboxytriazole (**3a**, **b**, **c** or **d**) in 8 mL of $SOCl_2$ was heated under reflux for 1 h. The reagent was distilled and the residue, consisting of the corresponding acyl chloride **4a**, **b**, **c** or **d**, was dissolved in 20 mL of anhydrous toluene. This solution was added dropwise to a solution of 2-hydroxy-5-chloroaniline **5** (0.430 g, 3.0 mmol) and TEA (1 mL, 7 mmol) in 15–20 mL of anhydrous toluene and the mixture was refluxed for 18–20 h. For the isolation of **7a** and **b**, the toluene solution, after cooling, separated a crystalline solid which was collected by filtration and treated with H_2O to dissolve the probably present TEA hydrochloride.

The insoluble material consisted of a first portion of **7a** or **b**. The toluene filtrate was evaporated in vacuo, the residue

Table 1
Physico-chemical properties

Compound	Yield (%)	Crystall. solvent	M.p. (°C)	Analyses (C, H, N)	IR, ν (cm^{-1})
2a	77	EtOAc/hexane	90–92	$C_{13}H_{14}N_3O_2Cl$	1704 (CO)
2b	85	Cyclohexane	53–55	$C_{13}H_{14}N_3O_2F$	1714 (CO)
2c	75	$CHCl_3$/hexane	107–108	$C_{14}H_{14}N_3O_2F_3$	1706 (CO)
2d	70	–	Oil		1726 (CO)
3a	82	Iso-PrOH	196–197	$C_{11}H_{10}N_3O_2Cl$	1704 (CO)
3b	90	EtOAc/hexane	178–179	$C_{11}H_{10}N_3O_2F$	1690 (CO)
3c	91	MeOH	203–205	$C_{12}H_{10}N_3O_2F_3$	1703 (CO)
3d	88	MeOH	188–190	$C_{12}H_{13}N_3O_3$	1682 (CO)
7a	65	MeOH/H_2O	256–258	$C_{17}H_{14}N_4O_2Cl_2$	1671 (CO); 3100b (OH); 3383 (NH)
7b	62	MeOH/H_2O	242–244	$C_{17}H_{14}N_4O_2ClF$	1673 (CO); 3250b (OH); 3384 (NH)
7c	65	MeOH/H_2O	217–220	$C_{18}H_{14}N_4O_2ClF_3$	1669 (CO); 3230b (OH); 3353 (NH)
7d	70	MeOH	244–245	$C_{18}H_{17}N_4O_3Cl$	1669 (CO); 3150b (OH); 3385 (NH)
8a	67	MeOH	243–245	$C_{18}H_{17}N_4O_2Cl$	1664 (CO); 3280b (OH); 3395 (NH)
8b	60	MeOH/H_2O	204–205	$C_{18}H_{17}N_4O_2F$	1652 (CO); 3300b (OH); 3398 (NH)
8c	70	MeOH/H_2O	197–200	$C_{19}H_{17}N_4O_2F_3$	1680 (CO); 3300b (OH); 3396 (NH)
8d	67	MeOH/H_2O	207–208	$C_{19}H_{20}N_4O_3$	1675 (CO); 3200b (OH); 3392 (NH)
12a	75	MeOH	142–144	$C_{17}H_{15}N_4OCl$	1670 (CO); 3288 (NH)
12b	73	MeOH	134–137	$C_{17}H_{15}N_4OF$	1666 (CO); 3315 (NH)
12d	78	MeOH	168–170	$C_{18}H_{18}N_4O_2$	1668 (CO); 3330 (NH)
13a	75	MeOH	146–148	$C_{17}H_{14}N_4OCl_2$	1672 (CO); 3251 (NH)
13b	69	MeOH/H_2O	138–140	$C_{17}H_{14}N_4OClF$	1672 (CO); 3365 (NH)
13d	73	MeOH	163–165	$C_{18}H_{17}N_4O_2Cl$	1681 (CO); 3375 (NH)
14a	78	MeOH	127–129	$C_{18}H_{17}N_4O_2Cl$	1682 (CO); 3370 (NH)
14b	72	MeOH	113–115	$C_{18}H_{17}N_4O_2F$	1672 (CO); 3351 (NH)
14d	68	MeOH	132–134	$C_{19}H_{20}N_4O_3$	1675 (CO); 3357 (NH)
15	60	MeOH/H_2O	173–175	$C_{17}H_{16}N_4O_2$	1651 (CO); 3130b (OH); 3360 (NH)
16	55	MeOH/H_2O	216–218	$C_{17}H_{15}N_4O_2Cl$	1666 (CO); 3370b (NH, OH)
17	60	MeOH/H_2O	188–190	$C_{17}H_{15}N_4O_2Cl$	1690 (CO); 3040b (OH); 3395 (NH)
18	58	MeOH/H_2O	216–218	$C_{17}H_{15}N_4O_2F$	1685 (CO); 3100b (OH); 3399 (NH)
19	55	MeOH/H_2O	237–239	$C_{17}H_{16}N_4O_3$	1635 (CO); 3160b (OH); 3374 (NH)
23a	75	MeOH/H_2O	130–132	$C_{18}H_{17}N_4OCl$	1648 (CO); 3330 (NH)
23b	72	MeOH/H_2O	128–130	$C_{18}H_{17}N_4OF$	1654 (CO); 3322 (NH)
23d	75	MeOH	163–165	$C_{19}H_{20}N_4O_2$	1649 (CO); 3340 (NH)
24a	70	MeOH	152–154	$C_{18}H_{16}N_4OCl_2$	1660 (CO); 3348 (NH)
24b	74	MeOH	137–139	$C_{18}H_{16}N_4OClF$	1655 (CO); 3298 (NH)
24d	73	MeOH	130–132	$C_{19}H_{19}N_4O_2Cl$	1665 (CO); 3314 (NH)
25a	65	MeOH	153–155	$C_{19}H_{19}N_4O_2Cl$	1660(CO); 3344 (NH)
25b	70	MeOH/H_2O	115–117	$C_{19}H_{19}N_4O_2F$	1669 (CO); 3317 (NH)
25d	75	MeOH	145–147	$C_{19}H_{20}N_4O_2$	1665 (CO); 3365 (NH)
26	50	MeOH	197–200	$C_{18}H_{18}N_4O_2$	1640 (CO); 3200b (OH); 3298 (NH)
27	54	MeOH	218–220	$C_{18}H_{17}N_4O_2Cl$	1638 (CO); 3300b (NH, OH)
28	52	MeOH	170–172	$C_{18}H_{17}N_4O_2Cl$	1628 (CO); 3180b (OH); 3283 (NH)
29	65	MeOH	190–192	$C_{18}H_{17}N_4O_2F$	1634 (CO); 3150b (OH); 3293 (NH)
30	62	MeOH/H_2O	223–225	$C_{18}H_{18}N_4O_3$	1634 (CO); 3290b (NH, OH)

