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Original article

Structural modifications of benzanilide derivatives, effective potassium channel openers. X.

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Abstract

Large-conductance calcium-activated potassium (BK) channels are involved in many fundamental cell functions. Consistently, the ability to activate BK channels by exogenous compounds is considered as a promising pharmacodynamic pattern for the potential treatment of several pathologies. In this perspective, the development of new and selective BK-openers can be considered as an actual field of research. This paper reports the synthesis and pharmacological evaluation of new benzanilides, useful for deepening the comprehension of the structure–activity relationships, emerged in previous studies on this class of BK-activators. From a structural point of view, these benzanilides belong to a general class of BK-activators, showing a common pharmacophoric model, consisting of two aryl groups linked through an appropriate ''spacer'' and the almost obligatory presence of a phenolic hydroxyl. In particular, a new series of benzanilides, in which the phenyl rings have been widely changed both on the acidic portion and the basic one of the amide spacer, were synthesised. Their vasorelaxing effects, induced through the activation of BK channels, were also evaluated. Although many compounds exhibited effects which could not be attributed to the activation of BK channels, two derivatives showed a clear profile of BK-activators with vasodilator activity comparable to or slightly lower than that recorded for the reference benzimidazolone **NS1619**. A further molecular modelling approach allowed us to obtain a molecular electrostatic potential feature which suggests a suitable interaction with the receptor site of the BK channel, from a tri-dimensional point of view. This approach seems to represent a further contribution for the development of new BK-activators, designed on the basis of the pharmacophoric model above-mentioned.

Keywords: BK channel; Benzanilide; BK-activator; Molecular modelling

1. Introduction

The large-conductance calcium-activated potassium (BK) channels are expressed in excitable as well as in non-excitable cells. They control several cell functions: in the nervous system, BK channels contribute to the shaping of action potential and modulate the neuronal excitability and the release of neurotransmitters; also, BK channels play a fundamental role in the regulation of the tone of smooth muscle cells [1,2].

The physiological activation of BK channels, induced mainly by two triggering signals, such as the rise of intracellular free calcium ions and membrane depolarisation, ensures the massive flow of potassium ions (with a single channel conductance of 150–300 pS) to the extracellular side of the plasmalemma, membrane hyperpolarisation and reduction of the cellular excitability. Conversely, the availability of exogenous compounds capable of activating BK channels can guarantee an innovative pharmacological tool for the clinical management of many pathological states, due to cell hyperexcitability,

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such as asthma, urge incontinence and bladder spasm, gastric hypermotility, neurological and psychiatric disorders [1,2].

As concerns the cardiovascular system, it is now widely accepted that BK channels ensure the predominant component of the outward K^+ current in vascular smooth muscle cells, accounting for the fundamental function of such ion channels in the modulation of the muscular tone of vessels [3,4]. Consequently, the vasorelaxing effects of exogenous BK-openers can furnish the pharmacological rational basis for the treatment of hypertension and/or other diseases related to an impaired contractility of vessels (for example, coronary vasospasm) [1,2].

In a previous work [5], we could observe that the synthesised 1-(2′-hydroxybenzoyl)-5-methyl-benzotriazole, showing structural analogies with the reference BK-openers **NS004** and **NS1619** (Fig. 1A) and exhibiting vasorelaxing effects probably due to the activation of vascular BK channels, was able to confer significant protection to the myocardial function, in isolated rat hearts submitted to ischemia/reperfusion cycles. This result (originally unexpected) can be now explained thanks to more recent experimental evidence showing that the activation of cardiac calcium-activated potassium channels could be involved in the cardioprotective mechanisms of ''ischemic preconditioning'' and that the administration of BK-openers, such as **NS1619**, could reduce cardiac injury following an ischemic event [6–8]. Of course, these reports let us foresee a further potential use of BK-activators in cardiovascular pharmacotherapy, as anti-ischemic drugs.

