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The Synthesis and Structure–Activity Relationships of 1,3-Diaryl 1,2,4-(4*H*)-triazol-5-ones: A New Class of Calcium-Dependent, Large Conductance, Potassium (Maxi-K) Channel Opener Targeted for Urge Urinary Incontinence

Piyasena Hewawasam,[a,*] Matthew Erway,[a] George Thalody,[b] Harvey Weiner,[b] Christopher G. Boissard,[b] Valentin K. Gribkoff,[b] Nicholas A. Meanwell,[a] Nicholas Lodge[b] and John E. Starrett, Jr.[a]

[a]*Department of Chemistry, The Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA*

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

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Abstract—A series of 1,3-diaryl 1,2,4-(4*H*)-triazol-5-ones was prepared and shown by electrophysiological analysis to activate a cloned maxi-K channel *mSlo* (or *hSlo*) expressed in *Xenopus laevis* oocytes. The effects of these structurally novel maxi-K channel openers on bladder contractile function were studied in vitro using isolated rat bladder strips pre-contracted with carbachol. Several 1,3-diaryl 1,2,4-(4*H*)-triazol-5-one derivatives were found to be potent smooth muscle relaxants but this activity did not completely correlate with maxi-K channel opening.

Urinary incontinence (UI) is defined clinically as an involuntary loss of urine that affects both men and women.[1] UI is a widespread condition with a significant social and hygienic impact and adverse effects on the quality of life.[2] The prevalence of UI among the general population is estimated to be about 10–20% worldwide and affects over 13 million people in the United States.[2] Current drug therapies are only modestly effective and suffer from poor side effect profiles.[3,4] UI has been classified into three major categories: urge incontinence (hyperactive bladder), stress incontinence, and overflow incontinence.[1,5] Urge urinary incontinence (UUI) is the most prevalent type of incontinence and considered to be most amenable to pharmacological intervention. UUI is characterized by abnormal spontaneous bladder smooth muscle contractions, which arise during bladder filling.

Currently, UUI is primarily treated with drugs that partially inhibit contractions of the bladder smooth (detrusor) muscle. Antimuscarinics have been widely prescribed for the treatment of bladder instability,[4,6] but, although effective, these drugs are not considered ideal because of their undesirable anticholinergic side effects and potential for the inhibition of normal contractile function.[6]

Large-conductance, Ca^{2+}-dependent potassium (maxi-K) channels are present in many excitable cell types, including neurons and various types of smooth muscle cell.[7,8] Because maxi-K 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 system and smooth muscle.[9,10] It has been demonstrated that rat and human urinary bladder smooth muscle cells express maxi-K channels and that they play a critical role in regulating excitability and contractility.[11,12] Thus, activation of maxi-K channels present in bladder smooth muscle represents a unique and attractive mechanism for inhibiting abnormal contractility because, as a consequence of their Ca^{2+} and voltage

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

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dependence, only depolarized (contracting) tissues are affected, thereby reducing the potential for undesirable side effects. An increase in K^+ conductance through the cell membrane would lead to hyperpolarization, thus inhibiting the influx of calcium ions through voltage-gated Ca^{2+} channels, reducing cell excitability and relaxing the smooth muscle cells.

Although the ATP-dependent potassium (K_{ATP}) channel opener, Cromakalim has shown some beneficial effect against bladder instability in humans, its clinical efficacy is severely limited by cardiovascular side effects.[13] However, another K_{ATP} opener, ZD-6169, discovered by AstraZeneca, has been claimed to show selectivity for bladder smooth muscle over cardiovascular smooth muscle in vivo.[14] Using patch-clamp methods on smooth muscle cells isolated from guinea pig bladder detrusor, it has been demonstrated that the maxi-K channel opener NS-004 exerts a robust augmentation of maxi-K currents at concentrations greater than 20 μM.[15] More recently, a novel detrusor-selective maxi-K channel opener, NS-8, discovered by scientists at Nippon Shinyaku, has been shown to exert a relaxant effect on isolated rat detrusor pre-contracted with KCl.[16] As part of our studies of the maxi-K channel opening pharmacophore associated with NS-004, we synthesized a series of disubstituted triazolones and evaluated them as openers of cloned maxi-K channels and as relaxants of bladder smooth muscle (Chart 1).

A series of triazolones was prepared as depicted in Scheme 1. Phenylglyoxylic acids **6a**–**g** were prepared by the addition of an ethereal solution of a Grignard reagent prepared from the aryl bromides **5a**–**g** to a cold (−78 °C) solution of diethyl oxalate in ether followed by saponification with aqueous NaOH. Condensation of the phenylglyoxylic acid derivatives **6a**–**g** with an arylhydrazine **7**[17] in refluxing EtOH gave the corresponding arylhydrazones **8a**–**g**. Curtius rearrangement of the carboxylate moiety of **8a**–**g** was effected using diphenylphosphoryl azide and Et_3N in refluxing toluene to generate the corresponding isocyanates, which spontaneously cyclized to give the triazolones **9a**–**g**. Demethylation of the methyl ether moiety of **9a**–**g** with BBr_3 afforded the triazolones **10a**–**g**, compiled in Table 1 along with relevant physicochemical data.

