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Structure–Activity Studies for a Novel Series of Tricyclic Dihydropyrimidines as K_{ATP} Channel Openers (KCOs)

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Abstract—A novel series of tricyclic dihydropyrimidines was synthesized and evaluated for activity as K_{ATP} channel openers. The functional activity of several compounds, for example **6A** ($EC_{50}=30$ nM) and its enantiomers exceeded cromakalim.

Potassium channels play an important role in regulating cell membrane excitability, action potential regulation and epithelial electrolyte transport. K_{ATP} channel openers are amongst the most widely explored channels with regard to their potential for the treatment of various diseases such as, for example, bladder overactivity.[1]

Bladder overactivity is a condition associated with the spontaneous contractions of its smooth muscle. K_{ATP} channels exist in the bladder and the ability of potassium channel openers to hyperpolarize cells and relax smooth muscle may provide a method to ameliorate or prevent bladder overactivity.

Since a small clinical study with cromakalim indicated potential usefulness of KCO's for the treatment of detrusor instability, several novel series of K_{ATP} openers were developed.[2] Amongst them ZD 6169,[3] ZM 244085,[4] WAY-133536, and WAY-151616,[5] have been reported to activate the bladder K_{ATP} channel in vitro and selectively inhibit bladder function in vivo.

Our effort was aimed at finding novel structures exhibiting K_{ATP} activity. The screening of our in house library of compounds identified pyrazolopyrimidine **1**, a compound with moderate potency. We reasoned that tricyclic dihydropyrimidines **2** (Scheme 1), being a structural hybrid of ZM 244085 and **1** may display enhanced K_{ATP} properties. The proposed structure **2** would have an electron deficient aromatic ring positioned orthogonally to a second ring with a hydrogen bond acceptor group. This perpendicular arrangement is hypothesized to be the pharmacophore responsible for the K_{ATP} activity of cromakalim-like compounds.[6] In this article we wish to report the synthesis and SAR studies of the novel series of tricyclic dihydropyrimidines **2** that were prepared based on this concept.

The synthesis of the proposed hybrid structure **2** was accomplished by a three component Hantzsch-type reaction as depicted in Scheme 2. A mixture of aldehyde **3**, 1,3-cyclohexanedione **4** and 3-aminopyrazole **5** was heated at reflux in ethanol. The resulting products: dihydropyrimidine (structure **A**) and dihydropyridine (structure **B**) were separated either by crystallization or chromatography on silica gel.

SAR studies were conducted by varying the three components of the reaction shown in Scheme 2. In the course of SAR studies on the left hand portion it was found that the ratio of the products formed depended on the ring size and the substitution of the starting diketone. In the case of five-membered rings, like 1,3-cyclopentanedione and tetrahydrothiophene-3-oxo-1,1-dioxide,[7] dihydropyridines (**B**) were the only products formed. Introduction of sulfur in the cyclohexanedione ring as in **23A** slightly diminished the ratio of dihydropyrimidines (**A**). Addition of gem-dimethyl groups to

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the cyclohexanedione ring as in **21A** and **22A** also lowered the ratio of **A**/**B**.

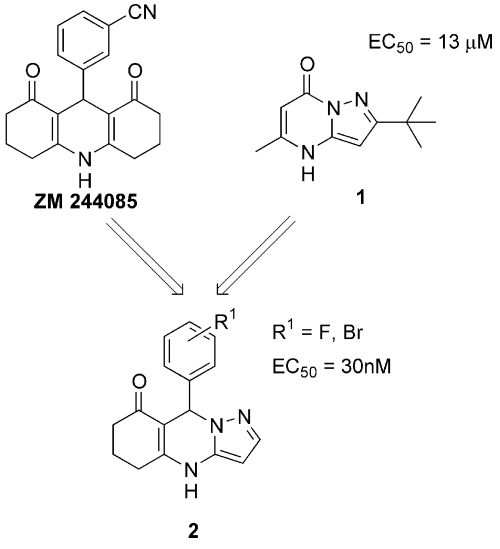
Optimization of the aromatic substitution was realized by using a variety of commercially available aldehydes. Replacement of 3-aminopyrazole with 3-amino-1,2,4-triazole or 5-aminotetrazole afforded **25** and **26**, respectively. Bromination of **6A** with bromine in dichloromethane yielded the bromo derivative **27**. The 3,4-dichlorosubstituted analogue **8** served as a starting material for the synthesis of various aryl and heterosubstituted derivatives by the Suzuki method (Scheme 3).

