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Design and synthesis of novel cyanoguanidine ATP-sensitive potassium channel openers for the treatment of overactive bladder

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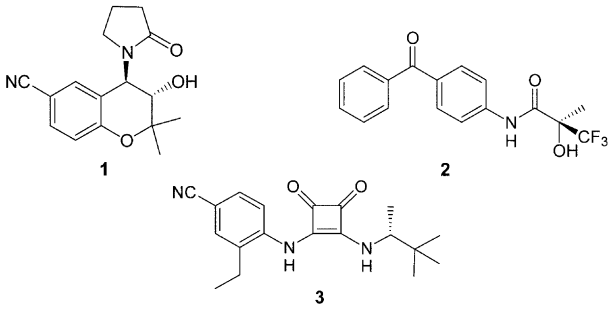
Abstract—Thiourea derivatives were identified as glyburide-reversible potassium channel openers through high-throughput screening. Based on these findings, a number of novel cyanoguanidines were designed and synthesized, which hyperpolarized human bladder K_{ATP} channels. These agents are potent full agonists in relaxing electrically-stimulated pig bladder strips. The synthesis, SAR and biological properties of these agents are discussed.

Overactive bladder (OAB) is a prevalent urological disorder in humans that results in an involuntary loss of urine. OAB has been hypothesized to arise primarily from functional changes in detrusor smooth muscle structure and function (myogenic etiology). It has been suggested that supersensitivity to agonists, increase in gap junctions and enhanced electrical coupling between smooth muscle cells could enable widespread dissemination of depolarization signals leading to spontaneous non-voiding contractions.[1,2] ATP-sensitive potassium channels (K_{ATPs}), present in bladder smooth muscle, play a critical role in controlling myogenic tone and excitability. Compounds that selectively open these channels hyperpolarize cells, decrease cellular (hyper)-excitability and diminish smooth muscle activity resulting in suppression of unstable bladder contractions.[3] Thus, K_{ATP} channel openers (KCOs) may be an attractive way of treating overactive bladder by inhibiting overactivity during the filling phase without affecting normal voiding contractions.

Clinical data, albeit limited, have provided support to the concept of a role for K_{ATP} channels for the treatment of overactive bladder.[4] A pilot clinical trial with the non-selective KCO, cromakalim **1**, demonstrated an improvement in the symptoms of bladder overactivity.[4] However, this agent showed serious side effects on the cardiovascular system. Clinically, no beneficial effects were seen in the bladder at doses that were already hypotensive.[5] It is clear that new agents are needed that can be more selective for the bladder than for the cardiovascular.

New KCOs[6] like ZD-6169[7] **2** and WAY133537[8] **3** have recently appeared and reported to activate the bladder K_{ATP} channels in vitro and selectively inhibit bladder function in preclinical animal models.[8,9]



Thiourea derivatives were identified as glyburide-reversible KCOs through high-throughput screening of our library. Thiourea **4**, an early analogue derived from a HTS hit, showed potent activity in vitro as K_{ATP} channel opener. Because of concerns about the potential toxicity associated with the thiourea and the trichloromethyl groups, chemistry efforts were first directed towards

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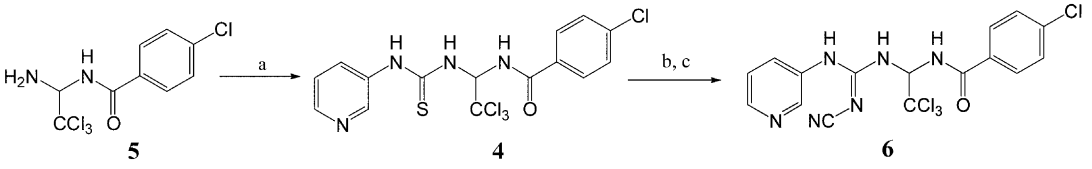
finding suitable replacements for these two moieties. We have synthesized a number of related aminals, which hyperpolarize human bladder K_{ATP} channels. In this study, the preparation, SAR and biological properties of these agents will be discussed.

