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N-BENZYLATED BENZIMIDAZOL-2-ONE DERIVATIVES: ACTIVATORS OF LARGE-CONDUCTANCE Ca^{2+}-DEPENDENT K^+ CHANNELS

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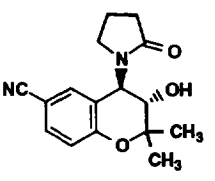
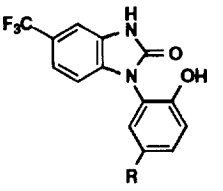
Abstract. A series of benzimidazol-2-ones structurally homologous to the known maxi-K opener NS-004 (**2**) were synthesized and evaluated by electrophysiological techniques as openers of the cloned maxi-K channel *m*Slo expressed in *Xenopus laevis* oocytes. The structure-activity relationships reveal tolerance in the topological relationship between the heterocycle and the phenol hydroxy and indicate the importance of an electron withdrawing substituent on the heterocycle for expression of maxi-K opening properties. The most efficacious activators of this channel were the 5-Cl derivative **4f** and the 5-NO_2 analogue **4i**.

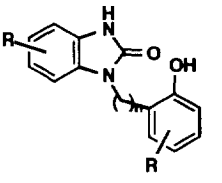
Potassium (K^+) channels are a widely distributed and structurally diverse family of membrane-spanning proteins that have emerged as important targets for therapeutic intervention in a number of disease states.[3-5] K^+ channels have been categorized based on the mechanism by which their activity is regulated and agents that selectively block many K^+ channels have been identified and used to elucidate the role of individual K^+ channels in cell function. In contrast, K^+ channel activators are less well known and have largely been restricted to activators of ATP-dependent potassium (K_{ATP}) channels. Cromakalim (**1**) is the prototype of a well established class of K_{ATP} channel opener that has been the subject of extensive pre-clinical and clinical study.[3-6] More recently, with the discovery of the benzimidazolone derivatives NS-004 (**2**) and NS-1619 (**3**),[7-10] the repertoire of K^+ channel openers has been extended to include the large-conductance calcium (Ca^{2+})-dependent K^+ channels, frequently referred to as maxi-K or BK channels. These channels, which are regulated by both voltage and Ca^{2+}, are found in many cell types[11] including neurons[12] and muscle cells, where they are thought to be important regulators of cellular excitability and function. Whilst the electrophysiological and biochemical pharmacological properties of NS-004 (**2**) and NS-1619 (**3**) have been examined in some detail,[7-10,13-15] structure-activity relationships for these compounds have not been described.



1, (±)-cromakalim



2, NS-004, R = Cl,
3, NS-1619, R = CF_3,



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In this article, we provide a survey of the maxi-K opening properties of a series of substituted benzimidazolone derivatives of general structure **4** that are structurally closely related to NS-004 (**2**) and NS-1619 (**3**), and which illuminate some of the fundamental aspects of structure-function relationships.

