New 3-Alkylamino-4*H*-thieno-1,2,4-thiadiazine 1,1-Dioxide Derivatives Activate ATP-Sensitive Potassium Channels of Pancreatic Beta Cells

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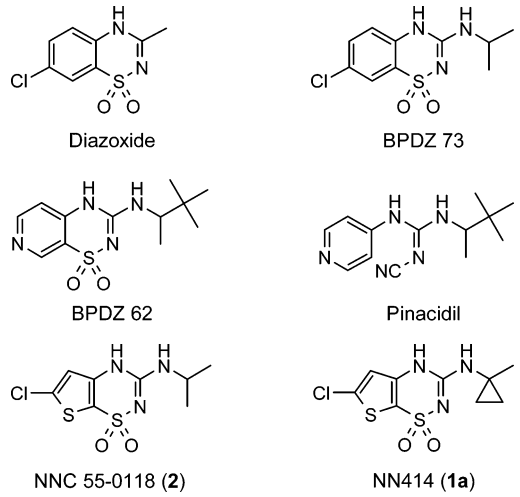
Compound **1a** (NN414) is a potent opener of Kir6.2/SUR1 K_{ATP} channels. Compound **1a** inhibits insulin release in vitro and in vivo and preserves beta cell function in preclinical animal models suggesting that such a compound could find use in treatment or prevention of type 1 and type 2 diabetes. The crystal structure and a convergent synthesis of **1a** are presented together with a range of new analogues of **1a**. Several compounds, e.g., 6-chloro-3-(1-methyl-1-phenylethyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**1h**), were found to be potent openers of Kir6.2/SUR1 K_{ATP} channels and were able to suppress glucose-stimulated insulin release from rat islets in vitro ($EC_{50} = 0.04 \pm 0.01$ μM) and in vivo after intravenous or peroral administration to hyperinsulinemic obese Zucker rats ($ED_{50} = 4.0$ mg/kg). Structural modifications of this series of K_{ATP} channel openers have provided compounds with promising pharmacokinetic properties indicating that brief periods of beta cell rest can be achieved.

Introduction

Activators (openers) of ATP-sensitive potassium (K_{ATP}) channels have been found to preserve and improve beta cell function under conditions of metabolic stress.[1−4] It is therefore hypothesized that orally active and beta cell selective K_{ATP} channel openers (K_{ATP}COs) will be useful in treatment and prevention of type 1 and type 2 diabetes.[5] Several studies have recently indicated that beneficial effects on beta cell function can be obtained after suppressing insulin release for only a short period each day.[1,6,7] This suggests that pharmacological induction of periods of brief beta cell rest could be beneficial in treatment and prevention of diabetes. The ATP-sensitive potassium channels are present in various types of tissues such as cardiac and smooth muscle, neurons, and pancreatic beta cells.[5,8−10] The open state probability of the K_{ATP} channels, which is regulated by intracellular nucleotides such as ATP and ADP, couples the metabolic state to cellular membrane potential. The K_{ATP} channel exists as an octameric complex of the sufonylurea receptor (SUR) and the pore-forming inwardly rectifying potassium channel (Kir) in a 4 + 4 stoichiometry. The genes for two closely related sulfonylurea receptors, SUR1 and SUR2, have been cloned, and two different splice variants of SUR2, SUR2A and SUR2B, have been reported. SUR1 combines with Kir6.2 to form the K_{ATP} channels of pancreatic beta cells, whereas the cardiac type consists of SUR2A and Kir6.2 and the smooth muscle type of SUR2B and Kir6.1 or Kir6.2. Activation of K_{ATP} channels of vascular smooth muscle causes relaxation of vasculature and a subsequent reduction in blood pressure. The K_{ATP} channels of beta cells are in the open state during fasting. In the presence of high glucose concentrations, the ATP/ADP ratio is increased and the K_{ATP} channels are closed. This will depolarize the cellular membrane and open the voltage-gated Ca^{2+} (L-type) channels to stimulate insulin secretion. Pharmacological activation of the K_{ATP} channels in the presence of high glucose concentration will prevent insulin secretion. The nonselective K_{ATP}COs diazoxide and BPDZ 73 and the SUR1-selective K_{ATP}COs BPDZ 62, **2** (NNC 55-0118), and **1a** (NN414) (Chart 1) inhibit insulin release.[11−14] The cyanoguanidine pinacidil, which relaxes smooth muscle to reduce blood pressure, is a potent and SUR2-selective K_{ATP}CO and inhibits insulin release only at high concentrations.[15] The potassium channel blockers and potassium channel openers interact with the sulfonylurea receptor in a compound-specific and complex manner, which involves both transmembrane regions and the two nucleotide binding folds.[16−18] Recently, we have shown that certain 6-chloro-3-alkylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide derivatives (e.g., **1a** and **2**) are potent activators of Kir6.2/SUR K_{ATP} channels,[13] which inhibit insulin release in vitro and in vivo to induce beta cell rest.[2,3,14] In this paper, we wish to present a series of novel K_{ATP}COs in order to analyze the structure−activity relationship (SAR) of derivatives of **1a**. In addition, we wish to present a report of the crystal structure of this compound as well as new synthetic procedures for the preparation of 3-alkylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**1**) and 3-alkylamino-4*H*-thieno-

Chart 1



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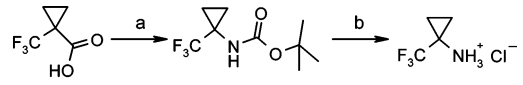
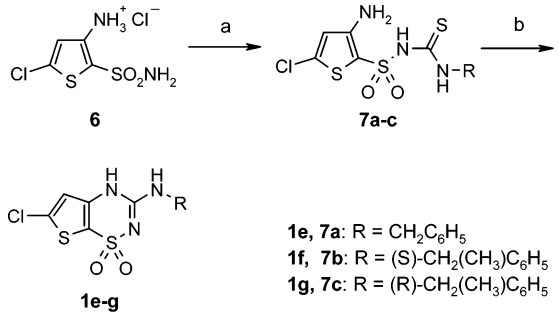
Scheme 1. Synthesis of 1-(Trifluoromethyl)cyclopropylamine Hydrochloride[a]



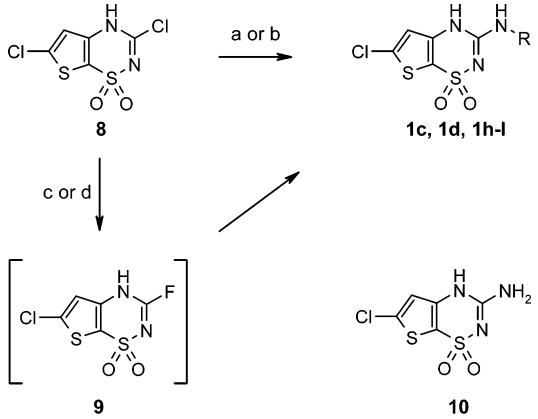
[a] Reagents: (a) DPPA, Et_3N, *t*-BuOH, reflux; (b) 1 N HCl, reflux.

Scheme 2. Synthesis of 6-Chloro-3-alkylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide Derivatives[a]



[a] Reagents: (a) *t*-BuOK, RNCS, DMF, 0 °C; (b) Et_3N, 1.93 M $COCl_2$ in toluene, THF, 0 °C.

Scheme 3. Synthesis of 6-Chloro-3-alkylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide Derivatives[a]



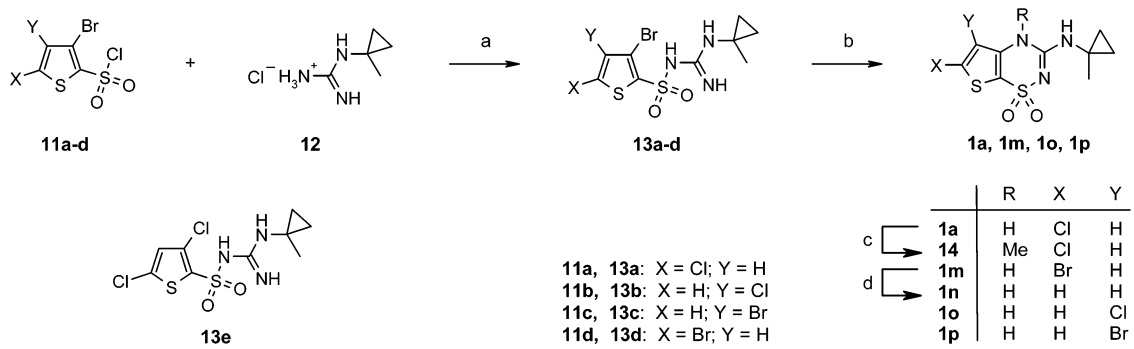
[a] Reagents: (a) RNH_2, 85−120 °C; (b) RNH_2, EtOH or THF, 120 °C; (c) (i) KF, hexadecyltrimethylammonium bromide, NMP, 120 °C and (ii) RNH_2·HCl, Et_3N, NMP, 75 °C; (d) RNH_2·HCl, TGME, KF, 2,6-dimethylpyridine, H_2O, 100 °C.

[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**18**) derivatives. Furthermore, we have characterized **1h** as a new potent inhibitor of insulin release, which potentially could be useful in generation of brief periods of beta cell rest.

