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Some structural changes on triazolyl-benzotriazoles and triazolyl-benzimidazolones as potential potassium channel activators. III

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Abstract

This paper reports the synthesis and pharmacological evaluation of some compounds, obtained by structural modifications of 1,2,3-triazolyl-benzotriazoles and 1,2,3-triazolyl-benzimidazolones, which had shown activity as potential activators of the big-conductance calcium-activated potassium channels (BK_{Ca}). Changes have concerned the introduction of a hinderer substituent in the 5-position of the benzimidazolone (**4a**, **b**) and benzotriazole (**5a**, **b**) rings, opening of the benzimidazolone ring (**7**) and substitution of the 1,2,3-triazole ring with a 2-hydroxyphenyl ring (**10**). Furthermore a series of 3-aryl-benzotriazin-4-one derivatives (**13a**–**e**) has been studied, which appears as a modification and/or combination of the benzimidazolone and benzotriazole rings. Only compound **10** shows interesting activity, while the other structural modifications either do not increase (compounds **4** and **5**) or reduce (compounds **7** and **13**) the pharmacological activity. However, these results provide useful information about structure–activity relationships.

Keywords: 1,2,3-Triazoles; Benzimidazolones; Benzotriazoles; Benzotriazin-4-ones; Potassium channels

1. Introduction

In a previous paper [1] we reported the synthesis and pharmacological evaluation as potassium channel activators, of a series of 1-triazolyl-benzimidazolones (A) and of the corresponding series of 1-triazolyl-benzotriazoles (B), bearing a substituent (Me, OMe, Cl, F and CF_3) in the 5-position (Fig. 1). These compounds had been prepared for their clear structural correlation with the benzimidazolone derivatives NS 004 and NS 1619 (Fig. 1), two known activators of the big conductance calcium-activated potassium channel subtype (BK_{Ca}) [2], considered an interesting target for therapeutic approaches in several cardiovascular, central nervous system and respiratory pathologies.

The pharmacological evaluation carried out on isolated vascular smooth muscle preparations had shown that these derivatives possessed a good vasodilator activity, probably induced by the potassium channel activation. The triazolyl-benzotriazole derivatives showed an effectiveness generally higher than that of the corresponding triazolyl-benzimidazolones and the activity of some compounds was comparable to that of NS 1619 tested as standard.

On the basis of these results, we took into consideration the introduction of some structural changes on the triazolyl-benzimidazolone (A) or triazolyl-benzotriazole (B) derivatives, in order to increase their effectiveness and/or potency towards potassium channels and to deduce some useful structure–activity relationships.

Thus, a methyl substituent in the 5-position of the benzimidazolone or benzotriazole ring [1] appeared more effective than the other experimented substituents (Cl, F, CF_3, OMe); therefore the first structural change consisted in an increase of both the steric hindrance and the lipophilicity of this substituent, by the introduction of two new substituents such as a *sec*-butyl and a phenyl group.

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In addition, a formal opening of the benzimidazolone ring led to the design of *N*-phenyl-*N*′-triazolyl urea moiety; while, the change of the 1,2,3-triazole substituent in the 1-position of the benzotriazole ring with a 2-hydroxyphenyl substituent furnished a 1-(2′-hydroxy)-phenyl-benzotriazole, closely related to the reference compounds NS 004 and NS 1619.

Finally, the replacement of the benzotriazole and benzimidazolone heterocycles by a benzotriazinone nucleus represented a structural change, possessing a partial combination of the two fundamental structures (benzotriazole and benzimidazolone) by an enlargement of the heterocyclic ring.

2. Chemistry

Starting from the 4-carboxamido-5-(4-*sec*-butyl-2-aminoanilino)-1,2,3-triazole (**3a**) (Scheme 1), previously described in the literature [3], the desired 5-*sec*-butyl-substituted derivatives **4a** and **5a** were obtained in good yield. The benzimidazolone compound **4a** was prepared by the reaction of **3a** with phosgene in pyridine solution, whilst the analogous benzotriazole compound **5a** by the diazotisation reaction of **3a**. For the synthesis of the corresponding 5-phenylsubstituted derivatives **4b** and **5b**, at first the new 2-nitro-biphenylazide (**1b**) was prepared by diazotisation of 2-nitro-biphenylamine [4] and treatment of the diazonium salt with sodium azide. The 1,3-dipolar cycloaddition reaction of **1b** to cyanacetamide provided the expected 5-(2-nitrobiphenylamino)-1,2,3-triazole derivative **2b**, which was reduced to the corresponding 5-(2-aminobiphenylamino)-1,2,3-triazole derivative **3b**, by catalytic hydrogenation. Starting from **3b**, as described above for **3a**, the 5-phenyl-benzimidazolone derivative **4b** was obtained by the reaction with phosgene, whilst the 5-phenyl-benzotriazole **5b** was obtained by a diazotisation reaction.