Chemistry

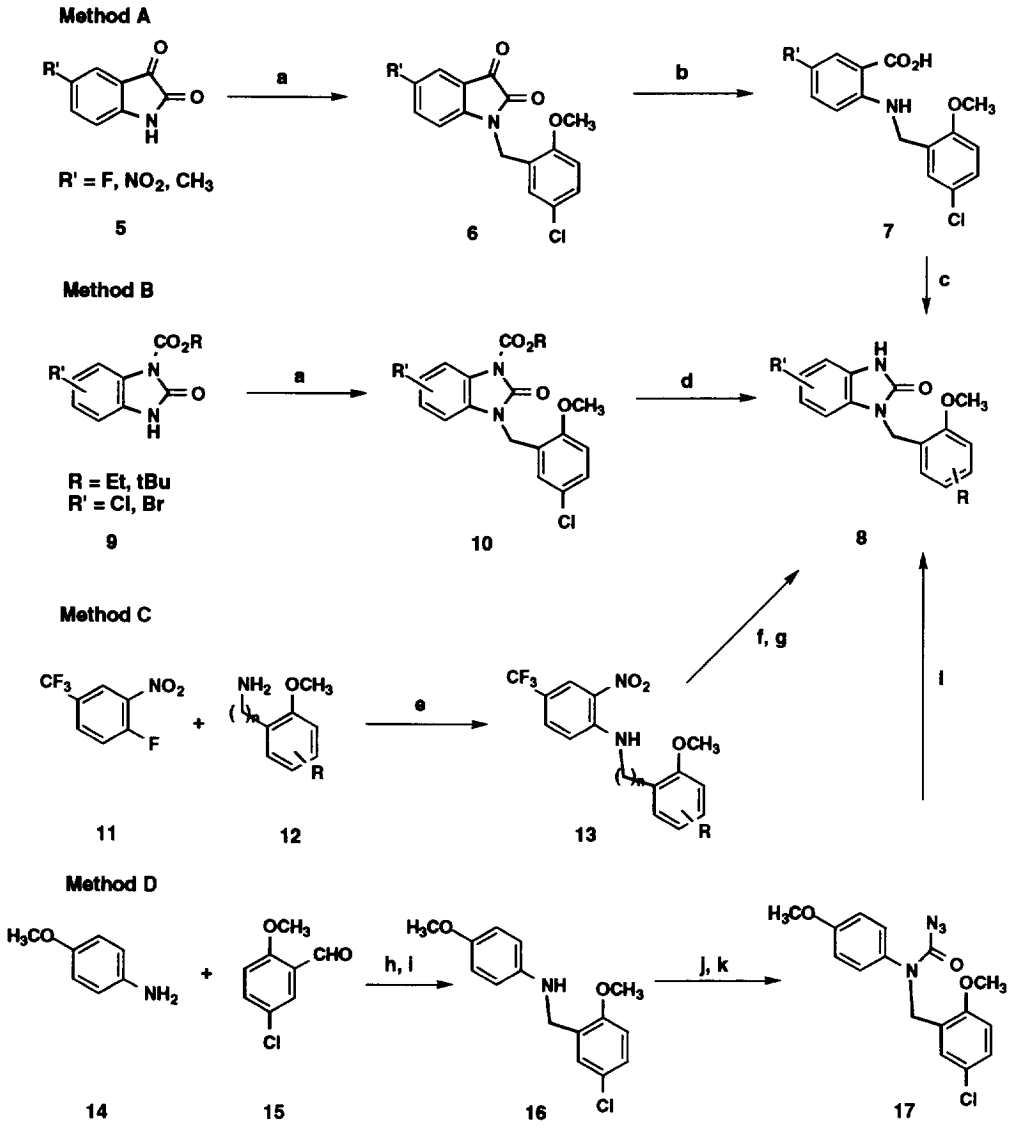
The four routes that were utilized to provide access to the methyl ether precursors **8** of target compounds **4** are summarized in Scheme 1. In Method A, alkylation of a 5-substituted isatin **5** with 2-(bromomethyl)-4-chloro-1-methoxybenzene afforded the N-benzyl derivatives **6** which were oxidatively cleaved to provide the corresponding anthranilic acids **7**.[16] Heating **7** with diphenylphosphoryl azide in toluene at reflux effected a Curtius rearrangement[17] with concomitant ring closure[18] to afford the corresponding benzimidazol-2-ones **8**. Method B entailed alkylation of mono-alkoxycarbonylated benzimidazolones **9** to provide **10**, which were deprotected to furnish **8** in excellent overall yield.[19] The 5-CF_3 analogues were synthesized from **11** which was heated with a benzyl- or phenethyl-amine (**12**) in the presence of K_2CO_3 to give **13**. Reduction of the nitro moiety using $SnCl_2$ in EtOH[20] was followed by ring closure to the benzimidazolones **8** by heating the phenylenediamine intermediate with carbonyldiimidazole[21] in THF (Method C). The 5-methoxy analogue was prepared by Method D that began with reductive amination of p-anisidine **14** with aldehyde **15** to afford **16**. Reaction of **16** with phosgene afforded the corresponding carbamoyl chloride which was exposed to NaN_3 in DMF to give azide **17**. Thermal decomposition of **17** in toluene at reflux provided **8** along with the corresponding indazole derivative, formed by rearrangement prior to reaction with the aryl ring.[22]

Removal of the methyl group from **8** to give the target compounds **4** was accomplished by treating with a 3-fold excess of BBr_3 in CH_2Cl_2[23] with the exception of the parent compound **4a**, which was demethylated by heating with excess pyridinium hydrochloride at 220 °C.[24] The compounds prepared as part of this study are compiled in the Table.[25]

Results and Discussion

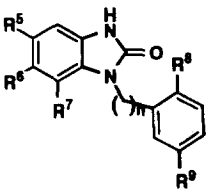
The target compounds **8a** and **4a-q** were evaluated for their maxi-K opening properties using two-electrode voltage clamp recording from *Xenopus laevis* oocytes that had been injected 2-6 days previously with *m*Slo[26] (*h*Slo[27] for compound **4m**) mRNA, as previously described.[28] The control outward current, defined as the iberiotoxin-sensitive component of total outward current, was established in the absence of drug. Voltage-clamp protocols consisted of +20 mV steps from a holding potential of -60 mV to a maximal potential of +140 mV. The experiment was repeated in the presence of drug, which was maintained at a concentration of 20 μM in the recording chamber. The poor solubility of the majority of these compounds in the required buffer systems precluded evaluation at higher concentrations. The increase in outward current in the presence of drug is reported in the Table as percent of drug free control and the data are an average of experiments conducted in at least 5 different oocytes. The results for NS-004 (**2**) are included for purpose of comparison.

