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DISCOVERY OF A NOVEL CLASS OF BK CHANNEL OPENERS: ENANTIOSPECIFIC SYNTHESIS AND BK CHANNEL OPENING ACTIVITY OF 3-(5-CHLORO-2-HYDROXYPHENYL)-1,3-DIHYDRO-3-HYDROXY-6-(TRIFLUOROMETHYL)-2*H*-INDOL-2-ONE

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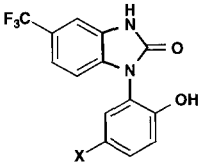
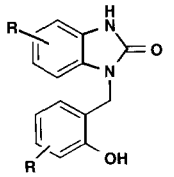
Abstract: 3-Aryl-3-hydroxyindol-2-ones have been identified as a novel class of BK channel openers. Synthesis of both racemic and chiral 3-aryl-3-hydroxyindolones is described along with their electrophysiological evaluation as activators of the cloned BK channel *mSlo* expressed in *Xenopus laevis* oocytes. The preliminary SAR data indicate the importance of both an electron-withdrawing substituent on the oxindole nucleus and the phenolic hydroxyl for expression of BK channel opening properties. Moreover, the dependence of BK channel opening activity on the absolute configuration of the chiral center in this pharmacophore has been demonstrated.

Potassium channels are a structurally diverse family of transmembrane proteins that are modulated by voltage, cell metabolism, calcium or receptor-mediated processes[3,4] and play a key role in the regulation of cell membrane potential and cell excitability.[3–7] Calcium-activated potassium (K_{Ca}) channels are a subgroup of these channels that share a dependence on intracellular calcium ion concentration for activity but are, in addition, also regulated by membrane potential and phosphorylation state.[8] On the basis of their single-channel conductance in symmetrical K^+ solutions, K_{Ca} channels have been broadly classified into three categories: the large conductance (BK or maxi-K) channels, which exhibit a single channel conductance of >150 pS, intermediate conductance channels (50–150 pS) and small conductance channels (<50 pS). BK channels are present in many excitable cell types including neurons and various types of smooth muscle cell.[8,9] Because BK channels are thought to be important regulators of cellular excitability and function, modulators of these channels have emerged as potentially useful agents in the therapy of various disease states associated with both the central nervous and smooth muscle.[10,11]



1 (NS-004), X = Cl
2 (NS-1619), X = CF_3



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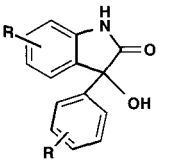

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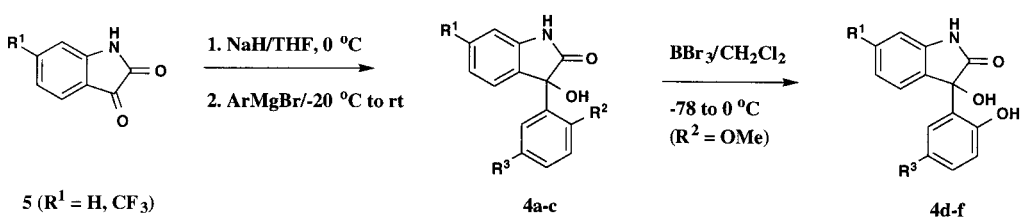
The range of synthetic and naturally occurring compounds that demonstrate BK channel opening activity and their associated electrophysiological and biochemical pharmacological properties has recently been reviewed.[10,11] Among these, the benzimidazolone derivatives NS 004 (**1**) and NS 1619 (**2**) are openers of BK channels[12–14] that have been studied in some detail, both in vitro and in vivo.[15–18] However, structure–activity relationships surrounding NS 004 (**1**) and NS 1619 (**2**) have not been described, although a recent study of structurally homologous N-benzylated benzimidazol-2-one derivatives (**3**) has illuminated some of the more fundamental aspects of this BK opening pharmacophore.[19] As part of an effort directed towards the identification of potent and selective activators of neuronal BK channels, we were interested in evaluating the effect on channel opening activity of variation of the three–dimensional relationships between key structural elements present in NS 004 (**1**). This aspect of SAR was probed by replacing the phenol-bearing N atom of the heterocycle with a carbon atom, thereby introducing an asymmetric center and facilitating an examination of the absolute stereochemistry associated with BK channel opening properties. In this article, we describe the preparation and electrophysiological evaluation of a series of 3-substituted 2*H*-indol-2-one derivatives **4** that provide unique insights into the BK opening pharmacophore originally discovered with NS 004 (**1**).

