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Original article

Benzoyl and/or benzyl substituted 1,2,3-triazoles as potassium channel activators. VIII

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Abstract

This paper reports the preparation of new benzoyl and/or benzyl substituted 1,2,3-triazole derivatives and their pharmacological evaluation as potential BK channel openers, as a part of a research program which hypothesized a pharmacophoric structure containing the 1,2,3-triazole ring. The synthetic procedures consist essentially with the 1,3-dipolar cycloaddition of aryl or benzyl azides to the asymmetric alkyne benzoylacetylene to give the wished 4-benzoyl-1,2,3-triazole isomers in larger amount. The pharmacological results show that the 1-(2-hydroxybenzyl)-4-benzyl-1H-1,2,3-triazole possesses high vasorelaxing activity involving the opening of the BK channels. Therefore the structure–activity relationships concerning this pharmacophoric structure confirm the usefulness of a phenolic function in the ortho position of the aromatic ring and would suggest a 1,2,3-triazole model bearing benzyl substituents. In addition such substituents appear more flexible and able to take different conformations with respect to phenyl groups which have higher trend to coplanar conformations.

Keywords: Potassium channels; Potassium channel openers; BK-activators; 1,2,3-Triazoles; Vasodilator activity

1. Introduction

The large conductance calcium activated potassium channels (also known as BK or MAXI-K channels) are a subtype of the large family of potassium channels and represent a potential therapeutic target for the synthesis of new drugs.

The activation of these channels allows a concentration-depending flow of potassium ions to the extracellular phase and consequent membrane hyperpolarization and reduction of the cellular excitability [1]. Therefore, compounds able to open selectively the BK channels, afford a new therapeutic approach for several pathological conditions involving cell hyperexcitability, such as asthma, urge incontinence and bladder spasm, gastric hypermotility, hypertension, coronary artery spasm, psychoses [1,2].

Following our research plain, addressed to synthesize 1,2,3-triazole derivatives as potential BK channel activators, belonging to the general formula **A** (Fig. 1), a previous paper [3]

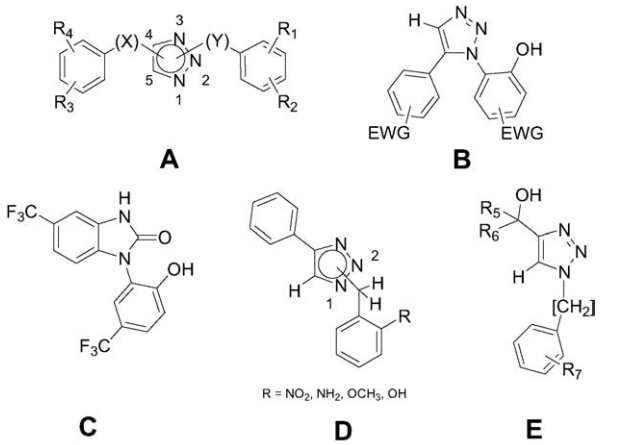

Fig. 1. General formula **A**: R_1, R_2, R_3, R_4 = hydrogen bond donors and/or acceptors and/or electron withdrawing groups. (X) and/or (Y), when present = CH_2, CHOH, CO groups. **B**: 1,5-diaryl-1,2,3-triazole derivatives. **C**: reference compound NS1619. **D**: 1,4- and/or 2,4-disubstituted-1,2,3-triazole derivatives. **E**: 1,2,3-triazole derivatives with a secondary or tertiary alcoholic function.

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reported 1,5-diarylsubstituted 1,2,3-triazole derivatives (**B**, Fig. 1), whose heterocyclic ring represents the spacer between the two phenyl rings; these compounds possessed a moderate vasorelaxing activity in addition, the structure–activity relationships indicated that a phenolic function in the ortho position of the aromatic ring was useful for the pharmacological activity, whilst a primary amino function strongly decreased it. Among these compounds, the 1-(2′-hydroxy-5′-nitrophenyl)-5-phenyl-1H-1,2,3-triazole derivative showed a good activity, exhibiting a lower efficacy parameter but a potency index comparable to that of NS1619 (**C**, Fig. 1), a BK-opener [4] chosen as reference compound.

A further study about 1,4- and/or 2,4-disubstituted-1,2,3-triazole derivatives, with/without a methylenic spacer between the heterocyclic ring and one of the phenyl substituents [5] (**D**, Fig. 1), suggested that a partially increased molecular flexibility, induced by the benzyl substituent, appeared as a deleterious requirement uneffective for the pharmacological activity. In any case the structure–activity relationships confirmed the importance of a phenolic function in the ortho position of the phenyl substituent and the negative influence of an amino function in the same position, suggesting the presence of a hydrogen bond donor with appropriate acid properties [6]. Also the introduction of a secondary or tertiary alcoholic function, as a neutral hydrogen bond donor placed adjoining to the four position of the 1,2,3-triazole ring [5] (**E**, Fig. 1), caused a loss of activity.

In order to give a deeper explanation of the hypotheses of structure–activity relationships, coming from the previous studies, in this third paper concerning 1,2,3-triazole derivatives, related to the general pharmacoforic structure **A**, we synthesized and tested new compounds bearing further spacers between the heterocyclic ring and one or both the phenyl substituents. These spacers consist of carbonyl and a methylenic groups, which confer to the structure a different molecular geometry. In particular, the planar carbonyl function, can induce coplanar conformations of the two bound rings, because of electronic resonance effects.