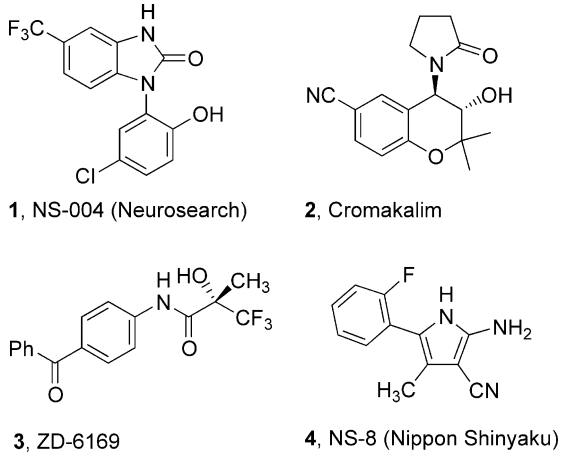
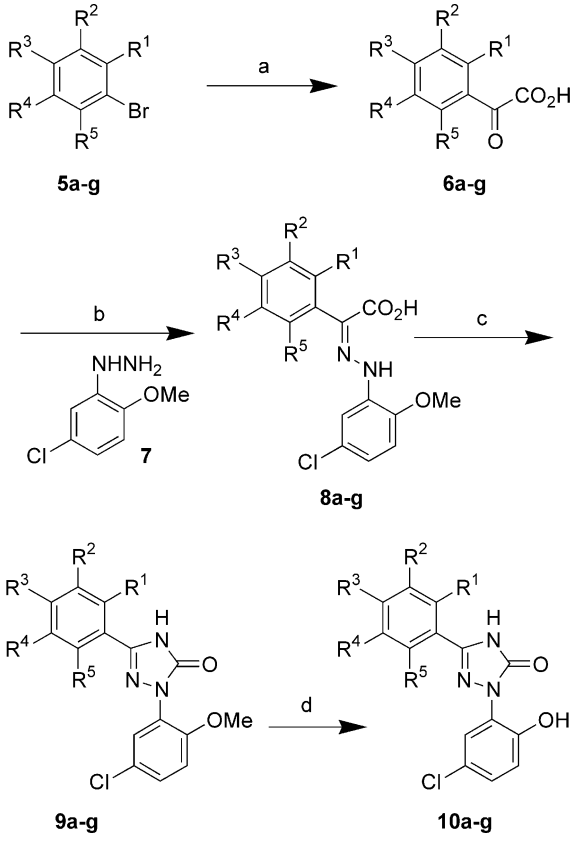


Chart 1.

The effect of the target compounds on outward K^+-current was determined by using two-electrode voltage clamp recording from *Xenopus laevis* oocytes expressing cloned *mSlo*[18] (or *hSlo*[19]) maxi-K channels, as described previously.[20]

The voltage clamp protocols consisted of 500–750 ms voltage steps in +20 mV increments from a holding potential of −60 mV to +140 mV which were conducted in the absence and presence of test compound. Compounds under evaluation were maintained at a concentration of 20 μM in the recording chamber and all experiments were concluded by incubation of the oocytes for 10 min with 50 nM iberiotoxin (IbTX). Voltage clamp protocols in the presence of this selective blocker of the maxi-K channel allowed quantification of *mSlo* or *hSlo* current expression. In all cases, a minimum of five different oocytes were used to evaluate the effect of a single drug concentration on channel current and the average percentage change in *mSlo* (or *hSlo*) current relative to drug-free control (100%) was determined for each compound tested. The results obtained are presented in Table 1 along with data for NS-004, which allows the efficacy of the triazolone derivatives to be compared with a prototypical maxi-K channel opener.



Scheme 1. (a) (1) Mg, ether; (2) $(CO_2Et)_2$, −78 °C; (3) NaOH, THF; >90%; (b) add **7**, EtOH, reflux; 95–100%; (c) Et_3N, $(PhO)_2P(O)N_3$, toluene, reflux, 90–95%; (d) BBr_3, DCM, 0 °C to rt; 90–95%.