Chemistry

The racemic 3-aryl-3-hydroxyindol-2-ones **4** were prepared in a straightforward fashion from the corresponding isatins **5**,[20] as depicted in Scheme 1. Addition of an aryl Grignard reagent to the sodium salt of isatin or 6-trifluoromethylisatin in THF gave the corresponding 3-aryl-3-hydroxyindol-2-ones **4a–c** in 80–90% yield. Demethylation of the methyl ether moiety of **4a** and **4c** with BBr_3 in CH_2Cl_2 afforded the corresponding phenols **4d** and **4e** respectively.

Scheme 1

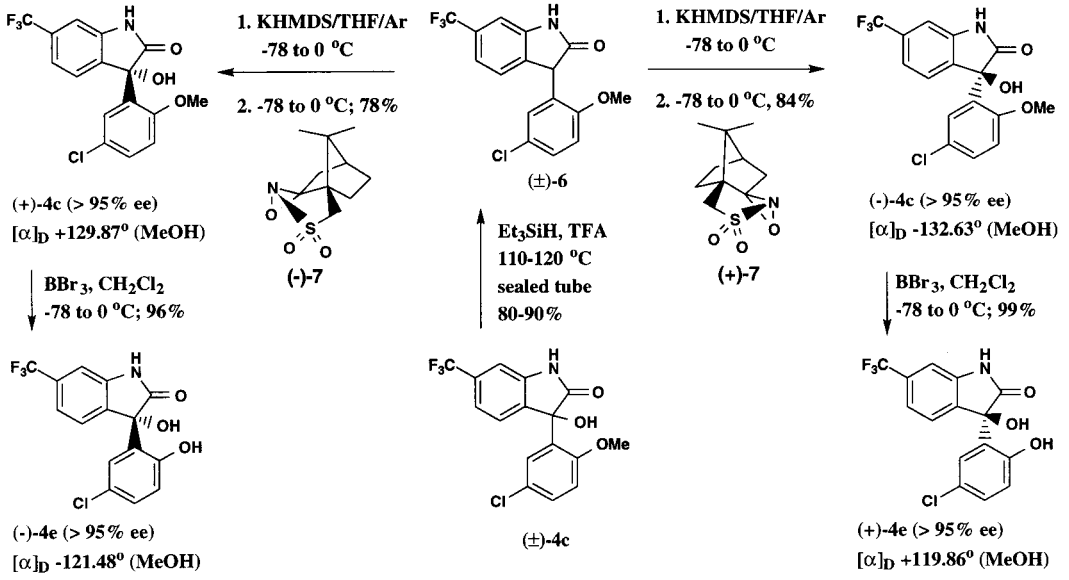


5 (R^1 = H, CF_3) 4a-c 4d-f

A chiral synthesis of 3-aryl-3-hydroxyindol-2-ones was accomplished by way of an asymmetric hydroxylation of the corresponding 3-arylindol-2-ones, as shown in Scheme 2. Direct oxidation of enolates has been used widely for the stereoselective synthesis of α-hydroxy carbonyl compounds. The method developed by Davis et al.[21] employs chiral oxaziridines as the oxidizing agent to asymmetrically hydroxylate enolate anions. Deoxygenation of racemic 3-hydroxyindolone **4c** with neat Et_3SiH and CF_3CO_2H in a sealed tube at 110–120 °C gave the indolone **6**. The potassium enolate of the indolone **6**, prepared with potassium *bis*(trimethylsilyl)amide in degassed dry THF under an argon atmosphere, with rigorous exclusion of oxygen,[22] was oxidized separately with