2. Chemistry

The 2-methoxy-phenylazide (**1a**) [7] (Fig. 2) reacted with benzoylacetylene (**2**) [8] in ethanol under reflux, to give the expected mixture of the triazole isomers 4-benzoyl-substituted **3a** and 5-benzoyl-substituted **4a** in 74% yield. The gas-chromatographic analysis showed a quantitative ratio **3a/4a** = 4:1 and a crystallization from ethyl acetate allowed the isolation (58% yield) and characterization of the isomer **3a**. Similarly the 2-methoxy-5-chloro-phenylazide (**1b**) [9] (Fig. 2) reacted with benzoylacetylene (**2**) [8], under the same experimental conditions, to give the expected mixture of isomers **3b** and **4b** in 86% yield and in a quantitative ratio 6:1 (by gas-chromatographic analysis). In this case also crystallization from ethyl acetate provided the isomer **3b** in 61% yield.

The structure of the 4-benzoyl-substituted isomers **3a** and **3b** was assigned upon the basis of previously acquired considerations for analogous reactions [10,11] and confirmed by analytical, physico-chemical and spectroscopic methods.

In fact, as known [12], the addition of azides to asymmetrical alkynes is regioselective, undergoing mesomeric and steric effects which, in this case, promoted formation of the 4-benzoyl-substituted isomer [10,11]. The gas-chromatographic analysis of the reaction mixtures confirmed the theoric hypothesis, indicating for **3a/4a** a quantitative ratio 4:1, with t_R = 13.52 and t_R = 7.88 min, respectively, and for **3b/4b** a ratio 6:1 with t_R = 11.12 and t_R = 14.77 min, respectively.

In addition, the 1,2,3-triazole, Refs. [12,13] shows that in the ^{1}H-NMR spectra of the isomer couples bearing substituents in the four and five positions, the triazole hydrogen in the five position resonates at fields lower than that of the triazole hydrogen in the four position. Examination of the ^{1}H-NMR spectra of the reaction mixtures containing the isomer couples **3a**, **4a** and **3b**, **4b**, shows clearly the presence of singlet signals attributable to the triazole hydrogens, in a quantitative ratio 4:1 and 6:1, respectively, at chemical shift values 9.20 and 8.36 ppm for the couple **3a**, **4a** and 9.23 and 8.40 ppm for the couple **3b**, **4b**. Therefore, the isomers present

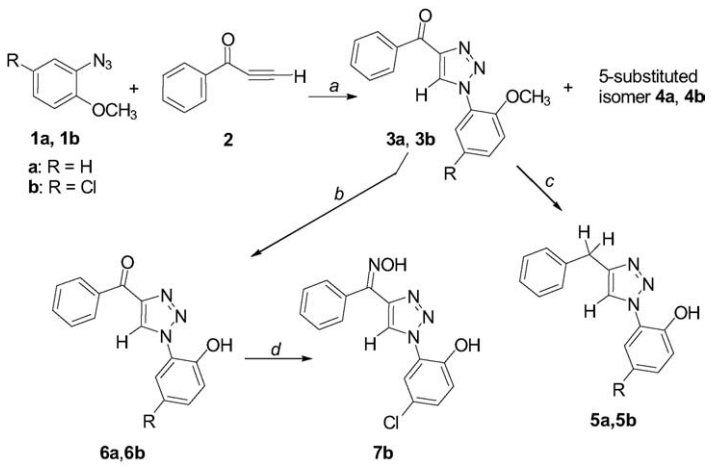

Fig. 2. a: Δ, EtOH; b: BBr_3; c: Wolff-Kishner reduction; d: $HONH_2$·HCl, pyridine, EtOH.

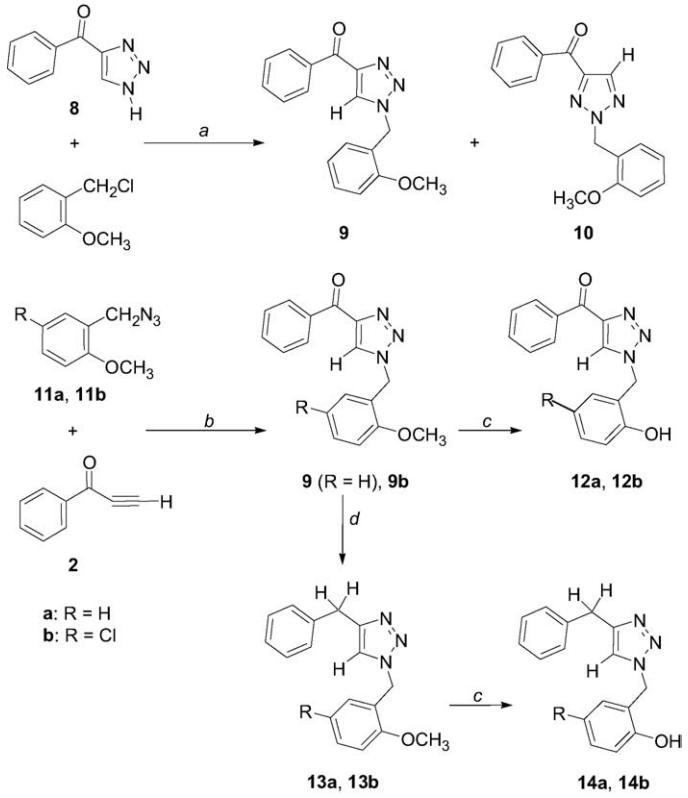

Fig. 3. a: Δ, K_2CO_3, Me_2CO; b: Δ, EtOH; c: BBr_3; d: Wolff-Kishner reduction.

in larger amount with chemical shifts 9.20 and 9.23 ppm correspond to the triazole compounds 4-benzoyl-substituted **3a** and **3b**.

