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3-Trifluoromethyl-4-nitro-5-arylpyrazoles are novel K_{ATP} channel agonists

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Abstract—This communication describes the discovery and synthesis of a series of 3-trifluoromethyl-4-nitro-5-arylpyrazoles as potent K_{ATP} channel agonists. The most potent compound reported is ca. 100-fold more potent than diazoxide and exhibits selectivity for the SUR1 K_{ATP} channel subtype. The 4-nitro substitutent on the pyrazole ring was required for activity, and limited SAR suggests that the de-protonated pyrazole maybe the active species.

1. Introduction

Potassium channels represent a subfamily of ion channels that selectively permit transport of potassium ions across the cellular membrane. A subset of this class of channels are the ATP-sensitive potassium channels (K_{ATP}), which have been identified in a number of tissues such as pancreatic β-cells,[1] smooth muscle,[2] cardiac muscle,[3] kidney,[4] and both peripheral and central neurons.[5] The K_{ATP} channels present in pancreatic β-cells serve to regulate glucose-mediated insulin secretion by coupling cellular glucose metabolism directly to membrane potential.[6] In this manner, β-cell K_{ATP} channels link plasma glucose levels (energy) to intracellular [Ca^{2+}] and ultimately insulin secretion.[7]

The β-cell K_{ATP} channel is comprised of two sub-units, the sulfonylurea receptor-1 (SUR1) and the potassium selective inward rectifier (Kir6.2).[8] The SUR1 protein is the molecular target for the sulfonylurea class of anti-hyperglycemic drugs such as glipizide, which have been widely used in the treatment of Type 2 diabetes mellitus.[9] These compounds are antagonists of the β-cell K_{ATP} channel and promote insulin secretion. In addition, a number of drugs act as agonists of K_{ATP} channels. Compounds such as cromakalim and pinacidil activate K_{ATP} channels found in vascular smooth muscle, comprised primarily of SUR2B/Kir6.1,[10] as well as channels found in the heart, consisting of SUR2A/Kir6.2.[11,12] Diazoxide activates the β-cell K_{ATP} channels thereby inhibiting pancreatic insulin release and is used by physicians to treat severe cases of hyperinsulinemia.[13] In addition, diazoxide has also shown beneficial results as a treatment for diseases associated with excessive insulin levels such as diabetes[14] and obesity.[15] However, the use of diazoxide is limited by side effects such as severe oedema,[16] and excessive hair growth,[17] which may be related to diazoxide's vasodilatory effects via activity at SUR2B/Kir6.1.[18]

To date, there are only a few reports of potent SUR1/Kir6.2 (β-cell K_{ATP}) agonists. Modifications to diazoxide have been reported which result in increased potency and selectivity for SUR1/Kir6.2.[19] Recently a series of potent and selective diazoxide analogues were described with potencies in the low nano-molar range.[20] Our desire was to find novel structures, distinct from the diazoxide template, that would serve as β-cell K_{ATP} channel agonists. In this effort, we used a high throughput screening assay, based on changes in membrane potential.[21] This resulted in the discovery of compound **1**. Since compound **1** represented a novel class of K_{ATP} channel agonist we were motivated to investigate. Herein, we report the activity of this new series of SUR1/Kir6.2 agonists and a limited SAR (Fig. 1).

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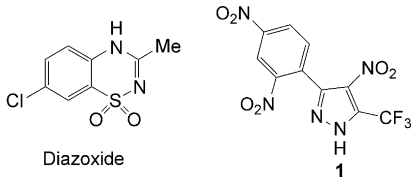
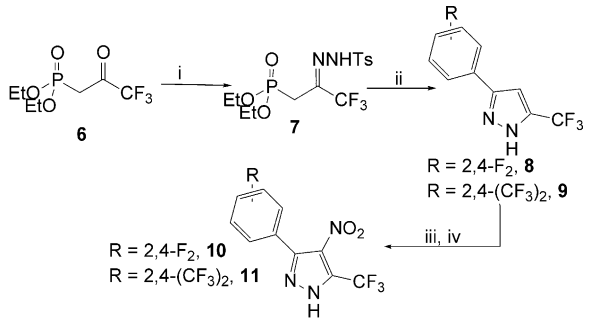


Figure 1.

