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1,5-Diarylsubstituted 1,2,3-triazoles as potassium channel activators. VI

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Abstract

As part of our program toward designing potassium channel openers, synthesis of a novel series of 1,5-diphenylsubstituted 1,2,3-triazoles, as potential activators of the large-conductance calcium-activated potassium channels (BK), as well as their vasorelaxant activity are presented. The functional effect of these potential structurally novel BK-openers on vascular contractile function were studied *in vitro*, using isolated rat aortic rings pre-contracted with KCl 20 mM. Among the target compounds, only **16** showed appreciable effectiveness, exhibiting an efficacy parameter (57%) lower than that of NS1619 and a comparable potency index (pIC_{50}: 5.58).
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1. Introduction

Large-conductance calcium-activated potassium channels (also known as BK or BK_{Ca} 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. In fact, the activation of these channels, allowing the concentration-depending passage of the potassium ions to the extracellular phase, causes membrane hyperpolarization and reduction of cellular excitability [1]. Therefore, compounds able to activate selectively 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].

Previously, we had synthesised and tested as potential potassium channel activators a series of 5-(4'-substituted-2'-nitro-anilino)-1,2,3-triazole derivatives [3] which showed a vasorelaxant activity, involving potassium channel opening and with a potency comparable to that of the reference compound NS 1619 [4]. Afterwards, a series of 5-substituted-triazolyl-benzimidazolones and the corre-

sponding series of 5-substituted-triazolyl-benzotriazoles were prepared [5]. Differently from the benzimidazolone derivatives, the benzotriazole derivatives generally showed full efficacy with vasorelaxing properties and potency parameters a little lower than that of the reference compound NS 1619. Some structural modifications of the 1,2,3-triazolyl-benzimidazolone and 1,2,3-triazolyl-benzotriazole derivatives [5] did not increase activity, even if they provided useful information about structure-activity relationships [6]. Finally, a series of new 5-substituted-1-(2-hydroxybenzoyl)-benzotriazole derivatives [7] showed full vasorelaxing efficacy and high potency values. The best compound in this series, further investigated in order to evaluate the possible mechanism of action involving BK channels, showed also an interesting cardioprotective activity [7].

On the basis of this experience of ours focused on the structural modifications of the prototypical NS004 and considering the chemical structure and pharmacophoric characteristics of potent BK activators recently investigated, bearing a disubstituted and five-membered terms heterocyclic ring [8-11], we planned the synthesis and pharmacological evaluation of new disubstituted monocyclic 1,2,3-triazole derivatives, corresponding to the general structure **A** (Fig. 1). In Figure 1, the comparison compounds NS004 and NS1619 (**B**) as well as their 1,2,4-triazol-3-one active analogue **C** [8] are reported. This first paper reports the results obtained from
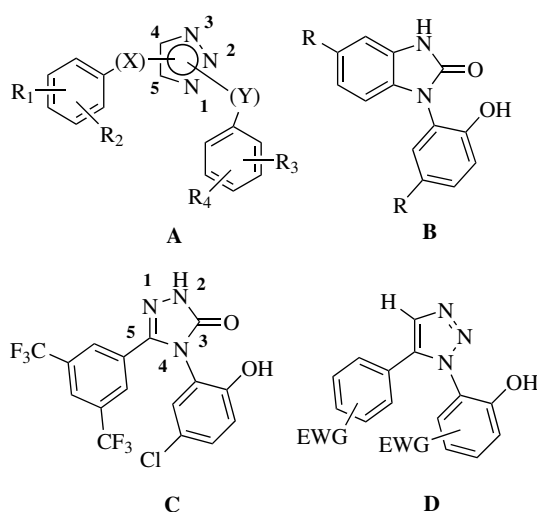
General formula **A**. R_1, R_2, R_3, R_4 = hydrogen bond donors and / or acceptors and /
or electron withdrawing groups. (X) and / or (Y), when present = CH_2, CHOH, CO groups.
Comparison benzimidazolones NS004 (R = Cl) and NS1619 (R = CF_3) (**B**), their deannulated
and structurally-related active analogue **C** [8] and general formula of target 1,2,3-triazoles (**D**),
where EWG indicates electron withdrawing groups.

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 NS004 (R = Cl) and NS1619 (R = CF_3) (**B**), their deannulated and structurally-related active analogue **C** [8]
and general formula of target 1,2,3–triazoles (**D**), where EWG indicates electron withdrawing groups.

the study of new 1,5-disubstituted 1,2,3-triazole derivatives
**D** structurally-related to the compounds **B** and **C** (Fig. 1).