The demethylation reactions of the methoxy group of **3a** and **3b**, carried out with boron tribromide in dichloromethane at −78 °C, gave the 1-(2-hydroxyphenyl)-triazole derivatives **6a** and **6b** in 69% and 79% yield, respectively. The chemical reduction of the ketone function of the benzoyl substituent of **3a** and **3b**, by the previously employed [10] Wolff-Kishner reaction, caused at the same time the cleavage of the methoxy substituent, to give the 4-benzyl-1-(2-hydroxyphenyl)-triazole derivatives **5a** and **5b** in 77% and 71% yield, respectively. Finally the benzoyltriazole **6b** was converted to the corresponding oxime **7b** by the usual treatment with hydroxylamine hydrochloride in ethanol in the presence of pyridine. Under such a condition, compound **7b** appeared to consist with the expected mixture of the geometrical isomers sin/anti, as shown by the melting range (195–230 °C) and ^{1}H-NMR spectrum with undoubled signals at 12.18 and 11.51 ppm for the oxime proton, 9.18 and 8.61 ppm for the H-5 triazole hydrogen and 11.02 and 10.96 ppm for the hydroxyl of the phenolic function.

The nucleophilic substitution reaction of 2-methoxybenzyl chloride with 4-benzoyl-1,2,3-triazole (**8**) [14] (Fig. 3), carried out in refluxing acetone in the presence of anhydrous potassium carbonate for 24 h, gave the mixture of the expected triazole isomers 1-*N*-benzyl-substituted **9** (t_R = 16.57 min) and 2-*N*-benzyl-substituted **10** (t_R = 9.95 min) in 71% total yield, with a quantitative ratio ≅ 1:1 (by gas-chromatographic analysis). The mixture was fractionated by flash-chromatography through silica gel and the triazole isomer compounds were isolated in 31% and 24% yield, respectively. The structure assignment to the two isomers was made easy by the obtainment of the same isomer **9** by the 1,3-dipolar cycloaddition reaction of 2-methoxy-benzylazide (**11a**) [15] to benzoylacetylene (**2**) [8] (Fig. 3). This reaction showed a quasi-regiospecific course, providing the 4-benzoyltriazole isomer **9** (t_R = 16.49 min) in 9:1 ratio regarding to the 5-benzoyl-substituted isomer. In this case the structure was unequivocally assigned upon the basis of the previous considerations (literature, R_f, t_R, ^{1}H-NMR) reported for the characterization of **3a** and **3b**, obtained by the same reaction. Compound **9** was easily isolated in high yield (81%) by crystallization of the reaction product from methanol.

Similarly the new 2-methoxy-5-chloro-benzylazide (**11b**), by reaction with benzoylacetylene (**2**) [8] (Fig. 3), gave the expected mixture of the 4- and 5-substituted triazole isomers in a quantitative ratio 12:1, from which the 4-benzoyl-substituted isomer **9b** (t_R = 15.29 min) was isolated in 75% yield by crystallization from ethyl acetate to petroleum ether.

The 1-(substituted-benzyl)-4-benzoyl-1,2,3-triazoles **9** and **9b**, treated with boron tribromide under the previously employed experimental conditions, provided the corresponding phenol derivatives **12a** and **12b** in 62% and 33% yield, respectively. The same 1-(substituted-benzyl)-4-benzoyl-1,2,3-triazoles **9** and **9b**, underwent a Wolff-Kishner reduction under the experimental conditions employed for the

Table 1
Experimental results: efficacy (E_{max} %) and potency (pIC50) values of the tested compounds

	E_{max} %	PIC50
5a	Ineffective	NC
5b	70 ± 13	6.75 ± 0.08
6a	Ineffective	NC
6b	54 ± 12	6.19 ± 0.09
7b	Ineffective	NC
12a	Ineffective	NC
12b	Ineffective	NC
14a	95 ± 4	7.56 ± 0.06
14b	98 ± 2	5.11 ± 0.19
NS 1619	97 ± 2	5.20 ± 0.08

NC indicates that the parameter could not be calculated because of the low efficacy (<50%).

analogous triazole derivatives **3a** and **3b**, to give the expected 1-(substituted-benzyl)-4-benzyl 1,2,3-triazoles **13a** and **13b** in good yield (59% and 55%, respectively). In this case, differently from what experimented with the reduction of **3a** and **3b**, only the ketone function was reduced whilst the cleavage of the ether function of the methoxyl did not occur. Therefore the methoxy substituent of **13a** and **13b** had to be demethylated by the usual reaction with boron tribromide to give the corresponding 2-hydroxybenzyltriazole derivatives **14a** and **14b** in 48% and 37% yield, respectively.

3. Pharmacology

The vasodilating effect of the novel potential BK-openers on vascular contractile function was studied in vitro using isolated rat aortic rings precontracted with KCl 20 mM (see later for the pharmacological details).

4. Results and discussion

Since a vasorelaxing activity on endothelium-denuded rat aortic rings is a pharmacodynamic property of well-characterized BK-activators [16], this experimental model has been chosen for a preliminary screening of the target compounds **5a**, **5b**, **6a**, **6b**, **7b**, **12a**, **12b**, **14a** and **14b**.

Compounds **5a**, **6a**, **7b**, **12a** and **12b** showed weak or none vasorelaxing efficacy, while **6b** exhibited a moderate, but significant (>50%) vasodilator efficacy, with a potency index in the micromolar range (Table 1).

