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Original article

1,4- and 2,4-substituted-1,2,3-triazoles as potential potassium channel activators. VII

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Abstract

New 1,4- and 2,4-substituted 1,2,3-triazole derivatives were synthesized and tested as potential BK_{Ca} channel openers, as a part of a research program, which hypothesizes a pharmacophoric structure containing the 1,2,3-triazole ring. The structure–activity relationships were studied introducing some structural changes concerning molecular geometry and the presence of a hydrogen bond donor as a primary amino group and a phenolic or alcoholic hydroxy function. The compounds were prepared by nucleophilic substitution on the 1,2,3-triazole ring and by 1,3-dipolar cycloaddition of azides to selected alkynes and to phenylacetone. The new compounds tested on rat aortic rings did not exhibit any significant vasorelaxing activity.

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

1. Introduction

As a development of a larger study [1–5], we have undertaken a research program regarding synthesis and pharmacological evaluation of new 1,2,3-triazole derivatives, corresponding to the general formula **A** (Fig. 1), as potential potassium channel (BK_{Ca}) activators. In Fig. 1 the reference benzimidazolones NS004 and NS1619 (**B**) as well as their 1,2,4-triazol-3-one active analog **C** [6] are also reported.

The open structure **A**, consisting of two substituted aromatic rings bonded to a heterocyclic linker, represents a pharmacophoric pattern for effective BK-activators [6–9]. The activation of potassium channels allows the concentration-dependent passage of potassium ions to the extracellular phase and, consequently, it causes membrane hyperpolarization and reduction of cellular excitability [10]. Therefore, compounds able to open selectively the BK channels, afford a new therapeutic approach for several pathological conditions such as asthma, urge incontinence and bladder spasm, gastric hypermotility, hypertension, coronary artery spasm, psychoses [11].

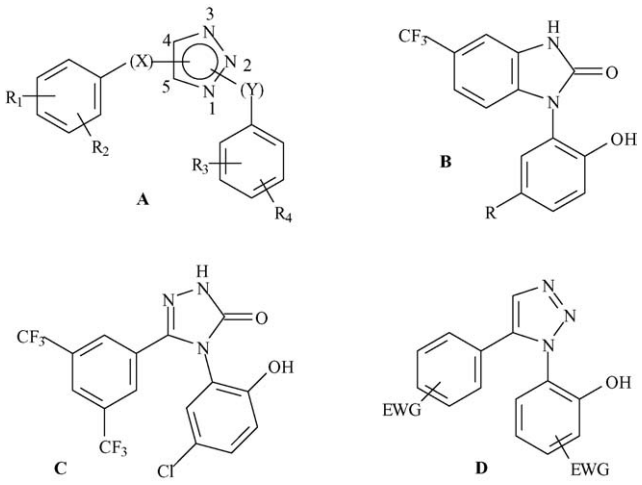

Fig. 1. General formula **A**: R_1, R_2, R_3, R_4 = hydrogen bond donors and/or acceptors and/or electron withdrawing groups. (X) and/or (Y): when present = CH_2, CHOH, CO groups. Comparison benzimidazolones **B**: NS004 (R = Cl) and NS1619 (R = CF_3), their deannulated and structurally-related active analog **C**. General formula of 4,5-diaryl-1,2,3-triazoles (**D**), where EWG indicates withdrawing groups.

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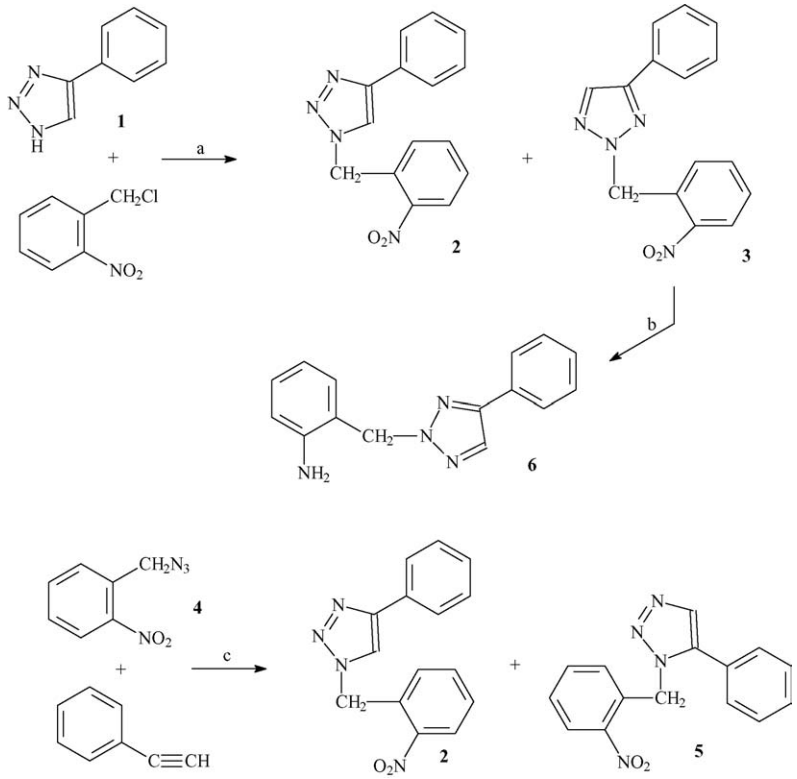
In the previous paper of this research program [12], 1,5-disubstituted-1,2,3-triazole derivatives **D** (Fig. 1) were studied. Only the 1-(2′-hydroxy-5′-nitrophenyl)-5-phenyl-1H-1,2,3-triazole showed appreciable effectiveness, exhibiting a lower efficacy parameter but a potency index comparable to that of the reference BK_{Ca} opener NS1619 [13]. In this paper new 1,2,3-triazole derivatives bearing aryl substituents in positions 1,4- or 2,4 respectively, with/without one methylene as a spacer group, are taken into consideration, to further evaluate the pharmacological effect of the 1,2,3-triazole ring as a heterocyclic linker between two aromatic or cycloalkyl substituents. These substituents are placed one on the nitrogen atom in the position 1 or 2, the other in the position 4 or 5 of the triazole ring respectively, so that two unsubstituted nitrogen atoms of the heterocyclic nucleus could act as hydrogen bond acceptors.

