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Synthesis and Evaluation of 7-Substituted-3-cyclobutylamino-4*H*-1,2,4-benzothiadiazine-1,1-dioxide Derivatives as K_{ATP} Channel Agonists

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Abstract—A series of 7-substituted-3-cyclobutylamino-4*H*-1,2,4-benzothiadiazine-1,1-dioxide derivatives has been synthesized and evaluated as K_{ATP} channel agonists using the inside-out excised patch clamp technique. The most active compounds were ~20-fold more potent than diazoxide in opening K_{ATP} channels. A linear relationship exists between the potency of the compound and the sigma value of the 7-substituent with electron-withdrawing groups exhibiting higher activity. These compounds may be useful in modulating insulin release from pancreatic β-cells and in diseases associated with hyperinsulinemia.
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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 control intracellular [Ca^{2+}] and ultimately insulin secretion.[7]

The β-cell K_{ATP} channel is comprised of two subunits, 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, diazoxide has certain liabilities which limit its potential as a therapeutic agent such as weak potency (ca.10 μM) at SUR1/Kir6.2 and poor selectivity versus the SUR2B subtype.[16]

To date there are only a few reports of potent and selective β-cell K_{ATP} agonists. Modifications made to the benzothiadiazine 1,1-dioxide core of diazoxide have resulted in analogues such as BPDZ 73 with enhanced potency and selectivity as compared to diazoxide (Fig. 1).[17]

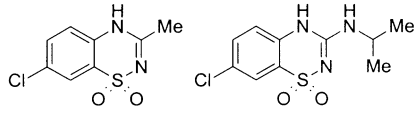

Figure 1.

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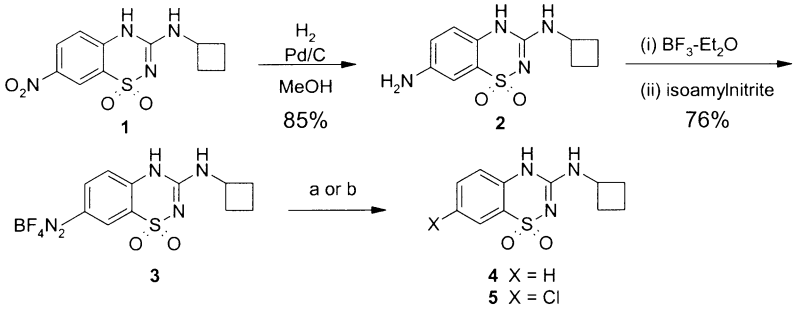
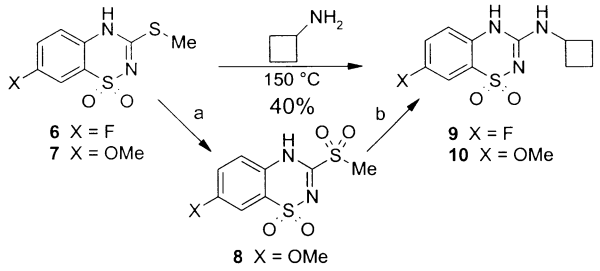


Scheme 1. (a) Refluxing MeOH, 75%; (b) CuCl, HCl, 74%.

Structure–activity relationships in these reports were determined on the ability of the compound to inhibit insulin secretion from glucose-stimulated rat islets in vitro. In order to gain a more thorough understanding of the structure–activity relationships within the diazoxide template, we decided to investigate the effects of both electron-withdrawing and electron-donating substituents at the C-7 position of the diazoxide core. We selected 3-cyclobutylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide as our template on the basis of the reported potency of related analogues.[17] However, unlike previous publications in this area, we measured 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 practice, ATP-sensitive K+ currents were recorded from inside-out macropatches pulled from Xenopus oocytes expressing the human SUR1/Kir6.2 channel.[18] 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}. This approach has several advantages over alternative methods of determining efficacy, including removing concerns that: (a) test compounds may indirectly affect K_{ATP} channels to alter membrane potential or insulin release and (b) test compounds do not permeate the cell. Unfortunately, this is a laborious assay that does not enable rapid testing of compounds.

