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2-(4-Methoxyphenoxy)-5-nitro-*N*-(4-sulfamoylphenyl)benzamide activates Kir6.2/SUR1 K_{ATP} channels

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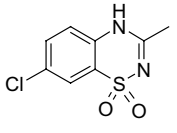
Abstract—2-(4-Methoxyphenoxy)-5-nitro-*N*-(4-sulfamoylphenyl)benzamide and close analogues inhibit glucose stimulated insulin release through activation of Kir6.2/SUR1 K_{ATP} channels of beta cells.

Only a few different structural types of activators of Kir6.2/SUR1 potassium channels have been described. These include the 1,2,4-thiadiazine 1,1-dioxide derivatives like diazoxide, BPDZ 62, BPDZ 73 and NN414[1–4] and certain cyanoguanidine derivatives, for example, compound **1**,[5,6] which all fit into the same pharmacophore model.[1] Recently a nitropyrazole (**2**) has been described as a K_{ATP} channel opener (K_{ATP}CO)[7] and we now wish to present a novel activator (2-(4-methoxyphenoxy)-5-nitro-*N*-(4-sulfamoylphenyl)benzamide) of the K_{ATP} channels of the Kir6.2/SUR1 type as well as the synthesis and characterisation of a small series of analogues in order to describe the structure activity relationship of this compound.

K_{ATP} channels are found in different tissues, such as the heart, vascular smooth muscle, central neurons and pancreatic beta cells.[8–10] Activators of K_{ATP} channels of smooth muscle (e.g., diazoxide and pinacidil) have been explored as drugs for treatment of cardiovascular diseases. Recently it has been suggested that activators of beta cell K_{ATP} channels can be used in the treatment of metabolic diseases through an inhibition of insulin release to induce beta cell rest.[11–13] It has been found that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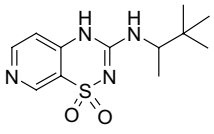
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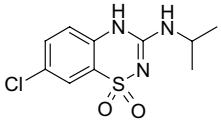
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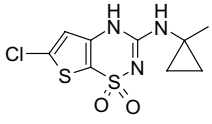
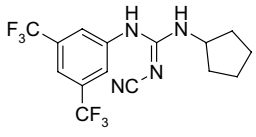

NN414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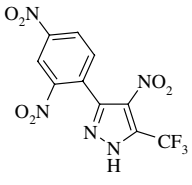
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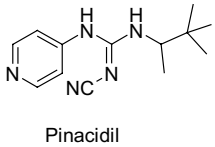
Pinacidil

Keywords: Diazoxide; K_{ATP} channels; Beta cell; NN414; Insulin release.
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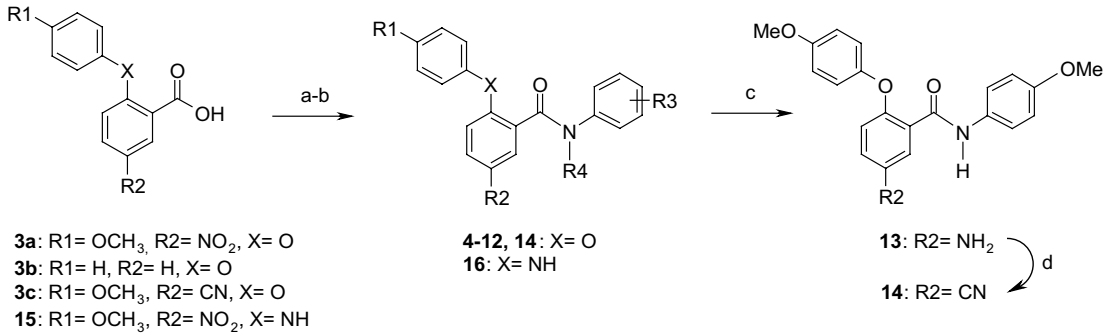

Scheme 1. Synthesis of benzamides **4**–**14** and **16**. Reagents and conditions: (a) $SOCl_2$, 25 °C; (b) aniline derivative, TEA, CH_2Cl_2, 25 °C; (c) **5**: H_2, Raney-Ni, DMF, EtOH; (d) HNO_2, $K_2Ni(CN)_4$, water.