Chemistry

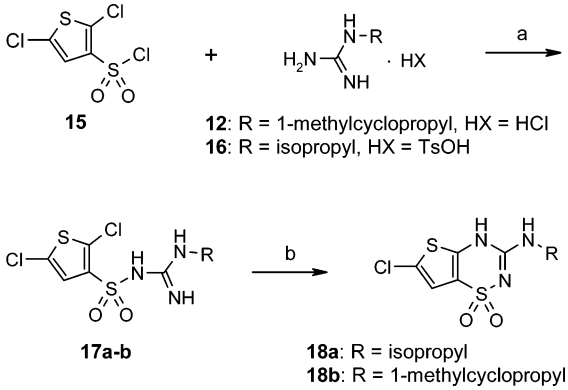
The alkylamines used as starting materials for this study were obtained commercially or prepared from the corresponding carboxylic acids by a modified Curtius rearrangement with diphenyl phosphorazidate (DPPA) and triethylamine in *tert*-butyl alcohol[19] with subsequent acidic cleavage of the Boc moiety. This procedure is shown in Scheme 1 for the conversion of carboxylic acid **3** to the *tert*-butyl carbamate **4** which afforded the 1-(trifluoromethyl)cyclopropylamine hydrochloride (**5**) after deprotection with hydrochloric acid. Most of the desired 6-chloro-3-alkylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxides (**1**) were prepared by our previously described routes,[14] as shown in Schemes 2 and 3. Thus, reaction of 3-amino-5-chlorothiophene-2-sulfonamide hydrochloride (**6**) with benzyl isothiocyanate and (*R*)- and (*S*)-1-phenylethyl isothiocyanate at 0 °C afforded the sulfonyl isothioureas (**7a**−**c**), which then were cyclized to the thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxides (**1e**−**g**) in low yields by treatment with phosgene (Scheme 2). Compounds **1d**, **1h**, **1j**, and **1k** were prepared in moderate to low yields from 3,6-dichloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**8**)[14] by displacement of the chlorine at the C-3 position with the appropriate amines (Scheme 3). For some deactivated amines possessing bulky or electron-withdrawing groups, the reaction was carried out via the activated 3-fluoro intermediate **9** in a two-step reaction (compound **1i**) or preferentially by a fluoride-catalyzed one-step amination process (compounds **1c** and **1l**) described previously for chloroimidazoles.[20] The introduction of the bulky 3-alkylamino side chains by halide displacement (Scheme 3) was achieved under conditions that often afforded reaction mixtures containing starting material and significant quantities of the dealkylated side product **10**. Generally, the methods outlined in Schemes 2 and 3 suffered from the disadvantage of multistep reactions with low overall yields when starting from commercially available compounds, and the methods also required potentially hazardous steps. Therefore, a new general approach was explored (Scheme 4) as an alternative to the above synthetic routes. Commercially available 3-bromo-5-chlorothiophene-2-sulfonyl chloride (**11a**) was reacted with *N*-(1-methylcyclopropyl)guanidine hydrochloride (**12**) which was prepared by a slight modification of the known guanylation method described by Bernatowicz et al.[21] The intermediate sulfonyl guanidine **13a** was obtained as the only isomer in high yield at room temperature in a heterogeneous mixture of aqueous sodium hydroxide and toluene. Cyclization of compound **13a** proceeded smoothly at reflux in aqueous 1-methyl-2-pyrrolidinone (NMP) in the presence of potassium carbonate and a catalytic amount of copper bronze affording the desired compound **1a** in good yield. Treatment of **1a** with iodomethane in acetone in the presence of potassium

Scheme 4. Synthesis of 3-(1-Methylcyclopropyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide Derivatives[a]



[a] Reagents: (a) 4 N NaOH/toluene; (b) copper bronze, K_2CO_3, DMF, 150 °C; (c) MeI, K_2CO_3, acetone; (d) (i) *n*-BuLi, THF, −78 °C and (ii) MeOH.

Scheme 5. Synthesis of 6-Chloro-3-alkylamino-4*H*-thieno[2,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide Derivatives[a]



[a] Reagents: (a) 1 N NaOH/Et_2O; (b) copper bronze, K_2CO_3, DMF, 150 °C, 4 h (**18a**) or Cs_2CO_3, 1-butanol, 110 °C, 48 h (**18b**).

carbonate gave the methylated derivative **14**. Compounds **1m**, **1o**, and **1p** were prepared in the same way as **1a** starting from the appropriate sulfonyl chlorides **11b**−**d**, which were obtained by chlorosulfonation of the corresponding thiophenes. Alternatively the cyclization procedure leading to **1a** could be carried out starting from *N*-[(3,5-dichlorothien-2-yl)sulfonyl]-*N*′-(1-methylcyclopropyl)guanidine (**13e**). The yields and reaction conditions were, however, inferior to those necessary for the ring closure of **13a**.[22] Compound **1m** was metalated with *n*-butyllithium and quenched with methanol to give the debrominated compound **1n**. Finally, treatment of 2,5-dichlorothiophene-3-sulfonyl chloride (**15**) with the appropriate guanidines **12** and **16** in a mixture of aqueous sodium hydroxide and diethyl ether gave the sulfonyl guanidines **17a** and **17b**. Subsequent cyclization in DMF in the presence of potassium carbonate and a catalytic amount of copper bronze or simply heating for 2 days in 1-butanol in the presence of cesium carbonate afforded a low yield of the 4*H*-thieno[2,3-*e*]-1,2,4 thiadiazines **18a** and **18b**, respectively (Scheme 5).

Optimization of the Ring Closure Reaction. Both *N*-[(3-bromo-5-chlorothien-2-yl)sulfonyl]-*N*′-(1-methylcyclopropyl)-guanidine (**13a**) and the 3-chloro analogue **13e** can be used as intermediates for the formation of **1a** via a ring closure reaction (Scheme 4). Different procedures for the cyclization of **13a** and **13e** have been investigated. Higher yields and a faster reaction can, in general, be achieved using **13a** as the starting material. The best procedure seems to be a reaction of **13a** in the presence of copper bronze as the catalyst and K_2CO_3 as the base in a mixture of NMP and water which afforded yields in the range of 80−90%.

Structure Analysis. Two crystal forms, polymorphs A and B of compound **1a**, have been identified. The crystal structure of the thermodynamically stable form at room temperature, polymorph A, was solved from single-crystal X-ray diffraction data. The solution confirms the molecular structure shown in Figure 1. Attempts to grow single crystals of polymorph B were not successful. The synthesis and SARs have previously been reported for the analogous K_{ATP}CO **2**, which differs from **1a** by having an isopropyl group instead of the cyclopropyl group.[14] Compound **2** has been found to crystallize in three modifications, A, B, and C, and the structures of polymorph A and form B, a solvate, have been solved from single-crystal X-ray diffraction data. The polymorphs A of the two compounds are isostructural with two molecules in the asymmetric unit of both the triclinic cell of **1a** and the monoclinic cell of **2**.

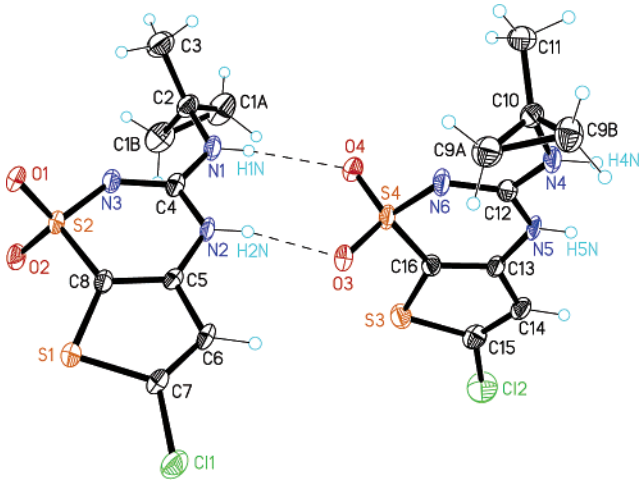


Figure 1. ORTEP plot (50% thermal ellipsoids) of **1a**. The thermal ellipsoids of the hydrogen atoms are not shown (cyan); instead, small spheres have been drawn.

Each independent molecule adapts the 4H-tautomeric form with the two N−H bonds pointing in the same direction. For pinacidil[23] the directions of two guanidine N−H bonds at the nitrogen atoms bridging to the pyridyl and alkyl groups, respectively, differ less than 20°. The 1,2,4-thiadiazine rings of **1a** and **2** are planar within experimental error. Thus, the structures of compounds **1a** and **2** in the solid state are analogous to those found for other K_{ATP}COs of the 1,2,4-pyridothiadiazine 1,1-dioxide[24−27] and the 1,2,4-benzothiadiazine 1,1-dioxide (e.g., diazoxide)[28] types. For **1a**, **2**, and diazoxide it is not possible to uniquely establish the 4H- or 2H-tautomerism by ^{1}H NMR spectroscopy, since there are no signals from neighboring atoms. The crystal structures are therefore pivotal to independently establish the tautomerism. It has in this study been possible to locate the 4N-hydrogen atomic positions and exocyclic amino hydrogen atomic positions by the difference Fourier method and to refine these independently. The difference Fourier maps showed no evidence of a hydrogen atom at the 2N position of the thiadiazine rings. The 4H-tautomeric form is also confirmed by the fact that the bond lengths of the "2N−3C" bonds (1.312(4), 1.322(4)) Å for **1a** and (1.320(2), 1.324(2)) Å for **2** are significantly shorter than the "4N−3C" bonds of (1.368(4), 1.355(4)) Å for **1a** and (1.362(3), 1.372(3)) Å for **2**.

Both the triclinic crystal structure of **1a** and the monoclinic crystal structure of **2** are supported by strong intermolecular hydrogen bonds from the thiadiazine 4H atom of one molecule to one oxygen atom in the sulfonyl group in the independent molecule above it. The hydrogen bond from the exocyclic amino hydrogen atom to the other oxygen atom in the sulfonyl group of the molecule above it is weak in both structures. Figure 1 shows the H-bonds for **1a**. This hydrogen-bonding motif extends throughout the structure almost parallel to the crystallographic *c*-axis of polymorph A of **1a** in a chain of molecules twisted approximately 90° (Figure 2). The *c*-axis is also the needle axis of the crystals of **1a**. Between the chains of molecules in the crystal only weak van der Waals interactions exist. In the polymorphs A of the two compounds, the angles between the thiophene ring and the 1,2,4-thiadiazine ring are small, 4.6(2)° and 5.7(2)° for the **1a** molecules and 1.9(2)° and 4.8(2)° for the **2** molecules. In form B of **2**, which represents a solvate in a rhombohedral crystal modification with one molecule in the asymmetric unit, **2** is also a 4H-tautomer and the thiophene and the thiadiazine rings are coplanar. The planar ring system is another characteristic of the pyridothiadiazine 1,1-dioxide[24−27]

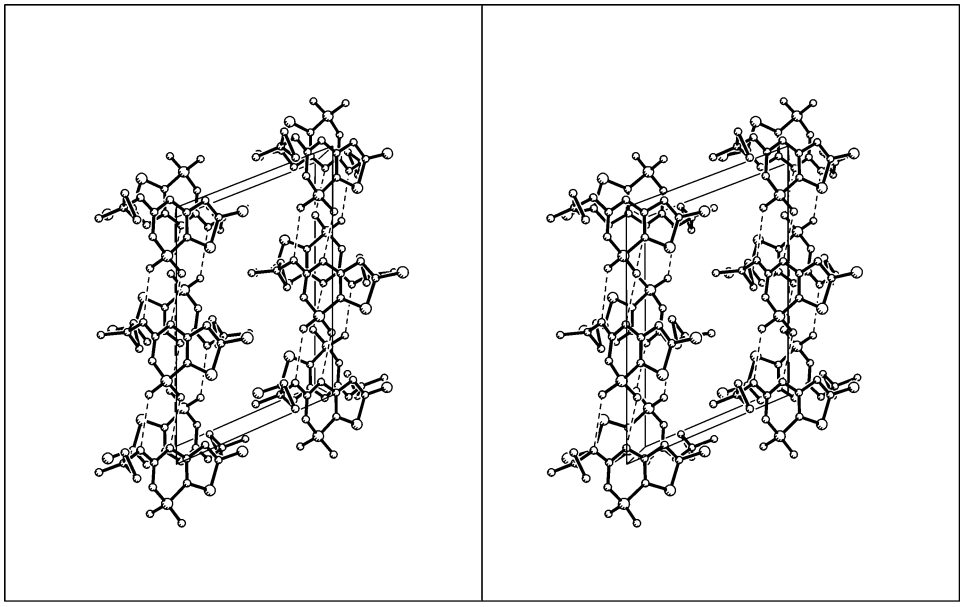


Figure 2. Stereoview of molecular crystal packing along the *b*-axis (**1a**). The four independent hydrogen bonds of type N−H···O−S along the crystallographic *c*-axis (vertical) are indicated. The *a*-axis is in the plane, in an oblique angle to the *c*-axis.

