6-Chloro-3-alkylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide Derivatives Potently and Selectively Activate ATP Sensitive Potassium Channels of Pancreatic *β*-Cells

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6-Chloro-3-alkylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide derivatives were synthesized and characterized as activators of adenosine 5′-triphosphate (ATP) sensitive potassium (K_{ATP}) channels in the *β*-cells by measuring effects on membrane potential and insulin release in vitro. The effects on vascular tissue in vitro were measured on rat aorta and small mesenteric vessels. Selected compounds were characterized as competitive inhibitors of [^{3}H]glibenclamide binding to membranes of HEK293 cells expressing human SUR1/Kir6.2 and as potent inhibitors of insulin release in isolated rat islets. 6-Chloro-3-(1-methylcyclobutyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**54**) was found to bind and activate the SUR1/Kir6.2 K_{ATP} channels in the low nanomolar range and to be at least 1000 times more potent than the reference compound diazoxide with respect to inhibition of insulin release from rat islets. Several compounds, e.g., 3-propylamino- (**30**), 3-isopropylamino- (**34**), 3-(*S*)-*sec*-butylamino- (**37**), and 3-(1-methylcyclopropyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**53**), which were found to be potent and *β*-cell selective activators of K_{ATP} channels in vitro, were found to inhibit insulin secretion in rats with minimal effects on blood pressure and to exhibit good oral pharmacokinetic properties.

Introduction

Diabetes is becoming a global health problem with an increasing number of people affected by either type 1 or type 2 diabetes. Whereas type 1 diabetes is characterized by rapid, autoimmune depletion of the insulin-producing pancreatic *β*-cells, type 2 diabetes is a slowly progressing disease affecting glucose and lipid homeostasis, frequently associated with obesity and elevated risk for cardiovascular diseases. A pivotal component of type 2 diabetes is dysfunctional insulin release including reduced first phase insulin release, hyperinsulinaemia, and impaired insulin pulsatility. Type 2 diabetes ultimately leads to *β*-cell degeneration resulting in insufficient insulin release and the need for administration of exogenous insulin.

In pancreatic *β*-cells, adenosine 5′-triphosphate (ATP) sensitive potassium channels (K_{ATP} channels) couple changes in blood glucose concentrations to insulin secretion.[1] K_{ATP} channels also have important functions in various other tissues, and their structure and function have been described in detail.[2−4]

The K_{ATP} channel exists as an octameric complex of the sulfonylurea receptor (SUR) and the pore-forming inwardly rectifying potassium channel (Kir) in a 4 + 4 stoichiometry. Intracellular nucleotides and different drugs regulate its activity. Whereas ATP and certain sulfonylurea and benzoic acid insulin secretagogues are inhibitors (blockers), MgADP (adenosine 5′-diphosphate) and potassium channel openers stimulate potassium currents.

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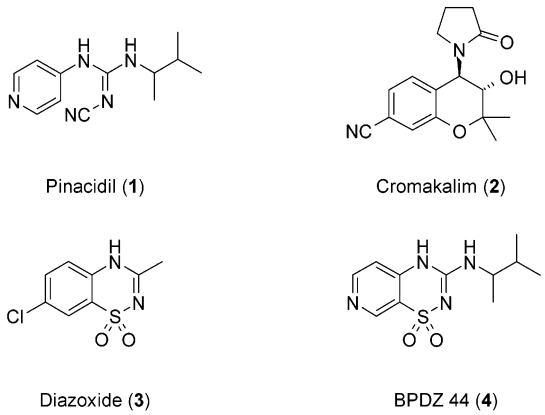
Chart 1



The genes for two closely related SURs, SUR1 and SUR2 have been cloned. 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 *β*-cells, whereas the cardiac and the skeletal muscle type consist of SUR2A and Kir6.2 and the smooth muscle type consists of SUR2B and Kir6.1 or Kir6.2.

Potassium channel openers, which activate the K_{ATP} channels of vascular smooth muscle, are well-described.[5] These include cyanoguanidines such as pinacidil (**1**), chromanes such as cromakalim (**2**), and 1,2,4-thiadiazines such as diazoxide (**3**) (Chart 1).[6−9] Whereas pinacidil and cromakalim have little or no effect on K_{ATP} channels of *β*-cells,[10−12] diazoxide nonselectively activates both K_{ATP} channels of *β*-cells (SUR1/Kir6.2) and

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those of smooth muscle (SUR2B/Kir6.2) and has weak stimulatory effects on K_{ATP} channels of the heart (SUR2A/Kir6.2).[13]

The ability of diazoxide to activate the K_{ATP} channels of pancreatic β-cells results in inhibition of glucose-stimulated insulin release.[14,15] Human studies have shown that diazoxide reduces glucose-stimulated insulin release in healthy individuals[16] and ameliorates the abnormal hyperinsulinaemia in patients suffering from insulinoma[17] and nesidioblastosis (PHHI, persistent hyperinsulinaemia and hypoglycemia of infancy).[18] Several preclinical and clinical studies have indicated that diazoxide can be used to reduce the hyperinsulinaemia commonly observed in obese, prediabetic individuals to reduce weight and to prevent the development of type 2 diabetes in animal models.[19,20] Recently, it has been suggested that administration of diazoxide in combination with intensive insulin treatment, to induce β-cell rest, will prevent or delay the progression of type 1 diabetes in newly diagnosed patients.[21] The use of diazoxide in this study was hampered by side effects such as severe oedema and headaches. Additional side effects associated with diazoxide include excessive hair growth,[22] which probably is related to the vasodilatory effect of the compound.[23]

Structural modifications of the diazoxide molecule have been described previously. These include variations of the size of the 3-alkyl substituent, in the substitution of position 7[24,25] and in the introduction of 3-alkylamino side chains to obtain hypotensive and antiinflammatory compounds.[26,27] By combining components from the structures of pinacidil and diazoxide, Pirotte et al.[28,29] have prepared a series of pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides, among which BPDZ 44 (**4**) (Chart 1) was reported to be a more potent and selective opener of K_{ATP} channels of pancreatic β-cells than diazoxide. These structures have subsequently been developed to include other potent benzo-1,2,4-thiadiazine 1,1 dioxide derivatives.[30]

