4-Aryl-3-(hydroxyalkyl)quinolin-2-ones: Novel Maxi-K Channel Opening Relaxants of Corporal Smooth Muscle Targeted for Erectile Dysfunction

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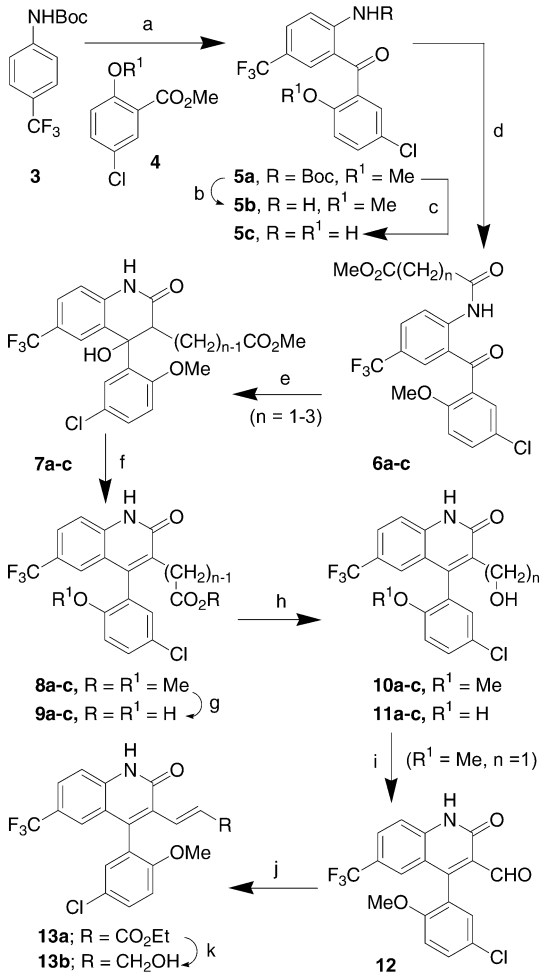
Abstract: Novel 4-aryl-3-(hydroxyalkyl)quinoline-2-one derivatives were prepared and evaluated as openers of the cloned maxi-K channel hSlo expressed in *Xenopus laevis* oocytes by utilizing electrophysiological methods. The effect of these maxi-K openers on corporal smooth muscle was studied in vitro using isolated rabbit corpus cavernosum. From this study, a potent maxi-K opener was identified as an effective relaxant of rabbit corporal smooth muscle and shown to be active in an in vivo animal model of male erectile function.

Introduction. Erectile dysfunction (ED) has been recognized as a common condition that affects approximately 30 million men in the U.S. and over 100 million worldwide.[1] ED is clinically defined as the persistent inability to achieve and maintain an erection adequate for satisfactory sexual activity.[1] The degree of dysfunction may range from moderate to complete ED depending on the age group and other medical conditions such as diabetes, hypertension, and heart disease.[2] In the past 2 decades, a variety of topical and oral drug therapies have been developed to treat male ED.[3,4] Sildenafil (Viagra) is a phosphodiesterase type 5 (PDE5) inhibitor that is a widely used treatment for male ED. However, there have been reports of cardiovascular and retinal side effects associated with this drug.[5,6] There continues to be a need for new and improved agents for the treatment of male ED.

Rationale and Background. It is widely believed that impaired relaxation of the corpus cavernosum smooth muscle is the primary cause of penile ED in a vast majority of impotent men.[7−9] As a consequence, precise modulation of cavernosal (corporal) smooth muscle tone is central to the development of effective and improved treatments for ED. Relaxation of corporal smooth muscle is accomplished by lowering of cytosolic calcium (Ca^{2+}), which is thought to be mediated by several mechanistic pathways.[10] One of the mechanisms involves hyperpolarization of corporal smooth muscle cells via activation of potassium channels.[10] It has been demonstrated that corporal smooth muscle cells express several different K^+ channels of which maxi-K channels were found to be the most prominent subtype.[11,12] Thus, activation of maxi-K channels present in corporal smooth muscle represents an important and attractive mechanism for controlling corporal smooth muscle function.[11−13] Activation of maxi-K channels would lead to membrane hyperpolarization, thereby closing the voltage-gated Ca^{2+} channels with a consequent lowering in cytosolic Ca^{2+} and smooth muscle relaxation. A variety of K_{ATP} channel openers such as cromakalim, pinacidil, and nicorandil have been shown to relax precontracted corporal muscle tissue isolated from both animals[14,15] and humans.[16]

As part of a broad-based effort directed toward the identification of orally bioavailable activators of maxi-K channels that would be useful in smooth muscle relaxation, we synthesized a series of 4-aryl-3-(hydroxyalkyl)-quinolin-2-one derivatives as shown in Schemes 1 and 2. 4-Aryl-3-hydroxyquinolin-2-ones (**1**) have been disclosed as maxi-K channel openers with antibacterial activity.[17] More recently, 4-aryl-3-aminoquinolin-2-ones