The synthesis of **4** is summarized in Scheme 1. Coupling of aminal **5**[10] with 3-pyridylisothiocyanate produced **4**. Trichloromethyl-aminal **6** was prepared from **4**, via activation of the thiourea group with EDCI followed by reaction with cyanamide in the presence of 2,6-lutidine and titanium isopropoxide in methylene chloride. Scheme 2 illustrates the synthesis of intermediates **8a–f** that contain replacements for the thiourea group. Thiomethyl ethers **7a–c** were prepared according to literature procedures.[11] Thiomethyl ethers **7d** and **7e** were obtained from the treatment of 3-pyridylisothiocyanate with the sodium salt of sulfamide or *N*,*N*-dimethylsulfamide followed by trapping of the sodium salt intermediate with methyl iodide. Thiomethyls **7a–e** were transformed to **8a–e** with ammonia in methanol.[12] *N*-Nitroguanidine **8f** was obtained by nitration of 3-pyridylguanidine dihydrochloride.[1]

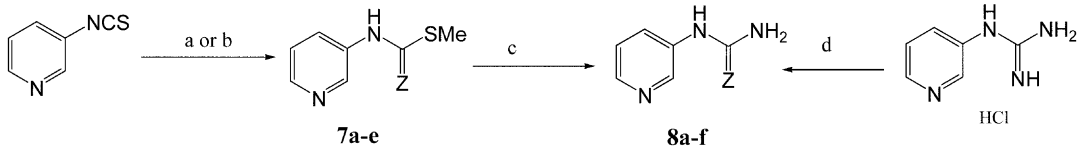
Preparation of **11** is outlined in Scheme 3. Reaction of 4-chlorobenzamide with 2,2-dimethylpropionaldehyde and benzotriazole afforded intermediate **9a**[14] ($R^4 = t$-Bu, $R^2 = Cl$), which was transformed to aminal **10** with ammonia in methanol. Condensation of aminal **10** with 3-pyridylisothiocyanate furnished thiourea **11**. Synthesis of **12a–q** was achieved by the two-step sequence depicted in Scheme 3. Reaction of substituted amides with the corresponding aldehyde[15] and benzotriazole in the presence of *p*-toluensulfonic acid[13] produced benzotriazole intermediates **9a–m**, which were coupled with amines **8a–f** to yield aminals **12a–q**.[16] Resolution of the most potent compound **12g** by preparative chiral HPLC with a Chiralcel AS column provided the levorotatory enantiomer **12g**-(*R*)-(−)[17] and the dextrorotatory enantiomer **12g**-(*S*)-(+). The absolute stereochemistry of the enantiomer **12g**-(*S*) was determined by X-ray crystallography.

The aminal analogues were assayed for K_{ATP} activity in cells expressing human bladder K_{ATP} channels (SUR2B17-/Kir6.2)[18] (K_{ATP}-FMP). Membrane potential changes were measured with a Fluorometric Imaging Plate Reader (FLIPR) using the fast membrane potential (FMP) dye.[19] These effects were reversed upon addition of glyburide, a K_{ATP} channel inhibitor, confirming a K_{ATP} mechanism. The most potent compounds were also evaluated in vitro for functional potassium channel opening activity using tissue strips obtained from Landrace pig bladders (LPD).[20] The SAR study reported herein is broken into three parts: (1) Investigation of bioisosteres of the thiourea group; (2) Replacement of the trichloromethyl; (3) Substitutions of the benzamide aromatic ring.

The SAR was initiated with the replacement of the trichloromethyl group by *tert*-butyl and investigation of bioisosteres of the thiourea group. The results are summarized in Table 1. The *t*-Bu compound **11** showed weaker activity than the parent analogue **4** suggesting that the inductive effect was important at the aminal carbon for K_{ATP} activity. In general deviation from the thiourea moiety resulted in a loss of activity in both cell and tissue based assays. The only active isosteres were cyanoguanidine and nitroguanidine. Nitroethylene and sulfonylamides were practically inactive. Although the cyanoguanidine **12a** was somewhat weaker than thiourea **11**, it is worthwhile to note that the cyanoguanidine group can be viewed as a more acceptable group than thiourea as a pharmaceutical entity. Therefore the next step was to optimize the aminal R^4 group having the cyanoguanidine as the preferred group. Table 1 illustrates attempts in this regard. Trichloromethyl compound **6** was prepared for comparison and not surprising was one of the most active compounds of this series. However, it is interesting that this cyanoguanidine showed comparable activity with thiourea **4**. Further substitution of one of the chlorine atoms by a methyl group resulted in dichloroethyl analogue **12g** and to a great extent K_{ATP} activity was retained. Larger groups such as diethylalkyl or gem dimethylalkyl were

