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Original article

Synthesis and biological activity of novel substituted benzanilides as potassium channel activators. V

Giuliana Biagi [a], Irene Giorgi [a], Oreste Livi [a], Antonio Nardi [a,*], Vincenzo Calderone [b], Alma Martelli [b], Enrica Martinotti [b], Oreste LeRoy Salerni [c]

[a] *Dipartimento di Scienze Farmaceutiche, Università di Pisa, via Bonanno 6, 56126 Pisa, Italy*
[b] *Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, Università di Pisa, via Bonanno 6, 56126 Pisa, Italy*
[c] *Butler University College of Pharmacy and Health Sciences, 4600 Sunset, Indianapolis, IN 46208, USA*

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Abstract

As part of our program toward designing potassium channel openers, the synthesis of a novel series of substituted benzanilides and their vasodilating activity are presented. The facile synthetic pathway generally involves coupling between the appropriate benzoyl chloride and commercial available anilines, followed by the selective or non-selective cleavage of methyl ether substituent(s), affording the corresponding phenol or bisphenol derivatives. The pharmacological evaluation of these structurally novel potential BK-openers on vascular contractile activity was studied in vitro, using isolated rat aortic rings pre-contracted with KCl 20 mM. Some derivatives were found to be potent smooth muscle relaxants and the vasodilation effects of these compounds were inhibited by tetraethylammonium (TEA) and iberiotoxin (IbTX), suggesting that the opening of BK channels is prevalent in the mechanism of action of these compounds. The best compound of the series was *N*-(2-hydroxy-5-phenyl)-(2-methoxy-5-chloro)-benzamide (**16b**) showing a full vasorelaxant efficacy and almost nanomolar potency index.

Keywords: Benzanilides; BK channels; BK-openers; Vasodilator activity

1. Introduction

Large-conductance calcium-activated potassium channels, also known as BK or Maxi-K channels, almost ubiquitously distributed among tissues, are expressed in both excitable and non-excitable cells, where they are involved in the regulation of cell excitability and function. Their physiological role has been especially studied in the nervous system [1–3], where they are key regulators of neuronal excitability and of neurotransmitter release, and in smooth muscle [4–8], where they are crucial in modulating the tone of vascular, broncho-tracheal, urethral, uterine or gastro-intestinal musculature. Given these implications, small agents with BK-opener properties, named BK-openers or BK-activators, could have a potentially powerful influence in the modulation and control of numerous consequences of muscular and neuronal hyperexcitability [1,9,10–16], such as asthma, urinary incontinence and bladder spasm, gastroenteric hypermotility, psychoses, post-stroke neuroprotection, convulsions and anxiety. As far as the cardiovascular system is concerned, since the activation of relatively few BK channels can have a strong influence on membrane potential, the physiological function of these ion channels represents a fundamental steady state mechanism, modulating vessel depolarisation, vasoconstriction and increases of intravascular pressure [17]. It has been clearly documented that the role and/or the expression of vascular BK channels is enhanced during chronic hypertension, acting as a protective mechanism triggered by the pathological status itself [17]. An increased role of BK channels in the vasodilator responses of several vascular beds has also been observed in hypercolesterolemic conditions. A decreased modulation by BK channels has been observed in the coronary vessels of aged humans and rats, a possible cause of senile coronary vasospasm [18].

Diabetes causes an approximate fourfold increased risk of coronary, cerebrovascular and further cardiovascular problems. In fact, diabetes represents a condition with impaired production of reactive oxygen species, determining a decreased availability of endogenous NO and a higher produc-

* Corresponding author.
E-mail address: nardi@farm.unipi.it (A. Nardi).