Scheme 1[a]



[a] (a) (1) ^{t}BuLi (2.2 equiv), THF, −78 to 40 °C; (2) add **4** at −40 °C then warm to 0 °C, 82%; (b) 3 N HCl, EtOH, reflux, 100%; (c) BBr_3, DCM, −78 to 0 °C, 98%; (d) $MeO_2C(CH_2)_nCOCl$, pyridine, DCM, 0−23 °C, 82−95%; (e) $KN(SiMe_3)_2$, THF, 23 °C, 1−16 h, 90−100%; (f) 35% HBr in HOAc, 78−82%; (g) pyridine·HCl, 200−210 °C, 15−20 min, 80−86%; (h) $Me_2S{\cdot}BH_3$, THF, 0−23 °C, 58−67%; (i) MnO_2, DCM, 23 °C, 54%; (j) $EtO_2CCH_2P(O)(OEt)_2$, NaH, DMF, 86%; (k) ^{i}Bu_2AlH, THF/hexanes, −78 to 23 °C, 87%.

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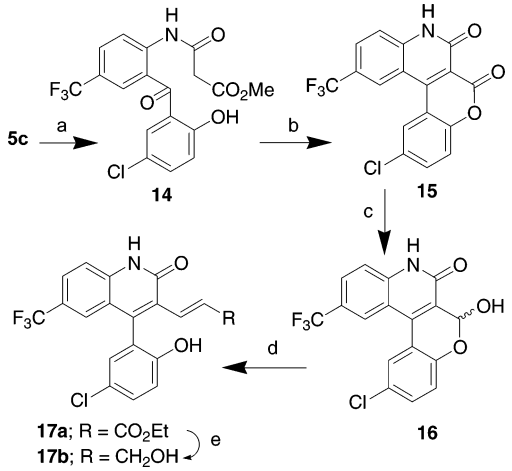
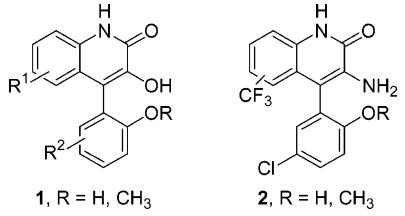
Scheme 2[a]



[a] (a) MeO_2CCH_2COCl, pyridine, DCM, 0−23 °C; (b) KO^{t}Bu, THF, reflux, 83% for two steps; (c) ^{i}Bu$_2$AlH, THF, −78 °C, 78%; (d) $EtO_2CCH_2P(O)(OEt)_2$, NaH, DMF, 72%; (e) ^{i}Bu$_2$AlH, THF/hexanes, −78 to 23 °C, 77%.

Chart 1. Structures of 4-Aryl-3-hydroxyquinolin-2-ones (**1**) and 4-Aryl-3-aminoquinolin-2-ones (**2**)



(**2**) have been identified as brain penetrable maxi-K channel openers with neuroprotective properties[18] (Chart 1).

Chemistry. The requisite 2-aminobenzophenone derivatives (**5a**−**c**) were prepared as described previously.[18] Acylation of **5b** with $MeO_2C(CH_2)_nCOCl$ (n = 1−3) gave the corresponding amides **6a**−**c**, which upon deprotonation with potassium bis(trimethylsilyl)amide in THF undergoes cyclization to afford hydroxyquinolones **7a**−**c**. Dehydration of **7a**−**c** with HBr in AcOH gave the quinolones **8a**−**c**. Simultaneous deesterification and demethylation of **8a**−**c** with pyridine hydrochloride afforded **9a**−**c**. Reduction of the carboxylic acid moiety of **8a**−**c** and **9a**−**c** with a borane−methyl sulfide complex provided the desired alcohols **10a**−**c** and **11a**−**c**, respectively. Oxidation of **10a** (n = 1) with MnO_2 provided the quinolone-3-carboxaldehyde **12**. Olefination of **12** using triethyl phosphonoacetate and NaH in DMF gave the ester **13a**, which was reduced with ^{i}Bu$_2$AlH to the desired allylic alcohol **13b** (Scheme 1).