Table 1. Structure and physical properties of 1,3-diaryl 1,2,4-(4*H*)-triazol-5-one derivatives **10a–g**, effect on maxi-K-mediated outward current in *Xenopus laevis* oocytes expressing the cloned maxi-K channels *mSlo* and relaxation of isolated rat bladder strips pre-contracted with carbachol

Compd	R^1	R^2	R^3	R^4	R^5	Mp (°C)[a]	% Increase in *mSlo* current @ 20 μM	% Inhibition of force @ 3 μM[c]
10a	H	H	H	H	H	276–278	Insol. @ 20 μM 91.4±5.9 @ 1 μM	35.0±6.3 89.4 @ 20 μM
10b	CF_3	H	H	H	H	167–170	117.1±4.3	34.0±3.0 90±2 @ 20 μM
10c	H	CF_3	H	H	H	240–245	161.5±4.6 @ 10 μM[b]	42 ($n=2$)[e]
10d	H	H	CF_3	H	H	252–255	196.8±17.7	87±3 @ 20 μM 22±7 @ 1 μM
10e	H	CF_3	H	CF_3	H	250–253	154.2±4.8	15 ($n=2$)
10f	H	Cl	CF_3	H	H	220–224	198.5±10.5	5 ($n=2$)
10g	H	H	CF_3	H	CF_3	270–275	175.5±9.4	46 ($n=2$) 94±1 @ 20 μM
1 (NS-004)							131.8±12.8[d]	8±6 @ 20 μM

[a]All new compounds exhibited spectroscopic and combustion data in accord with the designated structure.
[b]Data obtained from *Xenopus laevis* oocytes expressing *hSlo*.
[c]Percentage inhibition of isometric force in response to a test compound in isolated rat bladder strips pre-contracted with 10 μM carbachol.
[d]Reference compound **1** (NS-004) shown to have identical effects on *mSlo* and *hSlo*-mediated maxi-K currents..[20]
[e]Standard deviation not determined for $n=2$.

The structure–activity relationships described in Table 1 provide a basic understanding of the triazolone maxi-K channel opening pharmacophore. For this preliminary SAR investigation, the substitution pattern of the 1-aryl moiety was restricted to the *p*-chlorophenol element present in the prototypical maxi-K channel opener NS-004 and the optimal substitution pattern of 3-aryl moiety probed. From the results presented in Table 1, it appears that an electron withdrawing substituent is essential for the expression of maxi-K channel opening activity, a structure–activity relationship similar to that observed with a series of benzimidazolone derivatives.[21] The introduction of a CF_3 substituent markedly enhances channel opening efficacy as demonstrated by the three regioisomers **10b–d**. Furthermore, both the *para* regioisomer **10d** and the *meta* regioisomer **10c** were found to be superior to the *ortho* CF_3 substituted analogue **10b**. Similarly, both the 3,5-*bis*-CF_3 and the 2,4-*bis*-CF_3 derivatives **10e** and **10g**, respectively, exhibited good efficacy as did the 3-Cl, 4-CF_3 analogue **10f**.

The triazolones **10a–g** were evaluated in vitro for their ability to inhibit the contractile response induced by carbachol in rat bladder strips isolated from male Sprague–Dawley rats (250 to 350 g). The contractile response to 10 μM carbachol was measured twice and, following the second washout, the tissues were exposed to the test compound (1, 3, or 20 μM) for 1 h. A third contractile response to carbachol was then determined followed by washing away the test compound and assessing the contractile response to a fourth application of carbachol. For all experimental trials, one bladder strip per animal served as a paired vehicle control. The results are expressed as the percentage reduction of the second carbachol-induced response and were corrected for any spontaneous changes observed in the paired, vehicle control.

Several of the compounds in Table 1 produced greater than a 30% reduction of contractile force at concentration of 3 μM and four compounds, **10a**, **10b**, **10d**, and **10g**, inhibited the contractile response by almost 90% at 20 μM. Compound **10d** was found to be particularly potent with a significant relaxant effect recorded at concentrations as low as 100 nM. For comparison, reference compound **1** (NS-004) was not effective in this assay. However, as can be seen from the data presented in Table 1, the correlation between maxi-K channel opening activity and the ability to inhibit the force of carbachol-induced contractions in rat bladder strip assay is poor. In an attempt to reverse the rat bladder relaxation with iberiotoxin, a selective maxi-K channel blocker, only partial reversal was observed implicating a role for additional biochemical mechanisms in the tissue assay.

In summary, we have identified a novel class of maxi-K channel opener and demonstrated that channel opening activity is sensitive to both the nature and pattern of substitution of the 3-phenyl ring. The preference for electron-withdrawing substituents is reminiscent of SAR observed for a series of benzimidzolone-based maxi-K openers.[21] Whilst effective relaxants of pre-contracted rat bladder strips in vitro, the lack of correlation between this functional effect and maxi-K channel opening activity suggest that alternate mechanisms are operative. Nevertheless, these triazolone derivatives offer potential as agents for the treatment of urinary incontinence. A more detailed analysis of SAR for this series along with effects of these maxi-K channel openers on bladder contractile function will be described in future publications.

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