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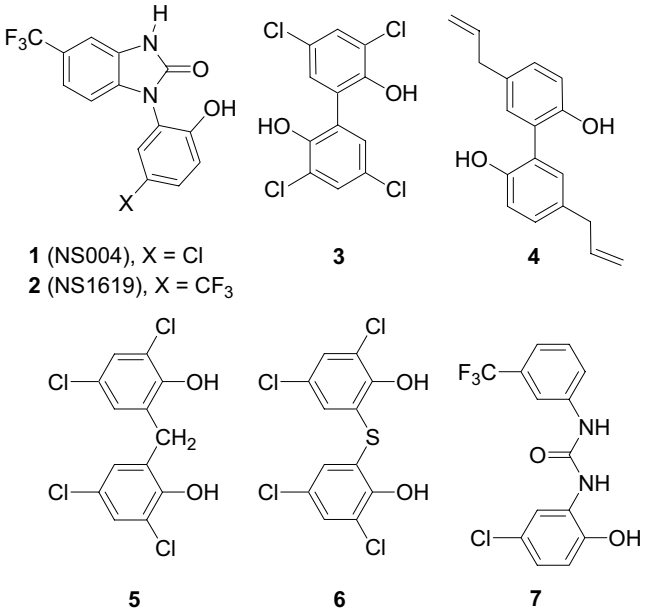
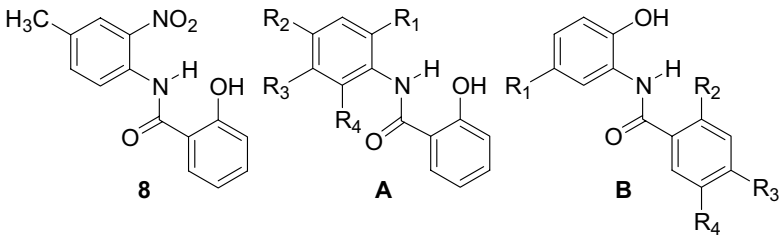


Fig. 1. Chemical structures of some BK-openers.

tion of peroxynitrite. Both these factors can play a major role in the inactivation of vascular BK channels, reported in several experimental models [17]. Given the above considerations, development of selective activators of BK channels appears to be a promising research field for the pharmacotherapy of vascular diseases, including hypertension, coronary diseases and vascular complications associated with diabetes or hypercolesterolemia.

The synthetic benzimidazolone derivatives NS 004 (**1**) and NS 1619 (**2**) (Fig. 1) are pioneer BK-activators [19,20] and represent the reference models, which led to the design of several chemically heterogeneous BK-openers [9]. Many of these compounds show a general pharmacophoric model, involving the presence of two phenyl rings, that may or may not be symmetrically, linked by a spacer unit, and at least two H-bond-donor sites [21,22]. One of the two phenyl rings is often represented by an *o*-hydroxyphenyl group; a halogen substituent, if present, is preferred in the *p*-position to the hydroxy function. The latter seems to be essential for BK-opener properties. The other aromatic ring, if not substituted by an *o*-hydroxyphenyl group, often displays electron withdrawing groups. The spacer unit, when present, may vary. It can be represented by a heterocyclic ring, that may or may not be fused to one of the two phenyls, or by an acyclic moiety. For the latter, chemically heterogeneous linkers of various lengths seems to be tolerated: the phenyl systems may be separated by one bond (no spacer), such as in the compound **3** [21] or **4** (magnolol) [23], two bonds, such as in the compounds **5** and **6** [21] that are, respectively, characterised by a methylene and a thioether spacer or even four, such as in the compound **7** (NS 1608) [24], where an urea linker is present (Fig. 1).

In the course of our studies focused on structural modification of the prototypical BK-opener NS 004, we elected to synthesise and evaluate a number of new potential BK-opener compounds [25–28]. On pursuing our researches, we sought, in an effort to explore the scope of the linker, to insert an amide moiety as a new three-bond-spacer unit between an *o*-hydroxyphenyl and a multi-substituted phenyl ring or between two multi-substituted phenol nuclei. This spacer, where the amidic NH could also represent one of the required H-bond-donor sites, has recently demonstrated to be a valid choice, as shown by the easy synthetic pathway and vasodilation properties of some 2-hydroxy-*N*-phenyl-benzamide derivatives (general formula **A**, R_1: NO_2, Fig. 2) [28] (in this series compound **8** is the most representative). These compounds represent key intermediates to the synthesis of some potassium channel openers, the 5-substituted-1-(2-hydroxybenzoyl)-benzotriazoles [28], which have been evaluated for activity as potential potassium channel activators. Thus, to further investigate this new scaffold, we planned a simple synthesis and pharmacological evaluation of some new benzanilides, the 2-hydroxy-*N*-phenyl-benzamides (general formula **A**, Fig. 2), bearing new substituents in the *N*-phenyl ring (compounds **12a–g**, Fig. 4) and new *N*-(2-hydroxyphenyl)-benzamides (general formula **B**, Fig. 2), represented by compounds **16a–b** and **17** (Fig. 6). Here, we also report the pharmacological evaluation of two related benzanilides (compounds **18a** and **18b**, Fig. 3), which



General formula A

R_1: OCH_3, OH, NO_2
R_2: OH, NO_2, H
R_3: Cl, NO_2, H
R_4: CH_3, H

General formula B

R_1: Cl, CH_3, H
R_2: OCH_3, OH, NO_2
R_3: Cl, H
R_4: CH_3, H

Fig. 2. Reference compound **8** and general formula **A** and **B** of the tested compounds.