was dissolved in $CHCl_3$ and the new solution, after washing with 5% $NaHCO_3$ and 10% HCl, was dried ($MgSO_4$) and evaporated to give a further amount of **7a** or **b**. The combined fractions were purified by crystallization (Table 1). For the isolation of **7c** and **d**, the toluene solution was evaporated and worked up as the previous filtrate (Table 1).

*5.1.4. N-(2-Hydroxy-5-methylphenyl)-1-(2-substituted-benzyl)-5-methyl-1H-1,2,3-triazol-4-carboxamides (**8a–d**)*

A solution of 3.0 mmol of the suitable carboxytriazole (**3a**, **b**, **c** or **d**) in 8 mL of $SOCl_2$ was heated under reflux for 1 h. The reagent was distilled and the residue, consisting of the corresponding acyl chloride **4a**, **b**, **c** or **d**, was dissolved in 20 mL of anhydrous toluene. This solution was added dropwise to a solution of 2-hydroxy-5-methylaniline **6** (0.369 g, 3.0 mmol) and TEA (1 mL, 7 mmol) in 20 mL of anhydrous toluene and the mixture was refluxed for 18–20 h. For the isolation of the title compounds **8a–d**, the toluene solution, after cooling, separated a crystalline solid. The reaction mixtures were worked up as described for **7a** or **b** (Table 1).