(1**S**)-(+)- and (1**R**)-(-)-(10-camphorsulfonyl)oxaziridines (**7**). Addition of a THF solution of (+)-**7** to a THF solution of the potassium enolate of **6** at -78 °C followed by gradual warming to 0 °C and then quenching with glacial acetic acid gave the desired 3-hydroxyindolone (-)-**4c**. The enantiomeric purity of (-)-**4c** was determined to be >95% by application of the NMR chiral-shift solvent (L)-trifluoromethylphenyl carbinol.[23] When the reaction was repeated with oxaziridine (-)-**7**, the desired opposite enantiomer (+)-**4c** was obtained in >95% ee. Since suitable single crystals of either enantiomer (+)-**4c** or (-)-**4c** could not be obtained, a chiral 3-hydroxyindolone containing the heavy atom bromine was prepared and the absolute configuration was determined by single crystal X-ray analysis.[24] In analogy with this stereochemical assignment, we tentatively assigned the absolute configurations of (+)-**4c** and (-)-**4c** as shown in Scheme 2. Finally, the methyl ether moieties of (+)-**4c** and (-)-**4c** were demethylated with BBr_3 in methylene chloride to afford the corresponding phenols (-)-**4e** and (+)-**4e**, respectively.

Scheme 2

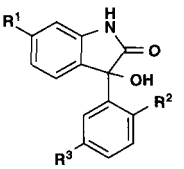


Results and Discussion

The target compounds were evaluated for their effects on outward K^+ current using two-electrode voltage clamp recording from *Xenopus laevis* oocytes expressing cloned *mSlo*[25] (*hSlo*[26] for compounds (±)-**4b**, (±)-**4c**, (+)-**4c**, and (-)-**4c**), as described previously.[27] Voltage clamp protocols consisted of 500–750 ms voltage steps in +20 mV increments from a holding potential of -60 mV to +140 mV in the absence and presence of test compound, which was maintained at a concentration of 20 μM in the recording chamber. All experiments were concluded by incubation (10 min) with 50 nM iberiotoxin (IbTx), a selective blocker of the BK channel, in order to quantify *m*Slo

current expression. A minimum of 5 different oocytes were used to evaluate a single drug concentration and for each compound tested the average percentage change in *m*Slo current relative to drug-free control (100%) was determined. The results obtained are listed in the Table along with data obtained using NS-004 (**1**), which provides a point of reference.

Table: Structure of 3-hydroxy-3-arylindol-2*H*-ones and effect on BK-mediated outward current in *Xenopus laevis* oocytes expressing cloned BK channels.



Cmpd. #	R^1	R^2	R^3	mp (°C)[a]	BK current @ 20 μM (% of control)	BK current @ 30 μM (% of control)
(±)-**4a**	H	OCH_3	Cl	205–207	Not tested	
(±)-**4b**	CF_3	H	Cl	268–270	96.7 ± 5.5[b]	
(±)-**4c**	CF_3	OCH_3	Cl	207–210	119.6 ± 3.5[b]	
(+)-**4c**	CF_3	OCH_3	Cl	243–245	117.0 ± 3.4[b]	
(-)-**4c**	CF_3	OCH_3	Cl	244–245	116.1 ± 4.0[b]	
(±)-**4d**	H	OH	Cl	216–218	95.3 ± 2.6	
(±)-**4e**	CF_3	OH	Cl	210–213	131.0 ± 6.6	200.8 ± 17.8
(+)-**4e**	CF_3	OH	Cl	200–201	123.9 ± 10.8	
(-)-**4e**	CF_3	OH	Cl	198–200	141.0 ± 9.1	
(±)-**4f**	CF_3	OH	H	209–211	90.4 ± 2.9	
1 (NS-004)					131.8 ± 12.8[c]	148.0 ± 7.8

[a]All new compounds exhibited spectroscopic and combustion data in accord with the designated structure.
[b]Data obtained from *Xenopus laevis* oocytes expressing *hSlo* . All other compounds evaluated using *mSlo*.
[c]Reference compound shown to have identical effects on *mSlo* and *hSlo*-mediated BK currents.[27]