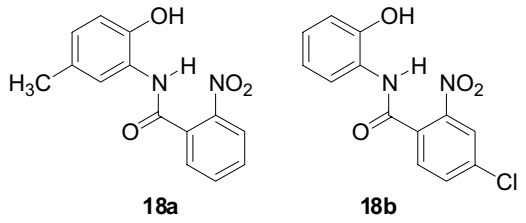
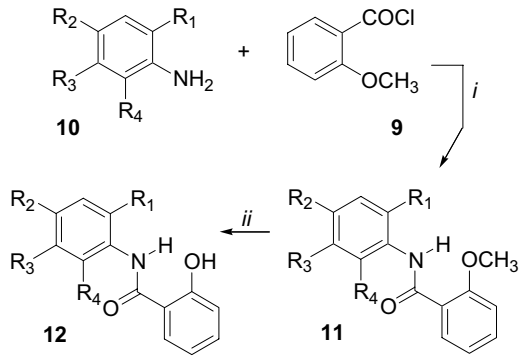


Fig. 3. Compounds **18a–b**, previously prepared and structurally correlated to **16a–b, 17**.

we previously prepared as intermediates to the synthesis of some benzotriazinones [27] whose vasodilating properties have not yet been investigated. The pharmacological study was performed on in vitro assays (rat aortic rings) by the pharmacological evaluation of their vasodilating activity and of the possible involvement with BK channels in the mechanism of action.



Cmpd	R_1	R_2	R_3	R_4
10 or 11a	NO_2	H	H	CH_3
10 or 11b	NO_2	OCH_3	H	H
10 or 11c	OCH_3	NO_2	H	H
10 or 11d	OCH_3	H	NO_2	H
10 or 11e	OCH_3	H	Cl	H
12a	NO_2	H	H	CH_3
12b	NO_2	OH	H	H
12c	OCH_3	NO_2	H	H
12d	OH	NO_2	H	H
12e	OCH_3	H	NO_2	H
12f	OH	H	NO_2	H
12g	OH	H	Cl	H

Fig. 4. Preparation of benzanilides **11a–e** and **12a–g**. Reagents and conditions: (i) toluene (100 °C); (ii) BBr_3.

2. Chemistry

A series of new 2-methoxybenzanilides (**11a–e**) was prepared in excellent yield, by the acylation reaction between the 2-methoxybenzoylchloride (**9**) [29] and commercial available anilines (**10a–e**) (see Table 1 for the experimental conditions). The methoxy derivatives (**11a–e**), on treatment with an excess of boron tribromide, underwent a demethylation reaction to give the corresponding salicylanilides (**12a–g**) (Fig. 4). (Compounds **12d** [30], **12f** [31], **12g** [32] were previously reported in literature but obtained by different procedures.) The demethylation reaction on benzanilides **11b–e**, obtained from anilines containing a methoxy group (**10b–e**), provided the expected dihydroxy compounds **12b, 12d, 12f, 12g**. In the case of dimethoxy derivatives **11c** and **11d**, bearing nitro substituents and a methoxy group in the *o*-position of the aniline ring, the treatment with boron tribromide at a temperature not higher than –70 °C, did not cleave the methoxy group in the *N*-aryl substituent and the monomethoxy derivatives **12c** and **12e** were isolated in good yields. Unequivocal proof of chemical structure of **12e** was demonstrated by its preparation by an alternate synthetic pathway, which involves reaction of the acetylsalicylic acid chloride (**13**) [33] with the 2-methoxy-5-nitroaniline (**10d**), as presented in Fig. 5. It is assumed that ester hydrolysis occurred upon evolution of HCl. Selective demethylation of some aryl methyl ethers by use of the analogue Lewis acid BCl_3 has been reported [34]. Therefore, under controlled experimental conditions, rapid and selective demethylation of the methoxy function in *o*-position to the carbonyl group takes place. This occurs even if in the presence of a large excess of BBr_3. Finally, in an effort to further explore this new chemotype, we prepared two other different amido derivatives, the *N*-(2-hydroxyphenyl)-2-methoxybenzamides **16a** and **16b** (Fig. 4), bearing a methoxy substituent on the acid part of the benzanilide and an hydroxy function in the *o*-position of the aniline ring. This was accomplished by reaction of 2-methoxy-5-chloro-benzoylchloride (**14**) [35] with 2-amino-4-chlorophenol (**15a**) or 2-amino-4-methylphenol (**15b**), respectively. The cleavage of methyl ether **16b**, again by treatment with boron tribromide, led to compound **17** in good yield (Table 2).