2. Chemistry

At first we undertook the preparation of new 1,2,3-triazole derivatives bearing a phenyl substituent in the 4 position and a benzyl substituent on one of the nitrogen atoms of the ring, in order to introduce a methylenic bridge as a further spacer group between the two aromatic rings. This spacer supplies better molecular flexibility, which was shown to be a valid structural feature in designing BK_{Ca}-openers [14]. In addition the nitro group on the benzyl substituent was selected planning to convert it into a hydroxyl function via reduction to amine, formation of the diazonium salt and its hydrolytic decomposition. Thus, we prepared the 4-phenyl-1,2,3-triazole (**1**) (Scheme 1), which had been reported in the literature by several synthetic procedures [15]. We made use of the 1,3-cycloaddition reaction between sodium azide and phenylacetylene [16] and, lengthening the reaction time, the product yield was improved. The 4-phenyltriazole (**1**) underwent a nucleophilic substitution reaction with 2-nitrobenzyl chloride in acetone in the presence of anhydrous potassium carbonate (Scheme 1) to give a mixture of the 1,2,3-triazole isomers 1-(2-nitrobenzyl)-substituted (**2**) and 2-(2-nitrobenzyl)-substituted (**3**) in 63% yield and in a 1:1.7 ratio respectively (by gas-chromatographic analysis). This mixture was fractionated by flash-chromatography through silica gel and elution with a 1:2 mixture of EtOAc/petroleum ether provided the isomer **3** (44% yield) followed from the isomer **2** (16% yield).

The attribution of the structure to the isomers **2** and **3** was performed by evaluation of their physical and spectroscopic properties and indirectly by a chemical demonstration. In fact from the nucleophilic substitution reaction, only two of the three expected isomers were isolated and upon the basis of steric hindrance considerations, the more probable isomers appeared to be those substituted on the 1,2,3-triazole ring in position 1 or 2 respectively. Therefore, as a chemical demonstration, we synthesized the mixture of 1-(2-nitrobenzyl)-triazole isomer (**2**) and 3-(2-nitrobenzyl)-triazole isomer (**5**) (Scheme 1), by 1,3-dipolar cycloaddition reaction of 2-nitrobenzyl azide (**4**) [17] to phenylacetylene.

This reaction is not regiospecific and provided the mixture of the isomers **2** and **5** in a ≈1:1 ratio, in high yield. The reac-



Scheme 1. (a) K_2CO_3, Me_2CO; (b) H_2, Pd/C; (c) Δ, toluene.