The 4-carboxamido-5-amino-1,2,3-triazole (**6**) [5], obtained in low yield from azidoformate and cyan-

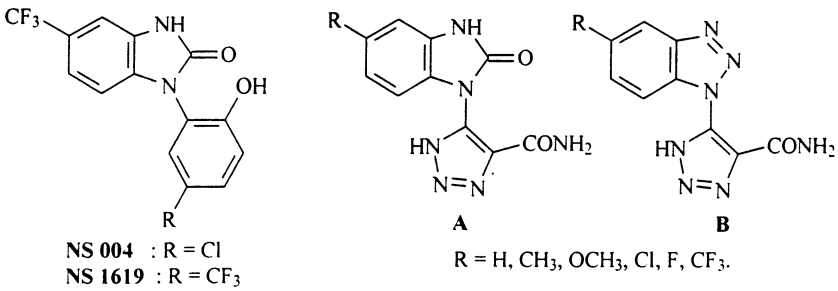
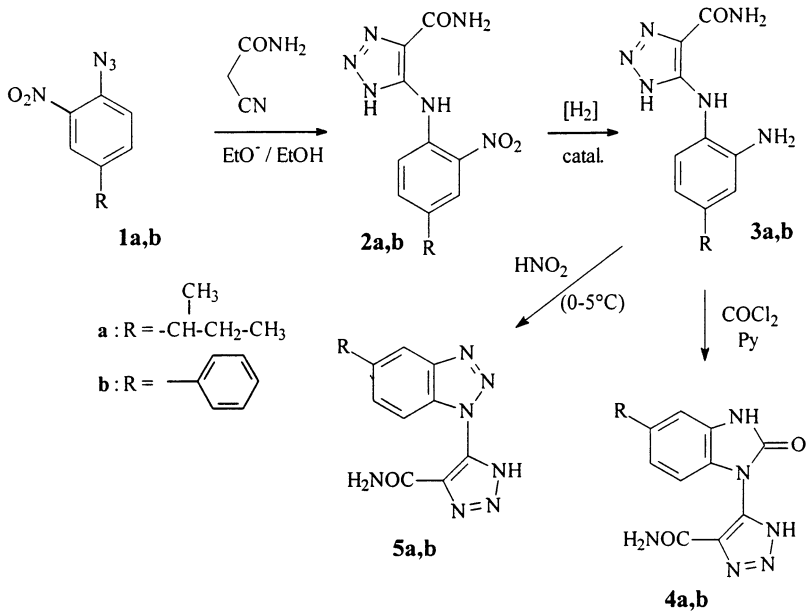
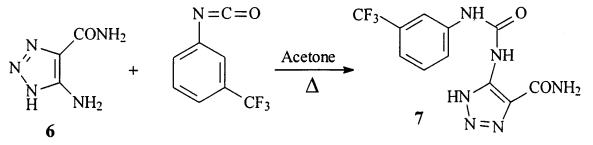


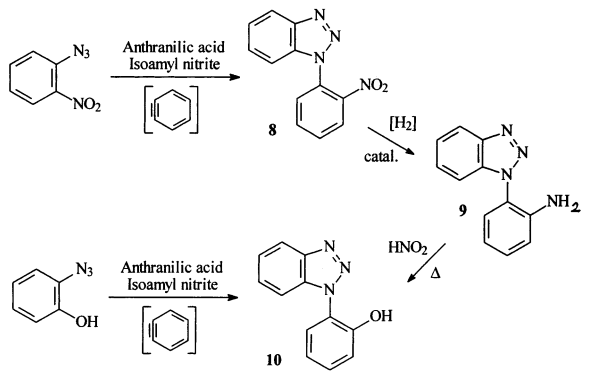
Fig. 1. Comparison and previously prepared compounds.



Scheme 1.



Scheme 2.



Scheme 3.

acetamide, was reacted with *meta*-trifluoromethyl-phenylisocyanate in refluxing acetone (Scheme 2). The expected asymmetrical disubstituted urea **7** was isolated in 31% yield together with the symmetrical *N*,*N*′-(*meta*-trifluoromethyl-phenyl)-urea as a by-product.

The 1-(2-hydroxyphenyl)-benzotriazole (**10**) was prepared by the following two routes (Scheme 3). The 2-nitrophenylazide [6] reacted with benzine, obtained in situ from anthranilic acid and isoamyl nitrite [7], to give the 1-(2-nitrophenyl)-benzotriazole (**8**) which was converted to the corresponding aminoderivative **9** by catalytic hydrogenation. The thermic decomposition of **9** diazonium salt in aqueous sulfuric acid provided the expected phenolic derivative **10** in 28% yield. The same compound was also obtained in 54% yield by a direct reaction between 2-hydroxyphenylazide [8] and benzine [7].

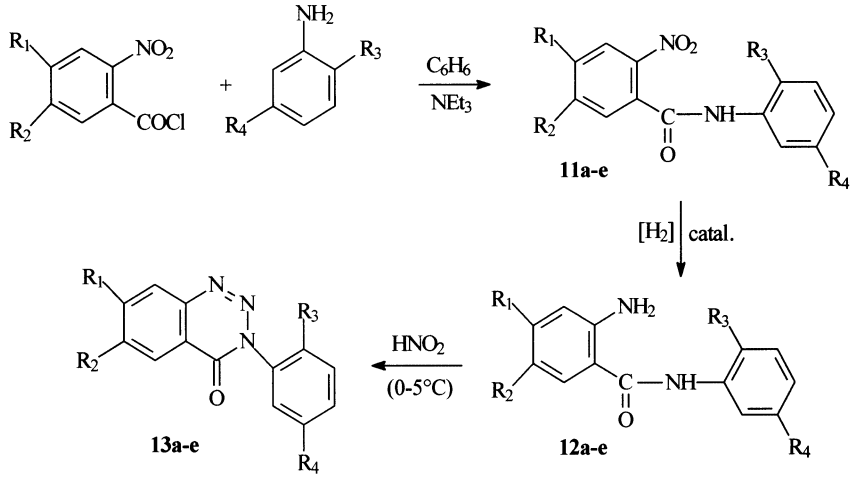
In Scheme 4 the synthesis of a series of 3-arylsubstituted-benzotriazin-4-one derivatives **13a**–**e** is reported. The synthesis was carried out according to a procedure described in the literature [9,10].

