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4-Aryl-3-(mercapto)quinolin-2-ones: novel maxi-K channel opening relaxants of corporal smooth muscle

Piyasena Hewawasam,[a,*] Wenhong Fan,[a] Deborah A. Cook,[b] Kimberly S. Newberry,[b] Christopher G. Boissard,[b] Valentin K. Gribkoff,[b] John Starrett[a] and Nicholas J. Lodge[b]

[a]*Department of Chemistry, The Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA*
[b]*Neuroscience/Genitourinary Drug Discovery, The Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA*

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Abstract—A novel series of 4-aryl-3-(mercapto)quinolin-2-one derivatives was 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*. In vivo efficacy has been demonstrated with a selective maxi-K opening relaxant in a rat model of erectile function.

Erectile dysfunction (ED) has been recognized as a common condition that afflict over 100 million men worldwide.[1] Clinically, ED is defined as the consistent inability to achieve and maintain an erection adequate for satisfactory sexual activity.[1] Severity of dysfunction can vary from moderate to complete ED depending on the age group and other health conditions such as diabetes, hypertension, and heart disease.[2] A variety of topical and oral drug therapies have been developed to treat ED during the past two decades.[3,4] Sildenafil (Viagra) and other phosphodiesterase type 5 (PDE5) inhibitors are effective oral agents for the treatment of male ED. However, there continues to be a need for new agents that have a reduced likelihood of cardiovascular or other side effects,[5,6] and that may treat patients refractory to PDE5 inhibitors.

It is generally believed that impaired relaxation of the corpus cavernosum smooth muscle is one of the major causes of penile erectile dysfunction in a great majority of impotent men.[7–9] Thus, precise modulation of corporal smooth muscle function plays a important role in developing effective and improved treatments for erectile dysfunction. There are several mechanistic pathways whereby lowering of cytosolic calcium (Ca^{2+}) leads to the relaxation of corporal smooth muscle.[10] However, in general the majority of these pathways depend upon the accumulation of cyclic nucleotides cAMP and cGMP or hyperpolarization of corporal smooth muscle cells via activation of potassium channels.[10] Maxi-K channels are the most prominent subtype among the several different K^+-channels that are present in corporal smooth muscle cells.[11,12] Therefore, activation of maxi-K channels represents an important and attractive mechanism for controlling corporal smooth muscle function.[11–13] Opening of maxi-K^+ channels would result in membrane hyperpolarization, thereby closing the voltage-gated Ca^{2+}-channels with consequent lowering in cytosolic Ca^{2+} and smooth muscle relaxation.

In pursuit of identifying orally bioavailable and water-soluble activators of maxi-K channels that would be useful in smooth muscle relaxation, we synthesized a series of 4-aryl-3-(mercapto)quinolin-2-one derivatives as shown in Scheme 1.

Prior to this work a series of 4-aryl-3-hydroxyquinolin-2-ones (**1**) has been disclosed as maxi-K channel openers with antibacterial activity.[14] A related series of 4-aryl-3-aminoquinolin-2-ones (**2**) has been identified as brain penetrable maxi-K channel openers with

* Corresponding author. Tel.: +1-203-677-7815; fax: +1-203-677-7702; e-mail: hewawasp@bms.com

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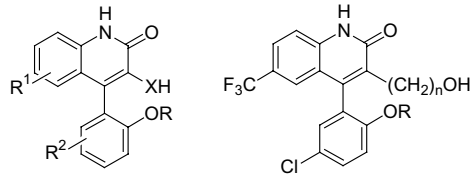
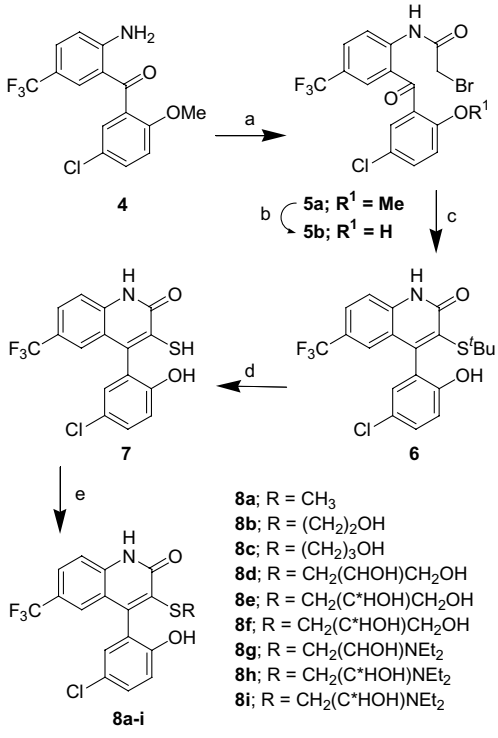


Chart 1.

neuroprotective properties.[15] More recently, a series of 4-aryl-3-(hydroxyalkyl)quinolin-2-ones (**3**) has been discovered as novel maxi-K channel opening relaxants of corporal smooth muscle[16] (Chart 1).