Structures of all the new compounds prepared were confirmed by analytical and spectroscopic methods. Table 3 reports the ^{1}H-NMR data.

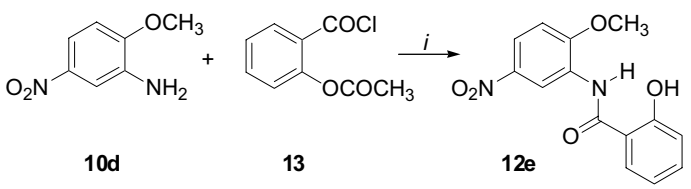


Fig. 5. Preparation of **12e** by one pot synthesis. Reagents and conditions: (i) toluene (100 °C).

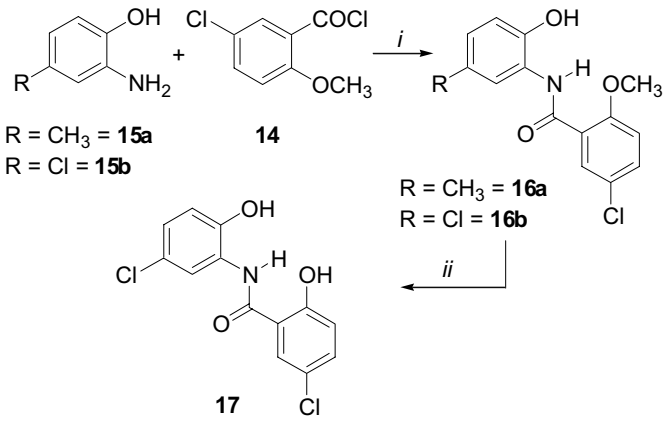


Fig. 6. Preparation of compounds **16a–b** and **17**. Reagents and conditions: (i) toluene (**16a**: 100 °C; **16b**: r.t.); (ii) BBr_3.

3. Pharmacology

The vasodilating effect of the novel potential BK-openers **12a–g, 16a–b, 17, 18a–b** on vascular contractile function was studied in vitro, using isolated rat aortic rings precontracted with KCl 20 mM (see later for the pharmacological details).

4. Results and discussion

The experimental results are summarised in Table 4. All the tested compounds showed a profile of full or nearly full vasodilation action, except for the compounds **12b** (Fig. 4), **18a** and **18b** (Fig. 3) which exhibited a modest vasodilating effect (<50%). Compound **12f** (Fig. 4), which is characterised by a value of efficacy of approximately 50%, and a value of potency lower than all the other compounds tested, proved to be comparable with that recorded for the reference compound NS 1619. Compound **12d** (Fig. 4) presented an index of potency slightly higher than that of NS 1619, while **12a, 12c, 12e, 12g** (Fig. 4) and **17** (Fig. 6) have shown a significant improvement (>10-fold) of potency. This parameter reached remarkable values for compounds **16a** and **16b** (Fig. 6), respectively, approximately 200- and 2000-fold higher than the value of potency of NS 1619. For this reason, compounds **16a** and **16b** were investigated further to identify the pharmacodynamic profile and a possible involvement of the potassium channels, especially the BK channels, in the vasodilating mechanism of action. In the presence of tetraethylammonium (TEA), a blocker of different subtypes of K_{Ca} channels, the dilating action of **16a** and **16b** was dramatically reduced. A higher inhibition was observed in the presence of iberiotoxin (IbTX), a selective BK channel blocker which significantly reduced the effects of these two compounds, suggesting that BK channels play a major role in the mechanism of action of **16a** and **16b**. The pharmacological results allow us to make some initial observations concerning structure–activity relationships. According to our previous studies [28], the 2-nitrosalicylanilide structures (general formula **A**, R_1: NO_2, Fig. 2) showed good vasodilating activity. In particular, compound **12a** (Fig. 4) was a more potent vasodilator (pIC_{50}: 6.60) than the reference compound NS 1619 (pIC_{50}: 5.18) and the isomeric compound **8** (Fig. 2), previously reported (pIC_{50}: 5.60) [28]. Compound **12b** (pIC_{50}: N.C.) (Fig. 4) showed that an introduction of an hydroxy function in the *m*-position to the nitro group on the anilino moiety of the salicylanilide, combined with the removal of the methyl substituent, caused a clear decrease of activity. On the contrary, the replacement of the nitro group in the *o*-position of the aniline moiety with a methoxy substituent, provided compounds with good vasodilating activity (**12c**, pIC_{50}: 6.72 and **12e**, pIC_{50}: 6.35, Fig. 4). The demethylation of the methoxy substituent of compounds **12c** and **12e** (Fig. 4) to give bisphenol derivatives **12d** (pIC_{50}: 5.84) and **12f** (pIC_{50}: 5.09) (Fig. 4) induced a remarkable lowering of the efficacy parameter. In contrast, the presence of a phenolic acid function in the 2 position of the aniline moiety and a methoxy group in the 2 position of the salicylic

