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The Discovery of a New Class of Large-Conductance Ca^{2+}-activated K^+ Channel Opener Targeted for Overactive Bladder: Synthesis and Structure–Activity Relationships of 2-Amino-4-azaindoles

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Abstract—2-Amino-4-azaindoles have been identified as a structurally novel class of BK_{Ca} channel openers. Their synthesis from 2-chloro-3-nitropyridine is described together with their in vitro properties assessed by $^{86}Rb^+$ efflux and whole-cell patch-clamp assays using HEK293 cells stably transfected with the BK_{Ca} α subunit. In vitro functional characterization of BK_{Ca} channel opening activity was also assessed by measurement of relaxation of smooth muscle tissue strips obtained from Landrace pig bladders. The preliminary SAR data indicate the importance of steric bulk around the 2-amino substituent.

Overactive bladder (OAB) is characterized by symptoms of increased urinary urgency, frequency, and/or incontinent episodes.[1] Until recently, urge urinary incontinence (UUI) has been the most noticeable manifestation of this condition although it represents only a subset of the potential patient population. In the US alone, estimates for patients who suffer from OAB range from 13–17 million.[2] Fewer than 50% of individuals consult their health care provider, and only about 20% of sufferers are on pharmacological therapy. Consequently, OAB is thought to be underestimated and insufficiently diagnosed due to lack of physician and patient awareness, limited practical and effective treatments, and patient embarrassment.

OAB is associated with the urodynamic finding of involuntary bladder contractions, referred to as detrusor instability. Detrusor instability may be of neurologic origin (e.g., Alzheimer's disease, Parkinson's disease or stroke) which is referred to as bladder hyperreflexia. Detrusor instability may also have myogenic origins that are idiopathic, or they may be caused by other conditions including urethral obstruction. Treatments employed to reduce bladder overactivity include oral drug therapy, instillation drug therapy and parasympathetic nerve ablation.

Existing pharmacological therapies for OAB include antimuscarinics that, although widely prescribed, are limited by the high incidence of side effects such as dry mouth.[3] The ATP-sensitive potassium channel (K_{ATP}) openers cromakalim[4] and ZD-6169[5] (see Fig. 1) have been shown to exert potent relaxant activity on bladder detrusor smooth muscle. However, the clinical utility of K_{ATP} openers has been limited as a result of hemodynamic side effects.

Recently, there has been growing interest in the therapeutic potential of modulators of large conductance calcium-activated potassium channels (BK_{Ca}).[6] These channels are regulated by both intracellular Ca^{2+} levels and membrane potential, and are present in many excitable cell types localized in the CNS and smooth muscle.[7] The role of BK_{Ca} channels in several disease states

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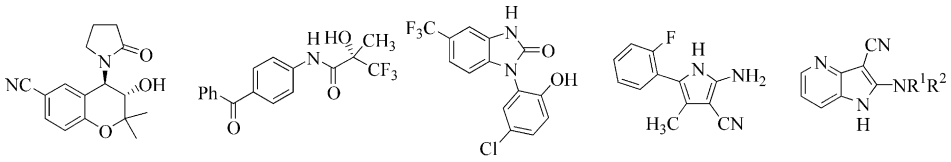


1 Cromakalim 2 ZD-6169 3 NS-004 4 NS-8 5

Figure 1.

has been investigated, including stroke, overactive bladder and sexual dysfunction.[8]

In vitro studies carried out with nonselective BK_{Ca} openers such as NS-004 (**3**, Neurosearch) showed that these compounds can relax bladder smooth muscle tissues.[9] Subsequently, NS-8 (**4**, Nippon Shinyaku), a pyrrole BK_{Ca} opener, has been shown to be efficacious in vivo in rat cystometry and in the rat hyper-reflexia model where it abolished isovolumetric contractions following intravenous and intravesicular administration.[10] Recently, a series of disubstituted triazolones have been reported with relaxant activity on isolated rat bladder strips.[11]