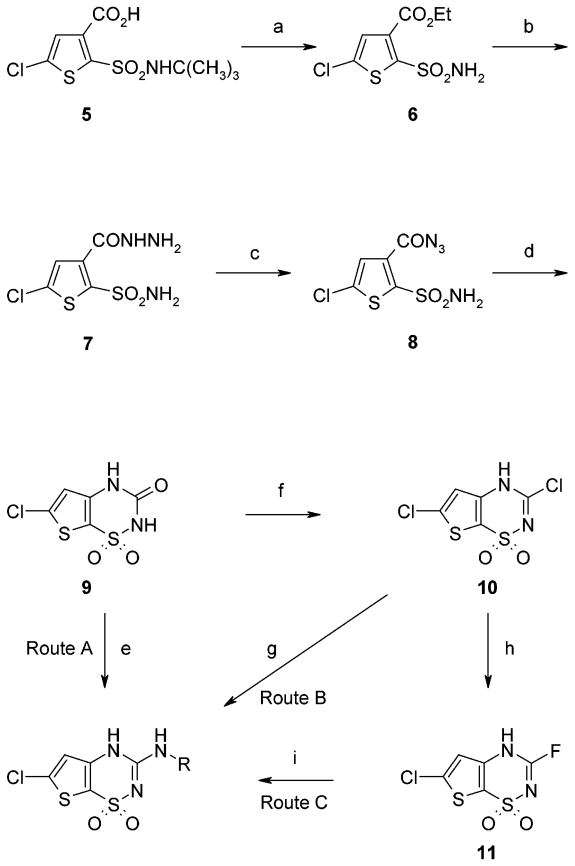
In analogy, pinacidil-like cyanoguanidine derivatives have recently been characterized as potent activators of β-cell K_{ATP} channels with little effects on vascular smooth muscle.[31]

On the basis of the above findings, we have synthesized a series of 3-alkylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxides in which we have varied the 3-alkylamino side chain to evaluate the structure−activity relationship based on activity of pancreatic β-cells and vascular smooth muscle in vitro and in vivo. It is expected that such compounds could be valuable tools for exploring the potential of reducing abnormal hypersecretion of insulin as a treatment of metabolic diseases. One compound of the series, described here, NNC 55-0118 (**34**), has previously been found to protect β-cells of rat islets when subjected to cytotoxic concentration of streptozotocin in vitro,[32] to prevent β-cell degeneration in the BB rat model of type 1 diabetes[33] and to prevent development of type 2 diabetes in the OLETF rat model of type 2 diabetes.[34]

Chemistry

The different synthetic routes used to prepare the 3-alkylamino-6-chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxides described in this paper are illustrated in Schemes 1−3. The strategy for the synthesis of key intermediate 6-chloro-2,3-dihydro-3-oxo-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**9**) (Scheme 1) was based on a previously reported synthetic approach to the imidazo analogue 4,5-dihydroimidazo[4,5-*e*]-1,2,4-thiadiazin-3(2*H*)-one 1,1-dioxide.[35] The starting material 2-(*tert*-butylamino)sulfonyl-5-chlorothiophene-3-carboxylic acid (**5**) was prepared in two steps from 2-chlorothiophene as described by Unterhalt and Moghaddam.[36] The carboxylic acid **5** was converted to the corresponding ethyl ester **6** with simultaneous cleavage of the *tert*-butyl group by heating in anhydrous ethanol saturated with hydrogen chloride. Treatment of **6** with excess hydrazine hydrate at room temperature gave the hydrazide **7**, which was converted to the carbonyl azide **8** in almost quantitative yield by reaction with nitrous acid. Because of the potential explosion hazard of acyl azides, crude **8** was cautiously dried[37] without further purification and then subjected to a Curtius rearrangement by heating in refluxing dry toluene. The intermediate isocyanate spontaneously cyclized to the desired

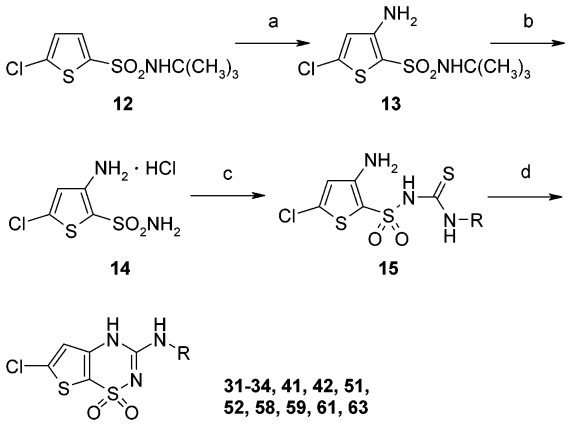
Scheme 1. Synthesis of 6-Chloro-3-alkylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide Derivatives (Routes A−C)[a]



Route A: **29, 34**
Route B: **27, 28, 30, 34-40, 43, 46-50, 53, 60, 64, 66, 67**
Route C: **53-57**

[a] Reagents: (a) HCl(g) in EtOH, reflux. (b) $NH_2NH_2 \cdot H_2O$, room temperature. (c) 1 N HCl, $NaNO_2$, H_2O, 0 °C. (d) Toluene, reflux. (e) P_2O_5, *N*,*N*-dimethylcyclohexylamine, $RNH_2 \cdot HCl$, 180 °C. (f) $POCl_3$, pyridine, 100 °C or $POCl_3$, pyridine·HCl, H_3PO_4, 100 °C. (g) RNH_2, Δ or RNH_2, R′OH (R′ = ^{i}Pr, Et), Δ or RNH_2, K_2CO_3, ^{i}PrOH, Δ. (h) CsF, DMSO, 150 °C or KF, hexadecyltrimethylammonium bromide, NMP, 130 °C. (i) $RNH_2 \cdot HCl$, TEA, EtOH, 50−100 °C.

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



[a] Reagents: (a) (i) *n*-BuLi, THF; (ii) *p*-toluenesulfonyl azide; (iii) hexadecyltributylphosphonium bromide, $NaBH_4$. (b) 12.5 N HCl, 60 °C. (c) *t*-BuOK, RNCS, DMF. (d) TEA, 1.93 M $COCl_2$ in toluene, THF.