and the benzothiadiazine 1,1-dioxide[28] molecules in the solid state. This is in contrast to pinacidil in the solid state where the guanidine moiety plane is twisted approximately 40° from the plane of the pyridine group.[23]

Results and Discussion

In Vitro Biology. Glucose-induced insulin release is mainly a consequence of closure of ATP-sensitive potassium channels mediated by changes in the ATP/ADP ratio in the pancreatic beta cells. The closure leads to beta cell plasma membrane depolarization, influx of Ca^{2+} through voltage-gated calcium channels, increase in cytoplasmic Ca^{2+}, and activation of exocytosis of insulin from the pool of secretory granules. Compounds that activate Kir6.2/SUR1 K_{ATP} will repolarize cell membranes of beta cells incubated in the presence of high glucose and will inhibit glucose-stimulated insulin release from beta cells and isolated islets. Initially, we have screened the compounds of this series for their ability to affect the membrane potential of βTC3 beta cells incubated in 11 mM glucose by measuring changes in $DiBAC_4(3)$ fluorescence and for their ability to inhibit insulin release from βTC6 cells incubated in 22 mM glucose.[14]

We have previously found that the 3-alkylamino side chain of the 6-chloro-3-alkylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide derivatives is very important. Specifically, we found that branching of the α-carbon could increase the potency of these derivatives as activators of K_{ATP} channels of the beta cells. Among the most interesting compounds thereof are the 1-methyl(cyclopropyl)amino (**1a**) and the 1-methyl(cyclobutyl)amino (**1b**) derivatives, which both potently repolarize the membrane potential of βTC3 cells and inhibit insulin release from βTC6 cells (Table 1).[14] The potency in these assays correlates well with the potency of the compounds in binding experiments and in patch clamp experiments, measuring ion currents through recombined Kir6.2/SUR1 channels expressed in HEK293 cells.[13,14]

Increasing the ring size from cyclopropyl (**1a**) to cyclobutyl (**1b**) increased potency, while further ring expansions to cyclopentyl or cyclohexyl resulted in a reduced potency.[14] Changing the methyl group to ethyl also caused a slight reduction in potency comparing the 1-methyl(cyclopropyl)amino derivative **1a** with the 1-ethyl(cyclopropyl)amino derivative.[14]

In the present series (Table 1) we found that substituting the methyl group with trifluoromethyl to get the 1-trifluoromethyl-(cyclopropyl)amino derivative **1c** only gave a minor reduction in potency and beta cell selectivity compared to that of **1a**. When **1c** was compared to the 2,2,2-trifluoroethylamino derivative **1d**, it was found that the cyclopropyl group provides an overall increase in potency and/or efficacy. Changing the methyl group of the 1-methyl(cyclopropyl)amino group of **1a** to a phenyl group (**1i**) did not cause a major change in potency and selectivity although a reduction in the effects on insulin release could be observed. If, alternatively, 4-methoxyphenyl was used (**1l**) an increase in vasorelaxation was observed, while the beta cell effect was retained, making the compound nonselective. To explore the importance of the cyclopropyl group additional analogues were prepared and examined. The 3-benzylamino derivative **1e** hyperpolarizes beta cell membranes and inhibits insulin release less potently and with lower efficacy compared to the 1-phenyl(cyclopropyl)amino derivative (**1i**). This again highlights the beneficial effects of the cyclopropyl group for the beta cell effect. Substituting the benzyl group to get the (*S*)-1-phenethylamino derivative (**1f**) further reduced beta cell activity. The antipode (**1g**), in contrast, was at least as potent as **1i** with respect to hyperpolarization of beta cell membrane and inhibition of glucose-stimulated insulin release. This stereoselectivity for the beta cell effects was not found for the vascular effects, which are in contrast to the observation that the (*R*)-form of pinacidil is approximately 50 times more potent than the (*S*)-form.[29] Increasing the branching of the α-carbon atom to get the 3-(1-methyl-1-phenylethyl)amino derivative (**1h**) considerably increases the beta cell potency in analogy with what previously has been observed when going from 3-isopropylamino to 3-*tert*-butylamino.[14] Compound **1h** only had minimal effects on rat aorta making the compound highly beta cell selective. The substitution on the thiophene ring is of considerable importance. Removing the halogen in position 6 to get compound **1n** significantly reduced beta cell activity compared with that of **1a**, while the effect on rat aorta was maintained at considerably reduced efficacy. Chlorine (**1o**) or

Table 1. Structures and in Vitro Biology Screening Data for Compounds **1a**−**p**, **14**, and **18a**,**b**

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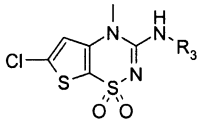

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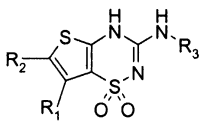
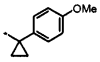


18

				Membrane potential βTC3 [a]	Insulin release βTC6[b]		Rat aorta[c]
Compound	R_1	R_2	R_3	EC_{50} (μM)	EC_{50} (μM)	Efficacy (%)	EC_{50} (μM)
1a	H	Cl		0.5 ± 0.1	0.25 ± 0.05	73 ± 3.0	119.2 ± 12.1[d]
1b	H	Cl		0.02 ± 0.002	0.02 ± 0.01	92 ± 1.84	6.5 ± 1.7[d]
1c	H	Cl		0.4 ± 0.2	1.4 ± 0.45	76 ± 3.07	40 ± 25.8
1d	H	Cl		3.1 ± 1.1	0.15 ± 0.12	36 ± 7	172.6 ± 15.8
1e	H	Cl		3.6 ± 0.5[e]	6.11 ± 1.31	30 ± 6.07	133 ± 40.5
1f	H	Cl		3.5 ± 0.6[e]	13 ± 2.11	28 ± 3.71	34.9 ± 11.99
1g	H	Cl		0.5 ± 0.2	0.99 ± 0.37	68 ± 4	24 ± 10.8
1h	H	Cl		0.13 ± 0.02	0.24 ± 0.07	76 ± 5.54	103 ± 64.2
1i	H	Cl		0.48 ± 0.09	2.67 ± 0.62	66 ± 6.75	183 ± 73.0
1j	H	Cl		2.0 ± 3	2.07 ± 1.18	69 ± 7.61	95 ± 12.4
1k	H	Cl		0.16 ± 0.04	13.36 ± 1.21	40 ± 6.21	13.9 ± 5.4
1l	H	Cl		0.5 ± 0.2	3.57 ± 0.51	69 ± 1.64	2.89 ± 1.33
1m	H	Br		0.14 ± 0.01	0.31 ± 0.10	81 ± 3.38	133 ± 11.3
1n	H	H		>30	0.19 ± 0.08	19 ± 8.9	6.87 ± 0.76[f]
1o	Cl	H		NA	0.28 ± 0.07	2 ± 1.65	NT
1p	Br	H		NA	6.7 ± 6.63	5 ± 5.08	NT
14	H	Cl		NA	0.74 ± 0.46	23 ± 16.23	NT
18a	H	Cl		5.0 ± 0.5	19.82 ± 8.04	16 ± 6.73	>300
18b	H	Cl		NA	NT	NT	NT
Diazoxide				13.7 ± 0.25	22.98 ± 2.83	25 ± 3.70	20.8 ± 6.1[d]

[a] Repolarization of *β*TC3 cells. Shown are means ± SEM ($n \geq 3$). [b] Inhibition of glucose-stimulated insulin release from *β*TC6 cells. Shown are means ± SEM ($n \geq 3$). [c] Relaxation of precontracted aorta rings. Shown are means ± SEM ($n \geq 3$). [d] Values from Nielsen et al. (ref 14). [e] Emax < 60%. NA = not active. NT = not tested. [f] Efficacy = 18%.

bromine (**1p**) in position 5 gave compounds with only minimal beta cell activity. In contrast, substituting chlorine in position 6 with bromine afforded **1m**, which potently hyperpolarizes beta cell membranes and inhibits insulin release with little effect on phenylephrin-induced relaxation of rat aorta. Similar findings have been made in a series of 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides, in which the 7-chlorine could be substituted with bromine or fluorine without significant loss of beta cell effect.[30] Methylation of N-4 gave **14**, which in analogy with previously findings for 3-alkylamino-4*H*-1,2,4-benzothiazine 1,1-dioxides,[25] resulted in loss of activity. Changing from 4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide to 4*H*-thieno[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxide and maintaining the 3-(1-methylcylopropyl)amino group abolished beta cell activity (**18b**). The corresponding 3-isopropylamino derivative **18a** was able to affect membrane potential ($EC_{50} = 5.0\ \mu M$) and inhibit insulin release ($IC_{50} = 19.8\ \mu M$), the latter however at low efficacy (16%). This makes the compounds considerably weaker than

Table 2. Effects on HEK293 Cells Expressing Human Kir6.2/SUR1 K_{ATP} Channels on Glucose-Stimulated Insulin Release from Rat Islets, Acute Effects on Plasma Insulin Levels after Intravenous Administration to Anesthetized Rats, and ID_{50} Values 2 h after Peroral Dosing to Zucker Obese Rats[a−d]

compd	membrane potential EC_{50} (μM)[a]	rat islets insulin release EC_{50} (μM)[b]	insulin release 3 mg/kg iv[c]	insulin release po ID_{50} (mg/kg)[d]
1a	0.19 ± 0.06	0.21 ± 0.08[e]	85 ± 14	1.5[e]
1c	0.53 ± 0.1	0.16 ± 0.13	90 ± 1	8.0
1h	0.18 ± 0.03	0.04 ± 0.01	86 ± 1	4.0
1i	0.81 ± 0.2	0.11 ± 0.07	NT	NT
1m	0.16 ± 0.04	0.02 ± 0 (n = 2)	97 ± 3	<3.0[f]
diazoxide	33 ± 11[g]	20.28 ± 8.82[g]	53 ± 8[e]	>30[e]

[a] Repolarization of HEK 293 cells expressing Kir6.2/SUR1 K_{ATP} channels. Shown are means ± SEM ($n \geq 3$). [b] Inhibition of insulin release from isolated rat islets; values are mean ± SEM of four experiments unless indicated; efficacy is above 77%. [c] Percent reduction of plasma insulin levels after iv administration of test compounds (3 mg/kg) onto anaesthetized Sprague Dawley rats. [d] ID_{50} for decrease in plasma insulin levels 2 h after peroral administration to Zucker obese rats. Values are mean ± SEM of 2−4 animals. [e] Data from Nielsen et al. (ref 14). [f] Maximal effect was obtained at 3 mg/kg. [g] Data from Schou et al. (ref 45).

the corresponding 4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide derivative **2**.[13,14]

On the basis of the in vitro screening of the present series several potent and selective compounds, i.e., **1c**, **1h**, **1i**, and **1m**, have been identified. These compounds have been subjected to additional investigations to substantiate their mechanism of action and their potential as drugs. Initially, all four compounds were tested for their ability to inhibit glucose-induced insulin release from rat islets and to affect ion currents through human K_{ATP} channels (Table 2). It was found that the compounds were able to inhibit insulin release from freshly isolated rat islet stimulated with 22 mM glucose with potency similar or higher than that of **1a**. Notable are **1h** with IC_{50} = 0.04 μM and **1m** with IC_{50} = 0.02 μM, which is 5−10 times more potent than **1a** and about 100 times more potent than diazoxide (IC_{50} = 20.28 μM).[14]

To measure direct effects on the K_{ATP} channels a newly developed procedure was used to measure K_{ATP}CO-induced changes in membrane potential in the presence of the K_{ATP} channel blocker tolbutamide.[31] Repolarization induced by the test compounds suggests that they either bind to the K_{ATP} channels to displace tolbutamide or increase ion currents by interaction with a site on the K_{ATP} channel different from the tolbutamide binding site. It was found that the compounds potently repolarize the cell membrane supporting a direct effect on Kir6.2/SUR1 (Table 2). The compounds were all quite potent being about 100 times as potent as diazoxide (IC_{50} = 33 μM).