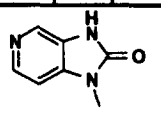
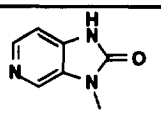
Scheme 1



Reagents: (a) 2-(bromomethyl)-4-chloro-1-methoxybenzene/K_2CO_3/cat. KI/CH_3CN/Δ or 110 °C/DMF; (b) H_2O_2/NaOH/H_2O; (c) DPPA/Et_3N/toluene/Δ; (d) NaOH or NaOMe/CH_3OH or CF_3CO_2H for R = *t*Bu; (e)K_2CO_3/CH_3CN/Δ or 110 °C/DMF; (f) $SnCl_2$/EtOH/Δ; (g) CDI/THF/Δ; (h) H^+/-H_2O; (i) $NaBH_4$/EtOH (j) $COCl_2$/Et_3N/$CHCl_3$; (k) NaN_3/DMF; (l) toluene/Δ;

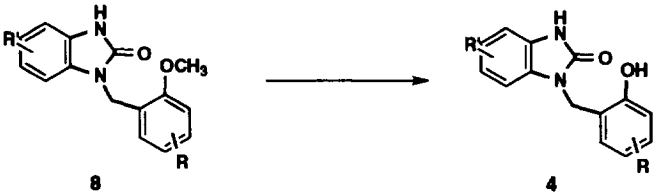
Table
Structure and cloned maxi-K channel opening properties of benzimidazol-2-one derivatives



Cmpd. #	R^5	R^6	R^7	R^8	R^9	n	Synthetic approach	mp (°C)	Outward current in the presence of test compound (20 μM) as % of control current
2 (NS-004)	CF_3	H	H	OH	Cl	0	B	203-205	131.8 ± 12.8
8a	H	H	H	OCH_3	Cl	1	C	203-204	104.2 ± 4.5
8b	CF_3	H	H	OCH_3	Cl	1	B	208-210	insoluble
4a	H	H	H	OH	Cl	1	C	234-236	110.9 ± 11.9
4b	CF_3	H	H	OH	Cl	1	C	262-265	133.4 ± 9.0
4c	CF_3	H	H	OH	H	1	C	215-217	126.0 ± 10.4
4d	CF_3	H	H	OH	H	2	C	188-191	119.5 ± 11.9
4e	F	H	H	OH	Cl	1	A	233-235	105.9 ± 15.6
4f	Cl	H	H	OH	Cl	1	B	240-243	150.1 ± 15.7
4g	Br	H	H	OH	Cl	1	B	244-247	135.7 ± 7.6
4h	I	H	H	OH	Cl	1	B	255-257	120.2 ± 5.0
4i	NO_2	H	H	OH	Cl	1	A	284-286	163.1 ± 29.5
4j	CH_3	H	H	OH	Cl	1	A	258-260	101.9 ± 11.0
4k	OH	H	H	OH	Cl	1	D	272-273	106.0 ± 9.8
4l	H	Cl	H	OH	Cl	1	B	280-283	116.3 ± 2.8
4m	H	Br	H	OH	Cl	1	B	298-300	107.9 ± 6.7[1]
4n	Cl	H	Cl	OH	Cl	1	B	246-248	118.3 ± 5.0
4o	Cl	Cl	H	OH	Cl	1	B	258-261	145.8 ± 9.2
4p				OH	Cl	1	B	276 (dec)	107.2 ± 6.8
4q				OH	Cl	1	B	280-282	100.8 ± 2.6

[1] data obtained from *Xenopus laevis* oocytes expressing h-*slo*.

Scheme 2



BBr_3/CH_2Cl_2/-78 °C to rt or pyridine.HCl/220 °C

NS-004 (**2**) and NS-1619 (**3**) possess several structural elements that could contribute to the observed maxi-K opening properties and with the series of compounds presented in the Table we have attempted to understand some of the more fundamental aspects of SAR. Compound **4b**, the closest structural homologue of NS-004 (**2**), is an equi-effective activator of the cloned *m*Slo K^+ channel, indicative of some flexibility in the relationship between the heterocycle and the phenolic hydroxyl. Further homologation of the linker was examined in the context of the des-chloro analogue **4d**, a compound less efficacious than its methylene analogue **4c** which, in turn, is a less effective maxi-K opener than **4b**. This suggests that the phenol hydroxyl is a part of the maxi-K opening pharmacophore although a more explicit examination of this facet of SAR was precluded by the poor solubility of the methyl ether **8b**. It is apparent from the data summarized in the Table that substitution of the heterocyclic nucleus with an electron-withdrawing substituent is essential for maxi-K opening properties, although the effects are subtle. Both the unsubstituted parent molecule **4a** and the 5-CH_3 derivative **4j** are inactive but introduction of Cl, Br, I or NO_2 at the 5 position in all cases restores maxi-K opening activity. A Cl or Br at the 6-position affords weakly active compounds, **4l** and **4m**, respectively, whilst the 5,6-di-chloro substitution pattern is more effective than the 5,7 arrangement presented by **4o**. Interestingly, an electron-deficient pyridine ring system in either of the topological relationships examined with **4p** and **4q** is not an effective substitute for a trifluoro-methylated benzene ring.

In summary, the structure-activity relationships summarized in the Table provide a basic insight into the maxi-K channel opening pharmacophore discovered with NS-004 (**2**) and NS-1619 (**3**). The data indicate the importance of both the nature and pattern of substitution of the heterocyclic nucleus and demonstrate flexibility in the relationship between the phenol and heterocyclic ring systems.

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