*5.1.5. N-(Phenyl)-1-(2-substituted-benzyl)-5-methyl-1H-1,2,3-triazol-4-carboxamides (**12a**, **b**, **d**)*

To a solution of 6.0 mmol of the appropriate azide (**1a**, **b** or **d**) and acetoacetanilide (1.06 g, 6.0 mmol) in 12 mL of anhydrous DMSO, 6–7 g of finely powdered anhydrous K_2CO_3 was added and the suspension was stirred at 100 °C for 48 h. The reaction mixture was diluted with H_2O to precipitate the title compounds, stirring was continued for 1 h then the

Table 2
^{1}H NMR in DMSO-d_6, ppm from TMS

2a	1.30 (t, 3H, CH_3); 2.50 (s, 3H, CH_3); 4.30 (q, 2H, OCH_2); 5.69 (s, 2H, CH_2); 6.96, 7.36, 7.53 (d, 1H, m, 2H, d, 1H, Ar).
2b	1.29 (t, 3H, CH_3); 2.51 (s, 3H, CH_3); 4.29 (q, 2H, OCH_2); 5.67 (s, 2H, CH_2); 7.13–7.46 (m, 4H, Ar).
2c	1.32 (t, 3H, CH_3); 2.45 (s, 3H, CH_3); 4.32 (q, 2H, OCH_2); 5.79 (s, 2H, CH_2); 6.82, 7.60, 7.83 (d, 1H, m, 2H, d, 1H, Ar).
2d	1.30 (t, 3H, CH_3); 2.50 (s, 3H, CH_3); 4.30 (q, 2H, OCH_2); 5.69 (s, 2H, CH_2); 6.96, 7.36, 7.53 (d, 1H, m, 2H, d, 1H, Ar).
3a	2.48 (s, 3H, CH_3); 5.68 (s, 2H, CH_2); 6.98, 7.37, 7.53 (d, 1H, m, 2H, d, 1H, Ar).
3b	2.49 (s, 3H, CH_3); 5.65 (s, 2H, CH_2); 7.12–7.48 (m, 4H, Ar); 13.0b (COOH).
3c	2.47 (s, 3H, CH_3); 5.79 (s, 2H, CH_2); 6.73, 7.49, 7.76 (d, 1H, m, 2H, d, 1H, Ar).
3d	2.47 (s, 3H, CH_3); 3.79 (s, 3H, OCH_3); 5.48 (s, 2H, CH_2); 6.91, 7.04, 7.32 (d, 2H, d, 1H, m, 1H, Ar); 12.8 (COOH).
7a	2.57 (s, 3H, CH_3); 5.73 (s, 2H, CH_2); 6.95, 7.37, 7.54, 8.30 (m, 3H, m, 2H, d, 1H, s, 1H, Ar); 9.5 (NH); 10.7 (OH).
7b	2.59 (s, 3H, CH_3); 5.71 (s, 2H, CH_2); 6.97, 7.18–7.46, 8.30 (m, 2H, m, 4H, s, 1H, Ar); 9.5 (NH); 10.7 (OH).
7c	2.52 (s, 3H, CH_3); 5.84 (s, 2H, CH_2); 6.85–8.20 (m, 7H, Ar); 9.8 (NH); 10.6b (OH).
7d	2.57 (s, 3H, CH_3); 3.81 (s, 3H, OCH_3); 5.54 (s, 2H, CH_2); 6.89–7.37, 8.30 (m, 6H, s, 1H, Ar); 9.5 (NH); 10.6 (OH).
8a	2.22 (s, 3H, CH_3); 2.56 (s, 3H, CH_3); 5.71 (s, 2H, CH_2); 6.76, 7.00, 7.37, 7.53, 8.07 (m, 2H, d, 1H, m, 2H, d, 1H, s, 1H, Ar); 9.5 (NH); 10.0 (OH).
8b	2.21 (s, 3H, CH_3); 2.57 (s, 3H, CH_3); 5.70 (s, 2H, CH_2); 6.78, 7.18–7.48, 8.06 (m, 2H, m, 4H, s, 1H, Ar); 9.5 (NH); 10.0 (OH).
8c	2.22 (s, 3H, CH_3); 5.82(s, 2H, CH_2); 6.80, 7.61, 7.83, 8.08 (m, 3H, m, 2H, d, 1H, s, 1H, Ar); 9.5 (NH); 10.0 (OH).
8d	2.22 (s, 3H, CH_3); 2.56 (s, 3H, CH_3); 3.81 (s, 3H, OCH_3); 5.53 (s, 2H, CH_2); 6.70–7.36, 8.08 (m, 6H, s, 1H, Ar); 9.5 (NH); 10.0 (OH).
12a	2.54 (s, 3H, CH_3); 5.74 (s, 2H, CH_2); 6.95, 7.08, 7.28–7.44, 7.54, 7.83 (d, 1H, t, 1H, m, 4H, d, 1H, d, 2H, Ar); 10.4 (NH).
12b	2.56 (s, 3H, CH_3); 5.71 (s, 2H, CH_2); 7.04–7.48, 7.83 (m, 7H, d, 2H, Ar); 10.4 (NH).
12d	2.54 (s, 3H, CH_3); 3.82 (s, 3H, OCH_3); 5.54 (s, 2H, CH_2); 6.93, 7.07, 7.32, 7.83 (d, 2H, m, 2H, m, 3H, d, 2H, Ar); 10.3 (NH).
13a	2.54 (s, 3H, CH_3); 5.73 (s, 2H, CH_2); 6.98, 7.36, 7.52, 7.88, (d, 1H, m, 4H, d, 1H, d, 2H, Ar); 10.6 (NH).