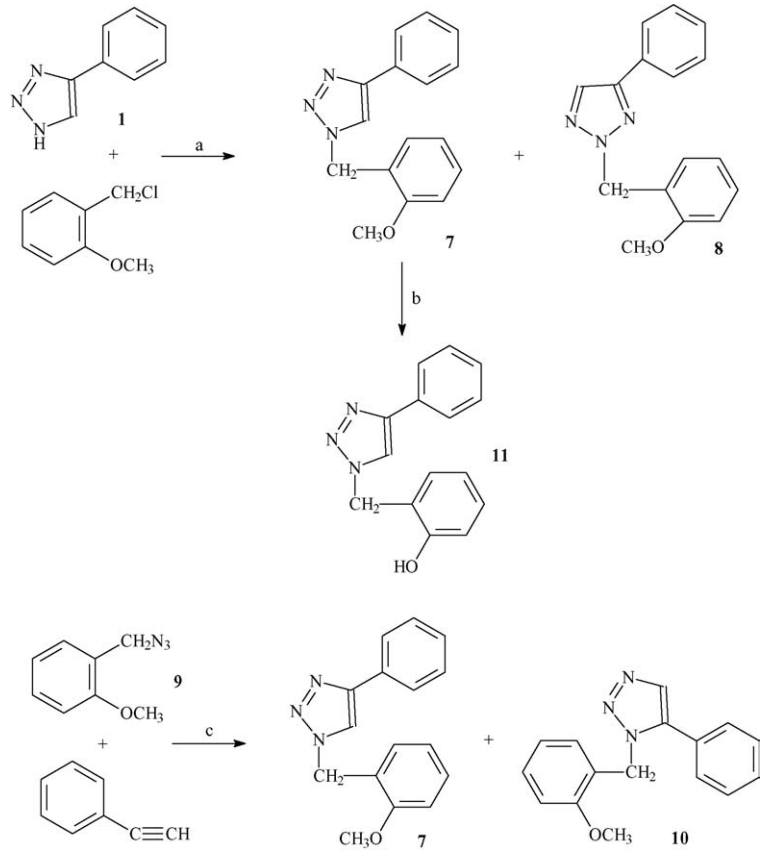
tion had been reported in the literature [18] and the Jordan authors also described the separation of the two isomers by preparative chromatography, although the physico-chemical properties of the two compounds are practically identical. Really the R_f values of the TLC analysis almost overlap as well as the chemical shift values of the H_5-triazole and H_4-triazole in the proton NMR spectra in $CDCl_3$. Nevertheless, examination of the same isomer mixture prepared by us, showed that 1- and 3-substituted 1,2,3-triazole compounds **2** and **5** have different retention time (t_R) in gas-chromatographic analysis, i.e. 14.18 min (amount 51%) and 8.82 min (amount 44%) respectively. Since the previously obtained isomers **2** and **3**, probably substituted on the nitrogen in the 1 or 2 position of the triazole ring, show t_R values corresponding to 14.38 min (amount 37%) and 8.28 min (amount 62%) respectively, this result allows us to assign the 1-substituted structure, corresponding to isomer **2**, to the compound with t_R 14.38 min. In addition the gas-chromatographic analysis of the isomer mixture coming from the nucleophilic substitution reaction shows, together with the peaks of compounds **2** and **3**, also the presence of a third peak (1.6%) with t_R 8.70 min, reasonably attributable to the triazole isomer substituted on position 3 of the ring, sterically hindered, which was not isolated. Therefore, the attribution of the 2-substituted structure to the isomer **3** could be deduced. A further confirmation of the previous structure attribution comes from the proton spectra in DMSO-d_6 of the isomers **2** and **3**. In fact the H_5-triazole values are easily identified at 8.62 δ and 8.35 δ respectively, whilst in $CDCl_3$ the values overlap at 7.98 δ. As known from the literature [19], the chemical shift value of the H_5-triazole resonates at fields lower than that of the H_4-triazole. Furthermore the UV spectra of isomers **2** and **3** show λ_{max} values at 248 nm and 256 nm respectively, indicating that the 2-substituted isomer **3** shifts towards greater λ. This agrees with the literature, which suggests a batochromic shift for the 2-substituted 1,2,3-triazole derivatives [20]. An attempt to prepare the 1-(2-nitrobenzyl)-5-phenyl-1H-1,2,3-triazole isomer (**5**), by ionic cycloaddition between 2-nitrobenzylazide (**4**) [17] and ethyl benzoylacetate to obtain the corresponding 4-carbethoxy-5-phenyl triazolester, which by hydrolysis and decarboxylation should give the expected compound **5**, failed. In fact, the ethyl benzoylacetate, under the basic experimental conditions required for the reaction, underwent the characteristic acid cleavage of the β-ketoesters. The nitrogroup of 2-(2-nitrobenzyl)-4-phenyl-2H-1,2,3-triazole (**3**) was reduced with stannous chloride or better by catalytic hydrogenation, to give the expected 2-benzylamino-triazole derivative (**6**). Some attempts at converting **6** to the corresponding phenol derivative failed. However, we thought it interesting to evaluate the pharmacological effect of the amino function, as a hydrogen bond donor, but with basic properties reversed to those of a phenol function, whose acid characteristics appear to have useful role for BK-channel activity [21]. The benzylamino derivative **6** showed a low vasorelaxing activity on rat aortic rings, so that, in consideration of the difficulty to obtain the corresponding phenol derivative by this route, the 1-(2-nitrobenzyl)-triazole isomer (**2**) was not treated.

Such a synthetic limitation was overcome by an analogs nucleophilic substitution reaction between 2-methoxybenzyl chloride and 4-phenyl-1,2,3-triazole (**1**) [16] (Scheme 2). The reaction was carried out in the usual manner, in acetone in the presence of anhydrous potassium carbonate, to give a 2:1 mixture (by gas-chromatographic analysis) of the triazole isomers **7** and **8** respectively. This mixture was fractionated by flash-chromatography through silica gel, obtaining the 2-substituted triazole isomer **8** in 21% yield, followed by the 1-substituted triazole isomer **7** in 41% yield. It is worth noting that this nucleophilic substitution reaction on the 1,2,3-triazole ring with 2-methoxybenzyl chloride shows a course different from that of the previous reaction with 2-nitrobenzyl chloride, confirming the involvement of mesomeric besides steric factors. In fact in the first case the isomeric ratio 1-substituted triazole **2**/2-substituted triazole **3** was 1:2, whilst in this case the isomeric ratio **7/8** was reversed to 2:1. Clearly the physico-chemical and spectroscopic properties of the two isomers were the same and for a comparison with the previous analogs substitution reaction they directed us towards a correct structure attribution.