K_{ATP}CO's (e.g., NN414) are able to preserve beta cell function in presence of high glucose[14,15] and inhibit apoptosis of human beta cells induced by glucose and IL-1β.[16]

The K_{ATP} channels are constructed as a 4 + 4 hetero-octamer made from the regulatory sulfonylurea receptor SUR and the inward rectifier Kir6.2 or Kir6.1. Different forms of SUR have been cloned and characterised.[17,18] In beta cells and neurons SUR1 combine with Kir6.2 to form the K_{ATP} channels, whereas SUR2B in combination with Kir6.1 and Kir6.2 form the channels of vascular smooth muscle and SUR2A in combination with Kir6.2 forms the channels of the heart.

N-Aryl-2-phenoxybenzamides were prepared as outlined in Scheme 1 starting from 2-phenoxybenzoic acid derivatives (**3a** and **3b**).[19] Thus treatment of **3a**,**b** with excess thionyl chloride at room temperature followed by evaporation in vacuo gave the corresponding benzoyl chlorides, which were reacted with equimolar amounts of substituted anilines in the presence of triethylamine to give the benzamides **4**–**12**. Reduction of the nitro group of compound **5** gave the amino derivative **13**, which could be converted to the corresponding cyano derivative **14** using standard Sandmeyer conditions but was preferably made from the benzoic acid **3c** through reaction with 4-aminobenzonitrile. Compound **3c** was prepared from 3-bromo-4-hydroxybenzonitrile through arylation with 4-methoxyphenylboronic acid followed by carboxylation with *n*-BuLi/CO_2. Reaction of 2-[(4-methoxyphenyl)amino]-5-nitrobenzoic acid **15**, prepared from 2-fluoro-5-nitrobenzoic acid and *p*-anisidine, using an analogous procedure as for the phenoxy derivatives gave compound **16**. Compounds **4**–**14** and **16** were characterised by ^{1}H NMR and LC–MS.

Compound **4** (NNC 55-0557) was initially identified by screening compound libraries in an assay aiming to find compounds, which were able to repolarise membrane potential of βTC3 beta cells depolarised by 10 μM glucose. In this assay[3–6,20] compound **4** was nearly 10 times more potent than diazoxide and only about 3 times less potent than NN414 (Table 1) (IC_{50}: 1.8, 13.7 and

Table 1. Effects of test compounds on beta cell membrane potential and insulin release

Compound	R1, R2, R3, R4	Membrane potential[a] βTC3 cells IC_{50} (μM)	Inhibition of insulin release[b] βTC6 cells	
			IC_{50} (μM)	Efficacy (%)
4	OCH_3, NO_2, 4-SO_2NH_2, H	1.8 ± 0.5	0.82 ± 0.16	79 ± 0.91
5	OCH_3, NO_2, 4-OCH_3, H	3.4 ± 1.5	1.6 ± 0.20	73 ± 3.95
6	OCH_3, NO_2, 4-F, H	7.2 ± 3.0	5.67 ± 3.67	52 ± 4.58
7	OCH_3, NO_2, 4-$COCH_3$, H	8.1 ± 3.7	1.12 ± 0.21	75 ± 1.63
8	OCH_3, NO_2, 3-SO_2NH_2, H	9.9 ± 3.1	20.53 ± 5.35	31 ± 4.94
9	OCH_3, NO_2, 2-SO_2NH_2, H	NS	10.37 ± 10.27	33 ± 7.23
10	OCH_3, NO_2, 4-Cl, CH_3	10.1 ± 1.2*	25.74 ± 13.52	33 ± 6.74
11	OCH_3, NO_2, 4-Cl, CH_2CH_3	8.3 ± 0.05*	10.4 ± 1.83	56 ± 3.25
12	H, H, 4-OCH_3, H	NS	NS	—
13	OCH_3, NH_2, 4-OCH_3, H	NS*	NS	—
14	OCH_3, CN, 4-OCH_3, H	2.5 ± 2.4	48.54 ± 45.55	17 ± 17
16	OCH_3, NO_2, 4-SO_2NH_2, H	NS	26.62 ± 7.36	1 ± 4.2
Diazoxide		13.7 ± 0.25[c]	22.98 ± 2.83[c]	25 ± 3.70[c]
BPDZ 73		0.25 ± 0.02	0.46 ± 0.11	75 ± 1.1
NN414[d]		0.6 ± 0.1	0.25 ± 0.05	73 ± 3.0

[a] Repolarisation of beta cell membrane potential in presence of 10 mM glucose. Values are mean ± SD of at least three experiments, unless otherwise noted (*: $n = 2$).
[b] Inhibition of glucose stimulated insulin release from βTC6 cells. Values are mean ± SEM of at least three experiments.
[c] Data from Tagmose et al.[6]
[d] Data from Nielsen et al.[4]