Novel analogues containing C-7 electron-donating groups (–OMe, $–NH_2$) and the unsubstituted adduct (-H), as well as electron-withdrawing groups ($–NO_2$, –Cl, –F)[19] were synthesized as outlined in Schemes 1 and 2.[20] Starting from **1**, which is prepared in three steps from commercially available 2-chloro-5-nitrobenzene sulfonamide,[21] catalytic hydrogenation of the nitro group using palladium on carbon and H_2 gave aniline **2** (Scheme 1). Treatment of **2** with BF_3–Et_2O in THF at 0 °C, followed by addition of isoamylnitrite yielded the diazonium salt **3**, which precipitated out of solution upon addition of hexanes. Thermal decomposition of **3** in refluxing MeOH produced the desired unsubstituted analogue **4**. Intermediate **3** was also converted to the chloride **5** using Sandmeyer conditions (CuCl in concentrated HCl at 80 °C). We attempted to convert **3** to the fluorinated analogue **9** by heating neat at 215 °C under N_2. Although the desired product was formed, purification proved to be difficult and we settled on preparing **9** by an alternate route.



Scheme 2. (a) mCPBA, acetone, 66%; (b) cyclobutylamine, 63%.

The syntheses of compounds **9** and **10** are shown in Scheme 2. Intermediates **6** and **7** have been reported and can be obtained in three steps from commercially available *p*-fluoroaniline or *p*-anisidine, respectively.[22] Heating **6** in cyclobutylamine at 150 °C in a sealed tube cleanly afforded **9** in moderate yield. For the case of **7**, the methylsulfide was first converted to the methylsulfone **8** using *m*-chloroperbenzoic acid in acetone at 0 °C. The sulfone, which is a better leaving group than methylthiol, was easily displaced with cyclobutylamine at room temperature to give **10**.

The relationship between the dose of a compound and K_{ATP} channel activity recorded from excised patches is illustrated in Figure 2 and 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 %Max refers to the percentage of maximal agonistic response elicited by the compound as compared to a fully efficacious effect of diazoxide (50 μM). Most of the compounds tested were more active than diazoxide with compounds **5** and **9** showing a greater than 20-fold improvement in potency. In addition, the maximal responses evoked by these two were greater than diazoxide by ~35%. The significant improvement in activity observed specifically for **5** and **9** suggests that electron-withdrawing groups at C-7 increase the ability of the compounds to directly activate β-cell K_{ATP} channels. Interestingly compound **1,** which bears the most electron-withdrawing substituent, showed improved potency (EC_{50} 0.53 μM) but only a 31% maximal

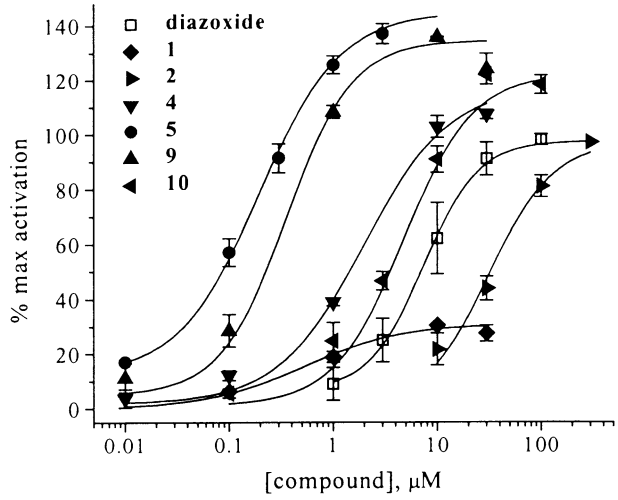


Figure 2. Compound dose–response curves for activation of K_{ATP} from excised patch recordings.

response at 30 μM as compared to diazoxide. Pirotte and Lebrun previously reported that a series of 7-nitro-3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide derivatives (including compound **1**) produced only weak inhibition of insulin secretion from glucose-stimulated rat islets.[21] They concluded that the 7-nitro group renders the proton at N-4 relatively acidic and therefore the compound is deprotonated to a significant extent at physiological pH (7.4). Previous data suggests that this proton makes important hydrogen bonds in a channel protein binding pocket and therefore compound potency would be diminished when the proton is absent.[17b] An alternative hypothesis that could not be excluded on the basis of their data is that the 7-nitro substituent greatly reduces the compound's ability to permeate the cell membrane. However, our finding that **1** has poor efficacy at K_{ATP} channels in excised patches where cell permeation is not a factor does not support this hypothesis.

Table 1. Compound activation of K_{ATP} from excised patch recordings

Compd	X	EC_{50}, μM[a,b]	%Max[a,c]	pK_a
Diazoxide		8.80 (±0.25)	100	8.62[23]
1	NO_2	0.53 (±0.07)	30.9 (±2.3)	7.89 (±0.02)
2	NH_2	31.0 (±5.0)	96.9 (±4.7)	
4	H	1.96 (±0.34)	109.0 (±7.4)	
5	Cl	0.196 (±0.018)	136.8 (±3.7)	9.36 (±0.02)
9	F	0.356 (±0.025)	135.5 (±.2)	9.57 (±0.01)
10	OMe	3.87 (±0.63)	121.8 (±3.8)	

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

Table 2. pEC_{50} and σ_p value for C-7 substituents

Compd	X	pEC_{50}[a]	σ_p[b]
2	NH_2	4.5	−0.66
4	H	5.7	0.00
5	Cl	6.7	+0.23
9	F	6.4	+0.06
10	OMe	5.4	−0.27

[a]Negative log of the EC_{50} value.
[b]Values taken from ref 23.