The non-selective K^+ channel blocker tetraethylammonium chloride (TEA) antagonized the effects of **6b**, indicating a possible role of these ion channels in the mechanism of action. The efficacy parameters reached an appreciable level for compound **5b**, which showed an interesting potency (about 40-fold higher then that of NS1619), and were almost full for compounds **14a** (which exhibited a potency about 200-fold higher than that of NS1619) and **14b** (with a potency parameter comparable with that of the reference benzimidazolone; Table 1).

Both **5b** and **14a** exhibited TEA-sensitive effects, suggesting that the vasorelaxing activity involves the activation of K^+ channels. Moreover, compound **14a** was also tested in the presence of IbTX (Iberiotoxin), selective blocker of BK channels. The toxin antagonized the effects of **14a**, indicating that BK channels probably are activated by this compound.

The analysis of the possible structure–activity relationships seems to indicate that the presence of a carbonyl spacer between the aromatic ring and position 4 of the triazole heterocycle is likely to be a deleterious requirement, and that a methylene spacer is preferable, as clearly suggested by a direct comparison of the couples of analogues **5b** vs. **6b** and **14a**, **14b** vs. **12a**, **12b**. The replacement of the carbonyl oxygen atom of **6b** with a hydroxyimino group (**7b**) seems to exert a dramatic impact.

The direct comparison of the most effective 1-*N*-benzyl substituted compound **14a** vs. its 1-*N*-phenyl substituted analogue **5a** seems to indicate that a more flexible conformation due to the benzyl group is more favorable than a phenyl substituent, which probably leads to a coplanar conformation.

Compounds showing a direct link between the 2-hydroxyphenyl group and the 1 nitrogen of the triazole moiety are potentiated by the presence of a chlorine atom in position 4 of such an aromatic ring (**5b** vs. **5a** and **6b** vs. **6a**). On the contrary, this structural requirement seems to have a detrimental impact on the 1-*N*-benzyl substituted triazoles (**14b** vs. **14a**). To date, the state of the art about our studies on diaryl-triazole derivatives does not allow us to recognize whether the influences of the chlorine atom are due to electronic factors or to aspects concerning a steric hindrance.

The future synthetic efforts will be addressed to the development of derivatives closely analogues to **14a**, in order to clarify the electronic and/or steric influences of different substituents on the two benzyl groups.

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 Varian Gemini 200 spectrometer in DMSO-d_6 or $CDCl_3$, in δ units, using TMS as internal standard. Mass spectra were performed with a Hewlett Packard GC/MS System 5988 A gas-chromatographic analyses were performed on a Shimadzu Mod. GC-17 A gas chromatography with a flame ionization detector, using a stationary phase SPB-5 [poly (5% diphenyl/95% dimethylsiloxane)] column (15 m × 0.53 mm × 0.5 μm film thickness). TLC data were obtained with Merck silica gel 60 F_{254} aluminum sheets, using the elution mixtures reported for the flash-chromatographies. Flash-column chromatographies were performed using Merck Kieselgel 60 (230–400 mesh). Elemental analyses (C, H, N) were within ± 0.4% of the theoretical values and were per-

formed on a Carlo Erba Elemental Analyzer Mod. 1106 apparatus. Petroleum ether corresponds to the fraction boiling at 40–60 °C.

*5.1.1. 1-(2-Methoxyphenyl)-4-benzoyl-1H-1,2,3-triazole (**3a**)*

A mixture of 2-methoxy-phenylazide (**1a**) (0.50 g, 3.35 mmol) and benzoylacetylene (**2**) (0.48 g, 3.69 mmol) in EtOH (10 ml) was stirred and heated under reflux for 22 h. After cooling, the dark solution was evaporated in vacuo and the resulting yellow solid (0.69 g, yield 74%; m.p. 80–84 °C) consisted of a mixture of the title compound **3a** and the 5-substituted isomer **4a** in a 4:1 ratio, respectively (by gas-chromatographic analysis). This mixture was separated by crystallization from EtOAc to achieve the isomer **3a** as pale yellow needles: 0.54 g, yield 58%, m.p. 99–101 °C, IR 1648 cm^{-1} (CO). Mass (*m/z*): 279 (M^+); 105 (100%).

^{1}H-NMR (DMSO-d_6): δ 3.88 (s, 3H, OCH_3), 7.19–8.30 (m, 9H, aromatics), 9.20 (s, 1H, H_5 triazole).

^{1}H-NMR ($CDCl_3$): δ 3.95 (s, 3H, OCH_3), 7.12–8.51 (m, 9H, aromatics), 8.87 (s, 1H, H_5 triazole).

Anal. for $C_{16}H_{13}N_3O_2$. Calc. %: C, 68.81; H, 4.69; N, 15.05. Found %: C, 69.12; H, 4.87; N, 15.23.

*5.1.2. 1-(2-Methoxy-5-chlorophenyl)-4-benzoyl-1H-1,2,3-triazole (**3b**)*

A mixture of 2-methoxy-5-chlorophenylazide (**1b**) (1.24 g, 6.78 mmol) and benzoylacetylene (**2**) (0.97 g, 7.46 mmol) in EtOH (25 ml) was stirred and heated under reflux for 24 h After cooling, the dark solution was evaporated in vacuo and the resulting yellow solid (1.83 g, yield 86%) consisted of the title compound **3b** and the 5-substituted isomer **4b** in a 6:1 ratio, respectively (by gas-chromatographic analysis). This mixture was separated by crystallization from EtOAc to give the isomer **3b** as pale brown plates: 1.30 g, yield 61%, m.p. 133–135 °C. Mass (*m/z*): 279 (M^+); 105 (100%).

^{1}H-NMR (DMSO-d_6): δ 3.88 (s, 3H, OCH_3), 7.40–8.28 (m, 8H, aromatics), 9.23 (s, 1H, H_5 triazole).