2. Chemistry

Our initial goal was to systematically remove the nitro groups on **1** to determine the contribution of each to the activity. The 3-trifluoromethyl-5-phenylpyrazole core, **2**, was prepared by condensation of the available 4,4,4-trifluoro-1-phenylbutane-1,3-dione with hydrazine mono-hydrate. As shown in Scheme 1, various nitration procedures were employed to give the desired nitrated products. Nitration at 0 °C gave a ca. 9:1 mixture of *para* and *meta* nitration products on the phenyl ring. Reaction at room temperature revealed the second nitration site to be the 4-position of the pyrazole ring, giving **4** in good yield. More vigorous conditions (100 °C) were required to achieve the third nitration in the synthesis of the original lead compound (**1**). For selective nitration on the pyrazole ring we turned to a two step method developed by Janssen.[22] Treatment of **2** with a preformed mixture of nitric acid and acetic anyhdride (CAUTION; pre-mix at 0 °C slowly) led to clean *N*-nitration of the pyrazole. Subsequent thermal rearrangement (chlorobenzene, 115 °C) gave the 4-nitropyrazole product **5**.

Replacement of the 2,4-dinitrophenyl group of **1** with other electron deficient phenyl groups is shown in Scheme 2. We desired a method which would allow for the use of readily available starting materials and quick construction of the pyrazole ring. The one-pot procedure reported by Santagostino[23] fulfilled these requirements. The diethyl phosphonate **6** was prepared from diethyl methylphosphonate and ethyl trifluoroacetate as described in the literature.[24,25] Treatment of **6** with tosylhydrazine in ethanol with a catalytic amount of TsOH afforded the hydrazone **7** in good yield.[26] Treatment of **7**



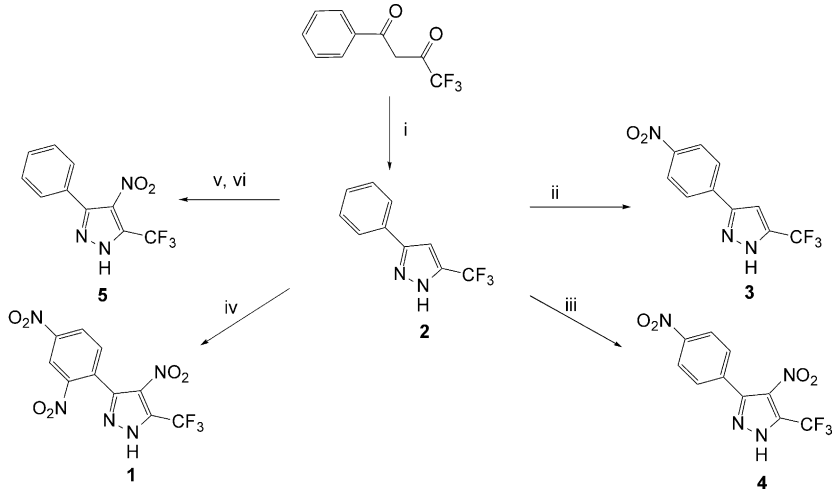
Scheme 2. Reagents and conditions: (i) NH_2NHTs, EtOH, TsOH–H_2O, rt, 91%; (ii) 2 equiv NaHMDS, −78 °C, ArCHO, rt 1 h, reflux 3 h; (iii) HNO_3, Ac_2O premix at 0 °C, HOAc, 100%; (iv) PhCl, 115 °C.

with two equivalents of NaHMDS follow by the required aldehyde, and subsequent heating effected the two-step process and gave the pyrazoles **8** or **9** in good yield (65 and 60%, respectively). The procedure proved to be quite general and a number of various benzaldehydes could be employed. The pyrazoles were nitrated at the 4-position using the two step procedure described earlier to give compounds **10** or **11**.