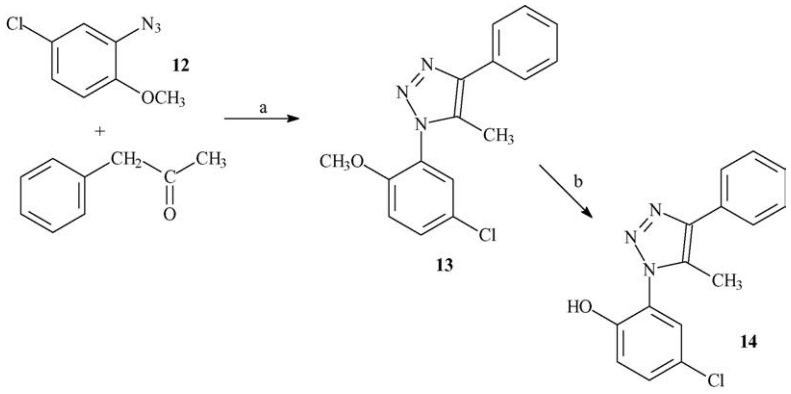
So it was observed that the 2-substituted triazole isomer **8** had a R_f 0.75 greater and a t_R 6.07 min lower than the 1-substituted triazole isomer **7**, with values of R_f 0.45 and t_R 10.88 min, respectively; the chemical shift values of the H_5-triazole in the ^{1}H-NMR spectra were 8.26 δ and 8.50 δ respectively. In addition, as previously reported, gas-chromatographic analysis of the original reaction mixture showed the presence of a third peak with t_R 7.33 min (4.0%), assignable to the 3-substituted triazole isomer **10** (Scheme 2), which had not been isolated.

On the other hand the thermic cycloaddition reaction between 2-methoxybenzylazide (**9**) [22] (Scheme 2) and phenylacetylene provided a mixture of the expected isomers 1-substituted triazole **7** and 3-substituted triazole **10** in a ratio 1.3:1 (by gas-chromatographic analysis), with t_R 10.86 min and t_R 7.53 min, respectively, to confirm unequivocally the structures assigned. The methoxy group of **7** was cleft by treatment with boron tribromide in CH_2Cl_2 at –78 °C to give the corresponding phenol derivative **11** in 56% yield. A modified structure without the methylenic spacer, but bearing the appropriate phenol function in the *ortho* position of the phenyl substituent and with a chlorine atom as a further substituent, was obtained by a regiospecific cycloaddition reaction (Scheme 3). Thus the 2-methoxy-5-chloro-phenylazide (**12**) [23] reacted with phenylacetone in ethanol in the presence of sodium ethoxide, to give in moderate yield the 1-(2-methoxy-5-chlorophenyl)-4-phenyl-5-methyl-1H-1,2,3-triazole (**13**), which was converted to the corresponding 2-hydroxy derivative **14** in the usual manner, by treatment with boron tribromide.

Finally a further substantial structural modification consisted with the change of position of the hydrogen bond donor function, which was introduced as an alcoholic hydroxyl in a position adjoining to the 4 position of the 1,2,3-triazole ring.



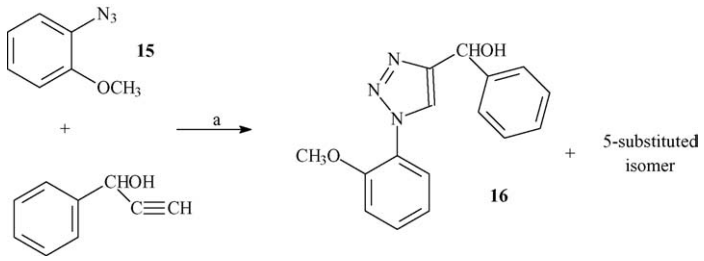
Scheme 2. (a) K_2CO_3, Me_2CO, (b) BBr_3; (c) Δ, toluene.



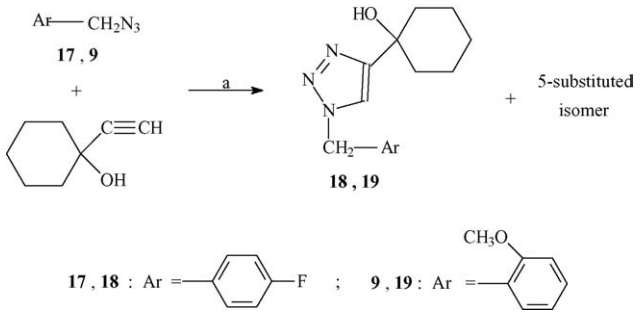
Scheme 3. (a) EtONa, EtOH; (b) BBr_3.

The reaction between 2-methoxy-phenylazide (**15**) [24] and α-ethynyl-benzyl alcohol (Scheme 4) led to the expected mixture (71% yield) of 4- and 5-substituted triazole isomers in 2.4:1 ratio respectively. Fractional crystallization of the mixture allowed isolation of the 4-hydroxybenzyl substituted isomer **16**, in 25% yield. In this compound the secondary alcoholic function represents a spacer inserted between the heterocyclic ring and the phenyl and it contributes flexibility to the molecular structure and to the hydrogen bond donor.