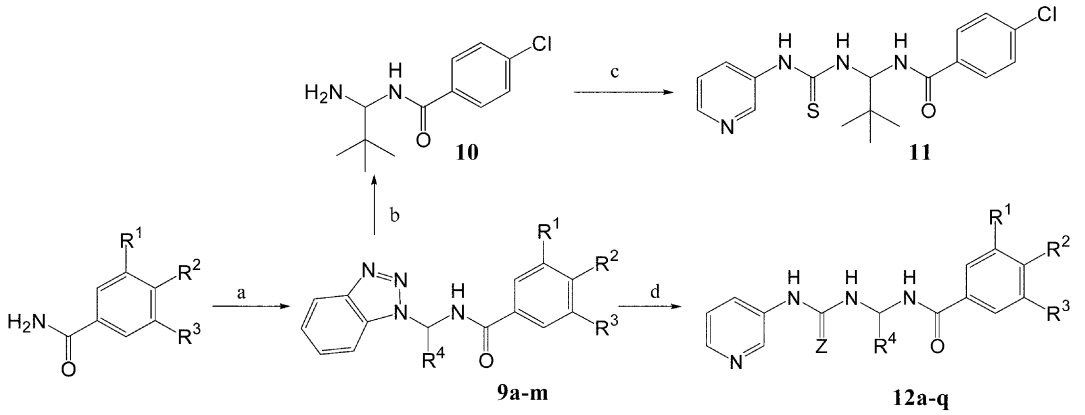


Scheme 1. Reagents and conditions: (a) 3-pyridylisothiocyanate, CH_2Cl_2, 60 °C; (b) EDCI, CH_2Cl_2; (c) 2,6-lutidine, NH_2CN, 3 Å molecular sieves, titanium isopropoxide, CH_2Cl_2, reflux.



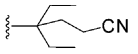
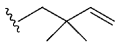
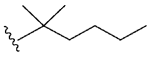
	a	b	c	d	e	f
Z	NCN	$CHNO_2$	$C(CN)_2$	NSO_2NH_2	$NSO_2(NCH_3)_2$	NNO_2

Scheme 2. Reagents and conditions: (a) sulfamide, NaH, THF, MeI, 70%; (b) *N*,*N*-dimethylsulfamide, NaH, THF, MeI, 77%; (c) NH_3, MeOH, 75–85%; (d) HNO_3, H_2SO_4, 66%.



Scheme 3. Reagents and conditions: (a) benzotriazole, R^2CHO, *p*TSOH, toluene, reflux; (b) NH_3, MeOH, 74%; (c) 3-pyridylisothioyanate, CH_2Cl_2, 60 °C, 33%; (d) **8a–f**, K_2CO_3, DMF, 25–60%.

Table 1. In vitro activity of compounds **2**, **4**, **6**, **11** and **12a–q**

Compd	*Z*	R^1	R^2	R^3	R^4	K_{ATP}-FMP (pEC_{50})[a]	LPD (pEC_{50})[b]
2	ZD-6169					6.4	5.6
4	S	H	Cl	H	CCl_3	6.1	5.7
11	S	H	Cl	H	$C(CH_3)_3$	5.6	5.1
12a	N–CN	H	Cl	H	$C(CH_3)_3$	5.2	4.7
12b	$N–NO_2$	H	Cl	H	$C(CH_3)_3$	4.5	5.1
12c	$CH–NO_2$	H	Cl	H	$C(CH_3)_3$	<4.0	
12d	$N–SO_2NH_2$	H	Cl	H	$C(CH_3)_3$	<4.0	
12e	$N–SO_2N(CH_3)$	H	Cl	H	$C(CH_3)_3$	<4.0	
12f	$C(CN)_2$	H	Cl	H	$C(CH_3)_3$	Antagonist	
6	N–CN	H	Cl	H	CCl_3	6.0	5.7
12g	N–CN	H	Cl	H	CCl_2CH_3	5.7	5.6
12g-(*R*)-(−)	N–CN	H	Cl	H	CCl_2CH_3	6.5	5.3
12g-(*S*)-(+)	N–CN	H	Cl	H	CCl_2CH_3	5.0	4.7
12h	N–CN	H	Cl	H		5.3	5.3
12i	N–CN	H	Cl	H		5.0	
12j	N–CN	H	Cl	H		4.7	
12k	N–CN	H	Cl	H		<4.0	
12l	N–CN	H	CH_3	H	CCl_2CH_3	5.6	
12m	N–CN	H	H	Cl	CCl_2CH_3	5.5	5.8
12n	N–CN	H	H	F	CCl_2CH_3	5.4	
12o	N–CN	F	H	F	CCl_2CH_3	5.4	5.3
12p	N–CN	H	H	CH_3	CCl_2CH_3	5.3	
12q	N–CN	H	Br	H	CCl_2CH_3	5.0	

[a] Values are means of two experiments.
[b] Values are means of four experiments.

dramatically weaker, indicative of the importance of the electronic effect of the halogen atoms over the steric effect. Examination of the enantiomers of **12g** demonstrated stereo differentiation in this series as the (*R*)-(−)-enantiomer of **12g** was 15-fold more active than the (*S*)-(+)-enantiomer. Furthermore, this (*R*) enantiomer showed in vitro K_{ATP} activity comparable to ZD-6169.