2. Chemistry

The 2-nitro-4-chloro-phenylazide **1** [12], chosen for the
chemical versatility of the nitro group, reacted with the
appropriate β-ketoester (**2a** or **2b**) (Scheme 1) in absolute
ethanol in the presence of sodium ethoxide at -10°C, to give
the expected 1,3-dipolar cycloaddition products, the 1,2,3-
triazolesters **3a** and **3b** respectively, in moderate yield, to-
gether with the corresponding acids **4a** [13] and **4b**. These
acids were the only reaction products when the reaction
mixture was directly hydrolized without isolation of the
esters **3a** and **3b** and were easily obtained by mild alkaline
hydrolysis of the same esters. The carboxylic acids **4a** [13]
and **4b**, by refluxing in DMF, were easily decarboxylated to
the 1,5-disubstituted triazole derivatives **5a** [13] and **5b**.
Reduction of the nitro group of **5a** with stannous chloride in
acidic medium gave the corresponding aminoderivative **6a**,
previously described in the literature [13]. A similar chemi-
cal reduction or a reduction by catalytic hydrogenation of **5b**,
involved also a reduction of the other nitrogroup to give the
diaminoderivative **6b** in moderate yield. The presence of a
primary amino function in the ortho position of the phenyl
substituent was considered interesting in order to evaluate its
pharmacological effect, because it can act as a hydrogen
bond donor. In fact, lots of BK_{Ca} channel openers, taken as
comparison compounds [4,8-11], generally bear a hydroxy
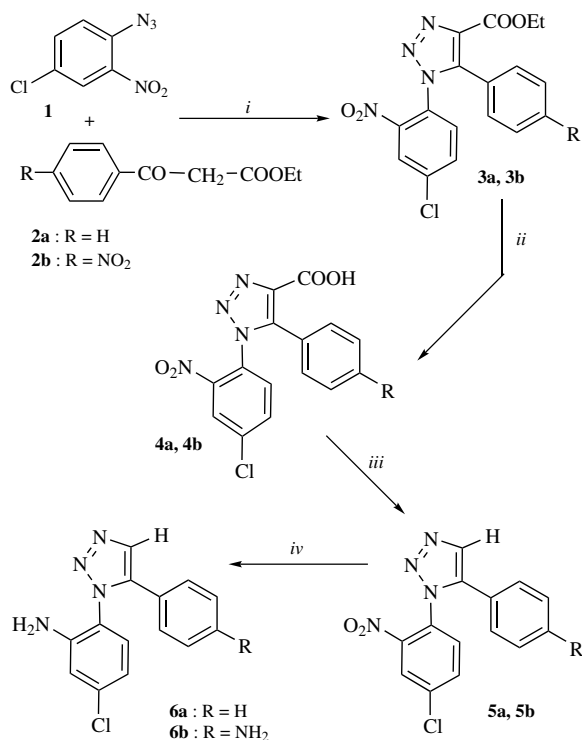
(*i*) EtONa / EtOH, r.t.; (*ii*) 4% KOH; (*iii*) DMF, reflux;
(*iv*) H_2/Pd/C or $SnCl_2$/H^+.

Scheme 1.
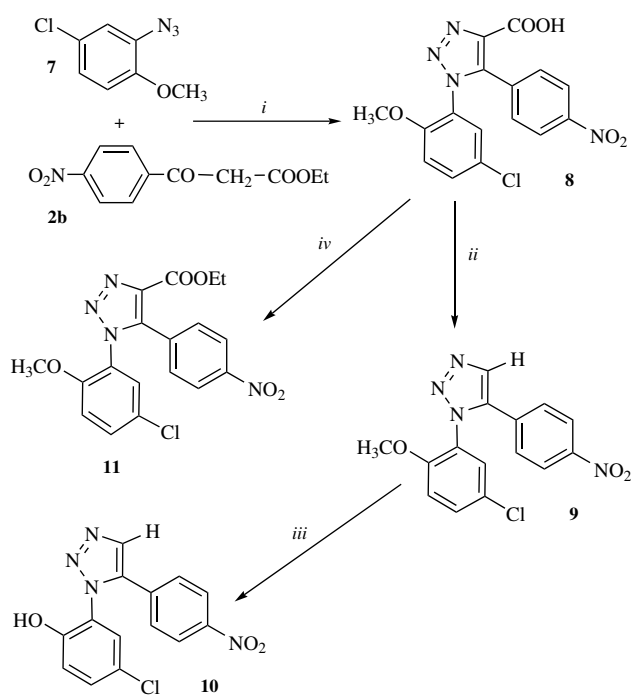
(*i*) EtONa / EtOH, 50°C; (*ii*) DMF, reflux;
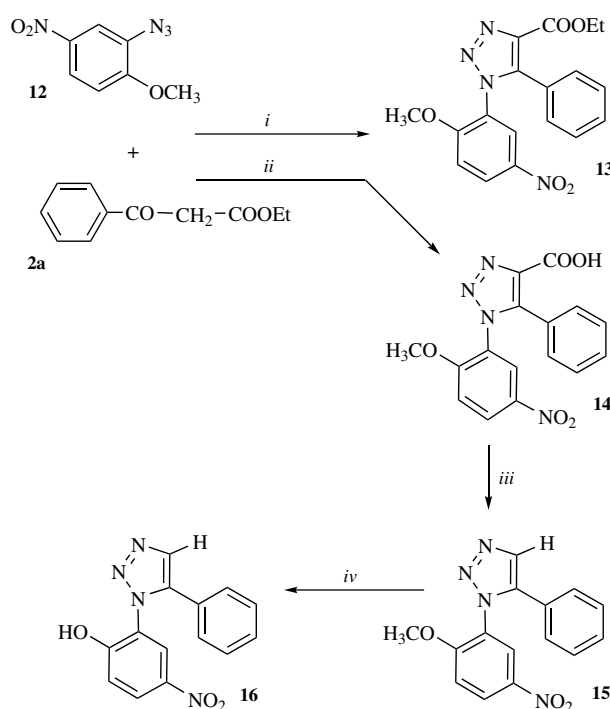(*iii*) BBr₃; (*iv*) EtOH, H⁺, reflux.

