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CYANOGUANIDINE BIOISOSTERES IN POTASSIUM CHANNEL OPENERS: EVALUATION OF 3,4-DISUBSTITUTED-1,2,5-THIADIAZOLE-1-OXIDES

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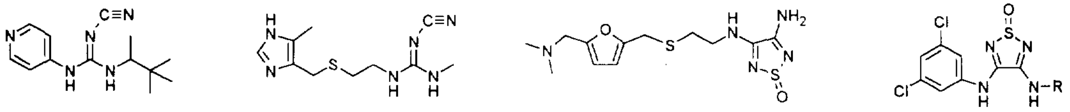
Abstract. Bioisosteric substitution of the cyanoguanidine group found in pinacidil (**1**) with a 3,4-diamino-1,2,5-thiadiazole-1-oxide moiety and replacement of the 4-aminopyridine group with a 3,5-dichlorophenyl group has resulted in a new structural class of potassium channel opener (PCO).

The cyanoguanidine moiety has been a major structural constituent of several drug discovery efforts, the most notable examples being the development of the antihypertensive pinacidil (**1**) and the H_2 antagonist cimetidine (**2**).[1,2] The former is a member of a class of drugs termed potassium channel openers (PCO's) which potentially offer additional clinical utility in areas of unmet medical need (i.e., incontinence), as well as in the treatment of asthma and hair growth related disorders such as *androgenetic alopecia.*[3]



1 2 3 4

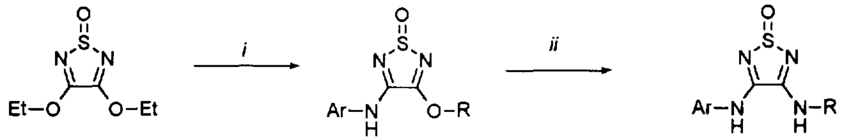
Figure 1.

Recent literature reports have suggested that exploitation of differences found in subtypes of the ATP sensitive potasium channel (K_{ATP}) may lead to more selective therapeutic agents.[3] Our interest in the development of more *tissue selective* potassium channel openers,[4] specifically selective peripheral vasodialators, prompted a search for structurally novel pinacidil analogues based on the bioisosteric modification the cyanoguanidine functionality found in **1**.

It is well known in the literature that a variety of H_2 antagonists have been reported that contain structurally distinct bioisosteres of the cyanoguanidine moiety found in both **1** and **2**.[1] Utilizing these reports, the 3,4-diamino-1,2,5-thiadiazole-1-oxide functionality was selected as a bioisosteric replacement for the cyano-guanidine moiety of pinacidil based on BMY-25271 (**3**), a thiadiazole-containing analogue of ranitidine.[5] Selection of this isostere was based on two criteria: retention of the planar relationship between the two pendant side chains and the preservation of the two nitrogen based hydrogen bond donors. In addition, a recent report by Morita et al.[6] suggested that the suggested that the pyridyl of **1** may be replaced with a 3,5-dichlorophenyl group with retention of PCO activity. The combination of these structural motifs resulted in the design of **4** as our synthetic target.

Chemistry. Aniline was added to 3,4-diethoxy-1,2,5-thiadiazole-1-oxide[7] (**5**) by refluxing in EtOH overnight. Alternatively, 3,5-dichloroaniline reacted with **5** at room temperature in the presence of sodium ethoxide.[8] Alkyl amines were readily added by stirring **6** (or **7**) and the amine at room temperature for 1 hour to 3 days, depending on the substituent. Primary unhindered alkyl amines reacted in good yields; primary α-substituted amines (i.e., *sec*-butylamine) required longer reaction times and afforded lower yields.[9]

SCHEME I



5

6 Ar = phenyl
7 Ar = 3,5-dichlorophenyl

8 Ar = phenyl
9 Ar = 3,5-dichlorophenyl

Reagents. *i* $ArNH_2$, EtOH/reflux or EtOH/NaOEt/rt. *ii* RNH_2, MeOH, rt.

In Vitro Biological Evaluation. Compounds **8** and **9** were evaluated for potassium-channel opening activity at three concentrations in an A-10 smooth muscle cell fluorescence-based in vitro assay that measured potassium channel modulating activity as a change in observed fluorescence (ΔF) over time (see Table 1).[10] For a potassium channel opener (hyperpolarizing agent) this effect was detected as a drop in the observed fluorescence. Consequently, a depolarizing agent caused an increase in observed fluorescence.