The survey of structure–activity relationships summarized in the Table extends and complements the previous study[19] and provides the first evidence for chiral discrimination by the protein that recognizes NS 004 (**1**). In an effort to probe the bound topology of BK channel openers, **4b** was designed to mimic a conformation of NS 004 (**1**) in which the phenol and benzimidazolone rings adopt an orthogonal relationship. That **4b** is inactive suggests a more planar arrangement although, because of reduced acidity, the 3-hydroxyl moiety of **4b** may not adequately substitute for the phenol hydroxyl of NS 004 (**1**). Introduction of a 2-hydroxyl substituent to the 3-phenyl ring of **4b** afforded a compound **4e** that in its racemic form increased outward current by the cloned BK channel to the same extent as NS 004 (**1**), at a concentration of 20 μM. Evaluation of the individual enantiomers of

(±)-**4e** indicated that the (-)-isomer was a more efficacious activator of the cloned BK channel than the (+)-isomer. The solubility properties of (±)-**4e** permitted evaluation at the higher concentration of 30 μM, where it demonstrated superior efficacy to NS 004 (**1**). However, higher concentrations of the separated enantiomers led to precipitation under the assay conditions, precluding a reliable evaluation of their efficacy at 30 μM.

The effect of variation of the substitution pattern of the 3-aryl ring was also examined in this series and indicated that a 2-methoxy group is compatible with BK channel opening activity, although (±)-**4c** exhibited lower efficacy than (±)-**4e**. However, in contrast to the observations noted above with **4e**, the activity of (±)-**4c** exhibits little dependence on chirality at the 3-position of the heterocycle since the resolved enantiomers demonstrate equivalent efficacy.

The BK channel opening properties of (±)-**4e** demonstrate a marked dependence to the nature of substitution on both the heterocyclic and the phenolic rings. Removal of the CF_3 moiety resulted in an inactive compound, **4d**, a result identical to that observed for the benzimidazolone class of BK channel opener.[19] However, the inactivity associated with the des-chloro analogue **4f** distinguishes this series of BK channel openers from the benzimidazolone derivatives where activity was considerably less sensitive to variation of the substitution pattern of the phenol ring.

In summary, we have identified a novel class of BK channel opener and demonstrated that channel opening activity is sensitive to both the nature and pattern of substitution and the absolute configuration of an asymmetric center. The preliminary structure-activity data for this series indicate the importance of both an electron-withdrawing substituent on the oxindole nucleus and the presence of a phenolic hydroxyl for effective expression of BK channel opening properties. A more detailed analysis of SAR for this series will be described in future publications.

Acknowledgment

We would like to thank Dr. Stella Huang for determining the enantiomeric excess of the chiral 3-hydroxyindolones and Dr. Qi Gao for determining the absolute stereochemistry of a chiral 5-bromo-3-hydroxyindolone analogue. We also express our gratitude to Dr. Graham Johnson for helpful discussions and encouragement.

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22. It was found that the enolate of **6** is quite sensitive to even trace amounts of dissolved oxygen and readily undergoes autooxidation, which results in signicficantly reduced asymmetric induction. In order to prevent hydroxylation by molecular oxygen, asymmetric hydroxylation of indolone **6** with the oxaziridines **7** was performed in degassed dry THF under an argon atmosphere.
23. Previously, chiral shift reagents have been used to determine the enantiomeric excess of chiral alcohols as described in reference 21. However, for this series of chiral alcohols, we have found that the use of the chiral solvent (L)-trifluoromethylphenyl carbinol can be used in place of chiral shift reagents to effectively determine the enantiomeric excess of **4c** and **4e**.
24. In order to establish the absolute stereochemistry of the asymmetric hydroxylation protocol, the absolute configuration of a chiral 5-bromo-3-hydroxyindolone analogue was determined by single crystal X-ray analysis. Thus, oxidation of the potassium enolate of 5-bromo-3-(5-chloro-2-methoxyphenyl)-1,3-dihydro-2*H*-indol-2-one with (1S)-(+)-(10-camphorsulfonyl)oxaziridine afforded (3R)-(-)-5-bromo-3-(5-chloro-2-methoxyphenyl)-1,3-dihydro-3-hydroxy-2*H*-indol-2-one.
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