Scheme 2.

(*i*) EtONa / EtOH, r.t.; (*ii*) EtONa / EtOH, 50°C;
(*iii*) DMF, reflux; (*iv*) BBr₃.

Scheme 3.

function in the same position. Anyhow we thought of converting the amino to a hydroxy function, via diazotization and hydrolytic decomposition of the diazonium salt. Unfortunately, the treatment of **6a** with sodium nitrite in sulfuric acid solution at 0-5°C and then decomposition of the diazonium salt either by heating or by stirring at room temperature (25°C) did not provide the expected phenolic derivative. The only product, isolated from the tarry reaction mixture in very low yield was the 5-chloro-benzotriazole [14], formed by the cleavage of the triazole ring owing to the intramolecular addition of the diazonium salt. In order to prevent this inconvenience, the 2-methoxy-5-chloro-phenylazide (**7**) [15] was similarly reacted with ethyl p-nitrobenzoylacetate (**2b**) (Scheme 2), under the usual experimental conditions. In this case, the 1,3-dipolar cycloaddition reaction provided directly the carboxylic acid **8** in 50% yield, which was, in its turn, decarboxylated by heating in boiling DMF, to give the 1-(2'-methoxy-5'-chloro)-5-(4'-nitrophenyl)-1H-1,2,3-triazole (**9**) in 63 % yield. The treatment of the methoxy derivative **9** with boron tribromide in dichloromethane at -78°C until it reached room temperature, caused the ether cleavage to give the desired phenolic derivative **10** in 35 % yield. The triazolester **11** was prepared (in moderate yield) by Fisher esterification of **8**.

Similarly, the 2-methoxy-5-nitro-phenylazide (**12**) [16], bearing in the 2 position the methoxy substituent convertible to a hydroxy function and in the 5 position a different and stronger electron withdrawing group, was employed in an

analogous reaction sequence (Scheme 3). Thus the ionic cycloaddition reaction of azide **12** to ethyl benzoylacetate (**2a**), carried out under mild experimental conditions, allowed isolation of the triazolester **13** in 36% yield. When the reaction was carried out under stronger experimental conditions, hydrolysis of the intermediate ester took place and the reaction mixture provided directly the triazole acid **14** in 70% yield. The acid was decarboxylated to **15** in the usual manner and this was converted to 1-(2'-hydroxy-5'-nitro)-5-phenyl-1H-1,2,3-triazole (**16**), by treatment with an excess of boron tribromide under the above reported experimental conditions.

In order to further change the arrangement of the electron withdrawing substituents on the two aromatic rings, a new synthetic procedure [17] was carried out to introduce a chlorine atom on the phenyl ring in the 5 position of the triazole (Scheme 4). Thus, the 2-methoxy-5-chloro-phenylazide (**7**) [15] reacted with an equimolar amount of 4-chlorobenzoyl-methylen-triphenylphosphorane (**17**) [18] in toluene (80°C) for three days, to give the 1-(2'-methoxy-5'-chloro)-5-(4'-chlorophenyl)-1H-1,2,3-triazole (**18**) in 68 % yield. This was then converted to the corresponding phenol derivative **19**, by treatment with boron tribromide.

The structure of all the new compounds obtained was assigned upon the basis of the mechanism of the well-known reactions employed. In fact, the 1,3-dipolar cycloaddition of azides to β-ketoesters is regiospecific [17] as well as the addition to α-keto-phosphorus ylides [14] and the following
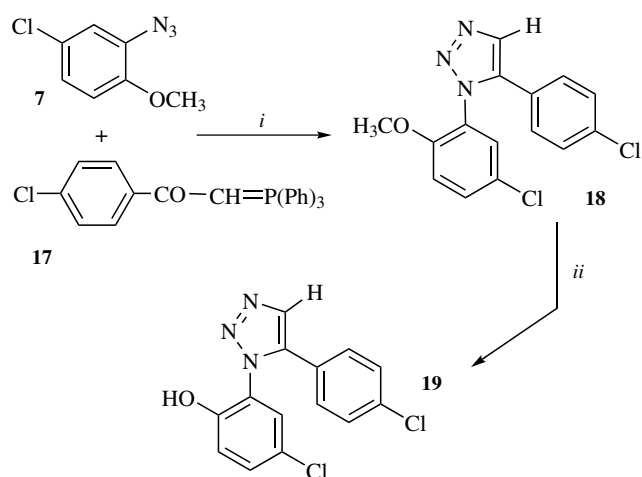
(*i*) benzene, reflux, three days; (*ii*) BBr$_3$