Similarly a tertiary alcoholic function bonded to a cycloalkyl substituent, more engaged and sterically hindered regarding the previous analog compound **16**, was introduced adjoining the 4 position of the 1,2,3-triazole ring (Scheme 5). Thus the 4-fluoro-benzylazide (**17**) [25] and 2-methoxy-benzylazide (**9**) [22] were reacted with 1-ethynyl-cyclohexanol to give the corresponding 1,2,3-triazole derivatives bearing substituents in the 1-position with different mesomeric properties and with more structural flexibility compared to compound **16**. The 4-fluoro-benzylazide (**17**) [25] provided the usual mixture (76% yield) of the 4- and 5-cyclohexyl substituted triazole isomers in 2:1 ratio respectively. The 1-(4-fluorobenzyl)-4-(1-hydroxycyclohexyl)-1H-1,2,3-triazole isomer (**18**) was present in a larger amount and was isolated in 36% yield by fractional crystallization. The 2-methoxy-benzylazide (**9**) [22] provided the expected mixture of the 4- and 5-substituted triazole isomers in 1.7:1 ratio



Scheme 4. (a) Δ, DMSO 90 °C.



Scheme 5. (a) Δ, toluene.

respectively. The 1-(2-methoxybenzyl)-4-(1-hydroxycyclohexyl)-1H-1,2,3-triazole isomer (**19**) was isolated in 35.5% yield by crystallization from ethyl acetate.

Structures of the new compounds **16**, **18** and **19**, coming from regioselective cycloaddition reactions, were assigned upon the basis of analytical and spectroscopic data as well as other chemical considerations, as above reported for analogous problems.

3. Biological results and discussion

The target compounds **2**, **3**, **6**, **11**, **16**, **18** and **19** can be viewed as the structural development of previously prepared 1,5-diaryl-1,2,3-triazoles showing, in some cases, a vasorelaxing activity probably due to the activation of potassium channels. Hence, they were submitted to a functional test, targeted to evaluate their possible vasodilator effects on KCl 20 mM precontracted rat aortic rings. Compounds **2**, **3**, **6**, **16**, **18** and **19** seem to differ from the casual structural features of the most common diarylheterocyclic BK-openers, described to date [7,8,26–28]. Typically these BK-activators show a hydroxy group in the 2 position of one of their aryl rings and probably this hydroxy group may be crucial for the pharmacological properties of such molecules. Therefore, it was not unexpected that compounds **2**, **3**, **6**, **16**, **18** and **19**, lacking this requirement, were devoid of any vasorelaxing effect and, consequently, of any BK-channel activating property. As concerns compounds **11** and **14**, their structures are relatively similar to those exibited by some recent compounds, such as 1-(2-hydroxy-4-chloro)-phenyl-3-phenyl-1,2,4-(4H)-triazol-5-one, which showed smooth muscle relaxant activity on a rat bladder, due to the activation of BK channels [7]. Nevertheless **11** and **14** did not exibit any vasorelaxing effect on rat aortic rings. The ineffectiveness of compounds **11** and **14** cannot be easily explained only by the lack of a carbonyl group in the heterocyclic spacer, because also some other 1,2,3-triazole derivatives, previously described by us, do not show this structural pattern, but possess vasorelaxing effects involving the activation of potassium channels [12].

In order to perform a deeper investigation on the structural requirements for the BK activating properties of diaryl-1,2,3-triazole and to define a suitable pharmacofore model, the development of new compounds modified in the position of the aryl groups and/or in their substituents borne by the aromatic rings is needed.

4. Experimental part

4.1. Chemistry

Melting points (m.p.) were determined on a Kofler hot-stage and are uncorrected. IR spectra in nujol mulls were recorded on a Mattson Genesis series FTIR spectrometer. UV spectra were obtained on a Perkin–Elmer Lambda 15 UV–Vis spectrophotometer in EtOH. ^{1}H-NMR spectra were recorded with a Varian Gemini 200 spectrometer in DMSO-d_6, 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 performed on a Carlo Erba Elemental Analyzer Mod. 1106 apparatus. Petroleum ether corresponds to the fraction boiling at 40–60 °C.

*4.1.1. 4-Phenyl-1,2,3-triazole (**1**)*

A mixture of phenylacetylene (5.0 ml, 45.57 mmol) and NaN_3 (16.0 g, 246 mmol) in 100 ml of anhydrous DMSO was heated at 120 °C under vigorous stirring for 11 days. Starvation of NaN_3 during the reaction led to its decomposition as already observed in the literature [29]. The reaction

mixture was poured into crushed ice (≈1 l), acidified (pH 3–4) with conc. HCl and stirred at room temperature for 30 min. The yellow precipitated solid was collected by filtration and repeatedly washed with H_2O (3.73 g). This solid was dissolved in 10% NaOH and the red solution was washed with CH_2Cl_2 (3 × 60 ml). The aqueous layer was then acidified (pH 3–4) and the title compound precipitated as pale yellow needles which were collected by filtration and dried: 3.39 g, yield 51%, m.p. 147–148 °C.