The separation of enantiomers of the most potent compound **6A** was accomplished by chiral chromatography on a Chiracel OD column. The absolute stereochemistry of the enantiomers was determined by X-ray crystallography.

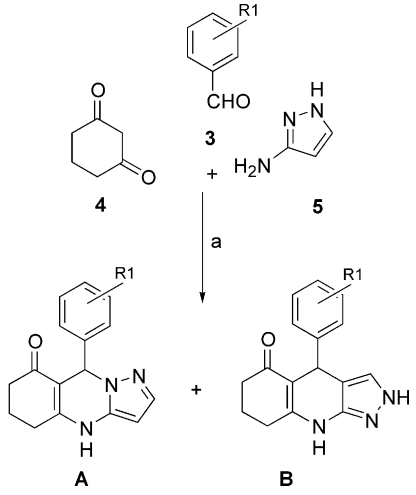
Compounds were evaluated for potassium channel opening activity using primary cultured guinea pig urinary bladder (GPB) cells.[8] Functional activity at potassium channels was measured by evaluating changes in membrane potential using DIBAC dye in a 96-well cell-based kinetic assay system, Fluorescence Imaging Plate Reader (FLIPR). Changes in fluorescence were measured by comparison to the effect elicited by P1075 (a potent KCO). The maximal response of each compound, expressed as % relative to P1075, was equal or above 80%. The observed effects were reversed by glyburide, confirming a K_{ATP} mechanism.

The more potent compounds were also evaluated in vitro using tissue strips obtained from Landrace pig bladders.[9] Tissues were stimulated by a low-frequency current that produced a stable twitch response. P1075 completely eliminated the stimulated twitch response in a dose-dependant fashion. The maximal efficacy of each compounds was expressed in comparison to P1075.

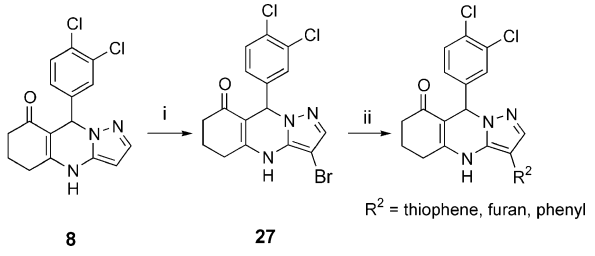
Table 1 summarizes the effect of various substituents on the aromatic ring. Electron withdrawing groups in the 3,4-position as in **6A**, **8**, **10**, **12**, and **17** enhanced the potency. Monosubstituted compounds **13**, **14**, **15**, and



Scheme 1.

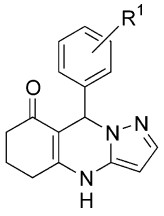


Scheme 2. Reagents and conditions: (a) EtOH, reflux.



Scheme 3. Reagents and conditions: (i) Br_2, CH_2Cl_2; (ii) Suzuki reaction.

Table 1. SAR of the aromatic substitution



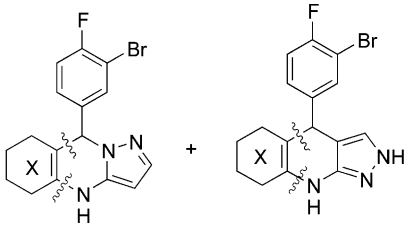
Compd	R^1	EC_{50} (nM)[a]
ZM244085	—	3800
(−)Cromakalim	—	425
6A[10]	3-Br, 4-F	30
6B[11]	3-Br, 4-F	>10,000
(*S*)-**6A**	3-Br, 4-F	262
(*R*)-**6A**	3-Br, 4-F	155
7	3-Cl, 4-F	256[b]
8	3,4-diCl	130
9	3-Cl	1100[b]
10	3-CF_3, 4-Cl	187[b]
11	3-Br	550[b]
12	3-CF_3, 4-F	290[b]
13	3-CN	2860
14	3-Me	>10,000
15	3-OCF_3	1700
16	4-F	7800
17	3-NO_2, 4-Cl	310

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

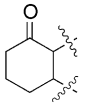
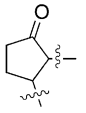
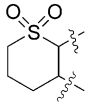
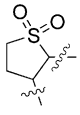
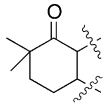
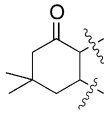
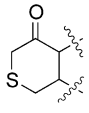
16 were significantly less active. This finding is in agreement with the previously described trends for the aromatic substitution around ZM 244085.[6]

Exploration of the core ring system was conducted with the optimized aromatic substitution (3-Br, 4-F). Table 2 summarizes the results of this effort. It should be noted that all the dihydropyridines of structure **B** were inactive irrelevant of the changes in the left-hand portion of the molecule. The following trends were observed in dihydropyrimidines **A**. Introduction of dimethyl groups α to the carbonyl group, as in **21A**, had no effect on potency, but when these groups were in the β position (**22A**) the activity dropped 300-fold. Replacement of keto-group with sulfone (**19A**) also lowered the potency.