Since demethylation of the methyl ether moiety of **13b** with a variety of demethylating agents led to the formation of multiple products, the alcohol **17b** was prepared by the route shown in Scheme 2. Acylation of 2-amino-2′-hydroxybenzophenone derivative **5c** with methyl malonyl chloride gave the amide **14**, which upon treatment with KO^{t}Bu in THF afforded the lactone **15**. Partial reduction of **15** with ^{i}Bu$_2$AlH in THF gave the lactol **16**. Alternatively, lactone **15** can be reduced to the alcohol **11a** (n = 1, Scheme 1) with ^{i}Bu$_2$AlH by simply changing the solvent to dichloromethane. Reaction of the lactol **16** with triethyl phosphonoacetate in the presence of NaH in DMF gave ester **17a** as the major product, which was reduced with ^{i}Bu$_2$AlH to afford the alcohol **17b** (Scheme 2). The 4-aryl-3-(hydroxyalkyl)quinolin-2-one derivatives synthesized by the methods illustrated in Schemes 1 and 2 are listed in Table 1 along with relevant physicochemical data.[19]

Table 1. Physical Properties of Compounds **10a**−**c**, **11a**−**c**, **13b**, and **17b**: Effect on Maxi-K-Mediated Outward Current in *Xenopus laevis* Oocytes Expressing the Cloned Maxi-K Channels hSlo and Relaxation of Isolated Rabbit Corpus Cavernosum Precontracted with Phenylephrine

compd	mp[a] (°C)	% increase in hSlo current @ 20 μM	% inhibition of force @ 10 μM[b]
10a (n = 1)[c]	232−235	114 ± 3	NT
10b (n = 2)	219−221	211 ± 20	26.8 ± 5.2 (N = 2)
10c (n = 3)	200−202	258 ± 26	35.7 ± 2.8 (N = 2)
11a (n = 1)	>250 (dec)	198 ± 8	36.0 ± 9.6 (N = 3)
11b (n = 2)	255−256	378 ± 35	83.7 ± 11.0 (N =3)
11c (n = 3)	257−259	292 ± 30	43.0 (N = 1)
13b	266−268	223 ± 9	31.8 ± 2.1 (N = 3)
17b	203−206	257 ± 18	46.0 ± 11.2 (N = 7)
NS-004		132 ± 13	31.0 ± 5.9 (N = 5)

[a] All new compounds exhibited spectroscopic and combustion data in accord with the designated structure.[19] [b] Percentage inhibition of isometric force in response to a test compound in isolated rabbit corpus cavernosum precontracted with phenylephrine (3 μM). Vehicle (DMSO) produced a 12.5 ± 6.7% inhibition of force. [c] This n refers to that in Scheme 1.

Results and Discussion. The ability of the target compounds to increase maxi-K-mediated whole-cell outward K^+ currents was determined by using two-electrode voltage clamp recordings from *Xenopus laevis* oocytes expressing cloned hSlo[20] maxi-K channels, as previously described.[21] All compounds were tested in a minimum of five different oocytes to evaluate the effect of a single drug concentration (20 μM) on outward K^+-current sensitive to iberiotoxin (IbTx). The average percentage change in hSlo current relative to drug free control (100%) was determined for each compound tested. The results obtained are presented in Table 1.

In this preliminary study, the optimal hydroxyalkyl group at C-3 was examined while restricting the substitution pattern of the quinoline nucleus to 6-CF_3 and the 4-aryl moiety to either *p*-chlorophenol or *p*-chloroanisole, an element present in prototype maxi-K openers **1** and **2**. As can be seen from the data presented in Table 1, all the compounds (except **10a**) were shown to be potent openers of maxi-K channels. In all examples, the *p*-chlorophenol derivatives (**11a**−**c**, **17b)** were more effective openers when compared to their methyl ether analogues, **10a**−**c**, and **13b**. Surprisingly, all the methyl ether derivatives (except **10a**) of this series exhibited good efficacy when compared to the corresponding methyl ether derivatives of quinolones, **1**,[17] and **2**.[18] The hydroxyethyl derivative **11b** was found to be the most effective opener identified from this study.

Effects of 4-Aryl-3-(hydroxyalkyl)quinolin-2-ones on Isolated Rabbit Corpus Cavernosum. A rabbit corpus cavernosal tissue strip assay was used to evaluate the functional effects of maxi-K openers on smooth muscle relaxation.[22] Corpus cavernosum strips were isolated from the rabbit penis and suspended in tissue baths containing warm (37 °C) physiological salt solution. Isometric force was measured using standard methods. Tissue strips were stimulated with the α-agonist phenylephrine (3 μM) and allowed to reach a steady level of force prior to the addition of test compounds.

The results are expressed as the percentage inhibition of phenylephrine-induced force compared to that of the vehicle control (Table 1).