Thus the appropriate 2-nitrobenzoyl chloride in refluxing benzene solution reacted with the suitable aniline to give the expected anilides **11a**–**e** in good yields. These nitroanilides were reduced to the corresponding aminoanilides **12a**–**e**, by catalytic hydrogenation at room temperature and pressure. Finally, by a diazotisation reaction, the aminoanilides **12a**–**e** underwent an intramolecular cyclisation to give the expected 3-aryl-benzotriazin-4-ones **13a**–**e** in high yield.

The structures of all the new compounds were assigned on the basis of the well-known reaction mechanisms [9–12], our previous evidence [1,3,13,14] and were confirmed by analytical and spectroscopic methods. ^{1}H NMR spectra of the compounds tested are reported in Table 3.

3. Pharmacology

Large conductance calcium-activated potassium channels (BK_{Ca}) play an essential function in modulating the vascular smooth muscle tone, as experimentally shown in different circulatory districts, such as the



	R_1	R_2	R_3	R_4
a	H	H	OH	CH_3
b	H	H	COOH	Cl
c	Cl	H	H	H
d	Cl	H	OH	H
e	H	Cl	H	H

Scheme 4.

Table 1
Physico-chemical properties of compounds **4**, **5**, **7**–**10**

Comp.	Yield (%)	Crystal solvent	M.p. (°C)	Analysis	Mass m/z	
					M^+	Base
4a	39	DMF–H_2O	203–205	$C_{14}H_{16}N_6O_2$	300	161
4b	53	AcOEt	150–153	$C_{16}H_{12}N_6O_2$	320	210
5a	66	MeOH	140–143	$C_{13}H_{15}N_7O$	229 ($M^+ - Bu$)	44
5b	72	MeOH–H_2O	190–196	$C_{15}H_{11}N_7O$	305	234
7	31	EtOH–H_2O	>350	$C_{11}H_9N_6O_2F_3$	314	161
8	48	*i*-PrOH	120–121	$C_{12}H_8N_4O_2$	240	50
9	90	MeOH	130–132	$C_{12}H_{10}N_4$	210	39
10	[a]	EtOH–H_2O	196–198	$C_{12}H_9N_3O$	211	154

[a] The yields are 28 and 54% with the procedures A and B, respectively.

Table 2
Physico-chemical properties of compounds **11**–**13a**–**e**

Comp.	Yield (%)	Crystal solvent	M.p. (°C)	Analysis	Mass m/z	
					M^+	Base
11a	81	MeOH	154–156	$C_{14}H_{12}N_2O_4$	272	122
11b	62	MeOH	262–265	$C_{14}H_9N_2O_5Cl$	320	150
11c	81	MeOH–H_2O	161–162	$C_{13}H_9N_2O_3Cl$	276	92
11d	60	EtOH–H_2O	215–216	$C_{13}H_9N_2O_4Cl$	292	108
11e	84	MeOH–H_2O	163–164	$C_{13}H_9N_2O_3Cl$	276	92
12a	95	MeOH–H_2O	131–134	$C_{14}H_{14}N_2O_2$	242	120
12b	93	MeOH–H_2O	234–236	$C_{14}H_{11}N_2O_3Cl$	290	120
12c	98	MeOH–H_2O	133–135	$C_{13}H_{11}N_2OCl$	246	92
12d	90	MeOH–H_2O	136–138	$C_{13}H_{11}N_2O_2Cl$	262	154
12e	95	MeOH–H_2O	150–153	$C_{13}H_{11}N_2OCl$	246	92
13a	95	EtOH	189–190	$C_{14}H_{11}N_3O_2$	253	105
13b	96	EtOH–H_2O	165–175 dec	$C_{14}H_8N_3O_3Cl$	301	121
13c	93	MeOH	184–187	$C_{13}H_8N_3OCl$	257	77
13d	87	EtOH	205–207	$C_{13}H_8N_3O_2Cl$	273	245
13e	93	MeOH	176–179	$C_{13}H_8N_3OCl$	257	77

rabbit pulmonary artery [15], the rat portal vein [16] and the rat aorta [17]. Indeed, the membrane hyperpolarisation induced by an opening of outward potassium channels is a crucial factor which determines the inactivation of voltage-operated calcium channels, the lowering of the concentration of free intracellular calcium and consequently a vasorelaxing effect. Thus, the functional evaluation of a vasorelaxing activity of the compounds tested on isolated rat aortae was chosen as a preliminary screening method, to unmask a possible potassium channel opening effect.