Scheme 4.

reactions (decarboxylation, nitro reduction, ether cleavage), involve functional groups placed in known positions. In addition the reaction accomplishment was confirmed by analytical and spectroscopic methods (Tables 1 and 2) .

3. Biological results and discussion

Target compounds **10, 16** and **19** underwent a functional evaluation of their vasorelaxing properties in *in vitro* assays as a preliminary screening test to point out their potential activation of potassium channels. Then, compounds **3-6a,b, 8, 9, 11, 13-15, 18** were also tested because of their clear chemical structural similarity with the target compounds and in order to explore and unmask a possible and new structurally-related chemical lead structure. The results of

the pharmacological study have been summarised in Table 3, where the values of the vasorelaxing potency and efficacy of the reference compound NS1619 and of the target compounds have been reported. In his Table, the pharmacological parameters of compound **3b** are also reported, as a unique non-target compound disclosed with significant and tetraethylammonium chloride (TEA)-sensitive vasorelaxant activity. Noteworthy, from a pharmacological point of view, **3b** is the best of all compounds tested, showing a potency about 10-fold higher than that of NS1619 but a not-full vasorelaxing efficacy. Besides, among the target compounds (**10, 16, 19**), only **16** showed appreciable effectiveness, exhibiting an efficacy parameter (57%) lower than than that of NS1619 and a comparable potency index (pIC$_{50}$: 5.58 *vs* pIC$_{50\ (NS1619)}$: 5.18). The vasorelaxing effects were significantly inhibited by TEA, suggesting the involvement of potassium channels.

Both the low efficacy parameters of **10** and **19** and the moderate parameter of **16** indicate that the viability of such deannulation of NS004 and the bioisosteric replacement of 4,5-diphenyltriazol-3-one with 1,5-diphenyl-1,2,3-triazole nucleus do not seem profitable and deserving of further synthetic and pharmacological investigation. As far as the low biological activity is concerned, one of the most important pharmacophore requirements which is not conserved in the 1,2,3-triazoles *vs* 1,2,4-triazol-3-ones seems to be the hydrogen bond donor capability on the heterocycle ring, supposing that the series of target compounds can interact at the same site as 4,5-diphenyltriazol-3-ones on BK channels. The paramount role of H-bond donors would be in agreement with the observation that in the vicinal and diphenyl substituted heterocyclic series the presence of at least two potential H-bond donor sites is crucial [8]. Again, a common general pharmacophore in lots of BK-openers [1] involves an H-bond donor moiety often placed on a cyclic or acyclic spacer linking two properly substituted phenyl rings, one of which fitted with a further potential H-bond donor such as a hy-

Table 1
Chemical and physical properties of the prepared compounds

Comp.[a]	Yield %	Crystall. Solvent	M.p. (°C)	Analyses (C, H, N)	IR v cm^{-1}
3a	36	MeOH/H$_2$O	132-134	C$_{17}$H$_{13}$N$_4$O$_4$Cl	1724 (CO)
3b	73	EtOH	173-175	C$_{17}$H$_{12}$N$_5$O$_6$Cl	1732 (CO)
4b	82	[b]	119-123	C$_{15}$H$_8$N$_5$O$_6$Cl	1716 (CO)
5b	81	EtOAc/Petr.Eth.	171-172	C$_{14}$H$_8$N$_5$O$_4$Cl	---------
6b	[c]	EtOH/H$_2$O	135-137	C$_{14}$H$_{12}$N$_5$Cl	3340, 3213 (NH$_2$)
8	50	EtOH	186-188	C$_{16}$H$_{11}$N$_4$O$_5$Cl	1701 (CO)
9	63	EtOH/H$_2$O	132-135	C$_{15}$H$_{11}$N$_4$O$_3$Cl	--------
10	35	EtOH	261-263	C$_{14}$H$_9$N$_4$O$_3$Cl	3448 (OH)
11	33	EtOH	228-231	C$_{18}$H$_{15}$N$_4$O$_5$Cl	1724 (CO)
13	36	EtOH/H$_2$O	228-230	C$_{18}$H$_{16}$N$_4$O$_5$	1728 (CO)
14	70	EtOH	165-167	C$_{16}$H$_{12}$N$_4$O$_5$	1715 (CO)
15	82	EtOH	180-182	C$_{15}$H$_{12}$N$_4$O$_3$	-------
16	32	EtOAc	278-280	C$_{14}$H$_{10}$N$_4$O$_3$	(OH)
18	68	MeOH/H$_2$O	154-157	C$_{15}$H$_{11}$N$_3$OCl$_2$	-------
19	40	EtOH/H$_2$O	273-274	C$_{14}$H$_9$N$_3$OCl$_2$	3441 (OH)

[a] : compounds 4a, 5a and 6a are described in the literature [10].
[b] : purified by dissolution in 10% NaOH, filtration and precipitation by acidification.
[c] : procedure A: 55%; procedure B: 76%.