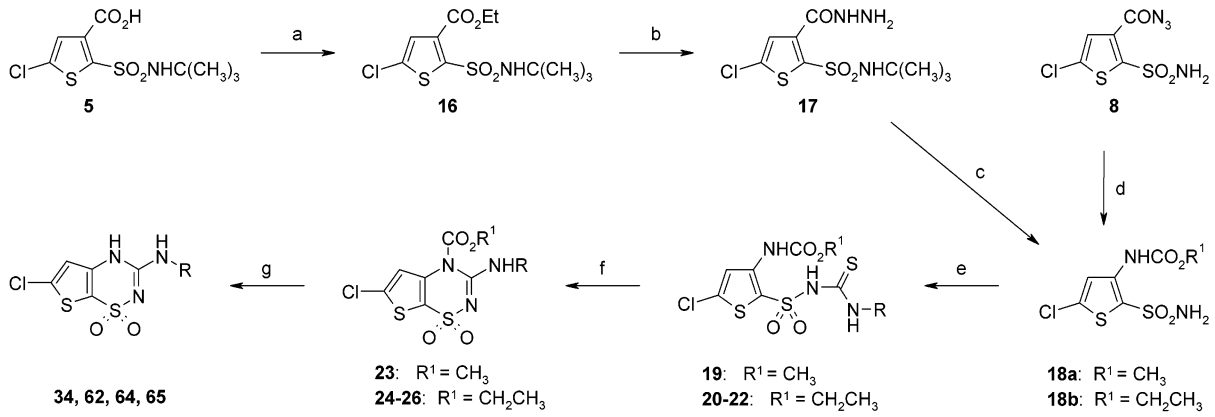
compound **9**. Our first attempt to obtain the target compounds by direct amination of compound **9** (route A) under conditions previously described for 2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxides[38] only resulted in low yields (21 and 11%, respectively) of the desired products **29** and **34**. In route B (Scheme 1), compound **9** was first chlorinated with phosphorus oxychloride in the presence of pyridine or preferable with activated phosphorus oxychloride[39] in the presence of pyridine hydrochloride and *ortho*-phosphoric acid, to afford 3,6-dichloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide (**10**). Nucleophilic aromatic substitution of **10** with the appropriate amines gave the desired products in varying yields (9−94%) by heating in a sealed flask. The reactions were performed either neat in a large excess of the amine, but preferentially with a few equivalents as an alcoholic solution, or with 1−2 equiv in 2-propanol in the presence of potassium carbonate. The best yields were obtained with aqueous ammonia (**27**, 94%) or with simple unbranched amines as, for example, methyl (**28**, 80%), while bulky amines generally resulted in low yields (e.g., **53**, 9%) due to incomplete conversion of **10** at the applied reaction conditions. The displacement reactions with bulky amine nucleophiles were slow even at elevated temperatures and had longer reaction times without improving the yields due to side reactions.

To increase the nucleophilic substitution rate, we changed the leaving group from chloro to fluoro as outlined in Scheme 1 (route C), where the labile 3-chloro substituent of **10** was displaced in a reaction with cesium fluoride or activated potassium fluoride[40] to give the more reactive fluoro derivative **11**. Compound **11** was either purified prior to amination (**53**−**56**) or aminated in situ (**57**) but in low yield despite the better leaving group. However, amination of **11** with methylcyclopropylamine actually doubled the yield of **53** to 54% as compared to that obtained with **10** as the starting material.

An alternative synthesis (route D) of 3-alkylamino-6-chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxides is shown in Scheme 2. The known *N*-(*tert*-butyl)-5-chloro-2-thiophenesulfonamide (**12**)[36] was converted to 3-amino-*N*-(*tert*-butyl)-5-chloro-2-thiophenesulfonamide (**13**) by ortho metalation with *n*-butyllithium followed by quenching with *p*-toluenesulfonyl azide and finally reduction with sodium borohydride in the presence of hexadecyltributylphosphonium bromide.[41−43] Cleavage of the *tert*-butyl group in sulfonamide **13** by heating in fuming hydrochloric acid afforded the desired key intermediate **14**. Compound **14** was reacted with the appropriate alkyl isothiocyanate at 0−25 °C to give the sulfonyl isothiourea derivative **15**, which was readily cyclized to the corresponding thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide by treatment with phosgene in toluene at 0 °C in the presence of triethylamine.

As shown in Scheme 3, it was possible to avoid direct handling of the potential hazardous azides used in the preparation of the required intermediates of routes A and D. The acid **5** was converted to the ethyl ester **16**, from which the hydrazide **17** was obtained by reaction with hydrazine hydrate in NMP. Compound **17** was reacted with isoamyl nitrite in an acid-catalyzed Curtius reaction in refluxing methanol followed by hydrochloric acid cleavage of the *tert*-butyl group to afford the methyl carbamate **18a** without isolation of the in situ-formed carbonyl azide. Alternatively, compound **18** could be made from **8** as exemplified by preparation of the ethyl carbamate **18b**. The key intermediate **18** of route E was then reacted with alkyl isothiocyanates in refluxing

Scheme 3. Synthesis of 6-Chloro-3-isopropylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (**34**) (Route E)[a]



[a] Reagents: (a) $CH(OEt)_3$, reflux. (b) $NH_2NH_2 \cdot H_2O$, NMP. (c) (i) $(CH_3)_2CHCH_2CH_2ONO$, MeOH, TFA, reflux; (ii) MeOH, 12.5 N HCl, reflux. (d) EtOH, reflux. (e) K_2CO_3, acetone, RNCS, reflux. (f) TEA, 1.93 M $COCl_2$ in toluene, THF. (g) (i) 2 N NaOH, reflux or 1 N NaOH, H_2O, EtOH at pH 9−10; (ii) 4 N HCl.

Table 1. pK_a, LogP, $LogD_{pH\ 7.4}$, and mLogP Values for Compounds **30**, **34**, **36**, **37**, and **47**

compd	pK_a	LogP	$LogD_{pH\ 7.4}$	mLogP
30	8.23	2.49	2.42	2.01
34	8.35	1.84	1.63	2.01
36	8.54	2.57	2.52	2.31
37	8.42	2.42	2.38	2.31
47	8.29	3.17	3.14	2.60

acetone followed by ring closure as described for **15** leading to the thieno[3,2-*e*]-1,2,4-thiadiazine carbamates **23**−**26** via the sulfonyl isothiourea derivatives **19**−**22**, respectively. Careful alkaline hydrolysis at pH 9−10 finally afforded the desired products **34**, **62**, **64**, and **65** in modest overall yields.