To further substantiate the effects of compound **1h** on K_{ATP} channels, the ability of the compound to displace radiolabeled glibenclamide from membranes of HEK293 cells expressing human Kir6.2/SUR1 channels was evaluated (Figure 3A−C).[14] In presence of 2 mM ATP, it was found that **1h**, like **1a**, competes with ^{3}H-glibenclamide in a biphasic manner giving rise to two inhibition constants, a high-affinity component with a potency (IC_{50}: 0.08 μM (**1h**); 0.286 μM (**1a**)) similar to that observed in the membrane potential assays and insulin release assays (Tables 1 and 2). Previously, we have shown that the high-affinity component correlates with the effects on, e.g., insulin release (in vitro).[14] The result not only supports that the effects of these compounds on beta cells are exerted through Kir6.2/SUR1 channels but also that **1h** is at least as potent as **1a** on the human K_{ATP} channels. Diazoxide competes with ^{3}H-glibenclamide in a monophasic manner supporting that **1h** interacts with the K_{ATP} channel in a way which is similar to that of **1a** but different from diazoxide. It has been shown that several binding sites for K_{ATP}COs exist on the sulfonylurea receptor,[17,32] but it cannot be concluded from the present data that the biphasic binding curves for, e.g., compound **1h**, reflect interaction with such binding sites.

Among the compounds of the present series there is in general a good correlation between the effects obtained in the different assays predicting opening of K_{ATP} channels of the beta cells. Previous studies have shown that compounds **1a** and **2** increase currents through Kir6.2/SUR1 K_{ATP} channels as measured by patch clamp. Neither **1a** nor **2** affects other targets to any significant level.[14] It is therefore most likely that the inhibition of insulin release exhibited by, e.g., **1h**, is mediated by an opening of the Kir6.2/SUR1 channels. A secondary effect on other targets, i.e., Ca^{2+} cannot be excluded.

In Vivo Biology. To evaluate their in vivo efficacy the compounds **1c**, **1h**, and **1m** were examined in rats. Initially, the pharmacokinetic profile after intravenous and peroral dosing was determined (Table 3). It was found that all three compounds exhibit good druglike profiles with high oral bioavailability and low clearance but with considerably different $t_{1/2}$ and t_{max} values. Whereas compound **1m** exhibits a pharmacokinetic profile characterized by a both long half-life after peroral and a C_{max} after 90 min, but a relatively low maximal plasma concentration achieved after 30 min (340 ng/mL, data not shown), compound **1h** has a short half-life and a high C_{max} achieved after 15 min. In comparison to both diazoxide and **1a**, compound **1h** has a shorter half-life but reaches a considerably larger C_{max} value. Using anaesthetized, postprandial adult Sprague Dawley rats as a screening model, we examined the effects of **1c**, **1h**, and **1m** for effects on insulin release upon intravenous administration of a fixed dose of 3 mg/kg for all compounds (Table 2). All three compounds were equally efficacious (>80%) in decreasing plasma insulin levels. In comparison, identical doses of diazoxide or **1a** gave a partial (53%) or comparable (85%) suppression of plasma insulin levels. The compounds (**1c**, **1h**, **1m**) have minimal blood pressure suppressing effect in the anaesthetized rats after intravenous dosing of 3 mg/kg (<30% maximal reduction of mean arterial blood pressure; data not shown). The effects of plasma insulin concentrations 2 h after peroral dosing of **1c**, **1h**, and **1m** to Zucker obese rats were determined in order to assess peroral activity and to establish a dose relationship. It was found that all three compounds were able to potently suppress plasma insulin levels with ID_{50} values of 8, 4, and <3 mg/kg, respectively. These compounds are therefore slightly weaker or comparable to **1a** (ID_{50} = 1.5 mg/kg) but considerably more potent than diazoxide (ID_{50} > 30 mg/kg). The relative peroral potencies of the three compounds roughly correlate to their relative in vitro potency and exposure as estimated in the PK study (Tables 1−3). It is, however, worth noting that maximal plasma concentrations of the three test compounds are obtained at very different times after administration (t_{max} (**1c**) = 30 min, t_{max} (**1h**) = 15 min, t_{max} (**1m**) = 90 min) and that maximal exposure and half-lives also are very

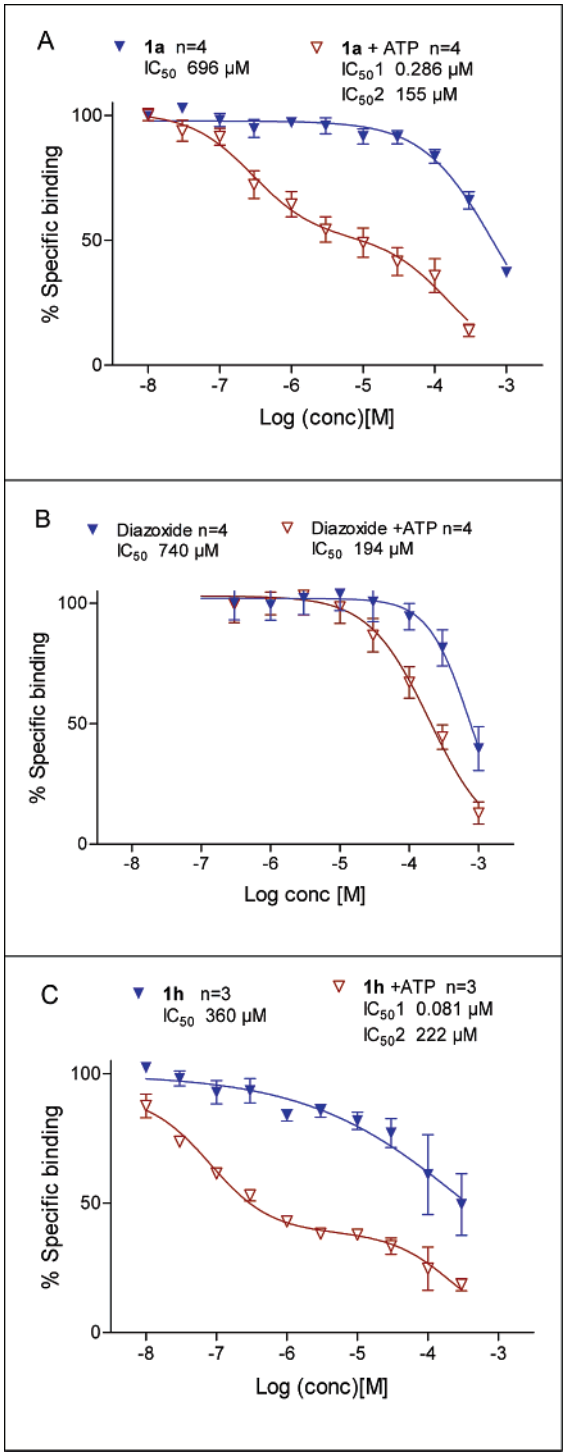


Figure 3. ^{3}H-Glibenclamide (1 mM) binding to a membrane preparation from HEK 293 cells stably expressing human Kir6.2/SUR1. Binding was measured in the presence (open symbols) or absence (closed symbols) of 2 mM ATP and 2 mM Mg^{2+} by **1a**, **1h**, and diazoxide.

different. This could affect the in vivo potency as determined by the assay used in this study.

Conclusion

The present study evaluates 3-alkylamino-4*H*-thieno-1,2,4-thiadiazine 1,1-dioxides as activators of ATP-sensitive potassium channels by elaborating on the SAR, the synthesis, and the crystal structure. By modifying the structure of the clinical candidate **1a** it has been possible to identify a new potent and beta cell selective K_{ATP} channel activator, **1h**. This compound, which inhibits insulin release in vivo after intravenous and peroral administration and which has a favorable pharmacokinetic profile to support brief beta cell rest, therefore could be useful in treatment and prevention of diabetes. A new and improved synthetic procedure based on a copper-catalyzed ring closure to compound **1a** and a range of analogues of **1a** has been developed (Scheme 4). The new procedure makes it possible to synthesize **1a** and its analogues in very few steps. Two crystalline polymorphs of **1a** have been observed. The crystal structure of the thermodynamically stable polymorph A has been solved, and the data confirm the molecular structure shown in Figure 1.

Table 3. Single-Dose Rat Pharmacokinetics after iv and po Administration

compd	C_{max} po (ng/mL)[a]	t_{max} (min)[b]	f_{po} (%)[c]	CL (mL/min/kg)[d]	$t_{1/2}$ po (min)[e]
1a	1232	30	103	7.02	250
1h	2587	15	82	7.3	52
1c	533	30	70	5	330
1m	885	90	96	15	156
diazoxide	1452	60	154	8.0	79

[a] Maximum concentration after dosing. [b] The time after dosing to reach C_{max}. [c] Fraction absorbed in percent oral bioavailability. [d] Total body clearance. [e] Terminal elimination half-life after peroral dosing.

Experimental Section

Chemistry. Melting points were determined in open capillary tubes on a Büchi B-545 apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker DPX 200 (200 MHz), a Bruker DRX 300 (300 MHz), or a Bruker AMX 400 (400 MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are given in ppm (δ), and splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet, and br, broad. The 70 eV EI solid mass spectra were recorded on a Finnigan MAT-TSQ 70 mass spectrometer. LC−MS analysis was performed on an HP1100 MSD equipped with a Waters Xterra MS C-18 X 3 mm column. Reactions were followed by thin-layer chromatography performed on silica gel 60 F254 (Merck) or ALUGRAMSIL G/UV_{254} (MACHEREY-NAGEL) TLC aluminum sheets. Elemental analyses (C, H, N) were within ±0.4%.