13b	2.56 (s, 3H, CH_3); 5.71 (s, 2H, CH_2); 7.17–7.50, 7.88 (m, 6H, d, 2H, Ar); 10.5 (NH).
13d	2.54 (s, 3H, CH_3); 3.82 (s, 3H, OCH_3); 5.54 (s, 2H, CH_2); 6.94, 7.07, 7.35, 7.88 (d, 2H, d, 1H, m, 3H, d, 2H, Ar); 10.5 (NH).
14a	2.56 (s, 3H, CH_3); 3.91 (s, 3H, OCH_3); 5.72 (s, 2H, CH_2); 6.92–7.13, 7.37, 7.53, 8.27 (m, 4H, m, 2H, d, 1H, d, 1H, Ar); 9.5 (NH).
14b	2.57 (s, 3H, CH_3); 3.90 (s, 3H, OCH_3); 5.70 (s, 2H, CH_2); 6.91–7.47, 8.28 (m, 7H, d, 1H, Ar); 9.5 (NH).
14d	2.57 (s, 3H, CH_3); 3.82 (s, 3H, OCH_3); 3.91 (s, 3H, OCH_3); 5.54 (s, 2H, CH_2); 6.94–7.36, 8.29 (m, 7H, d, 1H, Ar); 9.5 (NH).
15	2.55 (s, 3H, CH_3); 5.51 (s, 2H, CH_2); 6.71–7.36, 7.83 (m, 7H, d, 2H, Ar); 9.9 (NH); 10.3 (OH).
16	2.54 (s, 3H, CH_3); 5.51 (s, 2H, CH_2); 6.72–7.20, 7.37, 7.88 (m, 4H, d, 2H, d, 2H, Ar); 9.9 (NH);10.5 (OH).
17	2.58 (s, 3H, CH_3); 5.70 (s, 2H, CH_2); 6.76–6.96, 7.18, 7.49, 8.20 (m, 3H, m, 4H, d, 1H, Ar); 9.5 (NH); 10.2 (OH).
18	2.56 (s, 3H, CH_3); 5.71 (s, 2H, CH_2); 6.76–7.58 (m, 7H, Ar); 8.21 (d, 1H, Ar); 9.5 (NH); 10.2 (OH).
19	2.57 (s, 3H, CH_3); 5.50 (s, 2H, CH_2); 6.73–7.21, 8.20 (m, 7H, d, 1H, Ar); 9.5 (NH); 10.0b (OH); 11.3b (OH).
23a	2.49 (s, 3H, CH_3); 4.43 (d, 2H, CH_2); 5.68 (s, 2H, CH_2); 6.94, 7.18–7.43, 7.50 (d, 1H, m, 7H, d, 1H, Ar); 9.0 (t, NH).
23b	2.50 (s, 3H, CH_3); 4.42 (d, 2H, CH_2); 5.65 (s, 2H, CH_2); 7.12–7.47 (m, 9H, Ar); 9.0 (t, NH).
23d	2.49 (s, 3H, CH_3); 3.81 (s, 3H, OCH_3); 4.43 (d, 2H, CH_2); 5.49 (s, 2H, CH_2); 6.88–7.37 (m, 9H, Ar); 9.0 (t, NH).
24a	2.49 (s, 3H, CH_3); 4.51 (d, 2H, CH_2); 5.69 (s, 2H, CH_2); 6.99, 7.28–7.53 (d, 1H, m, 7H, Ar); 9.0 (t, NH).
24b	2.51 (s, 3H, CH_3); 4.51 (d, 2H, CH_2); 5.67 (s, 2H, CH_2); 7.16–7.47 (m, 8H, Ar); 9.0 (t, NH).
24d	2.49 (s, 3H, CH_3); 3.81 (s, 3H, OCH_3); 4.51 (d, 2H, CH_2); 5.50 (s, 2H, CH_2); 6.93, 7.05, 7.24–7.46 (d, 2H, d, 1H, m, 5H, Ar); 9.0 (t, NH).
25a	2.50 (s, 3H, CH_3); 3.83 (s, 3H, OCH_3);4.44 (d, 2H, CH_2); 5.69 (s, 2H, CH_2); 6.86–7.57 (m, 8H, Ar); 8.7 (t, NH).
25b	2.50 (s, 3H, CH_3); 3.81 (s, 3H, OCH_3); 4.41 (d, 2H, CH_2); 5.65 (s, 2H, CH_2); 6.83–7.47 (m, 8H, Ar); 8.7 (t, NH).
25d	2.49 (s, 3H, CH_3); 3.82 (s, 6H, OCH_3); 4.42 (d, 2H, CH_2); 5.50 (s, 2H, CH_2); 6.86–7.37 (m, 8H, Ar); 8.7 (t, NH).
26	2.49 (s, 3H, CH_3); 4.42 (d, 2H, CH_2); 5.46 (s, 2H, CH_2); 6.71–7.32 (m, 9H, Ar); 8.9 (t, NH); 10.0b (OH).
27	2.49 (s, 3H, CH_3); 4.50 (d, 2H, CH_2); 5.47 (s, 2H, CH_2); 6.70–7.46 (m, 8H, Ar); 8.9 (t, NH); 9.8b (OH).
28	2.49 (s, 3H, CH_3); 4.39 (d, 2H, CH_2); 5.68 (s, 2H, CH_2); 6.70–7.54 (m, 8H, Ar); 8.7 (t, NH); 9.6 (OH).
29	2.50 (s, 3H, CH_3); 4.39 (d, 2H, CH_2); 5.66 (s, 2H, CH_2); 6.69–7.47 (m, 8H, Ar); 8.7 (t, NH); 9.6 (OH).
30	2.48 (s, 3H, CH_3); 4.38 (d, 2H, CH_2); 5.46 (s, 2H, CH_2); 6.68–7.16 (m, 8H, Ar); 8.7 (t, NH); 9.9 (b, 2H, OH).