Table 1
Experimental conditions for the preparation of the benzanilides **11a–e** and **16a–b**

Compounds	Acyl chloride (mmol)	Amine (mmol)	Solvent (ml)	Temperature (°C)	Time (h)
11a	11.7	12.88	150	105	20
11b	5.86	5.86	150	85	20
11c	11.73	11.73	150	100	24
11d	5.86	5.86	150	85	20
11e	3.55	3.55	50	85	16
16a	10.71	10.71	150	20	16
16b	10.71	10.71	150	100	3

Table 2
Chemical and physical properties of all synthesised compounds

Compounds	Yield (%)	Crystallisation solvent	Melting point (°C)	Analyses (C, H, N)
11a	72	MeOH/H_2O	112–114	$C_{15}H_{14}N_2O_4$
11b	96	EtOH	148–150	$C_{15}H_{14}N_2O_5$
11c	85	DMF/H_2O	216–218	$C_{15}H_{14}N_2O_5$
11d	82	DMF/H_2O	257–259	$C_{15}H_{14}N_2O_5$
11e	70	EtOH	134–136	$C_{15}H_{14}NO_3Cl$
12a	94	MeOH/H_2O	143–145	$C_{14}H_{12}N_2O_4$
12b	90	EtOH	178–181	$C_{13}H_{10}N_2O_5$
12c	42	MeOH	205–206	$C_{14}H_{12}N_2O_5$
12d	61	MeOH/H_2O	197–200	$C_{13}H_{10}N_2O_5$
12e	[a]	DMF/H_2O	244–246	$C_{14}H_{12}N_2O_5$
12f	18 [b]	DMF/H_2O	267–270	$C_{13}H_{10}N_2O_5$
12g	34	EtOH/H_2O	192–195	$C_{13}H_{10}NO_3Cl$
16a	52	EtOH/H_2O	170–171	$C_{15}H_{14}NO_3Cl$
16b	81	EtOH	240–242	$C_{14}H_{11}NO_3Cl_2$
17	62	MeOH/H_2O	235–236	$C_{13}H_9NO_3Cl_2$

[a] Procedure A: 66%; procedure B: 84%.
[b] After recrystallisation (analytical sample).

moiety (compounds **16a**, pIC_{50}: 7.56 and **16b**, pIC_{50}: 8.49, Fig. 6), provided the most potent compounds of this series. However, poorly active compounds were obtained when the methoxy substituent was replaced with a nitro group (compounds **18a** and **18b**, efficacy <50%, Fig. 3). Substitution of a chloro for a nitro function, generally induced an increased vasodilation (compounds **16a** and **16b**; and **12g**: pIC_{50}: 6.87). Finally, the concurrent presence of a hydroxy group on both benzanilide rings caused a remarkable lowering of activity (compounds **12b, 12d, 12f** and **12g**, Fig. 4, and **17**, pIC_{50}: 6.43, Fig. 6). In this series, both the amido and phenolic function groups show acid properties and the biological data suggests that the introduction of another phenolic group, a third weakly acidic function, would be detrimental to BK channel activation.