The physicochemical properties of selected compounds have been examined (Table 1). The 3-alkylamino-6-chloro 4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxides were all weak acids with pK_a values around 8.3. This suggests that the compounds at physiological pH primarily will be in a neutral form. The water/octanol distribution coefficients $LogD_{pH\ 7.4}$ were measured and found to correlate to the calculated mLogP value. The $LogD_{pH\ 7.4}$ values were dependent on the hydrophobicity of the 3-alkylamino-side chain ranging from 1.63 (**34**) to 3.14 (**47**) all lying within an acceptable range for potential drugs.[44]

Results and Discussion

In Vitro Biology. On the basis of our aim to identify potent and β-cell selective potassium channel openers, we decided to initially screen our compounds in assays, which predict activation of K_{ATP} channels of β-cells and vascular smooth muscle (Tables 2−4).

Glucose-induced pancreatic β-cell insulin release is mainly a consequence of closure of ATP-regulated potassium channels mediated by changes in the ATP/ADP ratio. The closure leads to β-cell plasma membrane depolarization, influx of Ca^{2+} through voltage sensitive calcium channels, increase in cytoplasmic Ca^{2+}, and activation of exocytosis of insulin from their pool of secretory granules.

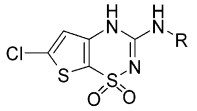
Qualitative changes in membrane potential caused primarily by changes in potassium fluxes were assessed in the glucose responsive insulin-producing murine cell line βTC3, by measuring $DiBAC_4(3)$ fluorescence. The probe is cell permeable and distributes between the cells and the extracellular medium depending on the membrane potential of the cells. Inside the cells, DiBAC associates with proteins and thereby enhances its fluorescence dramatically. After the cell is depolarized, DiBAC accumulates inside the cells, increasing their fluorescence. By repolarization, DiBAC escapes from the cells and their fluorescence decreases.

The ability of the compounds to inhibit the release of insulin from the mouse β-cell line, βTC6 incubated in the presence of 22 mM glucose and 0.1 mM IBMX to increase the insulin release, was determined. The vasorelaxing effects of the compounds were initially determined by their ability to relax phenylephrine-contracted rat aorta rings as previously described.[45]

In Table 2, the results from testing a series of 6-chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxides substituted in position 3 with linear alkylamino groups are compared to the 3-amino derivative **27** and diazoxide. The data support that diazoxide is a nonselective potassium channel opener, which hyperpolarizes membranes of βTC3 cells, inhibits insulin release of βTC6 cells, and relaxes phenylephrine-contracted aortic rings with approximately equal potency. This corresponds to previous studies, which have shown that diazoxide inhibits glucose-stimulated insulin release from rat islets ($IC_{50} = 24.7 \pm 3.4\ \mu M$) and relaxes KCl-induced contraction of rat aortic rings ($IC_{50} = 22.0 \pm 2.6\ \mu M$).[46,47] In comparison, we found that pinacidil is a potent vasodilator on rat aortic rings ($EC_{50} = 0.8 \pm 0.2\ \mu M$, data not shown) without exerting any effects on β-cell membrane potential or glucose-stimulated insulin release at concentrations up to 100 μM.

The 6-chloro-3-alkylamino-4*H*-1,2,4-thieno[3,2-*e*]thiadiazine 1,1-dioxides with primary alkylamino groups in position 3 as presented in Table 2 were in general able to activate K_{ATP} channels of β-cells as determined by the ability of the compounds to hyperpolarize membranes of βTC3 cells and to inhibit glucose-stimulated insulin release of βTC6 cells. The size of the 3-substituent has importance for the inhibition of insulin release from β-cells. The *n*-butyl (**31**) ($IC_{50} = 1.39 \pm 0.63\ \mu M$), the ethyl (**29**, $IC_{50} = 1.9 \pm 0.52\ \mu M$), the propyl (**30**, $IC_{50} = 2.4 \pm 0.44\ \mu M$), and the octyl (**33**, $IC_{50} = 1.5 \pm 0.5\ \mu M$) derivatives are approximately equipotent but considerably more potent than the unsubstituted aminoderivative (**27**, $IC_{50} = 21.1 \pm 17.4\ \mu M$), the methyl

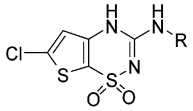
Table 2. Structures and Biology Screening Data for Compounds **27**−**33** and Diazoxide



		membrane[a] potential βTC3	insulin release in vitro[a] βTC6		relaxation[a] rat aorta rings
compd	R	EC_{50} (μM)	IC_{50} (μM)	E_{max} (%)	EC_{50} (μM)
27	H	NT[c]	21.1 ± 17.4	27 ± 10.2	262.0 ± 38.0
28	CH_3	>100	21.18 ± 9.61	35 ± 7.11	17.3 ± 8.8
29	CH_2CH_3	9 ± 4	1.9 ± 0.52	74 ± 1.3	135.5 ± 36.4
30	$(CH_2)_2CH_3$	2.0 ± 0.8	2.4 ± 0.44	65 ± 3.0	64.7 ± 9.2
31	$(CH_2)_3CH_3$	0.6 ± 0.6	1.39 ± 0.63	66 ± 4.0	13.4 ± 2.4
32	$(CH_2)_5CH_3$	0.2 ± 0.2	7.1 ± 1.6	67 ± 3.7	0.15 ± 0.15
33	$(CH_2)_7CH_3$	0.5 ± 0.1	1.5 ± 0.5	76 ± 2.2	176.0 ± 48
diazoxide		77.4 ± 4[b]	22.4 ± 3.7	23 ± 3.9	12.8 ± 2.5

[a] Shown are means ± SEM calculated from at least three independent experiments. [b] Data from Lebrun et al.[30] [c] NT, not tested.