solid compounds were collected by filtration, washed with H_2O and purified by crystallization (Table 1).

5.1.6. N-(4-Chlorophenyl)-1-(2-substituted-benzyl)-5-methyl-1H-1,2,3-triazol-4-carboxamides (**13a**, **b**, **d**)

To a solution of 6.0 mmol of the appropriate azide (**1a**, **b** or **d**) and 4-chloro-acetoacetanilide (1.27 g, 6.0 mmol) in 12 mL of anhydrous DMSO, 6–7 g of finely powdered anhydrous K_2CO_3 was added and the suspension was stirred at 100 °C for 48 h. The reaction mixture was diluted with H_2O and worked up as described above (Table 1).

5.1.7. N-(2-Methoxyphenyl)-1-(2-substituted-benzyl)-5-methyl-1H-1,2,3-triazol-4-carboxamides (**14a**, **b**, **d**)

To a solution of 6.0 mmol of the appropriate azide (**1a**, **b** or **d**) and 4-methoxy-acetoacetanilide (0.910 g, 6.0 mmol) in 12 mL of anhydrous DMSO, 6–7 g of finely powdered anhydrous K_2CO_3 was added and the suspension was stirred at 100 °C for 48 h. The reaction mixture was diluted with H_2O and worked up as described above (Table 1).