Discussion. A series of pinacidil analogues was prepared in which the cyanoguanidine functionality was replaced with a 3,4-diamino-1,2,5-thiadiazole-1-oxide moiety. At the outset, Ar = Ph was used to provide a model system to determine the experimental conditions required for this synthetic route, subsequently 3,5-dichloroaniline was incorporated onto the thiadiazole skeleton. Three classes of side chains were added: alkylamino, cycloalkylamino and alkylphenylamino.

Table 1

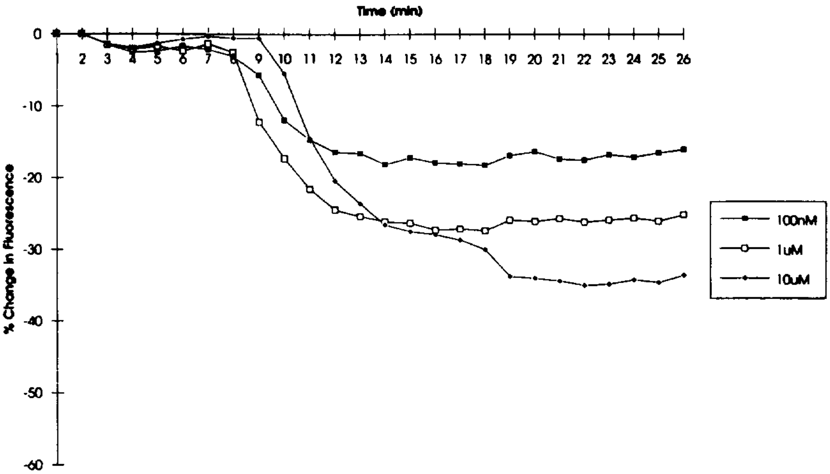
Product	Ar	R_1	Yield (%)	% Change in Fluorescence(SEM)[a]		
				10 μM	1 μM	0.1 μM
8c	Ph	*n*-propyl	53	-1.05(3.66)	-4.86(4.64)	-3.75(1.79)
8h	"	*n*-amyl	62	-5.23(3.23)	-7.68(1.78)	-3.76(4.74)
9a	3,5-dichloro-	H	62	-4.58(9.36)	-4.81(2.86)	+10.19(4.54)
9b	phenyl	CH_3	58	-33.56(2.00)	-25.12(2.18)	-16.03(0.66)
9c	"	*n*-propyl	51	-11.11(3.62)	+5.85(3.29)	+8.06(0.52)
9d	"	*i*-propyl	44	-9.36(0.92)	-5.37(0.48)	+2.12(2.85)
9e	"	*n*-butyl	92	-44.66(9.94)	-10.31(2.61)	+1.29(0.38)
9f	"	*i*-butyl	88	-10.83(6.19)	-3.59(2.37)	-1.32(1.75)
9g	"	*sec*-butyl	20	-18.97(9.32)	+1.42(4.49)	-0.43(0.82)
9h	"	*n*-amyl	34	-26.32(1.03)	-10.58(3.35)	+8.48(2.73)
9i	"	*neo*-pentyl	33	+1.84(4.83)	+5.58(3.43)	+1.77(0.58)
9j	"	*i*-amyl	55	-45.55(1.58)	-12.04(3.13)	+10.71(4.44)
9k	"	CH_2Ph	62	+0.84(2.87)	-0.91(4.03)	+7.83(1.33)
9l	"	$(CH_2)_2Ph$	67	-35.18(4.92)	-1.29(1.87)	-1.01(1.39)
9m	"	$(CH_2)_3Ph$	67	+14.09(5.31)	-19.73(1.01)	-16.02(5.95)
9n	"	$(CH_2)_4Ph$	70	+4.02(2.68)	-6.18(1.33)	-7.20(0.64)
9o	"	cyclopropyl	49	-3.96(4.23)	-1.10(3.61)	+0.95(3.86)
9p	"	cyclobutyl	66	-21.83(3.63)	-4.23(4.05)	-5.89(4.21)
9q	"	cyclopentyl	45	-4.17(5.85)	-1.36(0.68)	-5.82(1.80)
9r	"	cyclohexyl	37	-17.74(20.3)	-2.39(2.20)	+2.05(3.27)
1	4-pyridyl	$CH(CH_3)C(CH_3)_3$		-57.37(1.43)	-45.60(5.16)	-5.89(2.69)

[a]Mean of triplicate assays, assay values in arbitrary fluorescent units.