Table 2. SAR of the left-hand portion[a]



A B

X	Compd	Type A EC_{50} (nM)	Ratio **A**/**B**
	**6A**	30	3:1
	**18A**	—	B
	**19A**	910	4:1
	**20A**	—	B
	**21A**	35	2:1
	**22A**	9700	1:2
	**23A**	40[b]	1:1

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

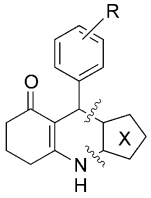
Insertion of sulfur in the cyclohexanone ring (**23A**) was well tolerated.

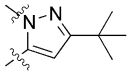
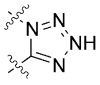
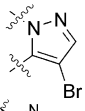
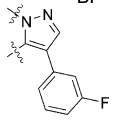
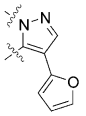
Cyclohexanone proved to be the optimal ring fusion for the core structure, affording the most potent compound in the series (**6A**). Resolution of the compound **6A** provided a set of enantiomers, both of which were active as KCOs.

With the aromatic substitution and left hand portion optimized we explored the limitations of the right-hand portion of the molecule. Judging by the striking difference in potency between **6A** and **6B** we surmised that the lone electron pair residing on the pyrazole nitrogen in **6A** mimics the effect of carbonyl group that is present in ZM 244085.

Generally, the right hand portion of the molecule proved to be more sensitive to changes. Steric bulk represented by a *t*-butyl group (as in **24**) diminished the K_{ATP} activity and introduction of additional nitrogen atoms in the ring (**25**, and **26**) led to a complete loss of activity. As is evident from Table 3, the only compound retaining activity, was bromo derivative **27**. A library of variously substituted phenyl and heterosubstituted compounds was prepared, (Scheme 3), however, none of the new compounds exhibited K_{ATP} activity.

Table 3. SAR of the right hand portion



Compd	R	X	EC_{50} (nM)[a]
24	3-Br, 4-F		12,000
25	3-Br, 4-F		>10,000
26	3-Br, 4-F		>10,000
27	3-Br, 4-F		394
28	3-Cl, 4-Cl		3200
29	3-Cl, 4-Cl		12,000

[a]Values are means of two experiments.

Table 4. Functional KCO activity in isolated bladder strips

Compd	Efficacy (%P1075)	PEC_{50}
(−)Cromakalim	95	6.34
6A	96	6.47
(R)-6A	100	6.67
(S)-6A	95	6.34
21A	75	6.0

The most potent compounds of the series were evaluated for their K_{ATP} activity in pig bladder strips. The results are presented in Table 4. The lead compound **6A** and its enantiomers demonstrate activity comparable to cromakalim.

In summary, we identified tricyclic dihydropyrimidines as a new class of potassium channel openers. The tricyclic core of **6A** was found to be an isostere of the acridinedione of ZM 244085 with the pyrazole nitrogen replacing the carbonyl group. The synthetic route utilized for the series allowed us a quick analysis of the optimal aromatic substitution and the steric and electronic limitations of the pharmacophore.

Acknowledgement

The authors would like to thank Rodger Henry for the X-ray structure determination.

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10. **6A** ^{1}H NMR (DMSO-d_6) δ 1.94 (m, 2H), 2.25 (m, 2H), 2.63 (m, 2H), 5.72 (d, 1H), 6.19 (s, 1H), 7.1 (m, 1H), 7.23 (t, 1H), 7.31 (d, 1H), 7.4 (dd, 1H), 10.55 (s, 1H).
11. **6B** ^{1}H NMR (DMSO-d_6) δ 1.89 (m, 2H), 2.19 (m, 2H), 2.58 (m, 2H), 5.08 (s, 1H), 7.12 (m, 1H), 7.19 (t, 1H), 7.33 (s, 1H), 7.39 (dd, 1H), 9.9 (s, 1H).