We then determined that the p*K*a of **1** = 7.89 using standard UV spectroscopic methods and the Henderson–Hasselbalch equation was used to calculate the percentage of protonated (HA) versus ionized (A-) species in solution at pH 7.2; the pH at which the excised-patch electrophysiology experiments were conducted. At this pH, 17% of the compound is deprotonated and therefore unable to hydrogen bond in the binding pocket. We next measured the p*K*a's of compounds **5** and **9**, which contain electron-withdrawing substituents and therefore might also be partially ionized at physiological pH. If compound **1** has weak activity at the K_{ATP} channel due solely to substantial deprotonation at N-4, then based on the increased potency of **5** and **9** relative to compound **1**, we would predict these two compounds to be significantly less acidic. Indeed, our p*K*a measurements found that both **5** and **9** are ~50-fold less acidic than **1** (Table 1) and should exist >99% in the protonated (HA) form at pH 7.2 (determined by Henderson–Hasselbalch calculations). Although partial ionization of **1** may account for a decrease in potency, it is likely there are other factors that contribute to the poor maximal agonistic response produced by the compound. The increased hydrophilicity or steric bulk of the nitro group (as compared to Cl or F) may induce conformational changes in the protein, which yield only partial agonism.

The importance of the N-4 proton on activity has thus far been implied in two ways: first, by the experiments described above, and second, methylation of the N-4 position leads to loss of K_{ATP} activity.[17b] The ability of the C-7 substituent to modulate the acidity of the N-4 proton, along with the observation that halogen substituents at C-7 increase compound activity at K_{ATP}, suggested that any change to the molecule that can enhance hydrogen bonding by stabilizing the partial negative charge on N-4 should increase potency. Thus electron-withdrawing groups at C-7 would increase potency over the unsubstituted analogue while electron-donating groups should reduce activity. The excised patch data in Table 1 reinforce this hypothesis, since the electron-deficient compounds **5** and **9** (X = Cl and F, respectively) show a greater than 5-fold increase in potency over **4** (X = H), whereas the activity of the electron-rich analogues **2** and **10** (X = NH_2 and OMe, respectively) is decreased relative to that of compound **4**. The correlation between compound pEC_{50} and the sigma value[23] (σ_p) for each substituent (Table 2) is plotted in Figure 3 and reveals the existence of a linear relationship with a high degree of correlation ($R^2 = 0.942$). The nitro compound **1** was omitted from the plot for reasons discussed above.

In conclusion, we have synthesized a series of 7-substituted-3-cyclobutylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide derivatives bearing both electron-donating and electron-withdrawing substituents that function as

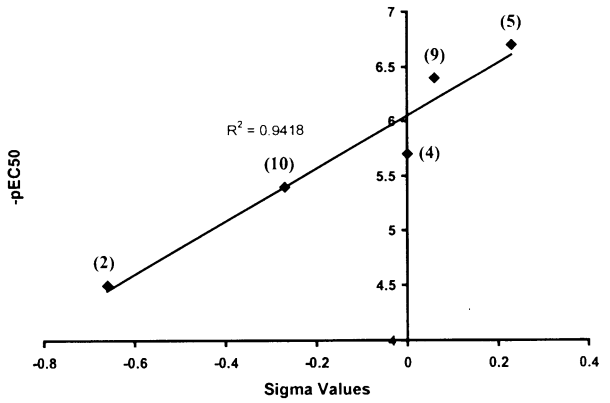


Figure 3. Plot of pEC_{50} versus sigma values.

K_{ATP} agonists in an excised patch clamp assay. The use of the excised patch clamp technique allowed measurement of the direct opening of the channel by the compound, thus removing factors which might confound interpretation of K_{ATP} activity such as indirect activation of the channel or lack of cell permeability of test compounds. Several of the compounds prepared were 20-fold more potent that diazoxide in opening K_{ATP} channels. Within this limited series of compounds, a linear relationship exists between the potency of the compound and the sigma value of the 7-substituent, with electron-withdrawing groups exhibiting higher activity. The exception is the nitro-bearing compound which renders the proton at N-4 sufficiently acidic to be deprotonated at physiological pH. Further investigations into compounds that modulate K_{ATP} activity are currently underway and will be reported in due time.

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