Table 3. Structures and Biology Screening Data for Compounds **34**−**48**



compd	R	membrane[a] potential βTC3 EC_{50} (μM)	insulin release in vitro[a] βTC6 IC_{50} (μM)	insulin release in vitro[a] βTC6 E_{max} (%)	relaxation[a] rat aorta rings EC_{50} (μM)
34	$CH(CH_3)_2$	0.6 ± 0.02	0.23 ± 0.02	85 ± 1.1	266.0 ± 59.8
35	(*RS*)-$CH(CH_3)CH_2CH_3$	1.3 ± 1.0	0.16 ± 0.03	74 ± 6.1	65.0 ± 10.9
36	(*R*)-$CH(CH_3)CH_2CH_3$	0.3 ± 0.02	0.24 ± 0.06	82 ± 2.3	70.0 ±9.1
37	(*S*)-$CH(CH_3)CH_2CH_3$	0.8 ± 0.1	0.41 ± 0.15	79 ± 3.3	51.0 ± 15.1
38	$C(CH_3)_3$	0.4 ± 0.3	0.17 ± 0.09	77 ± 5.0	169.0 ± 39.9
39	$C(CH_3)_2CH_2CH_3$	0.2 ± 0.1	0.03 ± 0.01	82 ± 7.5	11.8 ± 3.4
40	(*RS*)-$CH(CH_3)(CH_2)_2CH_3$	41 ± 150	0.22 ± 0.04	56 ± 3.84	83.0 ± 12.5
41	(*RS*)-$CH(CH_3)(CH_2)_4CH_3$	0.4 ± 0.05	24.95 ± 13.2	36 ± 3.82	2.0 ± 0.9
42	(*RS*)-$CH(CH_3)(CH_2)_3CH(CH_3)_2$	2.0 ± 1.1	4.89 ± 1.21	72 ± 2.31	25.2 ± 7.9
43	(*RS*)-$CH(CH_3)CH(CH_3)_2$	NT	0.17 ± 0.01	79 ± 2.6	34.8 ± 3.5
44	(*S*)-$CH(CH_3)CH(CH_3)_2$	14 ± 33	0.56 ± 0.26	80 ± 3.17	>300
45	(*R*)-$CH(CH_3)CH(CH_3)_2$	0.3 ± 0.3	1.01 ± 0.53	62 ± 4.23	75.3 ± 12.5
46	$CH_2CH(CH_2CH_3)_2$	>100	0.24 ± 0.09	38 ± 1.36	8.0 ± 4.2
47	(*RS*)-$CH_2CH(CH_3)CH_2CH_3$	2.0 ± 2.0	0.15 ± 0.03	48 ± 3.85	20.5 ± 6.8
48	(*RS*)-$CH(CH_3)C(CH_3)_3$	17 ± 30	0.75 ± 0.28	56 ± 5.3	2.0 ± 0.3

[a] Shown are means ± SEM calculated from at least three independent experiments; NT, not tested.

(**28**, IC_{50} = 21.18 ± 9.61 μM), and the hexyl (**32**, IC_{50} = 7.1 ± 1.6 μM) derivatives.

In addition, the compounds have been tested for their ability to potentiate the efflux of $^{86}Rb^+$ from preloaded RIN5F rat insulinoma cells. As expected for compounds that activate K_{ATP} channels of β-cells, there is an overall good correlation between the ability of the compounds to increase the ion currents of RIN5F cells, to hyperpolarize βTC3 cells, and to inhibit insulin release from βTC6 cells.

In comparison, these compounds were in general considerably less potent with respect to relaxation of rat aorta rings. Potency again seems to be dependent on the size of the alkyl group with the hexyl derivative **32** being the most potent (EC_{50} = 0.15 ± 0.15 μM) as compared to the relatively inactive ethyl (**29**, EC_{50} = 135.5 ± 36.4 μM), octyl (**33**, EC_{50} = 176.0 ± 48 μM), and 3-amino (**27**, EC_{50} = 262.0 ± 38.0 μM) derivatives. Compound **32** is more potent than diazoxide (EC_{50} = 12.8 ± 2.5 μM) and as potent as pinacidil (EC_{50} = 0.8 ± 0.2 μM).

In Table 3, a series of 6-chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxides substituted in position 3 with branched alkylamino groups are presented. As found for compounds having linear alkylamino substituents, size seems to be important for β-cell activity. α-Methyl substitution (for example, **34** as compared to **29** or **35** and **39** as compared to **30**) increases potency. In contrast, β-ethyl substitution (for example, **46** as compared to **31**) reduces potency. In this series of potassium channel openers, chirality of the side chain bears little importance for β-cell activity. This is in agreement with data generated on a pair of pyrido-1,2,4-thiadiazine 1,1-dioxides.[48]

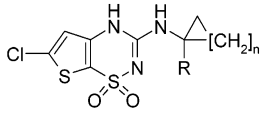
The lipophilic compounds **40**, **46**, and **47** are in general low efficacious inhibitors of glucose-stimulated insulin release from βTC6 cells and stimulate membrane hyperpolarization of βTC3 cells with low and variable potency. Nonspecific interactions due to the high lipophilicity or lack of aqueous solubility could contribute to these inconsistencies. Alternatively, these compounds could inhibit the insulin secretory process by interaction with other targets than the K_{ATP} channels.

Compounds **34**−**58** exhibit major differences with respect to vasodilatory activity. Among the compounds, there are some vasorelaxants such as, for example, **41** (EC_{50} = 2.0 ± 0.9 μM), but also compounds with little or no effects on rat aorta like **34** (EC_{50} = 266.0 ± 59.81 μM), **38** (EC_{50} = 169.0 ± 39.9 μM), and **44** (EC_{50} >300 μM). Overall, there is a dependency on chain length for activity on rat aorta rings. The most potent vasodilators are those having a six carbon side chain as in **32** (EC_{50} = 0.15 ± 0.15 μM) and **41** (EC_{50} = 2.0 ± 0.88 μM) with only small effect of a single methyl substituent (for example, **32** as compared to **41**). Reducing the length of the 3-alkylamino side chain to a 2-C or a 3-C entity considerably reduces potency, giving rise to compounds with considerable β-cell selectivity. This is in agreement with a series of *N*-alkyl-*N*′-pyrido cyanoguanidine (pinacidil) derivatives[49] and within a series of 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides[28] among which compounds with *N*-methyl and *N*-propyl groups are weak vasodilators.