Anal. for $C_{16}H_{12}ClN_3O_2$. Calc. %: C, 61.25; H, 3.86; N, 13.39. Found %: C, 61.28; H, 4.14; N, 13.31.

*5.1.3. 1-(2-Hydroxyphenyl)-4-benzyl-1H-1,2,3-triazole (**5a**)*

A mixture of benzoyltriazole **3a** (1.0 g, 3.58 mmol), 98% hydrazine hydrate (1.0 ml, 21.48 mmol) and diethylene glycol (15 ml) was heated at 100 °C for 1 h. After cooling, 1.4 g of KOH pellets were added and the suspension was warmed on a boiling water-bath until most of the pellets were dissolved. The flask was provided with an air-condenser and an internal thermometer then it was heated at 190 °C for 4 h. After cooling, the solution was poured, drop by drop, into H_2O (200 ml) then acidified (pH 6) with 37% HCl. The white solid precipitated was collected by filtration (0.83 g, yield 92%) and purified by crystallization from EtOAc: 0.69 g, yield 77%, m.p. 138–141 °C; IR 3401 cm^{-1} (OH), 1229 cm^{-1} (C-O). Mass (*m/z*): 250 (M^+); 118 (100%).

^{1}H-NMR (DMSO-d_6): δ 4.07 (s, 2H, CH_2), 6.92–7.59 (m, 9H, aromatics); 8.24 (s, 1H, H_5 triazole); 10.47 (s, 1H, OH, exchangeable).

Anal. for $C_{15}H_{13}N_3O$. Calc. %: C, 71.70; H, 5.21; N, 16.72. Found %: C, 71.66; H, 5.21; N, 16.77.

*5.1.4. 1-(2-Hydroxy-5-chlorophenyl)-4-benzyl-1H-1,2,3-triazole (**5b**)*

A mixture of benzoyltriazole **3b** (0.60 g, 1.92 mmol), 98% hydrazine hydrate (0.6 ml, 11.52 mmol) and diethylene glycol (15 ml) was heated at 100 °C for 1 h. After cooling, the yellow solution was added of KOH pellets (0.77 g) and the resulting suspension was warmed on a boiling water-bath until most of the pellets were dissolved. The flask was provided with an air-condenser and an internal thermometer then it was heated at 190 °C for 4 h. After cooling, the solution was poured, drop by drop, into H_2O (100 ml) then acidified (pH 6) with 37% HCl. The gray solid precipitated was collected by filtration (0.39 g, yield 71%) and purified by crystallization from MeOH: 0.32 g, yield 58%, m.p. 176–179 °C.

^{1}H-NMR (DMSO-d_6): δ 4.08 (s, 2H, CH_2), 7.08–7.69 (m, 8H, aromatics), 8.30 (s, 1H, H_5 triazole), 10.85 (s, 1H, OH, exchangeable).

Anal. for $C_{15}H_{12}ClN_3O$. Calc. %: C, 63.05; H, 4.23; N, 14.71. Found %: C, 63.3; H, 4.20; N, 14.81.

*5.1.5. 1-(2-Hydroxyphenyl)-4-benzoyl-1H-1,2,3-triazole (**6a**)*

To a stirred solution of **3a** (0.40 g, 1.43 mmol) in 20 ml of anhydrous CH_2Cl_2, cooled at –78 °C and under a nitrogen flow, a solution of BBr_3 (1.0 ml, 10.53 mmol) in 5 ml of anhydrous CH_2Cl_2 was added dropwise. Stirring was continued for 1 h, then the solution was left at room temperature overnight. Afterwards, the mixture was cooled again in an ice-salt bath and the reagent was decomposed by treatment with MeOH (7 ml) followed by H_2O (7 ml). The organic layer was washed with H_2O, dried ($MgSO_4$) and evaporated in vacuo, to give the title compound as a pale brown solid (0.32 g, yield 84%), which purified by crystallization from EtOAc appeared as shiny thin plates: 0.26 g, 69%, m.p. 187–189 °C. Mass (*m/z*): 265 (M^+); 77 (100%).

^{1}H-NMR (DMSO-d_6): δ 7.00–8.30 (m, 9H, aromatics); 9.17 (s, 1H, H_5 triazole); 10.74 (s, 1H, OH, exchangeable).

Anal. for $C_{15}H_{11}N_3O_2$. Calc. %: C, 67.92; H, 4.18; N, 15.84. Found %: C, 68.28; H, 4.20; N, 16.13.

*5.1.6. 1-(2-Hydroxy-5-chlorophenyl)-4-benzoyl-1H-1,2,3-triazole (**6b**)*

To a stirred solution of **3b** (0.90 g, 2.87 mmol) in 40 ml of anhydrous CH_2Cl_2, cooled at –78 °C and under a nitrogen flow, a solution of BBr_3 (2.0 ml, 21.06 mmol) in 10 ml of anhydrous CH_2Cl_2 was added dropwise. Stirring was continued for 2 h, then the solution was left at room temperature for 2 h. Afterwards, the mixture was cooled again in an ice-salt bath and the reagent was decomposed by treatment with MeOH (14 ml) followed by H_2O (14 ml). The organic layer

was washed with H_2O, then extracted with 10% NaOH. Acidification of the alkaline extract provided a yellow precipitate which was collected by filtration (0.79 g, yield: 92%). This solid was purified by crystallization from a mixture of MeOH/H_2O, to give **6b** as pale yellow needles: 0.68 g, yield 79%, m.p. 210–212 °C.

^{1}H-NMR (DMSO-d_6): δ 7.14–8.29 (m, 8H, aromatics); 9.20 (s, 1H, H_5 triazole); 11.08 (s, 1H, OH, exchangeable).