Exploration of the substitution of the benzamide was conducted with the 2,2-dichloroethyl at the aminal carbon as the preferred group. The preliminary SAR study for the benzamide showed that substitution at the *para* position seems to enhance K_{ATP} activity. This trend was observed for the 4-chloro (**12g**) and 4-methyl (**12l**) analogues but the data is limited and needs further exploration.

In conclusion, a diverse series of novel cyanoguanidine derivatives has been explored and several of which possess potent in vitro activity as K_{ATP} openers. These

compounds are also potent full agonists in relaxing pig bladder strips. Among these compounds, aminal **12g-(*R*)-(−)** was as potent as ZD-6169 in human bladder K_{ATP} channels. The steric and electronic requirements for the aminal carbon and the applicability of the cyanoguanidine group have been established for these derivatives. Further SAR studies to determine the relevance of the pyridine ring and in vivo studies as well as in vivo selectivity comparison with literature KCO standards are underway and will be presented in our full paper.

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References and notes

1. Brading, A. F. *Urology* **1997**, *50* (Suppl.), 57.
2. Mills, I. W.; Greenland, J. E.; McMurray, G.; McCoy, R.; Ho, K. M.; Noble, J. G.; Brading, A. F. *J. Urol.* **2000**, *163*, 646.
3. Quayle, J. M.; Nelson, M. T.; Standen, N. B. *Physiol. Rev.* **1997**, *77*, 1165.
4. Nurse, D. E.; Restorick, J. M.; Mundy, A. R. *Brit. J. Urol.* **1991**, *68*, 27.
5. Komersova, K.; Rogerson, J. W.; Conway, E. L.; Lim, T. C.; Brown, D. J.; Krum, H.; Jackman, G. P.; Murdoch, R.; Louis, W. *Brit. J. Clin. Pharmacol.* **1995**, *39*, 207.
6. Coghlan, M. J.; Carroll, W. A.; Gopalakrishnan, M. *J. Med. Chem.* **2001**, *44*, 1628.
7. (a) Grant, T.; Frank, C. A.; Kau, S. T.; Li, J. H.; McLaren, F. M.; Ohnmacht, C. J.; Russell, K.; Shapiro, H. S.; Trivedi, S. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2723. (b) Ohnmacht, C. J.; Russell, K.; Empfield, J. R.; Frank, C. A.; Gibson, K. H.; Mayhugh, D. R.; McLaren, F. M.; Shapiro, H. S.; Brown, F. J.; Trainor, D. A.; Ceccarelli, C.; Lin, M. M.; Masek, B. B.; Forst, J. M.; Harris, R. J.; Hulsizer, J. M.; Lewis, J. J.; Silverman, S. M.; Smith, R. W.; Warwick, P. J.; Kau, S. T.; Chun, Al.L.; Grant, T. L.; Howe, B. B.; Li, J. H.; Trivedi, S.; Halterman, T. J.; Yochim, C.; Dyroff, M. C.; Kirkland, M.; Neilson, K. L. *J. Med. Chem.* **1996**, *39*, 4592.
8. Butera, J. A.; Antane, M. M.; Antane, S. A.; Argentieri, T. M.; Freeden, C.; Graceffa, R. F.; Hirth, B. H.; Jenkins, D.; Lennox, J. R.; Matelan, E.; Norton, N. W.; Quangliato, D.; Sheldon, J. H.; Spinelli, W.; Warga, D.; Wojdan, A.; Woods, M. *J. Med. Chem.* **2000**, *43*, 1187.
9. (a) Chess-Williams, R.; Martin, S. W.; Korstanje, C.; Chapple, C. R. *Brit. J. Urol.* **1999**, *83*, 1050. (b) Zografos, P.; Li, J. H.; Kaus, S. C. *Pharmacology* **1992**, *45*, 216. (c) Martin, S. W.; Radley, S. C.; Chess-Willams, R.; Korstanje, C.; Chapple, C. R. *Brit. J. Urol.* **1997**, *80*, 405.