To determine the contribution of the pyrazole NH to the activity, the corresponding isoxazole **12** was prepared via treatment of 4,4,4-trifluoro-1-phenylbutane-1,3-dione with hydroxylamine, and subsequent nitration using conditions similar to that for compound **1**.[27]

3. Results and discussion

Previous investigators of K_{ATP} channel agonists have relied on either inhibition of insulin secretion from islets (or other insulin secreting cells) or whole cell Rb^+ efflux methods to quantitatively assess agonist activity. Here we desired a more direct measure of channel activity and therefore we determined compound potency and efficacy using the excised patch-clamp technique, in which a portion of the cellular membrane containing a functioning K_{ATP} channel is removed from the rest of the cell. In these studies ATP-sensitive K^+ currents were



Scheme 1. Reagents and conditions: (i) NH_2NH_2–H_2O, EtOH, 81%; (ii) HNO_3, H_2SO_4, 0 °C, 72%; (iii) HNO_3, H_2SO_4, rt, 65%; (iv) HNO_3, H_2SO_4, 100 °C, 90%; (v) HNO_3, Ac_2O premix at 0 °C, HOAc, 100%; (vi) PhCl, 115 °C, 78%.

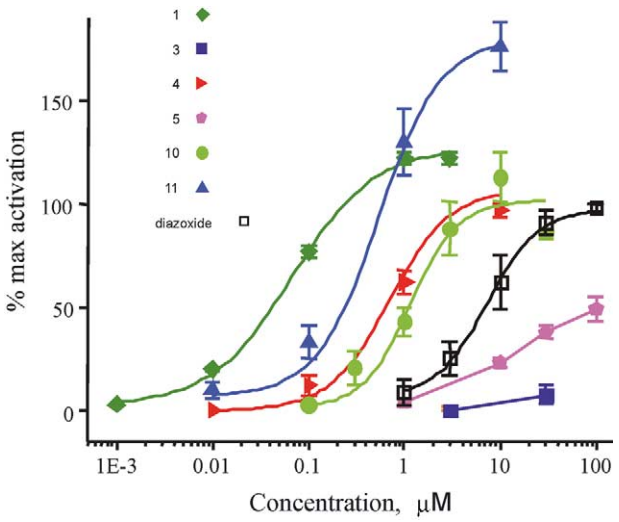


Figure 2. Compound dose–response curves for activation of K_{ATP} (SUR1/Kir6.2) from excised patch recordings.

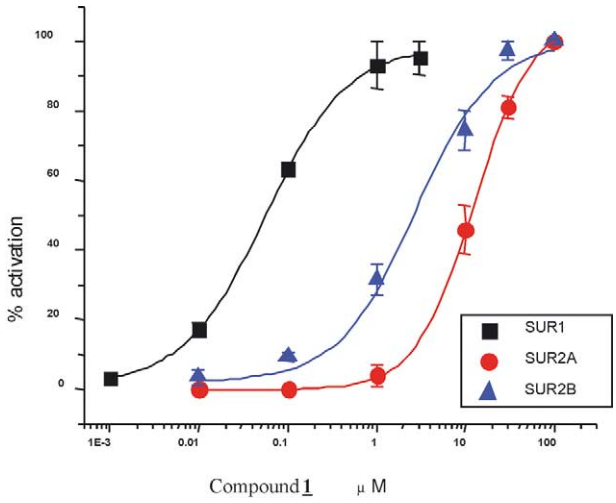


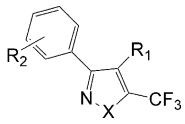
Figure 3. Dose–response curves for **1** versus K_{ATP} subtypes from excised patch recordings.

recorded from inside-out macropatches pulled from Xenopus oocytes expressing the human SUR1/Kir6.2 channel.[28] This technique enables the measurement of direct effects of the compound at the channel itself and thus provides an unambiguous determination of the activity of a compound at K_{ATP}.