Table 3
^{1}H-NMR spectra in DMSO-d_6 (δ)

11a	2.38 (s, 3H, CH_3); 3.97 (s, 3H, OCH_3); 7.06–7.82 (m, 7H, Ar); 10.13 (s, 1H, NH)
11b	3.86 and 4.05(2s, 6H, $2OCH_3$); 7.13–8.48 (m, 7H, Ar); 11.44 (s, 1H, NH)
11c	4.11 (s, 6H, $2OCH_3$); 7.14–7.82 (m, 7H, Ar); 11.02 (s, 1H, NH)
11d	4.11 and 4.13 (2s, 6H, $2OCH_3$); 7.16–8.41 (m, 6H, Ar); 9.39 (s.1H, Ar); 10.86 (s, 1H, NH)
11e	3.97 and 4.08 (2s, 6H, $2OCH_3$); 7.12–8.54 (m, 7H, Ar); 10.74 (s, 1H, NH)
12a	2.36 (s, 3H, CH_3); 6.95–7.97 (m, 7H, Ar); 10.51 and 11.81 (2s, 2H, NH and OH)
12b	6.93–8.13 (m, 7H, Ar); 11.31, 11.38 and 11.79 (3s, 3H, NH and 2OH)
12c	6.97–8.06 (m, 6H, Ar); 8.74 (d, 1H, Ar); 11.31 and 11.91 (2s, 2H, NH and OH)
12d	6.97–8.03 (m, 6H, Ar); 8.68 (d, 1H, Ar);11.27, 11.29 and 11.85 (3s, 3H, NH and 2OH)
12e	6.98–8.08 (m, 6H, Ar); 9.40 (d, 1H, Ar); 11.13 and 11.86 (2s, 2H, NH and OH)
12f	6.97–8.08 (m, 6H, Ar); 9.39 (d, 1H, Ar);11.09, 11.81 and 11.90 (3s, 3H, NH and 2OH)
12g	6.87–8.49 (m, 7H, Ar); 10.43, 10.91 and 11.74 (3s, 3H, NH and 2OH)
16a	2.23 (s, 3H, CH_3); 4.04 (s, 3H, OCH_3); 8.18–8.71 (m, 6H, Ar); 10.00 and 10.50 (2s, 2H, NH and OH)
16b	4.05 (s, 3H, OCH_3); 6.89–8.42 (m, 6H, Ar); 10.61 and 10.65 (2s, 2H, NH and OH)
17	6.88–8.45 (m, 6H, Ar); 10.50, 10.92 and 12.09 (3s, 3H, NH and 2OH)

Table 4
Experimental results: potency (pIC_{50}) and efficacy (E_{max} %) values of the tested compounds

Compounds	Efficacy (E_{max} %)	pIC_{50}
12a	100a	6.60 ± 0.052
12b	41.2 ± 9.7	N.C.
12c	100a	6.72 ± 0.23
12d	100a	5.84 ± 0.039
12e	100a	6.35 ± 0.17
12f	54.6 ± 10.6	5.09 ± 0.095
12g	90.2 ± 6.4	6.87 ± 0.37
16a	86.4 ± 6.6	7.56 ± 0.10
+TEA	27.7 ± 16.0*	N.C.
+IbTX	3 ± 4*	N.C.
16b	100a	8.49 ± 0.091
+TEA	15.8 ± 4.2*	N.C.
+IbTX	3 ± 2*	N.C.
17	89.8 ± 2.3	6.43 ± 0.11
18a	32.5 ± 16.6	N.C.
18b	24.0 ± 11.6	N.C.
NS 1619	100a	5.18 ± 0.055

The asterisk * indicates a significant difference from the control value. 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 S.E. could not be expressed.

5. Experimental protocols

5.1. 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.

5.1.1. General procedure for the N-(substitutedphenyl)-2-methoxybenzamides (11a–e)

Equimolar amounts of 2-methoxybenzoylchloride (**9**) and the appropriate substituted aniline (**10a–e**) (Fig. 4) in anhydrous toluene were reacted under the experimental conditions reported in Table 1. After cooling, the solvent was evaporated in vacuo and the residue was stirred with 10% NaOH, 10% HCl and H_2O. The residue obtained after every

decantation, consisting of the title compound, was collected by filtration and purified by crystallisation (Table 2).