Table 2
^{1}H-NMR spectra in DMSO-d$_6$ (δ)

3a	1.15 (t, 3H, CH$_3$); 4.24 (q, 2H, CH$_2$); 7.31-7.46 (m, 5H, Ar); 7.81-8.37 (m, 3H, Ar)
3b	1.17 (t, 3H, CH$_3$); 4.25 (q, 2H, CH$_2$); 7.68-8.43 (m, 7H, Ar)
4b	7.65-8.42 (m, 7H, Ar); 13.55 (bs, 1H, COOH)
5b	7.59-8.49 (m, 7H, Ar); 8.45 (s, 1H, H$_4$ triaz.)
6b	5.37 (bs, 2H, NH$_2$); 5.42 (bs, 2H, NH$_2$); 5.46-6.62 (m, 3H, Ar); 6.68-6.99 (m, 4H, Ar); 7.94 (s, 1H, H$_4$ triaz.)
8	3.49 (s, 3H, OCH$_3$); 7.10-8.24 (m, 7H, Ar); 13.25 (bs, 1H, COOH)
9	3.43 (s, 3H, OCH$_3$); 8.24-8.33 (m, 3H, Ar); 7.54-7.85 (m, 4H, Ar); 8.34 (s, 1H, H$_4$ triaz.)
10	6.98-8.25 (m, 7H, Ar); 8.33 (s, 1H, H$_4$ triaz.); 10.55 (bs, 1H, OH)
11	1.26 (t, 3H, CH$_3$); 3.50(s, 3H, OCH$_3$); 4.23 (q, 2H, CH$_2$); 7.10-8.26 (m, 7H, Ar)
13	1.16 (t, 3H, CH$_3$); 3.64 (s, 3H, OCH$_3$); 4.22 (q, 2H, CH$_2$); 7.30-8.66 (m, 3H, Ar); 7.35 (s, 5H, Ph)
14	3.65 (s, 3H, OCH$_3$); 7.35 (s, 5H, Ar); 8.36-8.57 (m, 3H, Ar); 13.10 (bs, 1H, COOH)
15	3.61 (s, 3H, OCH$_3$); 7.30-7.43 (m, 5H, Ar); 8.45-8.55 (m, 3H, Ar); 8.16 (s, 1H, H$_4$ triaz.)
16	7.12-8.34 (m, 7H, Ar); 8.15 (s, 1H, H$_4$ triaz.); 11.96 (bs, 1H, OH)
18	3.49 (s, 3H, OCH$_3$); 7.21-7.77 (m, 7H, Ar); 8.17 (s, 1H, H$_4$ triaz.)
19	6.95-7.65 (m, 7H, Ar); 8.16 (s, 1H, H$_4$ triaz.); 10.52 (bs, 1H, OH)

Table 3
Experimental results: potency (pIC$_{50}$) and efficacy
(Emax %) values of the tested compounds

Comp.	Efficacy (Emax %)	pIC$_{50}$
3b	56 ± 16	6.55 ± 0.03
10	24 ± 14	N.C.
16	57 ± 11	5.58 ± 0.25
19	38 ± 7	N.C.
NS1619	100a	5.18 ± 0.05

Compounds **3a, 4-6a,b, 8, 9, 11, 13-15, 18** are ineffective.
N.C. indicates that the parameter could not be calculated because of low efficacy (<50%).
The symbol a indicates that a full vasorelaxing effect was reached in all the experiments performed, hence the standard error could not be expressed.

droxy function. These useful observations have recently led us to the synthesis and pharmacological evaluation of new potent BK-opener benzanilides, bearing an amide moiety as a new acyclic H-bond donor spacer unit between an ortho-hydroxyphenyl group and a multi-substituted phenyl ring [19].

As also follows from the literature, the other crucial structural requirements of the diphenyltriazolone derivatives [8], as well as other synthetic [1,11] or natural [20] BK-openers, such as an electron deficient phenyl ring and an ortho-hydroxy-para-chlorophenyl substituent are conserved in the target 1,2,3-triazole series.

So, it follows that these latter pharmacophoric requisites appear not to be sufficient for biological activity and to be probably dependent on the chemical nature of their spacer unit. Finally, another problem could be their poor structural flexibility, as evidenced in some papers about small molecule BK-openers [21,22] and on some of our 1,2,3-triazole derivatives [23]. In these compounds the insertion of a benzyl

rather than a phenyl substituent on the heterocyclic ring enhanced flexibility and dramatically augmented the biological activity. In fact a better BK-opener activity had been pointed out in other more flexible diphenylheterocyclic derivatives [10,24,25].