*4.1.2. 1-(2-Nitrobenzyl)-4-phenyl-1H-1,2,3-triazole (**2**) and 2-(2-nitrobenzyl)-4-phenyl-2H-1,2,3-triazole (**3**)*

To a solution of 4-phenyl-1,2,3-triazole (**1**) (1.00 g, 6.89 mmol) and 2-nitrobenzyl chloride (1.20 g, 6.99 mmol) in 40 ml of anhydrous acetone, anhydrous K_2CO_3 (2.0 g) was added and the mixture was heated under reflux for 22 h. The reaction mixture was concentrated in vacuo and the residue was treated with H_2O and extracted with $CHCl_3$. The combined organic layers were dried ($MgSO_4$) and evaporated to give a semisolid residue consisting of a mixture of the title compounds **2** and **3**: 1.21 g, yield 63%. Gas-chromatographic analysis indicated a relative ratio 1:2 of the two isomeric compounds **2** and **3** respectively. This mixture was fractionated by flash-chromatography through a silica gel column. Elution with a mixture 1:2 of EtOAc/petroleum ether provided the 2-substituted isomer **3** followed by the 1-substituted isomer **2**.

2: 0.190 g, yield 16%; m.p. 143–145 °C from MeOH. GC: t_R = 14.38 min. TLC: R_f = 0.83. ^{1}H-NMR (DMSO-d_6): δ 6.02 (s, 2H, CH_2); 7.13–8.19 (m, 9H, aromatics); 8.62 (s, 1H, H triazole). UV (1.4×10^{-4} conc. in EtOH): λ_{max} 248 nm; log ϵ 3.745. Mass (*m/z*): 280 (M^+); 116 (100%).

Anal. for $C_{15}H_{12}N_4O_2$: C, H, N.

3: 0.520 g, yield 44%; m.p. 130–132 °C from MeOH. GC: t_R = 8.28 min. TLC: R_f = 068. ^{1}H-NMR (DMSO-d_6): δ 6.08 (s, 2H, CH_2); 7.11–8.17 (m, 9H, aromatics); 8.35 (s, 1H, H triazole). UV (1.4×10^{-4} conc. in EtOH): λ_{max} 256 nm; log ϵ 3.792. Mass (*m/z*): 280 (M^+); 145 (100%).

Anal. for $C_{15}H_{12}N_4O_2$: C, H, N.

*4.1.3. 2-(2-Aminobenzyl)-4-phenyl-2H-1,2,3-triazole (**6**)*

A) A mixture of 2-(2-nitrobenzyl)-4-phenyl-2H-1,2,3-triazole (**3**) (0.400 g, 1.43 mmol) and $SnCl_2$ $2H_2O$ (0.966 g, 4.28 mmol) in 30 ml of 24% HCl was heated under reflux for 4 h. The suspension obtained was filtered and the filtrate was treated with 10% NaOH until complete dissolution of the precipitated tin hydroxides. The new suspension was stirred for 3–4 h, then the white precipitate was collected by filtration and washed with H_2O: 0.123 g, yield 34%; m.p. 102–104 °C from MeOH/H_2O. IR (*ν*): 3430 and 3340 cm^{-1} (NH_2). ^{1}H-NMR (DMSO-d_6): δ 5.21 (s, 2H, NH_2); 5.54 (s, 2H, CH_2); 6.49–7.86 (m, 9H, aromatics); 8.28 (s, 1H, H triazole). Mass (*m/z*): 250 (M^+); 106 (100%).

Anal. for $C_{15}H_{14}N_4$: C, H, N.

B) To a solution of **3** (0.460 g, 1.64 mmol) in 20 ml of EtOH, 0.020 g of 5% Pd/C was added and the mixture was hydrogenated at room temperature and pressure. The catalyst was filtered off, washed with hot EtOH and the filtrate was evaporated under reduced pressure. The residue (0.280 g, yield 68%) consisted of **6**.

*4.1.4. 1-(2-Methoxybenzyl)-4-phenyl-1H-1,2,3-triazole (**7**) and 2-(2-methoxybenzyl)-4-phenyl-2H-1,2,3-triazole (**8**)*

To a solution of 4-phenyl-1,2,3-triazole (**1**) (1.00 g, 6.89 mmol) and 2-methoxybenzyl chloride (1.0 ml, 7.0 mmol) in 40 ml of anhydrous acetone, anhydrous K_2CO_3 (2.0 g) was added and the mixture was heated under reflux for 22 h. The reaction mixture was concentrated in vacuo and the residue was treated with H_2O and extracted with $CHCl_3$. The combined organic layers were dried ($MgSO_4$) and evaporated to give a semisolid residue (1.42 g) consisting essentially of a mixture of the title compounds **7** and **8** in a 2:1 ratio respectively (by gas-chromatographic analysis). This mixture was fractionated by flash-chromatography through a silica gel column, eluting with a mixture 1:3 of EtOAc/petroleum ether to give the 2-substituted isomer **8** followed by the 1-substituted isomer **7**.