5.1.2. N-*(2-nitro-6-methylphenyl)-salicylanilide (**12a**)*

To a stirred solution of monomethoxybenzanilide **11a** (0.400 g, 1.40 mmol) in 100 ml of anhydrous CH_2Cl_2, cooled at –60 °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 slowly added. The reaction mixture was left at –20 °C for 15 h, then again placed in an ice–salt bath, and finally the reagent was destroyed by the addition of MeOH and H_2O, drop by drop and with stirring. The organic layer was separated, washed with H_2O, then extracted with 7% NaOH. The combined alkaline extracts were acidified and again extracted with $CHCl_3$. The chloroform extracts, after washing with H_2O, were dried ($MgSO_4$) and evaporated to give **12a** as a solid residue which was purified by crystallisation (Table 2).

5.1.3. N-*(4-hydroxy-2-nitrophenyl)-salicylanilide (**12b**)*

To a stirred solution of dimethoxybenzanilide **11b** (0.600 g, 1.98 mmol) in 50 ml of anhydrous CH_2Cl_2, cooled at –78 °C and under a nitrogen flow, a solution of BBr_3 (2.0 ml, 21 mmol) in 8 ml of anhydrous CH_2Cl_2 was slowly added (≈40 min) and the temperature was raised to –60 °C. The reaction mixture was left at –20 °C for 15 h, then it was worked up as described for the preparation of **12a**. The title compound, precipitated by acidification as an orange solid, was collected by filtration (Table 2).

5.1.4. N-*(2-methoxy-4-nitrophenyl)-salicylanilide (**12c**)*

To a stirred solution of dimethoxybenzanilide **11c** (0.400 g, 1.32 mmol) in 150 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. The cooling-bath was removed when the temperature was raised to –50 °C and the reaction mixture was kept at –20 °C for 15 h, then at room temperature (r.t.) for 6 h. The reaction was worked up as described for the preparation of **12a** and the title compound, precipitated by acidification as a clear solid, was collected by filtration (Table 2).

5.1.5. N-*(2-hydroxy-4-nitrophenyl)-salicylanilide (**12d**)*

To a stirred solution of dimethoxybenzanilide **11c** (0.400 g, 1.32 mmol) in 80 ml of anhydrous CH_2Cl_2, cooled at –5 °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 slowly added. The reaction mixture was left at 4 °C for 15 h and at r.t. for 12 h, then it was worked up as described for the preparation of **12a**. The title compound, precipitated by acidification as a solid and consisting of a mixture of the expected didemethylated compound **12d** and ≈5% of the monodemethylated **12c**, was purified by fractional crystallisation from MeOH/H_2O, taking advantage of the lower solubility of **12c** (Table 2).

5.1.6. N-*(2-methoxy-5-nitrophenyl)-salicylanilide (**12e**)*

A) (Fig. 4) To a stirred solution of dimethoxybenzanilide **11d** (0.272 g, 0.90 mmol) in 120 ml of anhydrous CH_2Cl_2, cooled at –60 °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 slowly added (≈1 h). The reaction mixture was kept at –20 °C for 15–16 h, then it was worked up as described for **12a**, to give the title compound (Table 2).

B) (Fig. 5) A solution of acetylsalicylic acid chloride (**13**) (1.70 g, 8.61 mmol) and 2-methoxy-5-nitroaniline (**10d**) (1.45 g, 8.63 mmol) in 120 ml of anhydrous toluene was heated at 105 °C for 24 h. The solvent was evaporated in vacuo and the residue was stirred with 6% $NaHCO_3$, 10% HCl and H_2O. The new residue obtained after every decantation, consisting of **12e**, was collected by filtration and purified by crystallisation (Table 2).

5.1.7. N-*(2-hydroxy-5-nitrophenyl)-salicylanilide (**12f**)*

To a stirred solution of dimethoxybenzanilide **11d** (0.500 g, 1.65 mmol) in 150 ml of anhydrous CH_2Cl_2, cooled at –10 °C and under a nitrogen flow, a solution of BBr_3 (2.0 ml, 21 mmol) in 7 ml of anhydrous CH_2Cl_2 was slowly added. After stirring at r.t. for 24 h, the reaction mixture was worked up as described for **12a**. The title compound, precipitated by acidification as a brown gel, was collected by paper filtration. This gel had to be dissolved in hot MeOH and reprecipitated by the addition of H_2O as a crude brown solid, which was purified by treatment with EtOH/H_2O then recrystallised (Table 2).