Some years ago, we undertook a research program concerning the synthesis and pharmacological experimentation of new compounds such as BK channel openers. As a consequence 1,2,3-triazole derivatives [9–12], benzimidazoles [13,14] and benzotriazoles [5,13,14] were tested, providing good and encouraging results. High pharmacological activity was also detected in some appropriately substituted benzanilide derivatives [15].

On the basis of these results and the suggestions reported in the literature [16], a simple pharmacophoric model consisting of two suitably substituted phenyl rings bound to a linker of various kinds, was hypothesised (Fig. 1B).

In order to support this pharmacophoric model and, in particular, the effectiveness of the amidic linker, by a larger investigation of the structure–activity relationships, we decided to continue our research program developing the simple structures of the benzanilide derivatives.

Thus, considering that *N*-(2-hydroxy-5-chloro-phenyl)-2-methoxy-5-chloro-benzamide (Fig. 2A) was the most active compound as BK-opener, we began [17] with a modification of the acid moiety of the benzanilide, introducing a heterocyclic ring (furan, thiophene, pyrrole and pyridine) in place of the phenyl ring but leaving unaltered the basic anilino substituent (Fig. 2B). The next analogous structural modification considered the basic moiety of benzanilide, by the introduction of a heterocyclic ring (pyridine, thiazole, morpholine and pyrrolidine) in place of the aniline, leaving unaltered the benzoic acid substituent. In this case the useful phenol function was introduced by cleavage of the *ortho*-methoxy substituent on the acid moiety (Fig. 2C). The pharmacological results indicated that the presence of nitrogen heterocycles on the acid side of the amide linker seems to be a negative requirement, while furan and thiophene rings are well tolerated. On the contrary, the introduction of unsaturated heterocyclic rings (pyridine and thiazole) on the basic side of the amide linker led to satisfactory biological activity, while the presence of aliphatic heterocycles lowered the pharmacological effect. The presence of a phenolic function as a probable H-bond donor was confirmed.

In this new paper, concerning a further deepening of the structure–activity relationships of benzanilide derivatives previously studied as BK channel activators, some substitutions on the acid or basic moiety of the reference benzanilides, showing particular and specific properties from a mesomeric and/or steric point of view, have been taken into consideration.

2. Chemistry

Scheme 1 reports the preparation of three benzanilides **4a–c**, corresponding to the compounds previously prepared [5,15], in whose acid moiety a chlorine atom was introduced to increase the acid property of the phenolic function, whilst the substituents present on the basic moiety were maintained. Thus the 2-methoxy-5-chloro-benzoyl chloride (**1**) reacted with 2-nitro-4-methyl- (**2a**), 2-nitro-4-methoxy- (**2b**) or 2-methoxy-4-nitro-aniline (**2c**) in refluxing toluene in the presence of triethylamine, to give the corresponding benzanilides **3a–c** in good yield. The next reactions of demethylation of the methoxy substituent, carried out with excess of boron tribromide in dichloromethane at −20 °C overnight, caused the cleavage of the methoxy groups present in the *ortho* position of the benzoic moiety of the amides **3a–c**, because adjacent

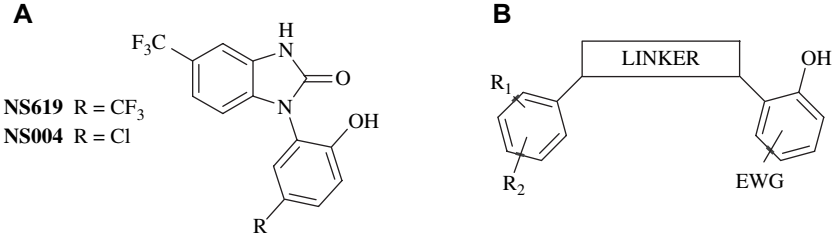

Fig. 1. A – Benzimidazolones **NS004** and **NS1619**. B – Generic pharmacophoric model for a BK-opener, consisting of two aryl rings, spaced by an appropriate heterocyclic or acyclic linker. EWG = electron-withdrawing group; R^1 and R^2 represent various kinds of possible substituents.