7: 0.743 g, yield 41%; m.p. 93–96 °C from MeOH. GC: t_R = 10.88 min. TLC: R_f = 0.45. ^{1}H-NMR (DMSO-d_6): δ 3.84 (s, 3H, OCH_3); 5.57 (s, 2H, CH_2); 6.90–7.89 (m, 9H, aromatics); 8.50 (s, 1H, H triazole). Mass (*m/z*): 265 (M^+); 91 (100%).

Anal. for $C_{16}H_{15}N_3O$: C, H, N.

8: 0.390 g, yield 21%; m.p. 61–64 °C from MeOH/H_2O. GC: t_R = 6.07 min. TLC: R_f = 0.75. ^{1}H-NMR (DMSO-d_6): δ 3.81 (s, 3H, OCH_3); 5.63 (s, 2H, CH_2); 6.87–7.87 (m, 9H, aromatics); 8.26 (s, 1H, H triazole). Mass (*m/z*): 265 (M^+); 91 (100%).

Anal. for $C_{16}H_{15}N_3O$: C, H, N.

*4.1.5. 1-(2-Hydroxybenzyl)-4-phenyl-1H-1,2,3-triazole (**11**)*

To a cooled (–78 °C) and stirred solution of **7** (0.300 g, 1.13 mmol) in 50 ml of anhydrous CH_2Cl_2, under a nitrogen flow, a solution of BBr_3 (1.5 ml, 15.8 mmol) in 8 ml of anhydrous CH_2Cl_2 was slowly added dropwise. Stirring was continued for 2 h, then the solution was left at –20 °C for 24 h. After 1 h at room temperature, the mixture was cooled in an ice–salt bath and the reagent was decomposed by treating with MeOH (10 ml) followed by H_2O (10 ml). The organic layer was washed with H_2O, then extracted with 10% NaOH. Acidification of the alkaline extract provided **9** as a white precipitate which was collected by filtration: 0.160 g, yield 56%; m.p. 209–211 °C from EtOAc/petroleum ether. ^{1}H-NMR (DMSO-d_6): δ 5.53 (s, 2H, CH_2); 6.75–7.87 (m, 9H, aromatics); 8.49 (s, 1H, H triazole); 9.92 (s, 1H, OH).

Anal. for $C_{15}H_{13}N_3O$: C, H, N.

*4.1.6. 1-(2-Methoxy-5-chlorophenyl)-4-phenyl-5-methyl-1H-1,2,3-triazole (**13**)*

To a stirred solution of EtONa (from 0.171 g, 7.45 mmol of sodium in 10 ml of absolute EtOH), a solution of 2-methoxy-5-chlorophenylazide (**12**) (1.36 g, 7.45 mmol) and

phenylacetone (1.00 g, 7.45 mmol) in 15 ml of absolute EtOH was added drop by drop and the mixture was heated at 50 °C for 4 h. The reaction mixture was evaporated in vacuo and the black oil residue was extracted with boiling petroleum ether (25 ml) to remove the unreacted azide. The new residue was then extracted with boiling EtOAc, which left a uncharacterized black semisolid. The combined organic layers were concentrated and purified by filtration through a silica gel column, eluting with EtOAc. Evaporation of the solvent gave the title compound as a white solid: 0.825 g, yield 37%; m.p. 99–102 °C from EtOAc/petroleum ether. ^{1}H-NMR (DMSO-d_6): δ 2.30 (s, 3H, CH_3); 3.83 (s, 3H, OCH_3); 7.35–7.79 (m, 8H, aromatics).

Anal. for $C_{16}H_{14}N_3OCl$: C, H, N.

*4.1.7. 1-(2-Hydroxy-5-chlorophenyl)-4-phenyl-5-methyl-1H-1,2,3-triazole (**14**)*

To a stirred solution of **13** (0.300 g, 1.0 mmol) in 25 ml of anhydrous CH_2Cl_2, cooled at –78 °C and under a nitrogen flow, a solution of BBr_3 (1.0 ml, 10.6 mmol) in 7 ml of anhydrous CH_2Cl_2 was slowly added dropwise. The cooling bath was removed and stirring was continued at room temperature for 15 h. To the reaction mixture, cooled in an ice-salt bath, was added MeOH (10 ml) followed by H_2O (15 ml) to destroy the reagent, then the organic layer was extracted with 10% NaOH. The combined aqueous extracts were paper filtered then acidified with conc. HCl to precipitate the title compound as a white solid, which was collected by filtration: 0.231 g, yield 81%; m.p. 287–290 °C from DMF/H_2O. ^{1}H-NMR (DMSO-d_6): δ 2.32 (s, 3H, CH_3); 7.14–7.80 (m, 8H, aromatics); 10.81 (s, 1H, OH).

Anal. for $C_{15}H_{12}N_3OCl$: C, H, N.