5.1.8. N-*(2-hydroxy-5-chlorophenyl)-salicylanilide (**12g**)*

To a stirred solution of dimethoxybenzanilide **11e** (0.600 g, 2.06 mmol) in 30 ml of anhydrous CH_2Cl_2, cooled at –5 °C and under a nitrogen flow, a solution of BBr_3 (2.0 ml, 21 mmol) in 8 ml of anhydrous CH_2Cl_2 was slowly added. The ice-bath was removed and the stirring continued at r.t. for 15–16 h. The reaction mixture was worked up as described for the preparation of **12a**, to give **12g** as a solid residue which was purified by crystallisation (Table 2).

5.1.9. N-*(2-hydroxy-5-methylphenyl)-(2-methoxy-5-chloro)-benzamide (**16a**) and* N-*(2-hydroxy-5-chlorophenyl)-(2-methoxy-5-chloro)-benzamide (**16b**)*

Equimolar amounts of 2-methoxy-5-chlorobenzoylchloride (**14**) and 2-hydroxy-5-methylaniline (**15a**) or 2-hydroxy-5-chloro-aniline (**15b**) in anhydrous toluene were reacted under the experimental conditions reported in Table 1. For the isolation of **16a** the solvent was evaporated and the residue was washed with 10% HCl, 6% $NaHCO_3$ and H_2O (Table 2). Compound **16b**, crystallised by the cooling of the reaction mixture, was collected by filtration (65% yield). A further fraction was obtained by the evaporation of the

filtrate and purification of the residue by the procedure described for **16a** (Table 2).

*5.1.10.*N-*(2-hydroxy-5-chlorophenyl)-(2-hydroxy-5-chloro)-benzamide (**17**)*

To a stirred solution of benzanilide **16b** (0.500 g, 1.68 mmol) in 160 ml of anhydrous CH_2Cl_2, cooled at –70 °C and under a nitrogen flow, a solution of BBr_3 (1.5 ml, 15.75 mmol) in 8 ml of anhydrous CH_2Cl_2 was slowly added (≈1 h). The reaction mixture was allowed to reach r.t. and stirring was maintained for a night, then again placed in an ice–salt bath, to destroy the excess of the reagent by the addition of MeOH and H_2O, drop by drop and under stirring. This mixture was extracted with 7% NaOH and the combined alkaline extracts were acidified. The white solid precipitated was collected by filtration and purified by crystallisation (Table 2).

5.1.11. 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 millimetres 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; $MgSO_4 \cdot 7H_2O$ 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 with an unirecord microdynamometer (Buxco Electronics). After an equilibration period of 60 min, the endothelial removal was confirmed by the administration of acetylcholine (ACh) (10 μM) to KCl (20 mM)-precontracted vascular rings. A relaxation <10% of the KCl-induced contraction was considered representative of an acceptable lack of the endothelial layer, while the organs, showing a relaxation ≥10% (i.e. significant presence of the endothelium), were discarded. From 30 to 40 min after the confirmation of the endothelium removal, the aortic preparations were contracted by 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. Preliminary experiments showed that the KCl (20 mM)-induced contractions remained in a stable tonic state for at least 40 min. In other sets of experiments, the non-selective potassium channel blocker tetraethylammonium chloride (TEA 10 mM) or the BK-selective blocker iberiotoxin (IbTX, 100 nM) were added, after the KCl (20 mM)-induced contraction, followed by the administration of selected compounds. The reference drug NS 1619 (Sigma) was dissolved (10 mM) in EtOH 95% and further diluted in Tyrode solution. Acetylcholine chloride (Sigma) was dissolved (100 mM) in EtOH 95% and further diluted in bidistilled water whereas KCl and TEA were both dissolved in Tyrode solution. Most of the synthesised derivatives (**12a–g, 16a, 18a–b**) were dissolved (10 mM) in NaOH 0.1 N whereas two of them (**16b** and **17**) were dissolved (10 mM) in DMSO; they all were further diluted in Tyrode solution. All the solutions were freshly prepared immediately before the pharmacological experimental procedures. Previous experiments showed a complete ineffectiveness of the vehicles. The vasodilating efficacy was evaluated as maximal vasodilating 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 vasodilating 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 $p\mathrm{IC}_{50}$, calculated as negative Logarithm of the molar concentration of the tested compounds, evoking a half reduction of the contractile tone induced by KCl 20 mM. The $p\mathrm{IC}_{50}$ 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 (S.E.), for 5–10 experiments. Student's *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: GraphPad Prism 3.0).

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