4. Results and discussion

In agreement with the previous experimental observations about the pharmacological properties of triazolyl-benzimidazolones and triazolyl-benzotriazoles [1], compounds **4a**, **b** and **5a**, **b** showed vasorelaxing effects (Table 4). The biological responses to the benzotriazoles **5a**, **b** consisted of an almost full abolition of the contractile tone induced by the depolarising stimulus (administration of 20 mM KCl), with potency orders of magnitude a little lower than that recorded for the reference compound NS 1619. Also the benzimidazolone **4b** induced an almost full vasorelaxation, but with a lower level of potency. Compound **4a** showed a very low efficacy ($\approx 50\%$) that made it impossible to calculate the potency value. The differences of activity between the two etherocyclic rings agreed with the previous results [1]. A preliminary investigation of the potential potassium channel opening mechanism of action was also performed on the isolated vessels. It is widely known that drugs, acting through potassium channel activation, undergo a dramatic reduction of potency and efficacy, when tested under experimental conditions of increased membrane depolarisation. This 'depolarisation-sensitive' vasorelaxing activity is a typical and characteristic profile of potassium channel openers, among the several classes of vasodilators [18].

Table 3
^{1}H NMR data (δ, ppm) in DMSO-d_6 for the compounds tested

4a	0.80–2.78 (m, 9H, *sec*-Bu), 7.10–8.14 (m, 5H, benzimidazolone + NH_2), 10.1 (NH)
4b	6.97–8.10 (m, 10H, Ph + benzimidazolone + NH_2), 8.8 and 10.7 (NH)
5a	0.72–2.95 (m, 9H, *sec*-Bu), 7.47–8.24 (m, 3H, benzotriazole), 7.25 (NH_2), 9.4 (NH)
5b	7.03–8.20 (m, 10H, Ph + benzotriazole + NH_2), 12.2 (NH)
7	7.43 (dd, 1H, 4-H), 7.53 (d, 1H, 2-H), 8.06 (d, 1H, 5-H), 9.68, 10.5, 15.2 (NH and NH_2)
10	7.00–7.28 (m, 2H, substituent), 7.41–7.68 (m, 5H, benzotriazole + substit.), 8.17 (m, 1H, benzotriazole), 10.4 (OH)
13a	6.90–7.24 (m, 3H, substituent), 7.90–8.33 (m, 4H, benzotriazine), 2.28 (s, 3H, CH_3), 9.7 (OH)
13b	7.76–8.38 (m, 7H, benzotriazine + substit.), 13.3 (COOH)
13c	7.53–7.68 (m, 5H, Ph), 8.01 (dd, 1H, 6-H), 8.31 (d, 1H, 5-H), 8.42 (d, 1H, 8-H)
13d	6.92–7.10 (m, 2H, substituent), 7.32–7.46 (m, 2H, substit.), 8.00 (dd, 1H, 6-H), 8.29 (d, 1H, 5-H), 8.41 (d, 1H, 8-H), 10.0 (OH)
13e	7.51–7.69 (m, 5H, Ph), 8.17 (dd, 1H, 7-H), 8.29 (d, 1H, 5-H), 8.31 (d, 1H, 8-H)

Therefore, compounds **4a**, **b** and **5a**, **b** were also tested on isolated aortae, whose contractile tone was induced by a higher level of membrane depolarisation (due to the administration of 60 mM KCl). Under these conditions, significant decreases of potency and efficacy could be observed, fitting the profile of response expected for a potassium channel opener. Moreover, the responses induced by compounds **5a**, **b** were markedly antagonised by the potassium channel blocker tetraethylammonium chloride (TEA, Table 4). As previously observed, the reference compound NS 1619 did not show any significant decrease of activity, when administered to preparations pre-contracted by 60 mM KCl. However, this anomalous behaviour is probably due to different ancillary mechanisms of actions, whose existence has already been suggested by the literature [17]. It is interesting to observe that the potency values of *sec*-butyl **5a** and phenyl **5b** substituted benzotriazoles were almost comparable; furthermore, they were not significantly different from that previously recorded for the methyl-substituted benzotriazole ($pIC_{50} = 4.86 \pm 0.21$; $E_{max} = 100$) [1].

The evaluation of the pharmacological properties of the asymmetrical disubstituted urea **7** suggested that the formal opening of the benzimidazolone ring led to a substantial decrease of activity.

Compound **10**, bearing the replacement of the triazolyl group in the 1-position of the benzotriazole moiety with a 2-hydroxyphenyl substituent, showed good vasorelaxing properties with full efficacy and satisfactory potency. Indeed, **10** was almost tenfold more potent than the analogous triazolyl-benzotriazole (with a hydrogen atom in the 5-position) previously tested ($pIC_{50} = 3.95 \pm 0.059$; $E_{max} = 100$) [1] and its activity was significantly reduced both by high levels of membrane depolarisation (60 mM KCl) and by TEA, indicating the possibly pharmacodynamic profile of potassium channel openers (Table 4).

On the contrary, all the benzotriazinone compounds **13a**–**e** failed to induce a significant vasorelaxing response, indicating a possible strong incompatibility between this heterocyclic ring and the required pharmacophoric model, well represented by the benzotriazole nucleus.