The relationship between the dose of a compound and K_{ATP} channel activity recorded from excised patches is illustrated in Figure 2 and calculated EC_{50} values are listed in Table 1. All compounds were tested at least three times over an appropriate range of four to five doses to determine EC_{50} values. The percent max refers to the percentage of maximal agonistic response elicited by the compound as compared to a fully efficacious effect of diazoxide (50 μM).

The lead compound **1** is ca. 100-fold more potent than diazoxide and is comparable to the potent diazoxide analogues reported by Nielsen.[20] The potency of this template is highly dependent on the 4-nitro substitutent on the pyrazole ring. Comparison of compound **4** with compound **3** reveals that removal of the pyrazole 4-nitro group results in a complete loss of activity. Activity is also dependent on the nature of the substitutents on the phenyl ring. Removal of the 2-nitro group on the phenyl ring results in a 10-fold loss of activity (**1** vs **4**) and removal of both phenyl nitro groups gives a compound (**5**) with minimal agonist activity. Activity can be restored by incorporation of other electron withdrawing groups on the phenyl ring as exemplified by compounds **10** and **11**. Within this limited compound set the electron withdrawing ability of the phenyl substitutents appears to correlate with potency [2,4-$(NO_2)_2$ > 2,4-$(CF_3)_3$ > 4-NO_2 > 2,4-F_2 > > H]. These initial results suggest that the ionization of the pyrazole N–H is critical to agonist activity. We determined the pK_a of **1** to be 4.78 (±0.02) using standard UV spectroscopic methods, which suggests the deprotonated species is the active agent at physiological pH. This hypothesis is also supported by the lack of activity of the oxazole analogue (**12**).

Table 1. Compound activation of KATP (SUR1/Kir6.2) from excised patch recordings



Compd	X	R1	R2	EC_{50}, μM [a,b]	%Max[a,c]
Diazoxide				8.80 (±0.25)	100
1	NH	NO_2	2,4-$(NO_2)_2$	0.07 (±0.01)	122 (±3)
3	NH	H	4-NO_2	> 30	8 (±4)[d]
4	NH	NO_2	4-NO_2	0.70 (±0.21)	94 (±9)
5	NH	NO_2	H	> 30	38 (±3)[d]
10	NH	NO_2	2,4-F_2	1.15 (±0.16)	113 (±12)
11	NH	NO_2	2,4-$(CF_3)_2$	0.54 (±0.15)	197 (±17)
12	O	NO_2	4-NO_2	> 30	

[a] Values are means from 3–6 experiments.
[b] Concentration which elicits 50% of the maximal response.
[c] Percent maximal agonist response as compared to 50 μM diazoxide.
[d] Maximal response at 30 μM.

We also examined the selectivity of the lead compound **1** for the SUR1/Kir6.2 channel versus other K_{ATP} channel subtypes. Shown in Figure 3 are the compound **1** dose–response curves for the three common K_{ATP} subtypes (SUR1, SUR2A, and SUR2B). The calculated EC_{50} values for **1** are 0.07, 12.30 and 2.74 micro-molar for SUR1, SUR2A, and SUR2B, respectively. Under the same assay conditions diazoxide was 2-fold selective for SUR1 over SUR2A and SUR2B (data not shown).

4. Conclusion

In conclusion, we have discovered a new class of SUR1/Kir6.2 (K_{ATP}) channel agonists, exemplified by compound **1**. The lead compound in this series is 100-fold more potent than diazoxide, and shows significant selectivity for the SUR1 K_{ATP} subtype. The limited SAR suggests that the deprotonated pyrazole maybe the active form. Further studies will expand the SAR of this series as well as investigate the SUR structural binding requirements.

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