Anal. for $C_{15}H_{10}ClN_3O_2$. Calc. %: C, 60.11; H, 3.36; N, 14.02. Found %: C, 60.31; H, 3.49; N, 13.86.

*5.1.7. [1-(5-Chloro-2-hydroxy-phenyl)-1H-1,2,3triazol-4-yl]-phenyl-ketone oxime (**7b**)*

A mixture of **6b** (0.30 g, 1.05 mmol), $NH_2OH^{\cdot}HCl$ (0.50 g, 7.19 mmol) and pyridine (1.0 ml) in EtOH (15 ml) was heated under reflux for 1 h. Evaporation in vacuo of the solution gave a yellow oil which solidified after addition of few drops of H_2O. The white solid was purified by crystallization from EtOH/H_2O, to give the title compound as white plates: 0.215 g, yield 65%, m.p. 195–230 °C.

^{1}H-NMR (DMSO-d_6): δ 7.10–7.79 (m, 8H, aromatics), 8.61 and 9.18 (s, 1H, H_5 triazole), 10.96 and 11.02 (broads, 1H, OH exchangeable), 11.51 and 12.18 (s, 1H, OH oxime, exchangeable).

Anal. for $C_{15}H_{11}ClN_4O_2$. Calc. %: C, 57.24; H, 3.52; N, 17.80. Found %: C, 57.14; H, 3.64; N, 17.92.

*5.1.8. 1-(2-Methoxybenzyl)-4-benzoyl-1H-1,2,3-triazole (**9**) and 2-(2-methoxybenzyl)-4-benzoyl-2H-1,2,3-triazole (**10**)*

To a solution of 4-benzoyl-1,2,3-triazole (**8**) (1.0 g, 5.77 mmol) and 2-methoxy-benzylchloride (0.83 ml, 6.0 mmol) in anhydrous acetone (35 ml), anhydrous K_2CO_3 (2.0 g, 14.47 mmol) was added and the suspension was heated under reflux for 22 h. The reaction mixture was concentrated in vacuo, the oily residue was dissolved in $CHCl_3$ and the solution was washed with H_2O. The combined organic layers were dried ($MgSO_4$) and evaporated in vacuo to give a semisolid residue (1.21 g, yield 71%), consisting of a mixture of the title compounds. The gas-chromatographic analysis indicated a 1:1 ratio of the two triazole isomers. Their separation was achieved by flash-chromatography through a silica gel column, eluting with a 1:4 mixture of EtOAc/petroleum ether. At first compound **10** was obtained, followed from the isomer **9**.

9: 0.53 g, yield 31%, m.p. 83–85 °C, TLC: R_f: 0.28. ^{1}H-NMR (DMSO-d_6): δ 3.80 (s, 3H, OCH_3), 5.63 (s, 2H, CH_2), 6.93–8.24 (m, 9H, aromatics), 8.81 (s, 1H, H_5 triazole).

Anal. for $C_{17}H_{15}ClN_3O_2$. Calc. %: C, 69.61; H, 5.15; N, 14.33. Found %: C, 69.66; H, 5.38; N, 14.38.

10: 0.40 g, yield 24%, m.p. 76–78 °C, TLC: R_f: 0.50. ^{1}H-NMR (DMSO-d_6): δ 3.80 (s, 3H, OCH_3), 5.74 (s, 2H, CH_2), 6.95–8.16 (m, 9H, aromatics), 8.40 (s, 1H, H_4 triazole).

Anal. for $C_{17}H_{15}ClN_3O_2$. Calc. %: C, 69.61; H, 5.15; N, 14.33. Found %: C, 69.58; H, 5.43; N, 14.58.

*5.1.9. 1-(2-Methoxybenzyl)-4-benzoyl-1H-1,2,3-triazole (**9**)*

A mixture of 2-methoxy-benzylazide (**11a**) (1.0 g, 6.13 mmol) and benzoylacetylene (**2**) (0.48 g, 3.69 mmol) in EtOH (30 ml) was stirred and heated under reflux for 24 h. After cooling, the solution was evaporated in vacuo to give a residue consisting of a mixture of the triazole derivative **9** and its 5-substituted isomer in a 9:1 ratio (by G.C. analysis). The title compound was obtained by crystallization from MeOH, as a yellow solid: 1.46 g, yield 81%, m.p. 83–85 °C. Mass (*m*/*z*): 293 (M^+); 91 (100%).

*5.1.10. 2-Methoxy-5-chloro-benzylazide (**11b**)*

A mixture of 5-chloro-2-methoxy-benzylchloride (0.90 g, 4.71 mmol), EtOH (15 ml), H_2O (1.0 ml) and NaN_3 (0.40 g, 6.15 mmol) was heated under reflux for 24 h. After cooling, the inorganic residue was filtered off and the resulting solution was concentrated, diluted with H_2O and extracted with $CHCl_3$. The combined organic extracts were dried ($MgSO_4$) and evaporated to give an oily residue (0.926 g) which was dissolved in EtOAc and purified by filtration through a short column of silica gel. Evaporation of the solvent left the title azide as a yellow oil, which was directly utilized: 0.766 g, yield 83%, IR 2102 cm^{-1} (N_3).

^{1}H-NMR (DMSO-d_6): δ 3.81 (s, 3H, OCH_3), 4.37 (s, 2H, CH_2), 7.06–7.42 (m, 3H, aromatics).