5. Experimental

5.1. Chemistry

Melting points (m.p.) were determined on a Kofler hot-stage and are uncorrected. IR spectra in Nujol

Table 4
Pharmacological effects of the tested compounds

Comp.	20 mM KCl		60 mM KCl		20 mM KCl + 1 mM TEA	
	pIC_{50}	Eff. (%)	pIC_{50}	Eff. (%)	pIC_{50}	Eff. (%)
4a	NC	54 ± 3	NC	20 ± 5	not tested	
4b	4.26 ± 0.16	100	3.24 ± 0.021	75 ± 2	not tested	
5a	5.01 ± 0.064	83 ± 1	4.41 ± 0.050	73 ± 2	4.63 ± 0.061	96 ± 3
5b	5.06 ± 0.084	100	3.76 ± 0.055	81 ± 3	4.51 ± 0.070	93 ± 4
7	4.10 ± 0.056	67 ± 1	NC	46 ± 1	not tested	
10	4.75 ± 0.036	100	4.07 ± 0.043	90 ± 2	4.26 ± 0.056	95 ± 3
13a–e	ineffective					
NS 1619	5.37 ± 0.14	100	5.41 ± 0.11	100	not tested	

The values of potency (pIC_{50}) are expressed as the mean ± SEM. The efficacy parameter indicates the maximal vasorelaxation, as a percentage of the contractile tone. The values of potency and efficacy, under a standard membrane depolarisation (20 mM KCl), under increased depolarisation (60 mM KCl), and in the presence of TEA are shown. The potency parameters of compounds possessing a low efficacy ($\approx 50\%$ or lower) could not be calculated (NC).

mulls were recorded in a Mattson Genesis series FTIR spectrometer. ^{1}H NMR spectra were recorded with a Varian Gemini 2000 spectrometer in DMSO-d_6 in δ units, using TMS as an internal standard. Mass spectra were performed with a Hewlett Packard MS/System 5988. Elemental analyses (C, H, N) were within $\pm 0.4\%$ of theoretical values and were performed in a Carlo Erba Elemental Analyser Mod. 1106 apparatus. TLC data were obtained with Riedel de Haen, 37360 DC-Karten $F_{254,}$ 0.2 mm, eluting with a ethyl acetate–petroleum ether 1:3 mixture. Petroleum ether corresponds to the fraction boiling at 40–60 °C.

*5.1.1. 2-Nitro-biphenylazide (**1b**)*

To an ice-cooled and stirred solution of 2-nitro-biphenylamine [4] (2.00 g, 9.34 mmol) in 100 ml of 75% H_2SO_4, a solution of $NaNO_2$ (0.84 g, 12.2 mmol) in 10 ml of H_2O was added. After 30 min, a solution of NaN_3 (0.79 g, 12.2 mmol) in 10 ml of H_2O was added drop by drop to the clear solution and stirring was continued for 2 h. The reaction mixture was diluted with H_2O, the acidity was reduced (pH 3–4) by addition of solid NaOH and after 1 h of stirring, **1b** was collected by filtration: 1.42 g, yield 63%; m.p. 102–104 °C from MeOH–H_2O. IR (ν, cm^{-1}): 2129 (N_3); 1531 and 1352 (NO_2). MS; m/z: 212 [$M^+ - N_2$], 102 [base peak]. *Anal.* ($C_{12}H_8N_4O_2$) C, H, N.

*5.1.2. 4-Carboxamido-5-(2-nitro-biphenylamino)-1,2,3-triazole (**2b**)*

To a stirred solution of sodium ethoxide (0.124 g, 5.39 mmol of Na) in 70 ml of absolute ethanol, 0.454 g (5.40 mmol) of cyanacetamide was added. After 20 min, the suspension was cooled in an ice-bath (-10 °C) and 1.00 g (4.16 mmol) of **1b** in 250 ml of absolute ethanol was added drop by drop keeping the temperature < 0 °C. After 1 h the ice-bath was removed and stirring continued at room temperature for 24 h. The solvent was evaporated under reduced pressure, H_2O and 8–10 ml of 10% NaOH were added and the mixture was heated under reflux for 20–30 min. After cooling, the precipitated 2-nitro-biphenylamine (0.73 g) was filtered off and the filtrate was acidified (pH 1–2) to give **2b** as an orange solid which was collected by filtration: 0.337 g, yield 25%; m.p. 162–166 °C from MeOH–H_2O. IR (ν, cm^{-1}): 3220 (NH); 1666 (CO); 1528 and 1376 (NO_2). MS; m/z: 324 [M^+], 221 [base peak]. *Anal.* ($C_{15}H_{12}N_6O_3$) C, H, N.

*5.1.3. 4-Carboxamido-5-(2-amino-biphenylamino)-1,2,3-triazole (**3b**)*

To a solution of **2b** (0.200 g, 0.62 mmol) in 100 ml of MeOH, 10% Pd/C (0.020 g) was added and the mixture was hydrogenated at room temperature and pressure. The catalyst was filtered off, washed with hot MeOH and the filtrate was evaporated in vacuo to give the title compound: 0.168 g, yield 92%; m.p. 181–185 °C from MeOH–H_2O. IR (ν, cm^{-1}): 3341 and 3189 (NH_2); 1662 (CO). MS; m/z: 294 [M^+], 44 [base peak]. *Anal.* ($C_{15}H_{14}N_6O$) C, H, N.