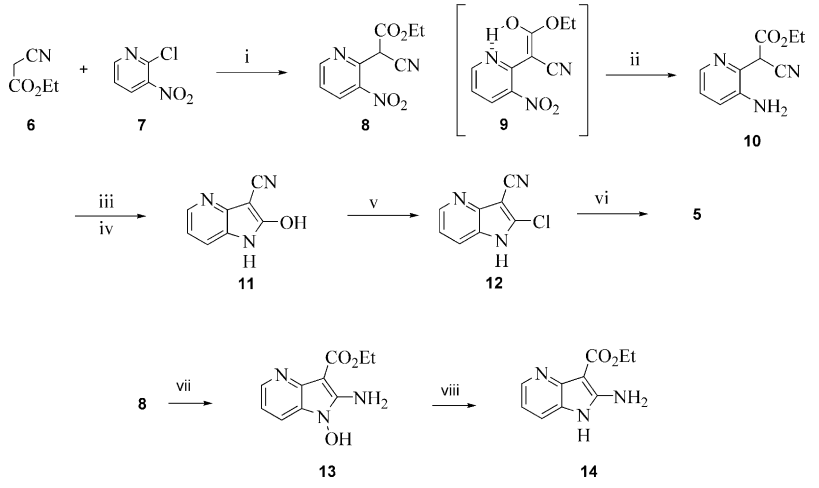
Using NS-8 as a starting point, we synthesized several different classes of fused pyrrole derivatives as potential BK_{Ca} openers. The 2-amino-4-azaindoles **5** were found to be active in $^{86}Rb^+$ efflux, whole-cell patch-clamp technique and pig bladder smooth muscle relaxation assays.

The synthesis of the 2-amino-4-azaindoles was accomplished by the route depicted in Scheme 1. The potassium enolate of ethyl cyanoacetate **6** was heated with 2-chloro-3-nitropyridine **7** to give the 2-adduct **8** which was recrystallized from methanol.[12] The ^1H NMR ($CDCl_3$) showed this to be completely in the enol tautomeric form **9** at room temperature. Catalytic hydrogenation of the nitro group using Pd-C gave the 3-amino product without any competitive reduction of the pyridyl ring. Thermal cyclization of **10** gave a crude mixture that was dissolved in NaOH and then treated with CO_2 (g) to precipitate the pure 2-hydroxy azaindole **11**. A number of different chlorinating agents ($SOCl_2$, PCl_3 and PCl_5) were examined but the best conversion to the key 2-chloro intermediate **12** was obtained with $POCl_3$ at 105 °C. Displacement of the *chloro* moiety with primary and secondary amines and anilines gave the 2-amino targets in moderate yields.

Surprisingly, when unrecrystallized **8** was catalytically reduced using the same conditions already described (Pd-C/EtOH), this afforded the *N*-hydroxy derivative **13** through partial nitro reduction and subsequent cyclization via addition to the nitrile. Cleavage of the N–O bond using Ra–Ni, in the presence of 3% NaOH solution, gave the 2-amino 3-ester analogue **14**. Both **13** and **14** were found to be inactive at BK_{Ca} channels.

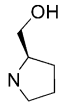
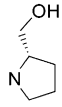
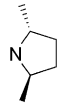
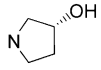
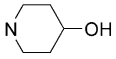
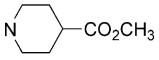
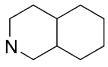
Compounds were evaluated for BK_{Ca} channel opening activity using a $^{86}Rb^+$ efflux assay in which HEK293 cells stably transfected with BK_{Ca} α subunits were treated with the test compound (10 μM).[13] The EC_{50} value was determined for compounds that showed increases in $^{86}Rb^+$ efflux > 40% relative to the positive control ionomycin (10 μM).

Further electrophysiological characterization of active compounds was performed using a whole-cell patch clamp technique. The HEK293 cells stably transfected with BK_{Ca} α subunits were voltage-clamped at a holding potential of −80 mV. The changes in ionic currents in the presence of compounds were measured at the testing potential of +40 mV for 400 ms.



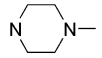
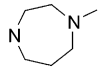
Scheme 1. Reagents and reaction conditions: (i) KOtBu, *i*-PrOH, rt, 30 min then 82 °C, 6 h (74%); (ii) Pd/C, H_2, EtOH, 3 h, 50 psi (99%); (iii) xylene, reflux, 20 h; (iv) NaOH then CO_2 (g) (46% over 2 steps); (v) $POCl_3$, 105 °C, 2 h (45%); (vi) R^1R^2NH, 110 °C, 18 h; (vii) Pd/C, H_2, EtOH, 3 h, 50 psi (48%); (viii) Ra/Ni, KOH, water (45%).