4. Experimental

4.1. Chemistry

All 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 on a Varian Gemini 2000 spectrometer in DMSO-d$_6$ in δ units, using TMS as an internal standard. Elemental analyses (C, H, N) were within + 0.4 % of the theoretical values and were performed in a Carlo Erba Elemental Analyser Mod. 1106 apparatus. Petroleum ether corresponds to the fraction boiling at 40-60°C.

4.1.1. Ethyl 1-(2'-nitro-4'-chlorophenyl)-5-phenyl-1H-1,2,3-triazole-4-carboxylate (3a)

To an ice-salt cooled and stirred solution of sodium ethoxide (from 0.460 g, 20.0 mmol of sodium) in 15 ml of absolute EtOH, a solution of 2-nitro-4-chloro-phenylazide (**1**) (2.00 g, 10.0 mmol) and ethyl benzoylacetate (**2a**) (2.32 g, 12.0 mmol) in 50 ml of absolute EtOH was slowly added. After 4 h, 80 ml of ice-water were added to the suspension and the mixture was extracted with CHCl$_3$. The combined organic layers were washed (H$_2$O), dried (MgSO$_4$) and evaporated *in vacuo* to give a solid residue (1.84 g) which was extracted with boiling petroleum ether (15 ml × 3) to dissolve the unreacted azide. The new residue, consisting of the title compound, was purified by crystallisation (Table 1). By acidification of the aqueous mother liquors, a little amount of the corresponding acid **4a** [13] precipitated.

4.1.2. Ethyl 1-(2'-nitro-4'-chlorophenyl)-5-(4'-nitro-phenyl)-1H-1,2,3-triazole-4-carboxylate (3b)

To an ice-salt cooled and stirred solution of sodium ethoxide (from 0.740 g, 32.2 mmol of sodium) in 15 ml of absolute EtOH, a solution of 2-nitro-4-chloro-phenylazide (**1**) (3.12 g, 15.7 mmol) and ethyl 4-nitrobenzoylacetate (**2b**) (4.52 g, 19.0 mmol) in 85 ml of absolute EtOH was slowly added. After 2 h the yellow solid precipitated was collected by filtration and purified by crystallisation (Table 1).

4.1.3. 1-(2'-Nitro-4'-chlorophenyl)-5-(4'-nitrophenyl)-1H-1,2,3-triazole-4-carboxylic acid (4b)

A solution of the triazolester **3b** (0.300 g, 0.72 mmol) in 4% KOH solution (22 ml of EtOH and 3 ml of H$_2$O) was stirred at room temperature for 24 h. The reaction mixture was then concentrated *in vacuo* at room temperature, diluted with H$_2$O (25 ml) and acidified with 10% HCl to precipitate **4b** as a yellow solid which was collected by filtration (Table 1).

4.1.4. 1-(2'-Nitro-4'-chlorophenyl)-5-(4'-nitrophenyl)-1H-1,2,3-triazole (5b)

A solution of the triazole acid **4b** (0.300 g, 0.77 mmol) in 1 ml of DMF was heated under reflux for 3 h. After cooling, the dark solution was diluted with H_2O and the fine precipitate was extracted with $CHCl_3$. The combined extracts were repeatedly washed with H_2O, then dried ($MgSO_4$) and evaporated *in vacuo* to give **5b** as a solid residue (Table 1).

4.1.5. 1-(2'-Amino-4'-chlorophenyl)-5-(4'-aminophenyl)-1H-1,2,3-triazole (6b)

A) A mixture of dinitrotriazole **5b** (0.345 g, 1.00 mmol) and $SnCl_2 \cdot 2 H_2O$ (1.35 g, 5.98 mmol) in 14 ml of 36% HCl and 7 ml of H_2O was refluxed for 3.5 h. After cooling, the reaction mixture was paper filtered and the filtrate was treated with an excess of 10% NaOH to precipitate the title compound **6b** which was collected by filtration and washed with H_2O (Table 1).

B) A solution of the triazole acid **5b** (0.210 g, 0.61 mmol) in 45 ml of MeOH was hydrogenated at room temperature and pressure, in the presence of 5% Pd/C (0.100 g). The catalyst was filtered off, washed with boiling MeOH and the combined filtrates were evaporated to give **6b** (Table 1).