*5.1.4. 1-(5-Carboxamido-1,2,3-triazol-4-yl)-5-sec-butyl-benzimidazolone (**4a**) and 1-(5-carboxamido-1,2,3-triazol-4-yl)-5-phenyl-benzimidazolone (**4b**)*

To an ice-cooled (0–5 °C) and stirred solution of 1.0 mmol of the appropriate compound **3a** or **3b** in 6 ml of anhydrous pyridine, a solution of 20% phosgene in toluene (0.8 ml, $\cong 1.5$ mmol) was added. The reaction mixture was stirred for 2 h, then the ice-bath was removed and stirring continued for 20 h. Dilution with H_2O and acidification (pH 3) with 10% HCl, caused precipitation of the title compounds which were collected by filtration after 2–3 h of further stirring (Table 1).

*5.1.5. 1-(5-Carboxamido-1,2,3-triazol-4-yl)-5-sec-butyl-benzotriazole (**5a**)*

To an ice-cooled (0–5 °C) and stirred solution of **3a** (0.275 g, 1.0 mmol) in 20 ml of 18% HCl, a solution of $NaNO_2$ (0.084 g, 1.2 mmol) in 10 ml of H_2O was added drop by drop. After 20 min, the ice-bath was removed and stirring continued at room temperature for 2 h. The precipitate obtained, consisting of the title compound, was collected by filtration and washed with H_2O (Table 1).

*5.1.6. 1-(5-Carboxamido-1,2,3-triazol-4-yl)-5-phenyl-benzotriazole (**5b**)*

To an ice-cooled (0–5 °C) and stirred solution of **3b** (0.294 g, 1.0 mmol) in 20 ml of 50% H_2SO_4, a solution of $NaNO_2$ (0.084 g, 1.2 mmol) in 10 ml of H_2O was added drop by drop. After 20 min, the ice-bath was removed and stirring continued at room temperature for 3 h. Solid $NaHCO_3$ was added to decrease the solution acidity (pH 3–4) and the precipitate obtained, consisting of the title compound, was collected by filtration and washed with H_2O (Table 1).

*5.1.7. N-(3-Trifluoromethylphenyl)-N′-(5-carboxamido-1,2,3-triazol-4-yl)-urea (**7**)*

To a solution of 3-trifluoromethyl-phenylisocyanate (0.630 g, 3.40 mmol) in 10 ml of anhydrous acetone, 0.432 g (3.40 mmol) of **6** was added and the mixture was refluxed for 10 h. The solvent was evaporated in vacuo and the solid residue was stirred with 10% NaOH ($\cong 20$ ml). The insoluble material consisting of di-(3-trifluoromethyl-phenyl)-urea was filtered off and the filtrate was acidified (pH 3) to precipitate **7** which was collected by filtration (Table 1).

*5.1.8. 1-(2-Nitrophenyl)-benzotriazole (**8**)*

A solution of 2-nitrophenylazide (1.64 g, 10.0 mmol) and isoamyl nitrite (1.6 ml, 12.0 mmol) in 50 ml of $CHCl_3$ was heated under reflux for 2 h. A solution of anthranilic acid (1.51 g, 11.0 mmol) in 15 ml of anhydrous acetone was slowly added drop by drop to the boiling solution ($\approx$ 2 h). The solvent was evaporated in vacuo and the tarry residue was extracted three times with 50 ml portions of boiling petroleum ether to remove unreacted azide ($\approx$ 3 h). The treatment of the new residue with 10–15 ml of isopropanol caused precipitation of the title compound as an orange solid which was collected by filtration (Table 1).

*5.1.9. 1-(2-Aminophenyl)-benzotriazole (**9**)*

To a solution of **8** (0.350 g, 1.5 mmol) in 30 ml of MeOH, 0.035 g of 10% Pd/C was added and the mixture was hydrogenated at room temperature and pressure. The catalyst was filtered off and the filtrate was evaporated to give the title compound (Table 1).

*5.1.10. 1-(2-Hydroxyphenyl)-benzotriazole (**10**)*

5.1.10.1. Procedure A. To a cooled (0–5 °C) and stirred solution of **9** (0.100 g, 0.5 mmol) in 10 ml of 40% H_2SO_4, a solution of $NaNO_2$ (0.041 g, 0.6 mmol) in 2–3 ml of H_2O was added drop by drop ($\approx$ 15 min). After 20 min of stirring the mixture was paper filtered and the filtrate was slowly heated up to 80 °C. When the evolution of N_2 gas ceased, the solution was extracted with $CHCl_3$ which in turn was extracted with 5% NaOH solution. Acidification (pH $\approx$ 4) of the alkaline layer with 36% HCl provided a precipitate which was isolated by extraction with $CHCl_3$. Evaporation of the solvent gave the title compound (Table 1).

5.1.10.2. Procedure B. A solution of 2-hydroxyphenylazide (3.50 g, 26.0 mmol) and isoamyl nitrite (4.2 ml, 31.0 mmol) in 100 ml of $CHCl_3$ was heated under reflux for 2 h. A solution of anthranilic acid (3.92 g, 28.6 mmol) in 40 ml of anhydrous acetone was slowly added drop by drop to the boiling solution ($\approx$ 2 h). The solvent was evaporated in vacuo and the tarry residue was extracted three times with 50 ml portions of boiling petroleum ether to remove unreacted azide ($\approx$ 2 h). The new residue was afterwards extracted five times with ether portions under reflux (300 ml, $\approx$ 3 h). The black residue was filtered off and the combined filtrates were evaporated to give a viscous reddish liquid which was dissolved in $\approx$ 30 ml EtOAc. This solution was filtered through a silica gel column and the filtrate was evaporated to give a semisolid residue which was triturated with 5–6 ml of ether. The title compound separated as a pale yellow solid which was collected by filtration (Table 1).