By contrast to the β-cell effects, the effects on vascular tissue (rat aorta) seem to be dependent on chirality. The (*S*) forms of the compounds (e.g., **37** as compared to **36** or **44** as compared to **45**) are equal or less potent than the corresponding (*R*) isomers. This is in accordance with previous reports on pyrido-1,2,4-thiadiazine 1,1-dioxide derivatives[48] and on pinacidil derivatives acting on vascular tissue.[48,50]

6-Chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxides substituted in position 3 with cycloalkylamino groups are presented in Table 4. The size of the carbocycle is important for potency with respect to hyperpolarization of β-cell membranes and inhibiton of insulin release with the cyclobutyl (**50**) and the cyclopentyl (**51**) being more potent than the cyclopropyl (**49**) and the cyclohexyl (**52**) derivatives. Increasing the branching at the α-atom as in compounds **53**−**55** and **57** leads to considerably increased potency. Among these

Table 4. Structures and Biology Screening Data for Compounds **49**−**57**



compd	R	*n*	membrane potential[a] βTC3 EC_{50} (μM)	insulin release in vitro[a] βTC6 IC_{50} (μM)	insulin release in vitro[a] βTC6 E_{max} (%)	relaxation[a] rat aorta rings EC_{50} (μM)
49	H	1	>30	7.38 ± 1.23	37 ± 4.5	28.2 ± 9.6
50	H	2	1.4 ± 1.8	0.49 ± 0.21	78 ± 4.64	27 ± 10.8
51	H	3	0.4 ± 0.4	1.15 ± 0.18	67 ± 3.0	>300
52	H	4	8 ± 6	25.57 ± 19.35	40 ± 2.76	6.8 ± 1.9
53	CH_3	1	0.6 ± 0.3	0.15 ± 0.03	69 ± 5.0	119.2 ± 12.1
54	CH_3	2	0.014 ± 0.6	0.02 ± 0.004	91 ± 1.1	6.5 ± 1.7
55	CH_3	3	0.08 ± 0.08	0.07 ± 0.03	79 ± 5.7	6.2 ± 0.7
56	CH_3	4	1.0 ± 1.0	5.33 ± 3.84	62 ± 3.0	1.4 ± 0.8
57	CH_2CH_3	2	0.1 ± 0.07	0.02 ± 0.01	81 ± 4.5	3.3 ± 2.0

[a] Shown are means ± SEM calculated from at least three independent experiments.

Table 5. Inhibition of [^{3}H]Glibenclamide Binding to HEK293 Cells Expressing SUR1/Kir6.2 and Glucose-Stimulated Insulin Release from Rat Islets; Relaxation of Precontracted Rat Mesenteric Arteries

compd	[^{3}H]glibenclamide binding[a] IC_{50} (μM) +ATP H	+ATP L	−ATP	insulin release rat islets[b] IC_{50} (μM)	vasodilation of mesenteric arteries[c] EC_{50} (μM)
30	2.27 ± 0.89	584 ± 229	924 ± 148	0.7 ± 0.17	23.3 ± 5.4
34	0.34 ± 0.16	187 ± 42	554 ± 64	0.18 ± 0.08	6.2 ± 2.1
36	0.64 ± 0.39	224 ± 79	487 ± 89	0.06 ± 0.03	25.2 ± 7.0
37	0.46 ± 0.31	309 ± 109	420 ± 48	0.07 ± 0.02 (2)	19.2 ± 8.4
38	0.23 ± 0.12	393 ± 169	1350 ± 885	0.06 ± 0.05 (2)	60.6 ± 26.6
39	0.18 ± 0.10	375 ± 93	899 ± 420	0.02 ± 0.01 (2)	29.4 ± 9.2
50	0.12 ± 0.04	146 ± 36	943 ± 536	0.05 ± 0.05 (2)	38.6 ± 0.9
51	NT	NT	NT	0.44 ± 0.07 (2)	10.8 ± 4.3
53	0.32 ± 0.14	165 ± 55	685 ± 110	0.21 ± 0.08	14.3 ± 5.0
54	0.003 ± 0.001	10.8 ± 5.0	859 ± 213	0.01 ± 0 (2)	7.2 ± 1.7
diazoxide		198 ± 36	750 ± 120	20.28 ± 11.7	20.8 ± 6.1

[a] IC_{50} values of K_{ATP} channel modulators from [^{3}H]glibenclamide competition studies in the absence or presence of 2 mM MgATP. In the presence of MgATP, [^{3}H]glibenclamide is displaced in a biphasic manner by K_{ATP} channel openers from a high (H) and low (L) affinity state. [b] Inhibition of glucose (22 mM)-stimulated insulin release from isolated Wistar rat islets. [c] Effects on Wistar rat mesenteric arteries precontracted with phenylephrine. Shown are means ± SEM of three or more independent experiments unless indicated (*n*); NT, not tested.

compounds, **54** with the methylcyclobutyl amino side chain is more potent (IC_{50} = 0.02 ± 0.004 μM) than the methylcyclopropyl **53** (IC_{50} = 0.15 ± 0.03 μM), the methylcyclopentyl **55** (IC_{50} = 0.07 ± 0.03 μM), or the methylcyclohexyl derivative **56** (IC_{50} = 5.33 ± 3.84 μM) with respect to inhibition of insulin release. In comparison, the ethylcyclobutyl derivative **57** (IC_{50} = 0.02 ± 0.01 μM) is more potent than the cyclobutylamino compound **50** (IC_{50} = 0.49 ± 0.21 μM) and equipotent with **54**. The increased β-cell activity of the branched cycloalkylamine derivatives is in accordance with the high potency of the *tert*-butyl and *tert*-amyl derivatives **38** and **39** as compared to, for example, the isopropyl derivative **34**. In this series, there is an overall good correlation between the effects on βTC3 cells and the effects on βTC6 cells. With respect to smooth muscle effects, the size of the cycloalkyl groups increases potency exemplified by **56** (methylcyclohexyl) (EC_{50} = 1.4 ± 0.8 μM), which is more potent than **55** (methylcyclopentyl) (EC_{50} = 6.2 ± 0.7 μM) and **54** (methylcyclobutyl) (EC_{50} = 6.5 ± 1.7 μM) and considerably more potent than **53** (methylcyclopropyl) (EC_{50} = 119.2 ± 12.1 μM). The ethylcyclopropyl derivative **57** (EC_{50} = 3.3 ± 2.0 μM) is slightly less potent than **56** and approximately equipotent with **54**.

The present results and previous reports indicate that the size and the shape of the alkylamino side chains of K_{ATP} channel openers of the cyanoguanidine and the thiadiazine 1,1-dioxide series are very important for the vascular activity. These lipophilic groups could occupy a pocket on the SUR2B subunit necessary for inducing ion channel activation.

On the basis of potency and β-cell selectivity, compounds **30**, **34**, **36**−**39**, **50**, **51**, **53**, and **54** were selected for extensive evaluation with respect to effects on recombined human SUR1/Kir6.2 K_{ATP} channels, freshly isolated rat islets and vascular tissue in vitro (Table 5), and on blood pressure and insulin release in vivo (Table 6).