Acylation of the 2-aminobenzophenone derivative **4**[16] with bromoacetyl bromide gave the corresponding amide **5a**. Demethylation of methyl ether moiety of **5a** with BBr_3 provided the corresponding phenol **5b**. Upon reaction of **5b** with *tert*-butyl mercaptan in the presence of NaH (2.2 equiv) in THF, initially formed *tert*-butylthioacetamide intermediate undergoes *cyclo*-dehydration to afford the quinolinone **6**. Treatment of **6** with BBr3 in DCM afforded the desired 3-(mercapto)quinolinone **7**. Selective S-alkylation of the 3-mercapto moiety with respective alkylating agents[17] in the presence of NaH in THF gave the quinolinone derivatives **8a**–**i** (Scheme 1). The 4-aryl-3-(mercapto)quinolin-2-one derivatives[18] prepared by the methods illustrated in Scheme 1 are listed in Table 1 along with solubility data.



Scheme 1. Reagents and conditions: (a) $ClC(O)CH_2Br$, pyridine, DCM, 0–23 °C; 96%; (b) BBr_3, DCM, −78–23 °C; 91%; (c) ^tBuSH, NaH, THF, 23–67 °C; 87%; (d) BBr_3, DCM, −78–0 °C; 100%; (e) RX (X = Br, Cl), NaH, THF, 23 °C, 16 h.

Table 1. Water solubility of compounds **6**, **7**, **8a**–**i**, 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 pre-contracted with phenylephrine

Compd	Solubility[a] mg/mL	% *hSlo* current @ 20 μM	% Inhibition of force @ 10 μM[b]
8a	<0.001	231 ± 23	22 (n = 2)
6	<0.001	Insol @ 20 μM 73 ± 7 @ 5 μM	NT
7	0.003	197 ± 5	NT
8b	0.004	348 ± 29	43 ± 10 (n = 5)
8c	0.003	242 ± 16	23 ± 5 (n = 3)
(±)-**8d**	0.194	246 ± 13	31 ± 10 (n = 6)
(*S*)-**8e**	0.092	223 ± 21	45 ± 10 (n = 6)
(*R*)-**8f**	0.097	256 ± 17	41 ± 7 (n = 5)
(±)-**8g**	>1.9	192 ± 11	55 ± 6 (n = 12) 34 ± 3 (n = 8) @ 1 μM
(*S*)-**8h**	>1.6	173 ± 9	59 ± 7 (n = 5) 33 ± 5 (n = 6) @ 1 μM
(*R*)-**8i**	>1.6	162 ± 11	45 ± 7 (n = 4) 30±6 (n = 6) @ 1 μM
NS-004		132 ± 13	31 ± 3 (n = 5)

[a] Solubility in HPLC-grade water as measured by UV absorptivity method.

[b] Percentage inhibition of isometric force in response to a test compound in isolated rabbit corpus cavernosum pre-contracted with phenylephrine (3 μM). Vehicle produced approximately 13% relaxation.

The effect of the target compounds on whole-cell outward K^+-currents was determined by using two-electrode voltage clamp recordings from *Xenopus laevis* oocytes expressing cloned *hSlo*[19] maxi-K channels, as described previously.[20] 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 a variation of 3-mercapto substitution was examined while restricting the quinoline nucleus to 4-(5-chloro-2-hydroxyphenyl)-6-(trifluoromethyl)quinolin-2-one, an element identified from prototype maxi-K openers **1**–**3**. In order to identify more soluble maxi-K openers polar groups such as alcohols and aminoalcohols were incorporated into the 3-mercapto moiety. As can be seen from the data presented in Table 1, all compounds including the parent 3-mercapto derivative (**7**) were shown to be effective openers of maxi-K channels. However, parent compound **7** and both *tert*-butyl and methyl thioethers of **7** showed extremely poor aqueous solubility. Hydroxyethyl derivative **8b** was the most effective maxi-K opener identified from this study. However, both hydroxyethyl and hydroxypropyl derivatives (**8b**,**c**) lacked adequate aqueous solubility. Incorporation of additional hydroxyl groups (**8d**–**f**) into the side chain somewhat improved the aqueous solubility (<100 μg/mL). Although replacement of terminal hydroxyl moiety with a dialkylamino group slightly diminished the maxi-K opening activity a significant enhancement in aqueous solubility (>1 mg/mL) was

observed. The racemic amino alcohol (**8g**) and individual enantiomers (**8h**,**i**) were equally effective openers with comparable aqueous solubility as their free base form. The different salt forms of these amino alcohols exhibited 40–100 times higher aqueous solubility.

The functional effects of maxi-K openers on smooth muscle relaxation were evaluated using a rabbit *corpus cavernosum* tissue strip assay.[21] *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 as compared to vehicle control (Table 1).

As shown in Table 1, a majority of maxi-K openers produced greater than a 30% reduction of contractile force at concentration of 10 μM. In direct comparison, a prototype maxi-K opener, NS-004 showed 31% reduction at 10 μM. In particular, racemic amino alcohol **8f** inhibited the contractile response by over 50% at 10 μM. The (*S*)-enantiomer **8h** was slightly more effective than (*R*)-enantiomer **8i** in rabbit corporal tissue assay. However, despite their robust maxi-K channel opening activity some of the compounds displayed poor inhibition of the contractile response. For an example, maxi-K openers (**8a**,**c**) were shown to be less effective as relaxants. 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. It is, therefore, plausible that other mechanisms contribute to the in vitro relaxant responses.