1-(Trifluoromethyl)cyclopropylamine Hydrochloride (5). A solution of 1-(trifluoromethyl)cyclopropanecarboxylic acid (6.52 g, 42.3 mmol) and dry triethylamine (5.9 mL, 42.3 mmol) in dry *tert*-butyl alcohol (25 mL) was stirred at room temperature in the presence of 0.4 nm molecular sieves. To the mixture was added 10.1 mL (46.5 mmol) of diphenyl phosphorazidate dropwise. The mixture was refluxed for 18 h under nitrogen and then concentrated in vacuo. The residue was stirred in Et_2O (100 mL) and filtered to give a biphasic solution. The ether layer was isolated, and the oily liquid was extracted with 4 × 50 mL of diethyl ether. The combined ether phase was washed with 5% citric acid (50 mL), saturated aqueous $NaHCO_3$ (2 × 25 mL), and brine (25 mL) and dried. Concentration gave 8.2 g (86%) of *tert*-butyl 1-(trifluoromethyl)-cyclopropylcarbamate (**4**), which was used without purification in the next step. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.95−1.05 (m, 2 H), 1.12−1.2 (m, 2 H), 1.37 (s, 9 H), 7.8 (br s, 1 H).

A suspension of crude **4** (8.2 g, 36.4 mmol) in 1 N HCl (300 mL) was stirred at reflux for 2 h and then at room temperature for 18 h. The solution was concentrated in vacuo at 80 °C for 1 h, and the residue was triturated with dry acetone (50 mL), at 0 °C, and filtered affording 2.99 g (49%) of the title compound **5** as white crystals. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.27−1.47 (m, 4 H), 9.66 (br s, 3 H). LC−MS: m/z 126 $(M + 1)^+$. Anal. ($C_4H_7ClF_3N$) C, H, N. The filtrate was concentrated in vacuo and triturated with ether affording a second crop of **5** (1.9 g, 32%).

***N*-(1-Methylcyclopropyl)guanidine Hydrochloride (12).** Triethylamine (773 mL, 5.55 mol) was added to a solution of

1-methylcyclopropylamine hydrochloride (284 g, 2.64 mmol) and 1*H*-pyrazole-1-carboximidamide hydrochloride (426 g, 2.81 mol) in dry DMF (2.2 L), and the mixture was stirred at room temperature for 24 h under nitrogen. The reaction mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. Diethyl ether (3.7 L) was added, and the precipitate was filtered and washed with diethyl ether. The crude product was dissolved in EtOH (885 mL) at reflux, EtOAc (5 L) was added, and the precipitated solid was isolated by filtration, washed with EtOAc, and dried in vacuo affording 315 g (79%) of compound **12**; mp 180−181 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.66−0.75 (m, 4H), 1.28 (s, 3H), 6.7−7.9 (very broad peaks, 4H), 8.35 (br s, 1H). LC−MS: *m*/*z* 114 (M + 1)$^+$.

6-Chloro-3-(1-methylcyclopropyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (1a). *N*-(1-Methylcyclopropyl)guanidine hydrochloride (**12**) (21.4 g, 143 mmol) was dissolved in a stirred mixture of 2 N NaOH (164 mL) and toluene (500 mL). A solution of 3-bromo-5-chlorothiophene-2-sulfonyl chloride (**11a**) (45.0 g, 152 mmol) in toluene (150 mL) was added to the mixture during 30 min at room temperature. The reaction mixture was stirred overnight and filtered. The precipitate was washed with toluene and dried affording 49 g (92%) of *N*-[(3-bromo-5-chlorothien-2-yl)sulfonyl]-*N*′-(1-methylcyclopropyl)guanidine (**13a**). ^{1}H NMR (DMSO): δ: 0.65 (br s, 4H), 1.3 (s, 3H), 7.32 (s, 1H), 7.9 (br s, 1H).

Crude **13a** (18.7 g, 50 mmol) was dissolved in NMP (95 mL). Potassium carbonate (7.07 g, 51 mmol), copper bronze (320 mg, 3 mmol), and water (280 mL) were added, and the mixture was heated at reflux with stirring. After 5 h an additional amount of potassium carbonate (3.5 g, 25 mmol) was added, and heating was continued for 20 min. The reaction mixture was cooled to room temperature, filtered, and slowly added to a 9% aqueous oxalic acid solution (50 mL) to pH = 6). Water (195 mL) was added, and the mixture was stirred overnight. The pH was adjusted from 6.8 to 6.0, and the product was isolated by filtration, washed with water, and dried to give 9.77 g (67%) of the title compound, identical with a previously prepared sample of **1a**.[14]

6-Chloro-3-{[1-(trifluoromethyl)cyclopropyl]amino}-4*H*-thieno-[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (1c). To a solution of 3,6-dichloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**8**)[14] (2.00 g, 7.78 mmol) and 2,6-dimethylpyridine (2.04 mL, 17.5 mmol) in triethylene glycol monomethyl ether (TGME) (5 mL) was added activated potassium fluoride[33] (0.451 g, 7.78 mmol), water (20 μL), and 1-(trifluoromethyl)cyclopropylamine hydrochloride (**5**) (1.51 g, 9.33 mmol), and the mixture was stirred for 21 h at 100 °C in a sealed flask. The cooled mixture was filtered, and the solid was washed with EtOAc. The organic filtrate was washed with 1 N HCl (50 mL), and the acidic aqueous phase was extracted with EtOAc (2 × 50 mL). The combined organic phase was washed successively with 1 N HCl (50 mL), water (50 mL), and brine (25 mL), dried, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel with EtOAc followed by recrystallization from EtOH/water (10:1) to give 0.89 g (34%) of the title compound; mp 284−286 °C dec. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.20−1.45 (m, 4H), 7.12 (s, 1H), 8.35 (br s, 1H), 11.05 (br s, 1H). LC−MS: *m*/*z* 346/348 (M + 1)$^+$. Anal. ($C_9H_7ClF_3N_3O_2S_2$) C, H, N.

6-Chloro-3-(2,2,2-trifluoroethyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (1d). A mixture of 3,6-dichloro-4*H*-thieno-[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**8**) (0.3 g, 1.17 mmol) and 2,2,2-trifluoroethylamine (2 mL) was stirred overnight at 85 °C in a sealed flask. The cooled mixture was concentrated in vacuo, and the residue was triturated with water (3 mL) and acidified to pH < 2 with 4 M HCl. The precipitate was filtered off and recrystallized from MeOH/water to give 199 mg (53%) of the title compound; mp 293−294 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 4.1(dq, 2H), 7.09 (s, 1H), 7.87 (br t, 1H), 11.44 (br s, 1H). LC−MS: *m*/*z* 320/322 (M + 1)$^+$. Anal. ($C_7H_5Cl\,F_3N_3O_2S_2$) C, H, N.

3-Benzylamino-6-chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (1e). Potassium *tert*-butoxide (0.494 g, 4.4 mmol) was added to a solution of 3-amino-5-chlorothiophene-2-sulfonamide hydrochloride (**6**) (0.5 g, 2.0 mmol) in dry DMF (5 mL) with stirring on an ice bath. The resulting mixture was stirred at 0 °C for 5 min before the dropwise addition of benzyl isothiocyanate (0.32 mL, 2.4 mmol). The mixture was stirred at 0 °C for 1 h and concentrated in vacuo at <50 °C. The residue was taken up in water (25 mL), treated with decolorizing charcoal, and filtered. The filtrate was acidified with AcOH to pH 3−4 and stirred at 0 °C until the product solidified. The solid was isolated by filtration affording 0.25 g (34%) of 3-amino-*N*-[(benzylamino)carbonothioyl]-5-chlorothiophene-2-sulfonamide (**7a**), which was used for the next step without further purification. Phosgene (0.32 mL of a 20% solution in toluene, 0.61 mmol) was added dropwise to a solution of crude **7a** (200 mg, 0.55 mmol) and dry triethylamine (0.154 mL, 1.1 mmol) in dry THF (3 mL) with stirring at 0 °C. The mixture was stirred for 2 h at 0 °C and then concentrated in vacuo. The residue was triturated with water, and the precipitate was isolated by filtration, washed with water, and crystallized from EtOH to give 61 mg (34%) of **1e**; mp 247−250 °C. ^{1}H NMR (DMSO-d_6, 200 MHz) δ 4.45 (d, 2H), 7.06 (s, 1H), 7.2−7.4 (m, 5H), 7.78 (t, 1H), 11.2 (s, 1H). EI-MS *m*/*z* 327/329 (M$^+$). Anal. ($C_9H_{12}ClN_3O_2S_2$) C, H, N.

(*S*)-6-Chloro-3-(1-phenylethyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (1f). Potassium *tert*-butoxide (0.494 g, 4.4 mmol) was added to a solution of 3-amino-5-chlorothiophene-2-sulfonamide hydrochloride (**6**) (0.5 g, 2.0 mmol) in dry DMF (5 mL) with stirring on an ice bath. The resulting mixture was stirred at 0 °C for 5 min before the dropwise addition of L-α-methylbenzyl isothiocyanate (0.37 mL, 2.4 mmol). The mixture was stirred at 0 °C for 1 h and concentrated in vacuo at <50 °C. The residue was taken up in water (25 mL), treated with decolorizing charcoal, and filtered. The filtrate was acidified with AcOH to pH 3−4 with stirring at 0 °C and then extracted with Et_2O (3 × 25 mL). The organic phase was washed with water (5 mL), dried, and concentrated in vacuo to give 727 mg (97%) of 3-amino-5-chloro-*N*-({[(1*S*)-1-phenylethyl]amino}carbonothioyl)thiophene-2-sulfonamide (**7b**) as an impure oil, which was used without further purification in the following step. Phosgene (1.0 mL of a 20% solution in toluene, 1.93 mmol) was added dropwise to a solution of the crude compound **7b** (700 mg, 1.86 mmol) and dry triethylamine (0.52 mL, 3.73 mmol) in dry THF (10 mL) with stirring at 0 °C. The mixture was stirred for 30 min at 0 °C and then concentrated in vacuo. The residue was triturated with water (2 × 25 mL) followed by Et_2O (25 mL) to give a solid which was isolated by filtration and recrystallized from EtOAc with decolorizing charcoal to give 75 mg (12%) of **1f**; mp 195−204 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.48 (d, 3H), 4.98 (quint, 1H), 7.10 (s, 1H), 7.22−7.45 (m, 5H), 7.79 (br s, 1H), 10.85 (br s, 1H). EI-MS *m*/*z* 341/343 (M$^+$). Anal. ($C_{13}H_{12}ClN_3O_2S_2$) C, H, N.