To measure the direct interaction of the compounds with recombined K_{ATP} channels of the β-cells, we examined the displacement of radiolabeled glibenclamide from membranes of HEK293 cells expressing human SUR1/Kir6.2 potassium channels. Glibenclamide potently binds to the SUR1 part of the β-cell K_{ATP} channels,[51] and it has previously been shown[52,53] that competition by diazoxide of [^{3}H]glibenclamide binding to SUR1/Kir6.2 is highly dependent on ATP and Mg^{2+}. This was found to be the case also for compounds **30**, **34**, **36**−**39**, **50**, **53**, and **54** (Figure 1, Table 5), whereas

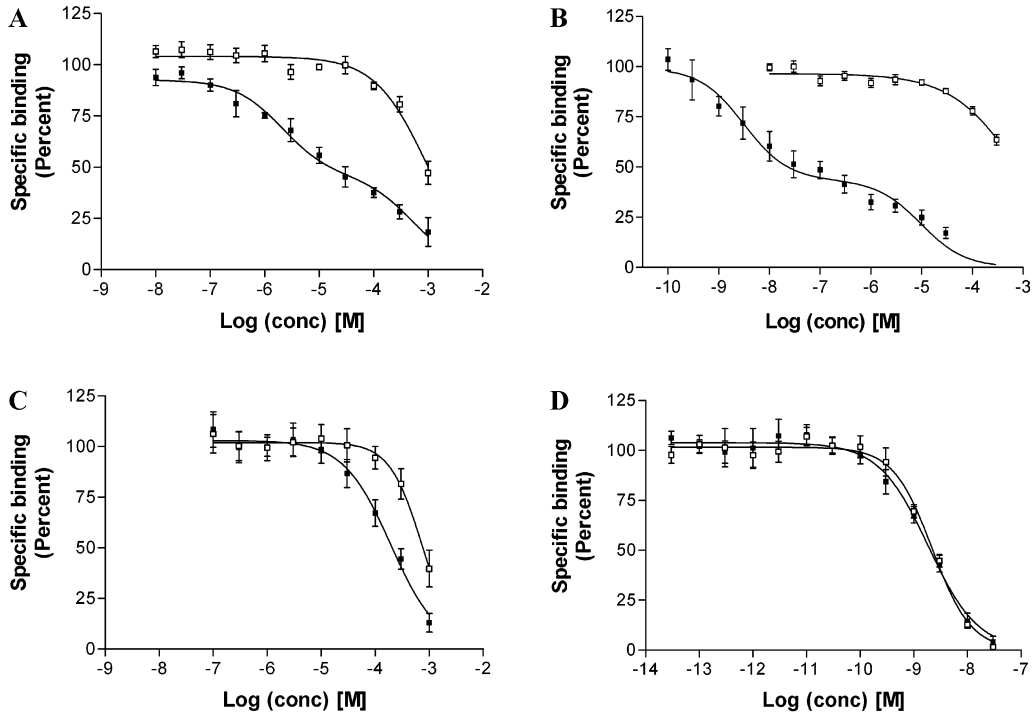


Figure 1. [^{3}H]Glibenclamide (1 nM) binding to membrane preparations from HEK293 cells stably expressing human SUR1/Kir6.2. Binding was displaced in the absence (open symbols) or presence (closed symbols) of 2 mM ATP and 2 mM Mg^{2+} by (A) compound **34**, (B) compound **54**, (C) diazoxide, and (D) glibenclamide. In the presence of MgATP, [^{3}H]glibenclamide is displaced in a biphasic manner by K_{ATP} channel openers **34** and **54** from a high (H) and low (L) affinity state. Values are mean ± SEM of three or more independent experiments.

competition by glibenclamide is independent of ATP and Mg^{2+} ($pIC_{50} = 8.73 \pm 0.06$ μM (+ATP); $pIC_{50} = 8.66 \pm 0.05$ μM (−ATP)) (Figure 1). The competition curves indicate that these compounds compete with glibenclamide in a biphasic manner, giving rise to two inhibition constants: a high affinity component, which largely correlates with the potency of the compounds in the β-cell membrane potential and the insulin release assays, and a low affinity component, which correlates with the inhibition by the present compounds in the absence of ATP and Mg^{2+}. In contrast, diazoxide exhibits a monophasic competition curve (Figure 1) suggesting that its mode of interaction with the K_{ATP} channel is different from the new 6-chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide derivatives. Selected compounds (i.e., **30** and **36**) were found also to inhibit binding of [^{3}H]glibenclamide in an ATP-dependent manner to membranes of HEK293 cells expressing SUR1 but not Kir6.2 (data not shown). This suggests that these compounds bind to the SUR part of the K_{ATP} channel complex to induce channel activation.

When measured by patch clamp in the whole cell configuration, the present compounds (e.g., **30**, **34**, and **53**) potently increase the ion current of human SUR1/Kir6.2, stably expressed in a HEK293 cell line and tranciently expressed in Xenopus oocytes, but do not activate ion currents of SUR2A/Kir6.2 or SUR2B/Kir6.2 channels (data not shown).[54]

When freshly isolated rat pancreatic islets are subjected to a high glucose concentration, insulin secretion is stimulated. Compounds **30**, **34**, **36**−**39**, **50**, **51**, **53**, **54**, and diazoxide dose dependently inhibit the insulin secretion with an order of potency similar to that found in the β-cell screening assay discussed above (Table 5). In general, the inhibitory effect on insulin release from rat islets occurs at concentrations 2−3 times lower than those inhibiting insulin release from the murine βTC6 cell line. The effect of diazoxide on rat islets ($IC_{50} = 20.28 \pm 11.7$ μM) is similar to that reported previously ($IC_{50} = 24.8 \pm 3.4$ μM).[47] The high potency of compound **54** in this assay ($IC_{50} = 0.01$ μM) corresponds to its potency on βTC6 cells ($IC_{50} = 0.002$ μM) and its ability to displace [^{3}H]glibenclamide from human SUR1/Kir6.2 channels ($IC_{50} = 0.003$ μM).