10. Weygand, F.; Steglich, W.; Lengyel, I.; Fraunberger, F.; Maierhofer, A.; Oettmeier, W. *Chem. Ber.* **1966**, *99*, 1944.
11. (a) Preparation of methyl *N*′-cyano-*N*-(3-pyridinyl)carbamimidothioate, **7a**: Schou, C.; Ottosen, E. R.; Petersen, H.; Bjoerkling, F.; Latini, S.; Hjarnaa, P. V.; Bramn, E.; Binderup, L. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3095. (b) Preparation of (1-methylsulfanyl-2-nitrovinyl)-pyridin-3-yl-amine, **7b**: Manley, P. W. *Quast, U. J. Med. Chem.* **1992**, *35*, 2327. (c) 2-(3-Pyridinyl)-2-methylmercapto-1-cyanoacrylnitrile, **7c**, was prepared according to: Yokoyama, M.; Tohnishi, M.; Kurihara, A.; Imamoto, T. *Chem. Lett.* **1982**, 1933.
12. Preparation of cyanoguanidine **8a**: Peterson, H. J. *J. Med. Chem.* **1978**, *21*, 773.
13. Dukat, M.; Abdel-Rahman, A. A.; Ismaiel, A. M.; Ingher, S.; Teitler, M.; Gyermek, L.; Gleenon, R. *J. Med. Chem.* **1996**, *39*, 4017.
14. Katritzky, A. R.; Drewniak, M. *J. Chem. Soc., Perkin Trans. 1* **1988**, 2339.
15. (a) 2,2-Dichloropropionaldehyde: De Buyck, L.; Verhe, R.; De Kimpe, N.; Courtheyn, D.; Schamp, N. *Bull. Soc. Chim. Belg.* **1980**, *89*, 441. (b) 3,3-Dimethyl-4-pentenal: Buchi, G.; Vogel, D. E. *J. Org. Chem.* **1983**, *48*, 5406. (c) 2,2-Dimethylhexanal was prepared from commercially available 2,2-dimethylhexanol employing the Swern oxidation: Swern, D.; Mancuso, A. J.; Huang, S. L. J. *Org. Chem.* **1978**, *43*, 2480.
16. *Z*-Geometry was established for the double bond of cyanoguanidines through X-ray crystal structure analysis of **12g-(+)**. Although, derivatives **12b–e** were obtained as single isomers, the geometry of the double bond was not determined.
17. **12g** was chromatographed over a Daicel Chiral Technologies Chiralcel AS chiral column (2.0 cm×25 cm) eluting with 5% ethanol/hexanes (flow rate = 10 mL/min) to provide (*R*)-(−)-4-chloro-*N*-[2,2-dichloro-1-(*N*′-cyano-*N*′-pyridin-3-ylguanidino)propyl]benzamide, **12g-(*R*)-(−)**; $[\alpha]_D^{23}$ −22° (*c* 0.19, DMSO); mp 188–190 °C; MS (ESI+) *m*/*z* 425 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 9.93 (s, 1H), 8.75 (d, 1H, *J* = 8.1 Hz), 8.52 (d, 1H, *J* = 2.0 Hz), 8.46 (d, 1H, *J* = 4.4 Hz), 7.85 (d, 2H, *J* = 8.5 Hz), 7.69 (m, 1H), 7.60 (d, 2H, *J* = 8.5 Hz), 7.47 (dd, 1H, *J* = 8.0, 4.4 Hz), 7.16 (d, 1H, *J* = 8.5 Hz), 6.55 (t, 1H, *J* = 8.5 Hz), 2.17 (s, 3H). Anal. calcd for $C_{17}H_{15}Cl_3N_6O$: C, 47.96; H, 3.55; N, 19.74. Found: C, 48.14; H, 3.64; N, 19.47.
18. Scott, V. E.; Davis-Taber, R.; Silvia, C.; Hoogenboom, L.; Choi, W.; Kroger, P.; Whiteaker, K. L.; Gopalakrishnan, M. *Eur. J. Pharmacol.*, in press.
19. Baxter, D. F.; Kirk, M.; Garcia, A. F.; Raimondi, A.; Holmqvist, M. H.; Flint, K. K.; Bojanic, D.; Distefano, P. S.; Custis, R.; Xie, Y. *J. Biomol. Screen* **2002**, *7*, 79.
20. Buckner, S. A.; Milicic, I.; Daza, A.; Davis-Taber, R.; Scott, V. E. S.; Sullivan, J. P.; Brioni, J. D. *Eur. J. Pharmacol.* **2000**, *400*, 287.