*5.1.11. 2-Nitrobenzoic acid-(2-hydroxy-5-methyl-anilide) (**11a**) and 2-nitro-4-chloro-benzoic acid-(2-hydroxy-anilide) (**11d**)*

A suspension of 10.0 mmol of the appropriate dried acid (2-nitrobenzoic or 2-nitro-4-chlorobenzoic) in 5 ml of $SOCl_2$ was heated under reflux for 2 h. The solvent was evaporated in vacuo and the residue, consisting of the acid chloride, was dissolved in 30 ml of anhydrous benzene. Et_3N (5 ml) and 12 mmol of the suitable phenolamine (2-hydroxy-5-methyl-aniline or 2-hydroxy-aniline, respectively) were added and the mixture was refluxed for 4 h. The solution was extracted with 10% HCl, 5% $NaHCO_3$ and 10% NaOH. The alkaline extract was paper filtered then acidified to precipitate the title compounds which were collected by filtration and washed with H_2O (Table 2).

*5.1.12. 2-Nitrobenzoic acid-(2-carboxy-5-chloro-anilide) (**11b**)*

A suspension of 0.835 g (5.0 mmol) of dried 2-nitrobenzoic acid in 4 ml of $SOCl_2$ was heated under reflux for 2 h. The solvent was evaporated in vacuo and the residue, consisting of the acid chloride, was dissolved in 30 ml of anhydrous benzene. Et_3N (6 ml) and 0.943 g (5.5 mmol) of 2-carboxy-5-chloro-aniline were added and the mixture was refluxed for 16 h. After one night the precipitate was collected by filtration, stirred for 1 h with 10% HCl and the insoluble material, consisting of **11b**, was collected and washed with H_2O (Table 2).

*5.1.13. 2-Nitro-4-chloro-benzanilide (**11c**) and 2-nitro-5-chloro-benzanilide (**11e**)*

A suspension of 2.02 g (10.0 mmol) of the suitable nitroacid (2-nitro-4-chlorobenzoic or 2-nitro-5-chlorobenzoic) in 10 ml of $SOCl_2$ was heated under reflux for 2 h. The solvent was evaporated in vacuo and the residue, consisting of the respective acid chloride, was dissolved in 40 ml of anhydrous benzene. Aniline (4.5 ml, 49.4 mmol) was added and the mixture was refluxed for 4 h. After one night the precipitate was collected by filtration and worked up as described for the preparation of **11b** (Table 2).

*5.1.14. 2-Aminobenzoic acid-(2-hydroxy-5-methyl-anilide) (**12a**) and 2-aminobenzoic acid-(2-carboxy-5-chloro-anilide) (**12b**)*

To a solution of 1.80 mmol of **11a** or **11b** in 100 ml and 300 ml, respectively, of MeOH, $\cong$ 0.100 g of wet Raney Ni was added and the mixture was hydrogenated at room temperature and pressure. The catalyst was filtered off, washed with hot MeOH and the filtrate was evaporated to give the title compounds (Table 2).

*5.1.15. 2-Amino-4-chloro-benzanilide (**12c**), 2-amino-4-chloro-benzoic acid-(2-hydroxy-anilide) (**12d**) and 2-amino-5-chloro-benzanilide (**12e**)*

To a solution of 2.50 mmol of the appropriate nitroderivative (**11c**, **11d** or **11e**) in 60–70 ml of MeOH, ≅0.100 g of wet Raney Ni was added. The mixture was then hydrogenated and worked up as described for the preparation of **12a** (Table 2).

*5.1.16. 3-(2-Hydroxy-5-methyl-phenyl)-benzotriazin-4-one (**13a**) and 3-(2-hydroxy-phenyl)-7-chloro-benzotriazin-4-one (**13d**)*

To an ice-cooled (0–5 °C) and stirred suspension of 2.00 mmol of the suitable 2-amino-benzanilide (**12a** or **12d**) in 50 ml of 10% HCl, a solution of 0.145 g (2.1 mmol) of $NaNO_2$ in 6 ml of H_2O was added drop by drop. After 4 h the precipitate newly formed, consisting of the title compounds, was collected by filtration and washed with H_2O (Table 2).

*5.1.17. 3-(2-Carboxy-5-methyl-phenyl)-benzotriazin-4-one (**13b**), 3-phenyl-7-chloro-benzotriazin-4-one (**13c**) and 3-phenyl-6-chloro-benzotriazin-4-one (**13e**)*

To an ice-cooled (0–5° C) and stirred suspension of 2.00 mmol of the suitable 2-amino-benzanilide (**12b**, **12c** or **12e**) in 16 ml of 18% HCl, a solution of 5.0, 3.4 and 3.6 mmol, respectively, of $NaNO_2$ in 12 ml of H_2O was added drop by drop. The reaction was then worked up as described for the preparation of **13a** (Table 2).