As can be seen from the data presented in Table 1, a majority of the compounds produced greater than a 30% reduction of contractile force at 10 μM. In general, relatively more efficacious maxi-K channel openers (i.e., *p*-chlorophenol analogues), **11a**−**c**, and **17b** were more effective relaxants when compared to the corresponding *p*-chloroanisole derivatives **10b**, **10c**, and **13b**. Thus, a weak correlation between maxi-K channel opening activity and the ability to relax phenylephrine-induced contractions in rabbit corporal tissue strip assay is observed for this limited series. Compound **11b** was found to be particularly effective with an inhibition of the contractile response by over 80% at 10 μM, which is comparable to the maximum relaxation observed with K_{ATP} openers Cromakalim (88%) and Pinacidil (87%) in a similar assay.[14] In a direct comparison, a prototype maxi-K opener NS-004 showed 31% reduction at 10 μM. Furthermore, **11b** produced a concentration-dependent relaxation (IC_{50} = 5.0 μM) that developed slowly over a period of 90 min. The ability of iberiotoxin (IbTx), a selective maxi-K channel blocker, to reverse the relaxation produced by **11b** was determined to assess the contribution of maxi-K opening to relaxation. Interestingly, the addition of IbTx (300 nM) to control tissues resulted in an augmentation of the existing α-agonist evoked force (30.6 ± 3.9% increase, n = 7 strips). This important observation provides direct evidence that maxi-K channels play a physiologically relevant role in the regulation of adrenergically mediated tone in the corpus cavernosum. Addition of IbTx to the corporal tissue strips treated with **11b** (3 μM) almost fully reversed the relaxation produced by this compound (compound + IbTx = 99.7 ± 5.8%, n = 7 strips; vehicle + IbTx = 104.1 ± 7.1, n = 6 strips), supporting the notion that **11b** induced relaxation by opening maxi-K channels.

Effects of 11b in a Rat Model of Erectile Function. The in vivo effects of **11b** on erectile function were evaluated by using a rat model that has been fully described in the scientific literature.[23] Male Fischer 344 rats (approximately 280−350 g) were anesthetized with sodium pentobarbital. A carotid artery was cannulated for the measurement of blood pressure, and both jugular veins were cannulated for the administration of test compound and the constant infusion of anesthetic (sodium pentobarbital). The trachea was cannulated to allow for artificial ventilation, and body temperature was maintained at 37 ± 0.5 °C using a heating blanket connected to a rectal probe thermistor. The right corpora cavernosa was exposed and cannulated for the measurement of intracavernous pressure. The cavernous nerve was isolated, and a stainless steel bipolar electrode was then placed around the nerve to allow for electrical stimulation. Mean arterial blood pressure and intracavernous pressure were continuously monitored. The cavernous nerve was electrically stimulated for 30 s at a frequency of 20 Hz, typically using 0.3 mA pulses of 0.22 ms duration. A second control stimulation was performed 15 min after the first stimulation, and then either vehicle (PEG 400) or compound was given intravenously. Repeated stimulations were performed at 5, 20, 35, 50 and 65 min after treatment (Figure 1). Statistics were performed using the Student *t*-test, and $p < 0.05$ was considered significant.

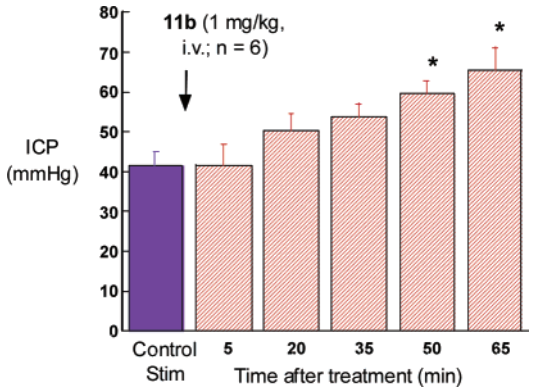


Figure 1. Effect of **11b** on intracavernous pressure (ICP) response elicited by electrical stimulation of the cavernous nerve at various time points following compound administration.

Administration of PEG 400 alone had no effect on either baseline or electrically stimulated increases in intracavernous pressure (data not shown). Intravenous injection of compound **11b** had no effect on basal intracavernous pressure but produced a potentiation of the electrically evoked increase in intracavernous pressure. This potentiation was observed at time points ≥20 min following compound administration and reached statistical significance 50 min postdose.

In summary, we have identified a novel series of maxi-K channel openers and demonstrated that channel opening activity is dependent on both the nature of the 3-(hydroxyalkyl) group and the 4-aryl moiety. Several of these maxi-K openers are effective relaxants of precontracted rabbit corpus cavernosal strips in vitro. However, a weak correlation between this functional effect and maxi-K channel opening activity was observed, implicating a role for additional biochemical mechanisms and tissue selectivity in the rabbit corporal tissue strip assay. Nevertheless, we have identified a potent maxi-K channel opener as an effective and maxi-K-mediated relaxant of corpus cavernosum tissue in vitro. Furthermore, we have demonstrated the in vivo efficacy of this maxi-K opener in a rat model of erectile function, indicating a potential utility in the treatment of male ED. In conclusion, we have demonstrated the efficacy of a maxi-K opener to elicit penile erection as a novel mechanism for the treatment of ED.

Supporting Information Available: Experimental data for various compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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