Table 1. Effect of 2-substitution of **5** on $^{86}Rb^+$ efflux in HEK293 cells stably transfected with BK_{Ca} α subunits

Compd	2-Substituent	$^{86}Rb^+$ Efflux BK-alpha (% response)	$^{86}Rb^+$ Efflux BK-alpha EC_{50} (μM)
11	OH	65	13.4
12	Cl	0	NA
15	$N(CH_3)_2$	60	5.13
16	$N(CH_2CH_3)_2$	57	6.94
17	$N(CH_3)(CH_2CH_2N(CH_3))_2$	63	6.75
18	$N((CH_2)_2CH_3)CH_2CH_2OH$	0	NA
19	NHPh	0	NA
20	$NHCH_2Ph$	0	NA
21		58	8.12
22		0	NA
23		0	NA
24		0	NA
25		0	NA
26		0	NA
27		65	3.02
28		65	5.07
29		28	15.1
30		55	17.0
31		61	3.34
32		0	NA
33		74	4.43
34		0	NA
35		62	5.86
36		62	3.86

(*continued on next page*)

Table 1 (*continued*)

Compd	2-Substituent	$^{86}Rb^+$ Efflux BK-alpha (% response)	$^{86}Rb^+$ Efflux BK-alpha EC_{50} (μM)
37		0	NA
38		0	NA

Values are the mean of two or three experiments—each performed in duplicate.
NA = Not active.

A selection of the more potent compounds was also evaluated for in vitro functional activity using tissue strips obtained from Landrace pig bladders.[14] Tissues were stimulated by a low-frequency (0.05 Hz) current that produced a stable twitch response. The potent K_{ATP} channel opener P1075[15] completely eliminated the stimulated twitch response in a dose-dependant fashion. The maximal efficacy of each compound was expressed relative to P1075.

SAR studies were conducted by varying the R^1/R^2 substitution of **5** as outlined in Table 1. For acyclic aliphatic groups, smaller substituents were generally favored (**15** > **16** > **18**). For cyclic aliphatics, the 6- and 7-membered rings were preferred over the 5-membered. The bridged analogue **33** was potent but the fused bicycle **34** was inactive. Replacement of the piperidine group with morpholine retained BK_{Ca} opening activity but the *N*-methyl piperazine and homopiperazines were inactive. All examples bearing an NH group at the 2-position (for example the aniline and benzylamine substitutions) were inactive.

The results of electrophysiological characterization of selected compounds are shown in Table 2. All three compounds showed clear increases in outward currents at 10 μM relative to the control with significantly greater responses than that observed with the standard BK_{Ca} channel opener NS-8.

Table 2. Electrophysiological assignment of BK_{Ca} opening activity

Compd	Outward current in the presence of compound (10 μM) as % over control
NS-8	21.7 ± 7.0%
15	75.5 ± 8.6%
28	107.8 ± 24.5%
31	104.8 ± 11.4%

Values are the mean of three experiments.

Table 3. Functional activity in isolated bladder strips

Compd	Efficacy (% P1075)	EC_{50} (μM)
(−) Cromakalim	95	0.257
NS-8	93	63.1
15	58	23.5
27	67	24.8
28	31	9.26
31	63	25.9

Values are the mean of four experiments.

The most potent compounds from $^{86}Rb^+$ efflux studies were also evaluated for their functional effects in isolated pig bladder detrusor strips (Table 3). Four compounds were effective in suppressing electrically stimulated contractions, with compound **28** displaying potency 7-fold greater than that of NS-8.

In summary, we have identified 2-amino-4-azaindoles as a novel structural class of BK_{Ca} channel openers. Their BK_{Ca} channel activity has been established using in vitro by evaluation in ^{86}Rb efflux and whole-cell patch-clamp assays. Their potential to act as relaxants of bladder smooth muscle has been demonstrated.

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