4.1.6. 1-(2'-Methoxy-5'-chlorophenyl)-5-(4'-nitrophenyl)-1H-1,2,3-triazole-4-carboxylic acid (8)

To a cooled (-10°C) and stirred solution of sodium ethoxide (from 0.160 g, 6.96 mmol of sodium) in 10 ml of absolute EtOH, a solution of 2-methoxy-5-chloro-phenylazide (**7**) (0.700 g, 3.81 mmol) and ethyl 4-nitro-benzoylacetate (**2b**) (1.08 g, 4.58 mmol) in 30 ml of absolute EtOH was slowly added. The ice-bath was removed and stirring was continued at room temperature for 2.5 h then the reaction mixture was heated at 50°C for 9 h. The suspension obtained was then concentrated *in vacuo* and the remaining EtOH was decanted and the residue treated with crushed ice. The resulting aqueous solution was washed with $CHCl_3$ then acidified with conc. HCl to precipitate **8** as a pale yellow solid which was collected by filtration (Table 1).

4.1.7. 1-(2'-Methoxy-5'-chlorophenyl)-5-(4'-nitrophenyl)-1H-1,2,3-triazole (9)

A solution of the triazole acid **8** (0.860 g, 2.29 mmol) in 6 ml of DMF was heated under reflux for 4 h. After cooling, the dark solution was diluted with H_2O to precipitate a brown solid which was collected by filtration and dissolved in $CHCl_3$. The organic solution was washed with 10% NaOH, dried ($MgSO_4$) and evaporated *in vacuo* to give **9** as a yellow solid residue (Table 1).

4.1.8. 1-(2'-Hydroxy-5'-chlorophenyl)-5-(4'-nitrophenyl)-1H-1,2,3-triazole (10)

To a stirred solution of **9** (0.500 g, 1.52 mmol) in 35 ml of anhydrous CH_2Cl_2, cooled at -10°C and under a nitrogen flow, a solution of BBr_3 (1.0 ml, 10.6 mmol) in 8 ml of anhydrous CH_2Cl_2 was added dropwise. Stirring was continued for 1 h, 10 ml of MeOH followed by 10 ml of H_2O were added dropwise, then the reaction mixture was extracted with CH_2Cl_2. The combined extracts were washed with H_2O, dried ($MgSO_4$) and evaporated to give a solid residue which was treated with 10% NaOH. After filtration the alkaline solution was acidified with 10% HCl to precipitate the title compound which was collected by filtration (Table 1).

4.1.9. Ethyl 1-(2'-Methoxy-5'-chlorophenyl)-5-(4'-nitrophenyl)-1H-1,2,3-triazole-4-carboxylate (11)

To a solution of triazole acid **8** (0.300 g, 0.800 mmol) in 20 ml of absolute EtOH, 2 drops of conc. H_2SO_4 were added and the mixture was heated under reflux for 2 h. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in $CHCl_3$. The chloroform solution was extracted with 4% NaOH, dried ($MgSO_4$) and evaporated to give the title compound (Table 1). From the alkaline extract, by acidification, 20% of the starting acid **8** was recovered (Table 1).

4.1.10. Ethyl 1-(2'-Methoxy-5'-nitrophenyl)-5-phenyl-1H-1,2,3-triazole-4-carboxylate (13)

To cooled (-10°C) and stirred solution of sodium ethoxide (from 0.100 g, 4.35 mmol of sodium) in 10 ml of absolute EtOH, a solution of 2-methoxy-5-nitro-phenylazide (**12**) (0.600 g, 3.09 mmol) and ethyl benzoylacetate (**2a**) (0.710 g, 3.71 mmol) in 30 ml of absolute EtOH was slowly added. After 2 h the ice-bath was removed and the mixture stirred at room temperature for 6 h. The reaction mixture was concentrated *in vacuo* at room temperature and the semisolid residue was treated with crushed ice, then extracted with $CHCl_3$. The combined organic layers were dried ($MgSO_4$) and evaporated to give a yellow oil which, by trituration with a small amount of boiling petroleum ether, left the title compound as a white solid (Table 1).

4.1.11. 1-(2'-Methoxy-5'-nitrophenyl)-5-phenyl-1H-1,2,3-triazole-4-carboxylic acid (14)

To a cooled (-10C) and stirred solution of sodium ethoxide (from 0.550 g, 23.9 mmol of sodium) in 20 ml of absolute EtOH, a solution of 2-methoxy-5-nitro-phenylazide (**12**) (2.00 g, 10.3 mmol) and ethyl benzoylacetate (**2a**) (2.74 g, 14.2 mmol) in 95 ml of absolute EtOH was slowly added. The ice-bath was removed and the mixture was heated at 60°C under stirring for 10 h. The reaction mixture was concentrated *in vacuo*, the semisolid residue was treated with H_2O, then extracted with $CHCl_3$. Acidification of the aqueous alkaline solution with 10% HCl gave acid **14** as a yellow precipitate which was collected by filtration (Table 1).

4.1.12. 1-(2'-Methoxy-5'-nitrophenyl)-5-phenyl-1H-1,2,3-triazole (15)

A solution of the triazole acid **14** (0.650 g, 1.91 mmol) in 5 ml of DMF was heated under reflux for 4 h. After cooling, the solution was diluted with H_2O and the brown precipitate was collected by filtration and washed with H_2O. This solid

was then treated with 10% NaOH, the mixture was paper filtered and the filtrate was acidified to give **15** as a precipitate which was collected by filtration (Table 1).