*5.1.11. 1-(2-Methoxy-5-chlorobenzyl)-4-benzoyl-1H-1,2,3-triazole (**9b**)*

A mixture of 2-methoxy-5-chloro-benzylazide (**11b**) (0.65 g, 3.31 mmol) and benzoylacetylene (**2**) (0.43 g, 3.31 mmol) in EtOH (20 ml) was stirred and heated under reflux for 24 h. After cooling, the solution was evaporated in vacuo to give a residue consisting with a mixture of the triazole derivative **9b** and its 5-substituted isomer. The title compound was obtained by crystallization from EtOAc/petroleum ether, as a yellow solid: 0.81 g, yield 75%, m.p. 117–120 °C. ^{1}H-NMR (DMSO-d_6): δ 3.83 (s, 3H, OCH_3), 5.65 (s, 2H, CH_2), 7.08–8.24 (m, 8H, aromatics), 8.88 (s, 1H, H_5 triazole).

Anal. for $C_{17}H_{14}ClN_3O_2$. Calc. %: C, 62.30; H, 4.31; N, 12.82. Found %: C, 61.94; H, 4.50; N, 12.65.

*5.1.12. 1-(2-Hydroxybenzyl)-4-benzoyl-1H-1,2,3-triazole (**12a**)*

To a stirred solution of **9** (0.53 g, 1.81 mmol) in 35 ml of anhydrous CH_2Cl_2, cooled at –78 °C and under a nitrogen flow, a solution of BBr_3 (1.4 ml, 14.31 mmol) in 8 ml of anhydrous CH_2Cl_2 was added dropwise. Stirring was continued for 1 h, then the solution was left at –20 °C overnight. Afterwards, the mixture was cooled in an ice-salt bath and the reagent was decomposed by treatment with MeOH (10 ml) followed by H_2O (15 ml). The organic layer was washed with H_2O, then extracted with 10% NaOH. Acidification of the alkaline extract provided **12a** as a white precipitate which was extracted with $CHCl_3$. The combined organic layers were then dried ($MgSO_4$) and evaporated in vacuo to give a yellow

solid residue which was purified by crystallization from EtOAc/petroleum ether. The title compound appeared as pale yellow needles: 0.312 g, yield 62%, m.p. 148–150 °C. Mass (*m*/*z*): 279 (M^+); 107 (100%).

^{1}H-NMR (DMSO-d_6): δ 5.62 (s, 2H, CH_2), 6.81–8.24 (m, 9H, aromatics), 8.78 (s, 1H, H_5 triazole), 9.98 (s, 1H, OH, exchangeable).

Anal. for $C_{16}H_{13}N_3O_2$. Calc. %: C, 68.81; H, 4.69; N, 15.05. Found %: C, 68.68; H, 4.80; N, 14.98.

*5.1.13. 1-(2-Hydroxy-5-chlorobenzyl)-4-benzoyl-1H-1,2,3-triazole (**12b**)*

To a stirred solution of **9b** (0.49 g, 1.50 mmol) in 30 ml of anhydrous CH_2Cl_2, cooled at –78 °C and under a nitrogen flow, a solution of BBr_3 (1.35 ml, 14.31 mmol) in 8 ml of anhydrous CH_2Cl_2 was added dropwise. The reaction was worked up as for the preparation of **12a**. Acidification of the alkaline extract provided **12b** as a white precipitate which was collected by filtration (0.34 g) and crystallized from MeOH/H_2O: 0.154 g, yield 33%; m.p. 273–276 °C.

^{1}H-NMR (DMSO-d_6): δ 5.68 (s, 2H, CH_2), 7.15–8.19 (m, 8H, aromatics), 7.98 (s, 1H, H_5 triazole), 10.78 (s, 1H, OH, exchangeable).

Anal. for $C_{16}H_{12}ClN_3O_2$. Calc. %: C, 61.25; H, 3.86; N, 13.39. Found %: C, 61.44; H, 3.79; N, 13.56.

*5.1.14. 1-(2-Methoxybenzyl)-4-benzyl-1H-1,2,3-triazole (**13a**)*

A mixture of benzoyltriazole **9** (0.70 g, 2.39 mmol), 98% hydrazine hydrate (0.70 ml, 14.34 mmol) and diethylene glycol (25 ml) was heated at 100 °C for 1 h. After cooling, the solution was added of KOH pellets (1.30 g) and the resulting suspension was warmed on a boiling water-bath until most of the pellets were dissolved. The flask was provided with an air-condenser and an internal thermometer then it was heated at 190 °C for 4 h. After cooling, the solution was poured, drop by drop, into H_2O (130 ml) and the resulting cloudy solution was left at room temperature overnight. The precipitate was collected by filtration, washed with H_2O and crystallized from EtOAc/petroleum ether to give the title compound as gray and transparent needles: 0.395 g, yield 59%, m.p. 89–91 °C.

^{1}H-NMR (DMSO-d_6): δ 3.80 (s, 3H, OCH_3), 3.97 (s, 2H, CH_2 in the four position), 5.47 (s, 2H, CH_2 in the one position), 6.88–7.37 (m, 9H, aromatics), 7.77 (s, 1H, H_5 triazole).

Anal. for $C_{17}H_{17}N_3O$. Calc. %: C, 73.10; H, 6.13; N, 15.04. Found %: C, 73.28; H, 6.16; N, 14.82.

*5.1.15. 1-(2-Methoxy-5-chlorobenzyl)-4-benzyl-1H-1,2,3-triazole (**13b**)*

A mixture of benzoyltriazole **9b** (1.00 g, 3.05 mmol), 98% hydrazine hydrate (1.0 ml) and diethylene glycol (25 ml) was heated at 100 °C for 1 h. After cooling, the solution was added of KOH pellets (1.50 g) and the resulting mixture was warmed on a boiling water-bath until most of the pellets were dissolved. The flask, provided with an air-condenser and an internal thermometer, was then heated at 190 °C for 4 h. After cooling, the solution was poured, drop by drop, into H_2O (150 ml) and the title compound precipitated as a gray solid which was collected by filtration and purified by crystallization from EtOAc/petroleum ether: 0.53 g, yield 55%, m.p. 146–147 °C.