(*R*)-6-Chloro-3-(1-phenylethyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (1g). d-α-Methylbenzyl isothiocyanate was treated according to the procedure of compound **1f** to provide compound **1g**, which was purified by column chromatography on silica with CH_2Cl_2/MeOH (19:1) (yield 17%); mp 218−220 °C (EtOAc). ^{1}H NMR (DMSO-d_6): δ 1.48 (d, 3H), 4.97 (quint, 1H), 7.10 (s, 1H), 7.2−7.4 (m, 5H), 7.73 (br, 1H), 10.81 (s, 1H). EI-MS *m*/*z* 341/343 (M$^+$). Anal. ($C_{13}H_{12}N_3Cl_1O_2S_2$) C, H, N.

6-Chloro-3-(1-methyl-1-phenylethyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (1h). A solution of 3,6-dichloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**8**) (1.0 g, 3.9 mmol) and cumylamine (1.06 g, 7.8 mmol) in EtOH (6 mL) was stirred for 31 h at 120 °C in a sealed flask. The cooled solution was concentrated in vacuo, and the residue was dissolved in 1 N NaOH (50 mL) followed by treatment with decolorizing charcoal. After filtration the clear solution was acidified to pH < 2 with 4 M HCl, and the precipitate was filtered off and recrystallized from EtOH to give 278 mg (20%) of the title compound; mp ca. 360 °C (decomposes gradually above 200 °C). ^{1}H NMR (DMSO-d_6, 300 Mz): δ 1.68 (s, 6H), 7.12 (s, 1H), 7.17−7.41 (m, 6H), 10.72 (br s, 1H). EI-MS: *m*/*z* 355/357 (M$^+$). Anal. ($C_{14}H_{14}ClN_3O_2S_2$) C, H, N.

6-Chloro-3-(1-phenylcyclopropyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (1i). A mixture of 3,6-dichloro-4*H*-thieno-

[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**8**) (1.0 g, 3.89 mmol), potassium fluoride (688 mg, 11.9 mmol), and hexadecyltrimethylammonium bromide (43 mg, 0.12 mmol) in dry NMP (4 mL) was stirred for 20 h at 120 °C under nitrogen to form 6-chloro-3-fluoro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**9**). The mixture was allowed to cool to room temperature and then reacted directly with 1-phenylcyclopropylamine hydrochloride (0.99 g, 5.84 mmol) and triethylamine (1.65 mL, 11.9 mmol) for 44 h at 75 °C in a sealed flask. The cooled mixture was triturated with water (40 mL), acidified to pH < 2 with 1 N HCl, and extracted with EtOAc (50 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated to dryness to give the crude product as brownish oil. Purification by column chromatography on silica with CH_2Cl_2/MeOH (9:1) gave 155 mg (11%) of the pure title compound; mp 279−280 °C. ^{1}H NMR (DMSO-d_6, 300 Mz): δ 1.37 (br s, 4H), 7.1(s, 1H), 7.13−7.36 (m, 5H), 8.2 (very br s, 1H), 10.9 (br s, 1H). LC−MS: *m*/*z* 354/356 $(M + 1)^+$. Anal. ($C_{14}H_{12}ClN_3O_2S_2$) C, H, N.

3-{[(1*S*)-1-(4-Bromophenyl)ethyl]amino}-6-chloro-4*H*-thieno-[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (1j). A solution of 3,6-dichloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**8**) (0.3 g, 1.17 mmol) in (1*S*)-1-(4-bromophenyl)ethylamine (2 mL) was stirred overnight at 120 °C in a sealed flask. The cooled solution was diluted with water (7 mL) and acidified to pH < 2 with 4 M HCl, and the precipitate was collected by filtration and washed with water. Recrystallization from MeOH/water afforded 337 mg (76%) of the pure title compound; mp 263−265 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.44 (d, J = 7.16 Hz, 3 H), 4.93 (quint, J = 7.16 Hz, 1 H), 7.08 (s, 1 H), 7.33 (d, J = 8.29 Hz, 2 H), 7.55 (d, J = 8.29 Hz, 2 H), 7.77 (br s, 1 H), 10.87 (br s, 1 H). LC−MS: *m*/*z* 420/422/424 $(M + 1)^+$. Anal. ($C_{13}H_{11}BrClN_3O_2S_2$) C, H, N.

6-Chloro-3-[1-(3-chlorophenyl)cyclobutylamino]-4*H*-thieno-[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (1k). A solution of 3,6-dichloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**8**) (257 mg, 1.0 mmol) and 1-(3-chlorophenyl)cyclobutylamine (545 mg, 3.0 mmol) in dry THF (2 mL) was stirred for 48 h at 80 °C in a sealed flask. The cooled solution was concentrated in vacuo, and the residue was stirred with a mixture of EtOAc (30 mL) and water (30 mL). The organic phase was isolated and washed with water (25 mL), dried, and evaporated to dryness. The residue was triturated with a small amount of EtOAc and dried to give 131 mg (33%) of the pure title compound; mp 272−273 °C. ^{1}H NMR (DMSO-d_6, 300 Mz): δ 1.75−1.93 (m, 1H), 1.96−2.12 (m, 1H), 2.47−2.56 (m, 4H), 7.06 (s, 1H), 7.25−7.50 (m, 4H), 8.02 (br s, 1H), 10.88 (br s, 1H). LC−MS: *m*/*z* 402/404 $(M + 1)^+$. Anal. ($C_{15}H_{13}Cl_2N_3O_2S_2$) C, H, N.

6-Chloro-3-[1-(4-methoxyphenyl)cyclopropyl]amino-4*H*-thieno-[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (1l). To a solution of 3,6-dichloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**8**) (1.0 g, 3.89 mmol) and 2,6-dimethylpyridine (1.36 mL, 11.7 mmol) in TGME (2 mL) was added activated potassium fluoride (0.068 g, 1.17 mmol) and 1-(4-methoxyphenyl)cyclopropylamine hydrochloride[34] (0.93 g, 1.2 mmol), and the mixture was stirred for 3 h at 100 °C in a sealed flask. The cooled mixture was filtered, and the solid residue was washed with EtOAc. The filtrate was washed with 1 N HCl (2 × 50 mL), and the organic phase was dried and concentrated in vacuo. The residue was purified by flash chromatography on silica gel with CH_2Cl_2/MeOH (19:1) followed by trituration with Et_2O to give 320 mg (21%) of the pure **1l**; mp 250−251 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.26 (br s, 4H), 3.72 (s, 3H), 6.87 (d, 2H), 7.10 (s, 1H), 7.14 (d, 2H), 8.27 (br s, 1H), 10.79 (br s, 1H). LC−MS: *m*/*z* 384/386 $(M + 1)^+$. Anal. ($C_{15}H_{14}ClN_3O_3S_2 \cdot 0.5\ CH_2Cl_2$) C, H, N.

6-Bromo-3-(1-methylcyclopropyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (1m). A solution of 3,5-dibromothiophene-2-sulfonyl chloride[35] (**11d**) (32 g, 94.1 mmol) in toluene (58 mL) was added to a solution of **12** (15.5 g, 0.1 mol) in a mixture of toluene (64 mL) and 4 N NaOH (59 mL) during 30 min at 10−15 °C. The solid that precipitated was filtered off, washed with water and toluene, and dried to provide 36 g (95%) of the intermediate *N*-[(3,5-dibromothien-2-yl)sulfonyl]-*N*′-(1-methylcyclopropyl)guanidine (**13d**) as off-white crystals. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.68 (broad s, 4H), 1.27 (s, 3H), 6.4−8.1 (three broad peaks, 3H), 7.42 (s, 1H). LC−MS: *m*/*z* 416/418/420 $(M + 1)^+$.

A stirred mixture of crude **13d** (15 g, 36 mmol), K_2CO_3 (7.0 g, 50.6 mmol), and copper bronze (60 mg) in a mixture of NMP (38 mL) and water (112 mL) was heated at reflux for 5 h under nitrogen. The reaction mixture was treated with decolorizing charcoal and filtered, and the filtrate was acidified with 1 N HCl to pH < 2. The brown solid that precipitated was collected, washed with water, dried, and flash chromatographed over silica gel with CH_2Cl_2/MeOH (95:5) followed by recrystallization from propan-2-ol/water to provide 2.4 g (20%) of the pure **1m**; mp 244−246 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.65−0.80 (m, 4H), 1.36 (s, 3H), 7.20 (s, 1H), 7.82 (br s, 1H), 10.73 (br s, 1H). LC−MS: *m*/*z* 336/338 $(M + 1)^+$. Anal. ($C_9H_{10}BrN_3O_2S_2$) C, H, N.

3-(1-Methylcyclopropyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (1n). A solution of 6-bromo-3-(1-methylcyclopropyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide **1m** (178 mg, 0.53 mmol) in dry THF (20 mL) was cooled to −78 °C, and *n*-BuLi (1.6 N in hexane, 2 mL) was added with stirring under nitrogen. The cooling bath was removed, and after stirring at room temperature for 30 min the mixture was again cooled to −78 °C followed by dropwise addition of MeOH (2 mL). The resulting suspension was stirred at this temperature for an additional period of 30 min and allowed to reach room temperature. The solvents were evaporated, and the residue was flash chromatographed over silica gel with CH_2Cl_2/MeOH (19:1) and finally triturated with EtOH/Et_2O to provide 61 mg (45%) of the title compound; mp 240−244 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.66−0.79 (m, 4H), 1.37 (s, 3H), 7.04 (br s, 1H), 7.71 (br s, 1H), 7.87 (d, 1H), 10.69 (br s, 1H), LC−MS: *m*/*z* 258 $(M + 1)^+$. Anal. ($C_9H_{11}N_3O_2S_2 \cdot 0.25$ EtOH) C, H, N.

5-Chloro-3-(1-methylcyclopropyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (1o). 3-Bromo-4-chlorothiophene[36] (5.33 g, 27 mmol) was added over 15 min to chlorosulfonic acid (5.4 mL, 81 mmol) with stirring at room temperature, and the resulting dark solution was stirred for 75 min prior to the addition of thionyl chloride (2 mL, 27 mmol). The reaction mixture was stirred overnight at room temperature and poured into a cooled mixture of water (100 mL) and toluene (100 mL). The organic layer was washed with water (4 × 50 mL), dried over Na_2SO_4, and evaporated to dryness to give a mixture containing 35% of 3-bromo-4-chlorothiophene-2-sulfonyl chloride (**11b**) and 57% of 4-bromo-3-chlorothiophene-2-sulfonyl chloride according to NMR and HPLC. A solution of the crude mixture in toluene (50 mL) was added to a stirred mixture of **12** (4.45 g, 30 mmol) in 4 N NaOH (18 mL) and toluene (20 mL). The mixture was stirred at room temperature for 3 h, and the precipitate was collected by filtration and washed with toluene and water to give the two isomeric sulfonyl guanidines contaminated with the corresponding sulfonic acids. The crude mixture in EtOAc (600 mL) was extracted with several portions of 1 M $NaHCO_3$, dried over Na_2SO_4, evaporated to dryness, and recrystallized from toluene to give 4.2 g of a pure mixture of the isomers *N*-[(3-bromo-4-chlorothien-2-yl)sulfonyl]-*N*′-(1-methylcyclopropyl)guanidine (**13b**) and *N*-[(4-bromo-3-chlorothien-2-yl)sulfonyl]-*N*′-(1-methylcyclopropyl)guanidine as yellow crystals.