The in vivo effects of racemic amino alcohol **8g** were evaluated using a rat model of erectile function as previously described in the literature.[22] Male Fischer 344 rats (280–350 g) were anesthetized with sodium pentobarbital. One carotid artery was cannulated to allow the measurement of mean arterial blood pressure (MABP). Both jugular veins were also cannulated for the administration of test compound as well as a constant infusion of sodium pentobarbital to maintain the plane of anesthesia. The trachea was cannulated to allow for artificial ventilation. 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 (ICP). The cavernous nerve was isolated and a stainless steel bipolar electrode was placed around the nerve to allow for electrical stimulation. MABP and ICP were continuously monitored. The cavernous nerve was electrically stimulated at a frequency of 20 Hz (typically 0.3 mA pulses of 0.22 ms duration) for a period for 30 s. A second control stimulation was given 15 min after the first and then either vehicle (PEG 400)[23] or compound was administered iv. Repeated stimulations were performed at 5, 20, 35, 50, and 65 min post treatment. Student's *t*-test was used for the analysis of data with $p < 0.05$ considered significant.

Amino alcohol **8g** (1 mg/kg iv) produced a significant increase in electrically-stimulated ICP (Fig. 1). The vehicle PEG400 had no effect on ICP ($n = 6$; not shown). MABP increased following injection of **8g** (Fig. 1). However, a similar increase was observed in a separate group of animals given only vehicle (Fig. 1). The α_1/α_2 adrenergic receptor antagonist phentolamine was evaluated as a comparitor; Vasomax (phentolamine mesylate) has been shown to be effective in the treatment of erectile dysfunction.[24] Phentolamine (0.03 mg/kg iv) did not affect electrically-stimulated ICP but produced a 13% fall in MABP ($n = 6$). A higher dose of phentolamine (0.3 mg/kg iv) produced a significant increase in ICP, but only at the 65 min post-dose time-point (Fig. 2). MABP in these animals decreased by 32% and then recovered slowly (Fig. 2). Saline did not affect blood pressure (separate group of animals; Fig. 2) or electrically-evoked ICP ($n = 6$; not shown). Thus, relative to phentolamine, **8g** produced an increase in ICP with a less effect on blood pressure.

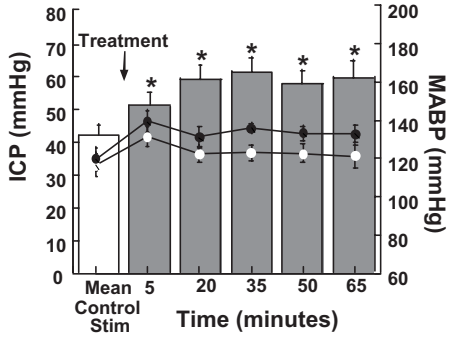


Figure 1. Effect of **8g** (1 mg/kg, iv) on intracavernous pressure (ICP) response elicited by electrical stimulation of the cavernous nerve at various time points following compound administration. ICP is indicated by the bars ($n = 6$). ∗ indicates $p < 0.05$. Blood pressure in response to **8g** (-●-) ($n = 7$) and PEG 400 vehicle (-○-) ($n = 6$).

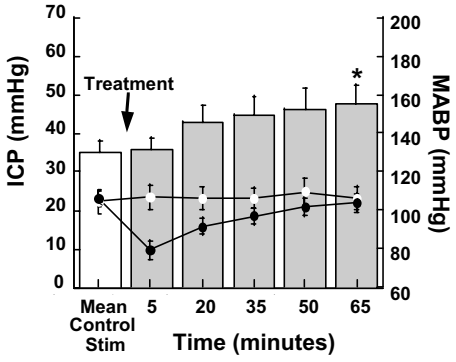


Figure 2. Effect of phentolamine on intracavernous pressure (ICP) response elicited by electrical stimulation of the cavernous nerve at various time points following compound administration. ICP is indicated by the bars ($n = 6$). ∗ indicates $p < 0.05$. Blood pressure in response to phentolamine (-●-) ($n = 6$) and saline vehicle (-○-) ($n = 6$).

In summary, we have identified a novel series of effective maxi-K channel openers and demonstrated that channel opening activity can be retained or enhanced by derivatizing the 3-mercapto moiety. A great majority of these maxi-K openers were shown to be effective relaxants of pre-contracted rabbit corpus cavernosal strips in vitro. Introduction of amino alcohol side chain imparts excellent water solubility to these maxi-K opening relaxants. Furthermore, we have demonstrated the in vivo efficacy of these maxi-K openers in a rat model of erectile function. In conclusion, we have identified a series of water-soluble maxi-K channel opening relaxants of corporal smooth muscle that offer potential as agents for the treatment of male ED.

Acknowledgements

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