5.2. 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 aortae of male normotensive Wistar rats (250–350 g).

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

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

Preliminary experiments showed that both the KCl (20 and 60 mM)-induced contractions remained constant in a stable tonic state for at least 40 min.

In other sets of experiments, the potassium channel blocker tetraethylammonium chloride (1 mM) was added, before the KCl (20 mM)-induced contraction, followed by the administration of selected compounds. Compounds **13c** and **13e** were dissolved (1 mM) in ethanol and further diluted in bi-distilled water.

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

5.2.1. Data analysis

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

The parameter of potency of the vasorelaxing effects was expressed as pIC_{50}, representing the negative logarithm of the vasodilator molar concentration determining a half reduction of the contractile tone induced by the contractile agent. The above parameters were calcu-

lated by means of non-linear regression analysis of the sigmoidal concentration–response curves (computer program: GRAPHPAD PRISM), and were expressed as the mean ± SEM of four to six experiments.

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

References

[1] B. Baragatti, G. Biagi, V. Calderone, I. Giorgi, O. Livi, E. Martinotti, V. Scartoni, Triazolyl-benzimidazolones and triazolyl-benzotriazoles: new potential potassium channel activators. II, Eur. J. Med. Chem. 35 (2000) 949–955.

[2] S.P. Olesen, E. Munch, P. Moldt, J. Drejer, Selective activation of Ca(2+)-dependent K+ channels by novel benzimidazolone, Eur. J. Pharmacol. 251 (1994) 53–59.

[3] G. Biagi, V. Calderone, I. Giorgi, O. Livi, V. Scartoni, B. Baragatti, E. Martinotti, 5-(4′-Substituted-2′-nitroanilino)-1,2,3-triazoles as new potential potassium channel activators. I, Eur. J. Med. Chem. 35 (2000) 715–720.

[4] F. Fichter, A. Sulzberger, Ueber das phenyl-benzochinon und einige derivate des byphenyls, Berichte 37 (1904) 878–881.

[5] J.R.E. Hoover, A.R. Day, Metabolite analogs. VI. Preparation of some analogs of 4-amino-5-imidazole-carboxamide, J. Am. Chem. Soc. 78 (1956) 5832–5836.

[6] P.A.S. Smith, J.H. Boyer, Benzofurazan oxide, in: R.S. Schreiber (Ed.), Organic Syntheses, Wiley, New York, 1951, pp. 14–16.

[7] G.A. Reynolds, The reaction of organic azides with benzine, J. Org. Chem. 29 (1964) 3733–3734.

[8] L.K. Dyall, G. L'Abbè, W. Dehaen, Rates of thermolysis of azidobenzenes in solutions: large stabilizations of transition states by charge transfer from electron-donor substituents, J.Chem. Soc., Perkin Trans. II (1997) 971–976.

[9] J.G. Erickson, P.F. Wiley, V.P. Wystrach (Eds.), The 1,2,3- and 1,2,4-Triazines, Tetrazines and Pentazines, Interscience, New York, 1956.

[10] H. Neunhoeffer, P.F. Wiley (Eds.), Chemistry of 1,2,3-Triazines and 1,2,4-Triazines, Tetrazines and Pentazines, Wiley, New York, 1978.

[11] P.N. Preston, D.M. Smith, G. Tennant (Eds.), Benzimidazoles and Congeneric Tricyclic Compounds, Part 1, Wiley, New York, 1981.

[12] G.P. Ellis (Ed.), Synthesis of Fused Heterocycles, Part 2, Wiley, New York, 1992.

[13] G. Biagi, I. Giorgi, O. Livi, V. Scartoni, S. Velo, P.L. Barili, New 4-(benzotriazol-1-yl)-1,2,3-triazole derivatives, J. Heterocycl. Chem. 33 (1996) 1847–1853.

[14] L. Bertelli, G. Biagi, V. Calderone, I. Giorgi, O. Livi, V. Scartoni, P.L. Barili, 1-(1,2,3-triazol-4-yl)-benzimidazolones, a new series of heterocyclic derivatives, J. Heterocycl. Chem. 37 (2000) 1169–1176.

[15] C. Vandier, P. Bonnet, Synergistic action of NS-004 and internal Ca^{2+} concentration in modulating pulmonary artery K^+ channels, Eur. J. Pharmacol. 295 (1996) 53–60.

[16] G. Edwards, A. Niederste-Hollenberg, J. Schneider, T. Noack, A.H. Weston, Ion channel modulation by NS 1619, the putative BKCa channel opener, in vascular smooth muscle, Br. J. Pharmacol. 113 (1994) 1538–1547.

[17] C.A. Sargent, G.J. Grover, M.J. Antonaccio, J.R. McCullough, The cardioprotective, vasorelaxant and electrophysiological profile of the large conductance calcium-activated potassium channel opener NS-004, J. Pharmacol. Exp. Ther. 266 (1993) 1422–1429.

[18] M. Magnon, V. Calderone, A. Floch, I. Cavero, Influence of depolarization on vasorelaxant potency and efficacy of Ca^{2+} entry blockers, K^+ channel openers, nitrate derivatives, salbutamol and papaverine in rat aortic rings, Naunyn-Schmiedebergs Arch. Pharmacol. 358 (1998) 452–463.