Water (28 mL) and K_2CO_3 (2.82 g, 20.4 mmol) were added to a solution of the obtained mixture (3.8 g, 10.2 mmol) in NMP (17 mL), and the reaction mixture was refluxed in the presence of copper bronze (1 g, 16 mmol) for 5 h under nitrogen and allowed to cool to room temperature. Copper was filtered off and washed with NMP (10 mL) and water (10 mL), and the aqueous filtrate was acidified with 1 M oxalic acid to pH 5 to give a greasy brown solid mainly consisting of *N*-[(4-bromo-3-chlorothien-2-yl)sulfonyl]-*N*′-(1-methylcyclopropyl)guanidine, which was removed by rapid filtration. Water (60 mL) was added to the filtrate to give a beige precipitate, which was isolated by filtration. A solution of the crude product in THF (4 mL) was purified by silica gel chromatography (EtOAc/heptane (1:1) to yield 0.67 g (9%) of the pure title compound **1o**; mp 219−220 °C. ^{1}H NMR (DMSO-d_6, 400 MHz):

δ 0.65−0.78 (m, 4H), 1.38 (s, 3H), 7.34 (br s, 1H), 8.07 (s, 1H), 10.5 (br s, 1H). EI-MS *m*/*z* 291/293 (M^+). Anal. ($C_9H_{10}ClN_3O_2S_2$) C, H, N.

5-Bromo-3-(1-methylcyclopropyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (1p). 3,4-Dibromothiophene (5.0 g, 20.7 mmol) was added dropwise during 20 min to chlorosulfonic acid (5.2 mL, 77 mmol) with stirring at 10−15 °C. After 1 h the resulting suspension was allowed to warm to room temperature and stirred for additional 1 h. Thionyl chloride (1.5 mL, 20.7 mmol) was added, and the reaction mixture was stirred overnight at room temperature and poured slowly into a cooled mixture of water (50 mL) and toluene (75 mL). The organic layer was washed with water (2 × 25 mL), dried over Na_2SO_4, and evaporated to dryness to give 6.0 g of crude 3,4-dibromothiophene-2-sulfonyl chloride (**11c**) as an orange solid, which was used for the next step without further purification. A solution of the crude mixture in toluene (35 mL) was added to a vigorously stirred mixture of **12** (3.41 g, 22.8 mmol) in 4 N NaOH (13 mL) and toluene (12 mL) to give a sticky mass, which was stirred at room temperature overnight. The resulting precipitate was collected by filtration, washed with toluene and water, and then partly dissolved in refluxing EtOAc (600 mL). The organic phase was allowed to cool to room temperature, washed successively with water (100 mL), 5% aqueous $NaHCO_3$ (3 × 50 mL), and water (2 × 100 mL), and concentrated to give 5.9 g of almost pure *N*-[(3,4-dibromothien-2-yl)sulfonyl]-*N*′-(1-methylcyclopropyl)guanidine (**13c**), which was used for the next step without further purification. Water (35 mL) and K_2CO_3 (5.17 g., 37.4 mmol) were added to a solution of the obtained intermediate (4.6 g, 11 mmol) in NMP (23 mL), and the mixture was refluxed in the presence of copper bronze (0.67 g, 10.5 mmol) for 12 h under nitrogen and allowed to cool to room temperature. The precipitate was collected by filtration, washed with NMP (8 mL) and water (80 mL), and the aqueous filtrate was acidified with 1 M oxalic acid to pH 6 to give a solid, which was isolated by filtration. The crude was stirred in 1 N NaOH (10 mL), filtered, and reprecipitated from the filtrate by addition of 1 M oxalic acid to pH 6. The solid product was dissolved in acetic acid, treated with decolorizing charcoal, filtered, and allowed to cool to give 1.09 g (20%) of pure title compound **1p**; mp 208−209 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.65−0.75 (m, 4H), 1.38 (s, 3H), 7.48 (br s, 1H), 8.14 (s, 1H), 10.2 (br s, 1H). Anal. ($C_9H_{10}BrN_3O_2S_2$·0.15 AcOH) C, H, N.

6-Chloro-4-methyl-3-(1-methylcyclopropyl)amino-4*H*-thieno-[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (14). A mixture of 6-chloro-3-(1-methylcyclopropyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**1a**) (0.525 g, 1.8 mmol) and K_2CO_3 (298 mg, 2.16 mmol) in dry acetone (5 mL) was treated with iodomethane (0.134 mL, 2.16 mmol) and stirred overnight at room temperature. Water (15 mL) was added, and the mixture was stirred for 30 min and filtered. The solid product was washed with water and recrystallized from EtOH to provide 235 mg (43%) of the title compound; mp 284−286 °C dec. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.64−0.82 (m, 4H), 1.37 (s, 3H), 3.38 (s, 3H), 7.43 (s, 1H), 7.89 (br s, 1H). LC−MS: *m*/*z* 306/308 $(M + 1)^+$. Anal. ($C_{10}H_{12}ClN_3O_2S_2$) C, H, N.

6-Chloro-3-isopropylamino-4*H*-thieno[2,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (18a). A solution of 2,5-dichlorothiophene-3-sulfonyl chloride (10.0 g, 39.8 mmol) in Et_2O (50 mL) was added to a stirred mixture of *N*-isopropylguanidine tosylate (**16**) (10.9 g, 39.8 mmol) in 1 N NaOH (80 mL) and Et_2O (50 mL), and the mixture was stirred at room temperature for 1 h. The two phases were separated, and the organic layer was left overnight to give a precipitate which was isolated by filtration, washed with Et_2O, and dried affording 7.8 g (62%) of *N*-[(2,5-dichlorothien-3-yl)sulfonyl]-*N*′-(isopropyl)guanidine (**17a**). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.06 (d, 6H), 3.8 (br s, 1H), 6.4−7.5 (broad peaks, 3H), 7.28 (s, 1H). The crude product was used in the next step without further purification. A mixture of *N*-[(2,5-dichlorothien-3-yl)sulfonyl]-*N*′-(isopropyl)guanidine (**17a**) (7.0 g, 22.1 mmol), K_2CO_3 (3.05 g, 22.1 mmol), and copper bronze (30 mg) in dry DMF (70 mL) was stirred at 150 °C for 4 h under nitrogen. The cooled mixture was evaporated to dryness, and the residue was partly dissolved in 1 N NaOH (50 mL) by gently heating. The mixture was treated with decolorizing charcoal, filtered, and the filtrate was acidified with 4 M HCl to pH < 2. The resulting dark solid was isolated by filtration, washed with water, and recrystallized from EtOH to give 2.45 g (39%) of the pure title compound; mp 271−272 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.16 (d, 6H), 3.85 (m, 1H), 7.23 (s, 1H), 7.48 (br d, 1H), 11.12 (s, 1H). EI-MS *m*/*z* 279/281 (M^+). Anal. ($C_8H_{10}N_3ClO_2S_2$) C, H, N.

6-Chloro-3-(1-methylcyclopropyl)amino-4*H*-thieno[2,3-*e*]-1,2,4-thiadiazine 1,1-Dioxide (18b). A solution of 2,5-dichlorothiophene-3-sulfonyl chloride (**15**) (2.51 g, 10 mmol) in Et_2O (15 mL) was added to a stirred solution of guanidine **12** (1.48 g, 10 mmol) in 1 N NaOH (20 mL) and Et_2O (15 mL), and the mixture was stirred at room temperature for 1 h. The solid that precipitated was isolated by filtration, washed with Et_2O, and dried affording 2.50 g (76%) of the intermediate *N*-(2,5-dichloro-3-thienylsulfonyl)-*N*′-(1-methylcyclopropyl)guanidine (**17b**); mp 136−138 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.65 (br s, 4H), 1.25 (s, 3H), 6.4−8.1 (broad peaks, 3H), 7.26 (s, 1H). LC−MS: *m*/*z* 328/330 $(M + 1)^+$. A mixture of crude **17b** (100 mg, 0.3 mmol) and Cs_2CO_3 (149 mg, 0.45 mmol) in dry 1-butanol (5 mL) was stirred at 110 °C for 48 h. The cooled mixture was treated with water (15 mL) and acidified with 1 N HCl to pH < 2 to give a sticky brown precipitate. The solvent was decanted, and the residue was taken up in EtOAc (25 mL) and washed with water, dried (Na_2SO_4), and filtered. The solvent was evaporated, and the solid residue was triturated with a small amount of EtOAc to provide 19 mg (22%) of the pure **18b**; mp 232−233 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.66−0.80 (m, 4 H), 1.34 (s, 3 H), 7.24 (s, 1 H), 8.11 (br s, 1 H), 11.09 (br s, 1 H). LC−MS *m*/*z* 292/294 $(M + 1)^+$. Anal. ($C_9H_{10}ClN_3O_2S_2$·0.25 H_2O) C, H, N.

X-ray Crystallography. A single crystal of polymorph A of **1a** was grown by slow cooling and evaporation from a concentrated acetic acid solution. A single crystal of polymorph A of **2** was grown by slow cooling and evaporation from a concentrated acetic acid solution with 5% v/v tetramethoxysilane. A single crystal of form B of **2** was grown by slow cooling and evaporation from a concentrated nitromethane solution. Single-crystal X-ray diffraction data for each sample were collected on a Siemens Smart CCD diffractometer (three-circle goniometer with a CCD area detector) with graphite-monochromated Mo Kα radiation, $\lambda = 0.71073$ Å. The samples were cooled by liquid nitrogen. No phase transitions were observed from room temperature to the data collection temperatures. Diffraction data were measured by ω-scan frames (hemisphere, $\Delta\omega = 0.3°$, 10 s per frame). The data were integrated and Lorentz- and polarization corrected using the program SAINT.[37] The structures were solved by direct methods using SHELXTL.[38,43] All non-hydrogen atoms were refined anisotropically using SHELXTL.[41,42] The hydrogen atoms of 4N and the alkyl-N atoms were located in the difference Fourier maps, while all other hydrogen atoms were in calculated positions. The structures were analyzed with PLATON,[39] and it was affirmed that no voids exist in the structures and that the symmetries are correctly assigned. The intramolecular bonds and angles agree well with those commonly observed for similar chemical entities.[40] Tables and figures have been prepared using the program SHELXTL.[38] Final *R*-factors are as follows: polymorph A of **1a**, $R(F) = 4.71\%$, $wR(F^2) = 7.77\%$, 3363 obs reflns, 325 parameters; polymorph A of **2**, $R(F) = 2.88\%$, $wR(F^2) = 7.02\%$, 3383 obs reflns, 305 parameters; form B of **2**, $R(F) = 2.65\%$, $wR(F^2) = 6.93\%$, 2201 obs reflns, 181 parameters. Details of the data collections and crystal structures including molecular geometry are given as Supporting Information. X-ray crystallographic data of **1a** will be deposited with the Cambridge Crystallographic Data Centre, U.K.