5.1.8. 1-(2-Substituted-benzyl)-5-methyl-1H-1,2,3-triazol-4-carboxanilides (**15–19**)

To a solution of 2.0 mmol of the suitable methoxy derivative (**12d**, **13d**, **14a**, **b** or **d**) in 100–120 mL of anhydrous CH_2Cl_2, cooled at −30 °C, a solution of BBr_3 (≈2 mL, ≈20 mmol; ≈3 mL, ≈30 mmol for **14d**) in 10–12 mL of anhydrous CH_2Cl_2 was added drop by drop, under stirring. After 1 h at this temperature, the reaction mixture was left at −20 °C overnight. The excess of the reagent was decomposed by the cautious addition of MeOH (10 mL) and H_2O (40 mL). The organic phase, after washing with H_2O, was extracted with

Table 3
Parameters of vasorelaxing efficacy (E_{max} %) and potency (pIC_{50}) exhibited by the synthesized compounds and by the reference drug NS1619

Compound	E_{max} (%)	pIC_{50}
7a	39 ± 13[a]	NC
7b	25 ± 2[a]	NC
7c	Ineffective	–
7d	37 ± 6[a]	NC
8a	Ineffective	–
8b	Ineffective	–
8c	Ineffective	–
8d	43 ± 8[a]	NC
15	89 ± 2	4.75 ± 0.03[a]
15 + TEAC	37 ± 4[b]	NC
16	53 ± 8[a]	NC
17	52 ± 2[a]	NC
18	35 ± 2[a]	NC
19	81 ± 9	4.69 ± 0.04[a]
26	48 ± 4[a]	NC
27	51 ± 3[a]	NC
28	49 ± 2[a]	NC
29	24 ± 3[a]	NC
30	76 ± 5	4.66 ± 0.03[a]
NS1619	94 ± 4	5.29 ± 0.03

NC indicates that the potency value could not be calculated because of the low efficacy (≤55%).
[a] Significantly different from the corresponding value exhibited by NS1619.
[b] Significantly different from the corresponding value recorded in the absence of tetraethylammonium chloride (TEAC, 10 mM).

10%NaOH. Compounds **15**, **16** and **19** precipitated by acidification (36% HCl) of the alkaline extract, were then collected by filtration and purified by crystallization (Table 1). For the isolation of **17** and **18**, the treatment with 10% NaOH caused precipitation of the respective sodium salt which was collected by filtration, suspended in H_2O and acidified. A further small amount of the compound precipitated from the alkaline filtrate by acidification (Table 1).

*5.1.9. N-(Benzyl)-1-(2-substituted-benzyl)-5-methyl-1H-1,2,3-triazol-4-carboxamides (**23a**, **b**, **d**); N-(2-chlorobenzyl)-1-(2-substituted-benzyl)-5-methyl-1H-1,2,3-triazol-4-carboxamides (**24a**, **b**, **d**) and N-(2-methoxybenzyl)-1-(2-substituted-benzyl)-5-methyl-1H-1,2,3-triazol-4-carboxamides (**25a**, **b**, **d**)*

A solution of the suitable acyl chloride (**4a**, **b** or **d**), prepared from 3.0 mmol of the corresponding carboxytriazole (**3a**, **b** or **d**) in 20 mL of anhydrous toluene (see compounds **7a–d**), was added dropwise to a solution of 3.0 mmol of the appropriate benzylamine [benzylamine (**20**), 2-chlorobenzylamine (**21**) or 2-methoxybenzylamine (**22**)] and TEA (1 mL, 7 mmol) in 20 mL of the same solvent and the mixture was refluxed for 18–20 h. The reaction mixture was evaporated in vacuo and the residue was treated with H_2O to dissolve the TEA hydrochloride and extracted with $CHCl_3$. The organic solution, after washing with 10% HCl and 10% NaOH, was dried ($MgSO_4$) and evaporated to give the title compounds which were purified by crystallization (Table 1).