4.1.13. 1-(2'-Hydroxy-5'-nitrophenyl)-5-phenyl-1H-1,2,3-triazole (16)

To a stirred solution of **15** (0.400 g, 1.35 mmol) in 30 ml of anhydrous CH_2Cl_2, cooled at -78°C and under a nitrogen flow, a solution of BBr_3 (0.90 ml, 9.50 mmol) in 7 ml of anhydrous CH_2Cl_2 was added dropwise. The reaction mixture was kept for a night at -20°C and 1 h at 4°C then 10 ml of MeOH and 10 ml of H_2O were added dropwise and basified with 10% NaOH. The aqueous layer was acidified with 10% HCl and extracted with $CHCl_3$. The combined extracts were dried ($MgSO_4$) and evaporated *in vacuo* to give the title compound as a solid residue (Table 1).

4.1.14. 4-Chlorobenzoyl-methylen-triphenylphosphorane (17)

To a solution of ω-bromo-acetophenone (5.00 g, 21.4 mmol) in 50 ml of anhydrous THF, triphenylphosphine (5.62 g, 21.4 mmol) was added. After few minutes a fine solid began to precipitate and after 15 min the suspension was heated under reflux for 6 h. The reaction mixture was cooled in an ice-salt bath, then the precipitate was collected by filtration. This solid (10.15 g) was treated with 500 ml of 0.25% NaOH, the suspension was stirred for a night, then the precipitate, consisting of **17**, was collected by filtration and washed with H_2O: 8.25 g, yield 93 % ; m.p. 196-198°C (lit. [18] 195-197°C).

4.1.15. 1-(2'-Methoxy-5'-chlorophenyl)-5-(4'-chlorophenyl)-1H-1,2,3-triazole (18)

A solution of 2-methoxy-5-chloro-phenylazide (**7**) (0.370 g, 2.0 mmol) and α-keto-phosphorus ylide **17** (0.830 g, 2.0 mmol) in 25 ml of anhydrous toluene was heated (80°C) for 3 days. The solvent was evaporated *in vacuo* and the brown oil residue, triturated with petroleum ether, provided a brown solid (0.590 g) which was dissolved in $CHCl_3$. This solution was washed with 10% HCl and H_2O, then dried ($MgSO_4$) and evaporated to give a new solid residue (0.500 g) which had to be purified by crystallisation (Table 1).

4.1.16. 1-(2'-Hydroxy-5'-chlorophenyl)-5-(4'-chlorophenyl)-1H-1,2,3-triazole (19)

To a stirred solution of **18** (0.320 g, 1.0 mmol) in 50 ml of anhydrous CH_2Cl_2, cooled at -70°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 added very slowly (1 h). After 3 h of stirring the reaction mixture was kept at –20°C for a night. The mixture was transferred into an ice-salt bath, 10 ml of MeOH and 10 ml of H_2O were added dropwise, stirred for 15 min, then the organic phase was separated, washed with H_2O and extracted with 5% NaOH. The combined alkaline layers were acidified (10% HCl) to precipitate the title com-

pound as a white solid which was collected by filtration (Table 1).

4.2. Pharmacology

All the experimental procedures were carried out following the guidelines of the European Community Council Directive 86-609. To determine a possible vasodilator mechanism of action, the compounds were tested on isolated thoracic aortic rings of male normotensive Wistar rats (250-350 g). The rats were sacrificed by cervical dislocation under light ether anaesthesia and bled. 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; $MgSO_4$ $7H_2O$ 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 to a unirecord microdynamometer (Buxco Electronics).

After an equilibration period of 60 minutes, 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 endothelium), were discarded. From 30 to 40 minutes after confirmation of the endothelium removal, the aortic preparations were contracted by treatment with a single concentration of KCl (20 mM) and when the contraction reached a stable plateau, 3-fold increasing concentrations of the 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 minutes. In other sets of experiments, the non-selective potassium channel blocker TEA (10 mM) was added, after 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 nM) in EtOH 95% and further diluted in bidistilled water whereas KCl and TEA were both dissolved in Tyrode solution. Some of the synthesised derivatives (**4b, 10, 16, 19**) were dissolved (10 mM) in NaOH 0.1 N whereas all the other tested compounds in DMSO (10 mM); they were all further diluted in Tyrode solution. All the solutions were freshly prepared immediately before 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 a 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 the negative Logarithm of the molar concentration of the tested compounds, evoking a half reduction of the contractile tone induced by KCl 20 mM. The pIC_{50} could not be calculated for those compounds showing an efficacy parameter lower than 50%. The parameters of efficacy and potency were expressed as mean ± standard error, for 5-10 experiments. Student t test was selected as statistical analysis, $P < 0.05$ was considered representative of a significant statistical difference. Experimental data were analysed by a computer fitting procedure (software: Graph-Pad Prism 3.0).

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