*4.1.8. 1-(2-Methoxyphenyl)-4-(α-hydroxybenzyl)-1H-1,2,3-triazole (**16**)*

A stirred solution of 2-methoxy-phenylazide (**15**) (0.770 g, 5.16 mmol) and α-ethynylbenzyl alcohol (1.1 ml, 8.9 mmol) in 18 ml of DMSO was heated at 90 °C for 4 h. After cooling, the brown solution was diluted with H_2O (60 ml) and the mixture was extracted with Et_2O (3 × 90 ml). The combined organic layers were washed with H_2O (4 × 100 ml), dried ($MgSO_4$) and evaporated to give the expected mixture of the 1,2,3-triazole isomers as a dark oil: 1.030 g, yield 71%; relative ratio of the two isomers 1:2.4 by gas-chromatographic analysis (t_R 9.19 and 11.47 min respectively). The mixture was dissolved in EtOAc and cooled at –20 °C to give the title compound as pale yellow needles which were collected by filtration: 0.360 g, yield 25%, m.p. 124–125 °C from EtOAc; GC: t_R = 11.48 min. ^{1}H-NMR (DMSO-d_6): δ 3.84 (s, 3 H, OCH_3); 5.89 (d, 1 H, CH); 6.09 (d, 1 H, OH); 7.07–7.62 (m, 9 H, aromatics); 8.21 (s, 1 H, H_5 triazole). Mass (*m*/*z*): 281 (M^+); 77 (100%).

Anal. for $C_{16}H_{15}N_3O_2$: C, H, N.

*4.1.9. 1-(4-Fluorobenzyl)-4-(1-hydroxycyclohexyl)-1H-1,2,3-triazole (**18**)*

A solution of 4-fluoro-benzylazide (**17**) (0.606 g, 4.00 mmol) and 1-ethynyl-cyclohexanol (0.512 g, 4.12 mmol) in 25 ml of toluene was heated under reflux for 24 h. The solvent was evaporated in vacuo and a semisolid residue, consisting with the expected mixture of the 1,2,3-triazole isomers, was obtained: 0.839 g, yield 76%; relative ratio of the two isomers 1:2 by gas-chromatographic analysis (t_R 8.48 and 8.99 min respectively). This residue was refluxed with 20 ml of petroleum ether, which, after cooling, was decanted off. The new residue, as a white crystalline solid (0.650 g, yield 59%), was dissolved in EtOAc. The solution was paper filtered and cooled at –20 °C to give the title compound as big colorless dipyramidal crystals: 0.394 g, yield 36%, m.p. 119–122 °C from EtOAc; GC: t_R = 8.48 min. ^{1}H-NMR (DMSO-d_6): δ 1.19–2.00 (m, 10 H, cyclohexyl); 4.84 (s, 1 H, OH); 5.53 (s, 2 H, CH_2); 7.16–7.43 (AA′BB′, 4 H, aromatics); 7.92 (s, 1 H, H_5 triazole). Mass (*m*/*z*): 275 (M^+); 109 (100%).

Anal. for $C_{15}H_{18}N_3OF$: C, H, N.

*4.1.10. 1-(2-Methoxybenzyl)-4-(1-hydroxycyclohexyl)-1H-1,2,3-triazole (**19**)*

A solution of 2-methoxy-benzylazide (**9**) (2.0 g, 12.25 mmol) and 1-ethynyl-cyclohexanol (1.53 g, 12.32 mmol) in 60 ml of toluene was heated under reflux for 48 h. The solvent was evaporated in vacuo and the yellow oil residue, consisting with the expected mixture of the 1,2,3-triazole isomers, underwent a gas-chromatographic analysis: relative ratio of the two isomers 1:1.7 with t_R = 10.93 and 11.93 min respectively. This residue was refluxed with 20 ml of petroleum ether, which, after cooling, was decanted off. The new residue, as a white crystalline solid (2.350 g, yield 67%), was crystallized from EtOAc to give the title compound as white plates: 1.250 g, yield 35. %, m.p. 120–122 °C from EtOAc; GC: t_R = 10.98 min. ^{1}H-NMR (DMSO-d_6): δ 1.32–1.85 (m, 10 H, cyclohexyl); 3.83 (s, 3 H, OCH_3); 4.64 (s, 1 H, OH); 5.48 (s, 2 H, CH_2); 6.90–7.38 (m, 4 H, aromatics); 7.76 (s, 1 H, H_5 triazole). Mass (*m*/*z*): 287 (M^+); 121 (100%).

Anal. for $C_{16}H_{21}N_3O_2$: C, H, N.

4.2. 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 a 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 intimal surface of the vessels with a hypodermic needle. Five mm 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 an unirecord microdynamometer (Buxco Electronics).

After an equilibration period of 60 min, the endothelial integrity 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, threefold increasing concentrations of the tested compounds or of the reference drug NS 1619 (a well-known BK-activator) were added cumulatively. 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 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 whereas KCl was dissolved in Tyrode solution. Most of the synthetized derivatives (**2**, **6**, **14**, **16**, **18**) were dissolved (10 mM) in DMSO whereas one of them (**11**) was dissolved (10 mM) in NaOH 0.1 N; 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. Compounds exhibiting an efficacy level < 20% were considered as ineffective. Experimental data were analysed by a computer fitting procedure (software: GraphPad Prism 3.0).

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