^{1}H-NMR (DMSO-d_6): δ 3.79 (s, 3H, OCH_3), 3.98 (s, 2H, CH_2 in the four position), 5.47 (s, 2H, CH_2 in the one position), 7.05–7.41 (m, 8H, aromatics), 7.83 (s, 1H, H_5 triazole).

Anal for $C_{17}H_{16}ClN_3O$. Calc. %: C, 65.07; H, 5.14; N, 13.39. Found %: C, 65.40; H, 5.01; N, 13.18.

*5.1.16. 1-(2-Hydroxybenzyl)-4-benzyl-1H-1,2,3-triazole (**14a**)*

To a stirred solution of **13a** (0.27 g, 0.966 mmol) in 30 ml of anhydrous CH_2Cl_2, cooled at –78 °C and under a nitrogen flow, a solution of BBr_3 (0.8 ml, 8.46 mmol) in 8 ml of anhydrous CH_2Cl_2 was added dropwise. Stirring was continued for 1 h, then the solution was left at –20 °C overnight. Afterwards, the mixture was cooled again in an ice-salt bath and the reagent was decomposed by treatment with MeOH (5 ml) followed by H_2O (7 ml). The organic layer was separated and the aqueous phase was washed several times with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and evaporated in vacuo to give an oily residue which, by refluxing with a few ml of hexan, provided a solid residue which was collected by filtration (0.17 g, yield 63%). This latter was purified by crystallization from MeOH/H_2O, to give the title compound as colorless plates: 0.13 g, yield 48%; m.p. 112–115 °C.

^{1}H-NMR (DMSO-d_6): δ 3.97 (s, 2H, CH_2 in the four position), 5.43 (s, 2H, CH_2 in the one position), 6.73–7.33 (m, 9H, aromatics), 7.73 (s, 1H, H_5 triazole), 9.87 (s, 1H, OH, exchangeable).

Anal. for $C_{16}H_{15}N_3O$. Calc. %: C, 72.43; H, 5.70; N, 15.84. Found %: C, 72.52; H, 5.69; N, 15.95.

*5.1.17. 1-(2-Hydroxy-5-chlorobenzyl)-4-benzyl-1H-1,2,3-triazole (**14b**)*

To a stirred solution of **13b** (0.50 g, 1.59 mmol) in 30 ml of anhydrous CH_2Cl_2, cooled at –78 °C and under a nitrogen flow, a solution of BBr_3 (1.35 ml, 14.31 mmol) in 8 ml of anhydrous CH_2Cl_2 was added dropwise. Stirring was continued for 1 h, then the solution was left at room temperature overnight. Afterwards, the mixture was cooled again in an ice-salt bath and the reagent was decomposed by treatment with MeOH (10 ml) followed by H_2O (15 ml). The organic layer was washed with H_2O, then extracted with 10% NaOH. Acidification of the alkaline extract provided **14b** as a white precipitate which was collected by filtration and purified by crystallization from MeOH/H_2O: 0.177 g, yield 37%; m.p. 265–266 °C.

^{1}H-NMR (DMSO-d_6): δ 3.87 (s, 2H, CH_2 in the four position), 5.45 (s, 2H, CH_2 in the one position), 7.15–7.68 (m, 8H, aromatics), 8.11 (s, 1H, H triazole), 10.72 (s, 1H, OH, exchangeable).

Anal. for $C_{16}H_{14}ClN_3O$. Calc. %: C, 64.11; H, 4.71; N, 14.02. Found %: C, 64.20; H, 4.69; N, 14.11.

5.1.18. 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 a light ether anesthesia, 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 intimal surface of the vessels with a hypodermic needle. Five millimeters 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 isometric transducer (Grass FTO3), connected with a unirecord microdynamometer (Buxco Electronics).

After an equilibration period of 60 min, the 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 the endothelium), were discarded. From 30 to 40 min after the confirmation of the endothelium removal, the aortic preparations were contracted by KCl (20 mM) and when the contraction reached a stable *plateau*, threefold increasing concentrations of the tested compounds or of the reference drug NS 1619 (a well-known BK-activator) were added cumulatively.

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

In other sets of experiments, the non-selective potassium channel blocker TEA (10 mM) or the BK-selective blocker Iberiotoxin (IbTX, 100 nM) were added, after the KCl (20 mM)-induced contraction, followed by the administration of selected compounds.

The reference drug NS 1619 (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, KCl was dissolved (2 M) in Tyrode solution, TEA (Sigma) was dissolved (10 mM) in Tyrode solution, IbTX (Sigma) was dissolved (100 nM) in bidistilled water. All the synthesized derivatives (**5a**, **5b**; **6a**, **6b**; **7b**; **12a**, **12b**; **14a**, **14b**) were dissolved (10 mM) in DMSO; they all were further diluted in Tyrode solution. All the solutions were freshly prepared immediately before the pharmacological experimental procedures. Previous experiments showed a 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 0.1 mM (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 negative Logarithm of the molar concentration of the tested compounds, evoking a half reduction of the contractile tone induced by KCl 20 mM. Those compounds showing an efficacy parameter lower than 50% were considered uneffective. 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 analyzed by a computer fitting procedure (software: Graph Pad Prism 3.0).

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