Effects on Membrane Potential in βTC3 Cells. Measurement of $DiBAC_4(3)$ fluorescence was made using the procedure described by Lebrun et al.[41] For each compound and concentration (0.01, 0.1, 1, 10, and 100 μmol/L) a total of four individual wells were used to construct the data sets underlying the values in Table 1. Briefly, data were normalized to the initial value for each well and expressed

as the fractional fluorescence change during the 45 min incubation period using Excel (Microsoft, U.S.A.). A four-parameter nonlinear curve fit was then made to extract EC_{50} values and SEM using MacCurveFit 1.3 (Kevin Raner Software, Australia).

Inhibition of Insulin Release in Vitro. a. Inhibition of Glucose-Induced Insulin Release in βTC6 Cells. βTC6 cells[42] were cultured at 5×10^4 cells/microtiter well in DMEM + 10% FCS, 11 mM glucose, 1% Glutamax, and 20 mM HEPES for 3 days (95% humidity, 37 °C, 5% CO_2). Cells were washed twice with NN buffer (all in mM: NaCl, 114; KCl, 47; KH_2PO_4, 1.21; $MgSO_4$, 1.16; $NaHCO_3$, 25.5; $CaCl{\cdot}2H_2O$, 2.5; HEPES, 10) supplemented with 0.1% BSA and incubated 60 min in this buffer. All wells were aspirated, and the cells incubated for 3 h with NN buffer, 22 mM glucose, 0.1 mM IBMX, and serial dilutions of the compounds. A reference compound (BPDZ 73, 7-chloro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide)[41] served as positive control. A test for responsiveness toward a series of glucose concentrations was included in every assay to ensure functionality. The supernatant from each well was harvested, and insulin content was measured by an in-house ELISA using guinea pig antiinsulin antibodies and a rat insulin as standard. The results were analyzed in Prism (GraphPad Software) and expressed as IC_{50} and E_{max} (the maximum inhibition obtained at 10 μM of the compound). SEM was calculated for all compounds.

b. Inhibition of Glucose-Induced Insulin Release in Wistar Rat Islets. Islets were isolated by collagenase and gradient centrifugation in Ficoll gradient (40−13%). Isolated islets were incubated in bulk overnight in RPMI, 10% FCS, 11 mM glucose. The islets were handpicked and placed at 10 islets/microtiter well and cultured overnight in DMEM, 10% FCS, 3 mM glucose. Essentially the islets were tested as described for the βTC6 but with no addition of IBMX. The insulin content was measured in the same ELISA as used for the βTC6 cells.

Relaxation of Precontracted Rat Aorta in Vitro. Female Wistar rats weighing approximately 150−200 g were killed by cervical dislocation, and the thoracic aorta was removed. Aorta and bladder were cut into rings approximately 5 mm wide. Ring preparations were mounted in 5 mL (Danish Myo Technology, Aarhus, Denmark) or 10 mL organ baths (Schuler Organ Bath 809, Hugo Sachs Elektronik, Germany) with a resting tension of 2 g and bathed in Krebs ringer solution with the following composition (mM): NaCl, 118.5; $NaHCO_3$, 25.0; KCl, 4.7; $CaCl_2$, 6.8; $MgCl_2$, 2.4; glucose, 11.1 in double-distilled water. The ringer solution was continuously aerated with 95% O_2/5% CO_2 at 37 °C. Cumulative concentration response curves (0.1−300 μM) were constructed for all test compounds on top of a precontraction induced by 0.3 μM phenylephrine. All test compounds were freshly dissolved in dimethyl sulfoxide. Potency of a compound (EC_{50}), with regard to producing smooth muscle relaxation was defined as the concentration required to obtain a half-maximal dilating effect. EC_{50} was estimated for individual concentration response curves by four-parameter nonlinear, logistic regression using Myodata software (Danish Myo Technology, Aarhus, Denmark).

Cell Culture. HEK293 stably expressing human SUR1 and Kir6.2 were cultured at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 in Dulbecco's modified Eagles medium with 4.5 g/L glucose supplemented with 10% FCS, penicillin (100 units/mL), streptomycin (0.1 mg/mL) plus 44 μg/mL hygromycin and 0.6 mg/mL G418 as selection medium.[43] Cells are seeded out in polylysine-coated microtiter plates at 50 000 cells/well and cultured for 2 days in 100 μL/well culture medium before the day of the experiment.

Membrane Potential Studies in HEK 293 Cells Expressing Kir6.2/SUR1. K_{ATP} channel activity was monitored by evaluating changes in membrane potential, using a membrane potential kit (Molecular Devices, U.S.A.). Assays were carried out in black clear-bottomed 96-well plates at 34 °C on a NovoStar machine (BMG, Germany), and K_{ATP} channels were activated by the addition of the K_{ATP} channel opener in a mixed assay medium (loading buffer and Hanks balanced salt solution (HBSS, Molecular Devices and Gibco) plus 10 μM tolbutamide to suppress constitutive K_{ATP} channel activity. Changes in fluorescence were measured using an excitation wavelength of 490 nm and an emission wavelength of 550 nm, before and 5−10 min after addition of test compounds.

^{3}H-Glibenclamide Binding. HEK 293 cells stably expressing SUR1/Kir6.2 were grown in DMEM with 1% penicillin/streptomycin, 0.6 mg/mL G418, and 10% fetal calf serum. Cells were washed with PBS and harvested in PBS. The cells were centrifuged at 48 000*g* for 10 min. The cell pellet was homogenized in buffer (30 mM Tris, pH 7.4) using an Ultra Turrax homogenizer, then centrifuged at 48 000*g* for 10 min. The pellet was homogenized again in buffer and centrifuged at 48 000*g* for 10 min. The pellet was suspended in buffer with 250 mM sucrose by homogenization. Total protein was determined using a BioRad kit. The receptor preparation was kept in small aliquots (5 mg protein/mL) at −80 °C until use. Receptor preparation (100 μg/mL) was as follows: ^{3}H-glibenclamide (1 nM, New England Nuclear, NET1024), test compound, and assay buffer (30 mM HEPES, 2 mM $MgCl_2$, 2 mM Na_2ATP, pH 7.4) were mixed to a final volume of 250 μL. All test compounds were dissolved in DMSO and diluted in assay buffer. Glipizide (10 μM) was used to determine nonspecific binding. The assay was incubated for 1 h at 37 °C. Incubation was terminated by dilution with 4 mL of ice-cold 0.9% NaCl solution, followed by rapid filtration through Whatman GF/B filters. Filters were washed with 2 × 4 mL of ice-cold 0.9% NaCl solution. The radioactivity retained on the filters was dissolved with scintillation cocktail and counted using a Packard scintillation counter. IC_{50} values were calculated by nonlinear regression analysis (sigmoid two-site or one-site) of binding data using Prism for Windows 3.02, GraphPad Software, San Diego, CA.

Beta Cell Activity Studies after Intravenous Administration to Rats. Postprandial, adult male Sprague Dawley rats, weighing 280−320 g (M&B Breeding Center, Ll. Skensved, Denmark) were anaesthetized with a mixture of hypnorm/dormicum receiving supplemental anesthetic every 20 min.[44] Body temperature was maintained at 37 °C by a homeothermic blanket system (Harvard homeothermic blanket system, Harvard Apparatus Ltd., U.K.). A polythene cannula (PE50) was inserted into the jugular vein for drug infusion and blood sampling. After a stabilization period, the test substances were injected as a bolus lasting for 20 s. Blood samples were drawn at 5−10 min intervals, from 15 min preinjection until 30 min postinjection. Compounds were dissolved in NaOH (0.15%) and saline and were administered in a fixed dose of 3 mg/kg rat. Plasma insulin levels were measured with in-house ELISA using guinea pig antiinsulin antibodies and rat insulin as standard.

Pharmacokinetic Properties in Rat. The pharmacokinetic rat experiments were performed on Sprague Dawley rats, body weight about 180 g, using the cassette approach with two compounds per cassette ($n = 2$). Blood sampling was done by heart puncture, and each single data point originates from one rat. The doses administered were selected in order to obtain linear pharmacokinetics. For intravenous administration 1.08 mg/kg of **1m**, 1.22 mg/kg of **1c**, 1.24 mg/kg of **1h**, 0.89 mg/kg of **1a**, and 0.50 mg/kg of diazoxide were given. For oral administration 2.48 mg/kg of **1m**, 2.19 mg/kg of **1c**, 2.30 mg/kg of **1h**, 1.89 mg/kg of **1a**, and 2.00 mg/kg of diazoxide were used. Dosing vehicle for compounds **1c**, **1h**, and **1m** were 10% Tween 20, 5% ethanol in 0.1 M phosphate buffer, pH 7.0. **1a** was dissolved in 5% ethanol and 0.1 M phosphate buffer, pH 8.0, and diazoxide was prepared in 40% poly(ethylene glycol) 300 (PEG 300), 10% Cremophor, and water.

Blood samples were collected from Sprague Dawley rats at predetermined time points. Sampling was done post dose at the following time points: iv dosing at 5, 15, 30, 60, 120, 240, and 360 min; po dosing at 15, 30, 60, 90, 120, 240, and 360 min. EDTA plasma was isolated by centrifugation. Plasma samples were assayed by dedicated and selective LC−MS/MS methods. Concentration−time data were analyzed by noncompartmental pharmacokinetic methods.

Potency and Efficacy after Peroral Dosing to Zucker Obese Rats. Conscious, fasted male Zucker obese rats (400−700 g) were dosed (by gavage) with test compounds dissolved in NaOH (1.5%)

and suspended in a 1:1:4:4 mixture of glycerol, water, gelatin, and CMC. Administered doses were from 0.3 to 100 mg/kg rat (**1m**: 3−100 mg/kg) (n = 4/group). Before dosing and up to 8 h post dose, a blood sample was collected by puncture of the tail vein. Plasma insulin was measured with a rat-specific ELISA, and the results were reported as percent decrease in insulin levels from the rat's own baseline. The maximal efficacy was, in pilot experiments, established to be at 2 h, and that data was used to calculate an ID_{50} value for the compounds tested (Table 2).

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Supporting Information Available: Screening experiments for the optimization of the ring closure reaction leading to compound **1a**; details of crystal structure data and refinement of **1a**, polymorph A; details of crystal structure data and refinement of **2**; elemental analyses of compounds **1c**−**p**, **5**, **14**, and **18a**,**b**. This material is available free of charge via the Internet at http://pubs.acs.org. CCDC 609647 contains the supplementary crystallographic data for compound **1a**. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk./data_request/cif.

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