0.6 μM, respectively). The compound was subsequently found to inhibit glucose stimulated insulin release[4] from βTC6 beta cells (Table 1) (IC_{50} = 0.8 μM) and freshly isolated rat islets (IC_{50} = 0.73 ± 0.39 μM (n = 6); 80% max efficacy), which is approximately 30 times more potent than diazoxide (βTC6: IC_{50} = 22.98 μM and islets: IC_{50} = 20.28 ± 8.82 (n = 7), 36% max efficacy) but less potent than NN414 (βTC6: IC_{50} = 0.25 ± 0.05 μM and islets: IC_{50} = 0.18 ± 0.07 μM (n = 8), 72% max efficacy).

The ability of compound **4** to hyperpolarise the beta cell membrane and inhibit glucose stimulated insulin release strongly suggests that it activates K_{ATP} channels although inhibition of insulin release can be mediated through other mechanisms.[21] To substantiate a direct effect on the channel compound **4** was examined for effects on HEK 293 cells expressing human Kir6.2 and human SUR1. It was found that **4** potently repolarised the cell membrane that was depolarised with the K_{ATP} channel blocker tolbutamide. The potency (IC_{50} = 1.3 ± 0.7 μM, (n = 4), 100% max efficacy), which is similar to the effects observed on beta cell membrane potential and insulin release, is considerably improved to that of diazoxide (33 ± 11 μM) and similar to that of BPDZ 73 (0.8 ± 0.01 μM).[22]

To further explore the mechanism of action of **4**, the effects of the compound were examined using the patch clamp technique in the whole cell configuration (Fig. 1).[4] It was found that **4** (0.1, 1 and 10 μM) potently increases the ion current through the Kir6.2/SUR1 channel with an efficacy at 10 μM similar to that of diazoxide (300 μM). Together these studies strongly suggest that **4** inhibits glucose activated insulin release through an activation of Kir6.2/SUR1 channels on the beta cell.

To make a preliminary evaluation of the structure activity relationship of derivatives of compound **4**, compounds **5**–**14** and **16** were examined for their ability to hyperpolarise βTC3 cell membranes and to inhibit glucose stimulated insulin release from βTC6 cells (Table 1). It was found that changing the 4-sulfamoyl group to either 4-OMe (**5**), 4-F (**6**) or 4-acetyl (**7**) only had minor effect. Moving the 4-sulfamoyl group to position 3 (**8**) or position 2 (**9**), however, considerably reduced potency. Changing the 4-sulfamoyl group to 4-Cl and

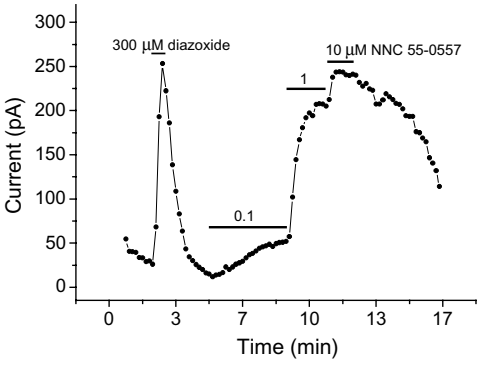

Figure 1. Effects of compound **4** (0.1, 1.0 and 10 μM) on Kir6.2/SUR1 on patch clamp, whole cell recordings.

the amide NH to NCH_3 (**10**) or NCH_2CH_3 (**11**) also gave significantly active but less potent compounds compared to **4**.

Reduction of the nitro group of compound **5** gave the inactive aniline **13**. Transforming **13** to the cyano derivative (**14**) regained some of the beta cell activity. The efficacy of **14** in the membrane assay was low (49%) compared to that of **4** (100%, data not shown), highlighting the importance of the electron withdrawing nitro group. This is substantiated by the complete lack of activity of 2-phenoxy-*N*-(4-methoxyphenyl)benzamide (**12**). The 4-methoxyphenylaminobenzamide derivative **16** was inactive.

Conclusion

2-(4-Methoxyphenoxy)-5-nitro-*N*-(4-sulfamoylphenyl)-benzamide has been identified as a new activator of Kir6.2/SUR1 K_{ATP} channels. Although the compound or its close analogues are not drug candidates due to, for example, low solubility, multiple hydrogen bond acceptors and high polar surface area, they do provide leads for further optimisations.

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