The alkylamino side chains were chosen to evaluate straight-chain and branched alkyl groups in this template. Three of these analogues (**9b**: R=methyl; **9e**: R=*n*-butyl; **9j**: R=*i*-amyl) showed modest hyperpolarization at 10 μM. Of the cycloalkyl analogues prepared, two (**9p**: R=cyclobutyl; **9r**: R=cyclohexyl) showed low to moderate activity which was not sustained at 10 μM. The alkylphenyl analogues examined the effects of a phenyl ring "tethered" at various lengths from the thiadiazole moiety. Activity was lost at 10 μM when R=benzyl (**9k**) and R=phenylbutyl (**9n**). Moderate activity was seen at 10 μM when R=phenylethyl (**9l**). A low to moderate hyperpolarization effect was seen at 1 μM when R=phenylpropyl (**9m**). Interestingly, at 10 μM a slight *depolarizing* effect was observed. The biochemical relevance of this observation is unclear. The most active analogues at 10 μM from this series were **9e** (R=*n*-butyl) and **9j** (R=*i*-amyl), both with a ΔF of -45%. These analogues compared favorably in activity with pinacidil (ΔF= -60% @ 10 μM). However, **9e** and **9j** did not retain this activity at lower doses. Pinacidil (**1**) also displayed this steep dose-response between 1 μM and 0.1 μM in this assay. Of the thiadiazoles evaluated, **9b** exhibited the most consistent potassium channel opening activity across the 100-fold concentration range tested. Figure 2 graphically depicts the fluorescence assay results comparing **9b** and **1**. At 0.1 μM **9b** was actually more active than pinacidil.

It is clear that a rigorous, detailed structure-activity relationship cannot be developed from the results of this three concentration screen. The objective of this effort was the demonstration of the utility of the 3,4-diamino-1,2,5-thiadiazole-1-oxide as a bioisosteric replacement of the cyanoguanidine group. The significance of the these data lie in the confirmation of this hypothesis. Additionally, the utility of the 3,5-dichlorophenyl group as a replacement of the 4-pyridyl moiety found in **1** was confirmed.

In summary, a new structural class of K_{ATP} openers has been prepared which offer insight to the requirements for binding to the ATP sensitive potassium channel in vascular smooth muscle.[3] Further studies regarding the more comprehensive evaluation of these compounds in various tissues as well as the further use of the bioisosteric replacement technique will be reported in due course.



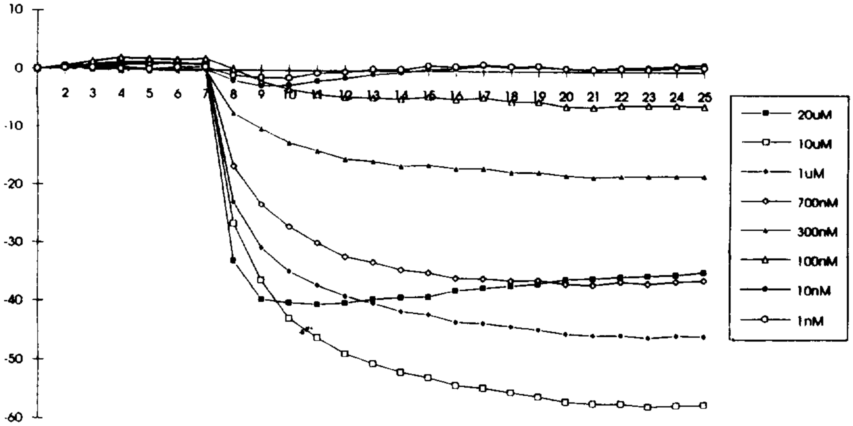


Figure 2. Comparison of **9b** (upper) and **1** (lower) at various concentrations in the A-10 smooth muscle cell assay. Drug added at 7 min.[10] Results are reported as a percent change in fluorescence (ΔF) where 30 mM KCl, by definition, induces a 100% change.

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