*5.1.10. N-(Benzyl)-1-(2-hydroxybenzyl)-5-methyl-1H-1,2,3-triazol-4-carboxamide (**26**) and N-(2-chloro-benzyl)-1-(2-hydroxy-benzyl)-5-methyl-1H-1,2,3-triazol-4-carboxamide (**27**); N-(2-hydroxy-benzyl)-1-(2-substituted-benzyl)-5-methyl-1H-1,2,3-triazol-4-carboxamides (**28–30**)*

To a solution of 1.6 mmol of the suitable methoxy derivative (**23d**, **24d**, **25a**, **b** or **d**) in 100–120 mL of anhydrous CH_2Cl_2, cooled at −30 °C, a solution of BBr_3 (≈1.6 mL, ≈16 mmol; ≈2 mL, ≈20 mmol for **25d**) in 6–8 mL of anhydrous CH_2Cl_2 was added drop by drop, under stirring. After 1 h at this temperature, the reaction mixture was left at −20 °C overnight, then worked up as described for the preparation of **15–19**. Compounds **26–28** and **29** were isolated by acidification of the alkaline extract, collected by filtration and purified by crystallization (Table 1). For the isolation of the dihydroxyderivative **30**, the alkaline extract appeared as a suspension caused by the partial precipitation of the sodium salt, but the compound was similarly isolated by direct acidification (Table 1).

5.2. Pharmacology

All the experimental procedures were carried out following the guidelines of the European Community Council Directive 86-609. A possible vasodilator mechanism of action was investigated by testing the effects of the compounds on isolated thoracic aortic rings of male normotensive Wistar rats (250–350 g). After light ether anaesthesia, the rats were sacrificed by cervical dislocation and bleeding. The aortae were immediately excised and freed of extraneous tissues. The endothelial layer was removed by gently rubbing the intima surface of the vessels with a hypodermic needle. Five millimeter wide aortic rings were suspended, under a preload of 2 g, in 20 mL organ baths, containing Tyrode solution (composition of saline in mM: NaCl 136.8; KCl 2.95; $CaCl_2$ 1.80; $MgSO_4$ 1.05; NaH_2PO_4 0.41; $NaHCO_3$ 11.9; glucose 5.5), thermostated at 37 °C and continuously gassed with a mixture of O_2 (95%) and CO_2 (5%). Changes in tension were recorded by means of an acquisition data software (BioPac MP 100).

After an equilibration period of 60 min, endothelial removal was confirmed by the administration of acetylcholine (Ach, 10 μM) to KCl (20 mM)-precontracted vascular rings. A relaxation <10% of the KCl-induced contraction was considered representative of an acceptable lack of the endothelial layer, while the organs showing a relaxation ≥10% (i.e. significant presence of endothelium) were discarded. From 30 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 (10 nM–30 μM) of the tested compounds or of the reference drug NS1619 (a well-known BK-activator) were added cumulatively. For the selected compound **15**, the same protocol was also carried out in the presence of the potassium channel

blocker tetraethylammonium chloride (TEAC; 10 mM), which was incubated for 20 min before the addition of KCl 20 mM. Each compound was tested in 5–10 experiments. Preliminary experiments showed that the KCl (20 mM)-induced contractions remained in a stable tonic state for at least 40 min. The reference drug NS1619 (Sigma) was dissolved (10 mM) in EtOH 95% and further diluted in Tyrode solution. Acetylcholine chloride (Sigma) was dissolved (100 mM) in EtOH 95% and further diluted in bidistilled water, whereas KCl was dissolved in Tyrode solution. TEAC was diluted in bidistilled water. All the synthesized derivatives were dissolved (10 mM) in DMSO, and then diluted in Tyrode solution. All the solutions were freshly prepared immediately before the pharmacological experimental procedures. Previous experiments showed complete ineffectiveness of the vehicles. The vasorelaxing efficacy was evaluated as maximal vasorelaxing response, expressed as a percentage (%) of the contractile tone induced by KCl 20 mM. When the limit concentration 30 μM (the highest concentration, which could be administered) of the tested compounds did not reach the maximal effect, the parameter of efficacy represented the vasorelaxing response, expressed as a percentage (%) of the contractile tone induced by KCl 20 mM, evoked by this limit concentration. The parameter of potency was expressed as $pIC_{50,}$ calculated as a negative Logarithm of the molar concentration of the compounds tested, evoking a half reduction of the contractile tone induced by KCl 20 mM. The pIC_{50} value could not be calculated for those compounds that showed an efficacy value <55%. Compounds exhibiting an efficacy level <20% were considered as ineffective. The parameters of efficacy and potency were expressed as mean ± standard error, for 5–10 experiments. Student's *t*-test was selected as a statistical analysis, $P < 0.05$ was considered representative of a significant statistical difference. Experimental data were analysed by a computer fitting procedure (software: GraphPad Prism 3.0).

Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejmech.2008.02.032.

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