All of the selected compounds of this series induced concentration-dependent maximal vasodilatation of small mesenteric arteries that were precontracted with phenylephrine (Table 5). The relative high potency with respect to dilatation of mesenteric arteries of all of the tested compounds is surprising in view of the observed effects in isolated rat aorta. Recent studies, however, have suggested that the K_{ATP} channels of some vascular tissues consist of SUR2B and Kir6.1 instead of Kir6.2.[55,56] This potentially could lead to the discrepancy between the effects on aorta and mesenteric arteries. To further examine the effects of these compounds on smooth muscle, **30**, **36**, and **37** were added to rat bladder rings that were precontracted with the muscarinic agonist bethanechol. The compounds were only very weak dilators of bladder tissue (**30**, $IC_{50} = 146.3 \pm 46.9$ μM; **36**, $IC_{50} = 204.1 \pm 70.2$ μM; and **37**, $IC_{50} > 300$ μM; data not shown). Overall, the 6-chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxides presented in Table 5 exhibit a high degree of β-cell selectivity as measured by their ability to inhibit insulin release from rat islets as compared to their vasorelaxant potency. In contrast, diazoxide affects rat islets and mesenteric vessels with equal potency.

The in vitro results strongly indicate that the present potassium channel openers, e.g., **30**, **34**, **36**, **37**, **53**, and

Table 6. Acute Effects on Plasma Insulin Levels and Blood Pressure,[a] and Pharmacokinetic Properties[b] in Rats

compd	max reduction in plasma insulin (%)[c]	reduction in plasma insulin, 30 min (%)[c]	max change in MAP (%)[d]	MAP_{AUC} (%)[d]	pharmacokinetics	
					f_{po} (%)[b]	$t_{1/2}$ (min)[b]
30	100 ± 0	16 ± 5	13 ± 2	ND	69	72
34	96 ± 1	96 ± 1	−19 ± 4	−1187 ± 314	65	164
36	97 ± 2	94 ± 2	−17 ± 2	−1133 ± 923	90	163
37	96 ± 2	94 ± 6	−7 ± 4	−362 ± 930	49	58
38	85 ± 8	71 ± 12	−36 ± 13	−7205 ± 2574	NT	NT
39	87 ± 7	77 ± 16	−34 ± 9	−7870 ± 2676	47	99
50	100 ± 0	100 ± 0	5 ± 4	−1237 ± 572	49	113
51	99 ± 1	22 ± 9	29 ± 5	3133 ± 997	NT	NT
53	85 ± 14	77 ± 23	−4 ± 3	−268 ± 240	103	250
54	82 ± 8	76 ± 13	−48 ± 7	−10950 ± 3277	NT	NT
diazoxide	53 ± 8	53 ± 8	−26 ± 2	−3008 ± 195	154	79

[a] Effects of compounds (3 mg/kg, except for compound **54**, 0.3 mg/kg) on plasma insulin levels and mean arterial blood pressure (MAP) after iv administration to anaesthetized Sprague−Dawley rats. Values are mean ± SEM of 2−4 animals. [b] Oral bioavailability (f_{po}) and half-life ($t_{1/2}$) after oral administration to Sprague−Dawley rats. The values are means of two animals. [c] Maximal reduction in plasma insulin and reduction in plasma insulin 30 min postinjection (calculated as percentage reduction of the level prior to administration). [d] Maximal change in MAP and area under the MAP curve measured over 5 min; ND, not determined; NT, not tested.

54 through binding to the SUR1 receptor, are potent activators of K_{ATP} channels of pancreatic β-cells with minimal effects on vascular K_{ATP} channels. On the basis of studies on recombined ATP-activated potassium channels (SUR2A/Kir6.2, data not shown), it is unlikely that these compounds will exert potassium channel opening effects on cardiac tissues. When subjected to a PanLab in vitro pharma screen, **34** and **53** (10 μM) did not show any significant effects on a series of receptors and ion channels. In contrast to diazoxide,[57] **34** does not potentiate the effect of glutamate on AMPA receptors (data not shown).

Among the tested compounds, **54** stands out as being the most potent activator of β-cell K_{ATP} channels ever described. With respect to all parameters tested in vitro, this compound is at least 1000 times more potent than diazoxide. This is the first example of a compound that is able to activate the SUR1/Kir6.2 K_{ATP} channels in the low nanomolar range. Only K_{ATP} channel blockers of SUR1/Kir6.2 channels and activators of SUR2A/Kir6.2 and SUR2B/Kir6.2 channels have previously been described to act with similar potency.[58]

In Vivo Biology. Diazoxide has been shown to inhibit insulin release in several species including humans[21,59,60] with a long lasting effect that can largely be attributed to the high bioavailability and long half-life.[61] Diazoxide induces a significant hypotensive response when given either intravenously (iv) or per os (po).[59] Using anaesthetized, postprandial adult Sprague−Dawley rats as a screening model, we examined the effects of selected 6-chloro-3-alkylamino-4*H*-thieno-[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide derivatives (**30**, **34**, **36**−**39**, **50**, **51**, **53**, **54**, and diazoxide) for effects on insulin release and blood pressure upon iv administration of a fixed dose of 3 mg/kg for all compounds except **54**, for which 0.3 mg/kg was used (Table 6). All of the selected 6-chloro-3-alkylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide derivatives were equally efficacious (>80%) in decreasing plasma insulin levels although the effects of **30** and **51** were of shorter duration (measured as percent decrease in plasma insulin at 30 min). In comparison, an identical dose of diazoxide (3 mg/kg iv) only gave a partial (53%) but sustained reduction of the concentration of insulin in plasma.

In the present study, diazoxide (3 mg/kg iv) was able to reduce mean arterial blood pressure when determined both as percent maximal change from baseline and as the area under the curve measured during the first 5 min after administration. Compounds **30**, **37**, **50**, and **53** had minimal effects on blood pressure while **34** and **36** decreased blood pressure less potently than diazoxide. Compounds **38**, **39**, **51**, and **54** markedly affected blood pressure. The potent hypotensive effect of **54** at 0.3 mg/kg was retained at 0.1 mg/kg iv (data not shown) indicating that the compound in vivo is a considerably more potent hypotensive drug than expected from the in vitro data. In analogy, the hypotensive effects of **38** and **39** and the hypertensive effect of **51** were not predicted from the screening assays and highlight that both in vitro and in vivo tests are needed to verify the selectivity of the present compounds.

Pharmacokinetic studies in Sprague−Dawley rats using cassette dosing (Table 6) have shown that compounds **30**, **34**, **36**, **37**, **39**, **50**, and **53** all have good oral bioavailability (f_{po}, 47−103%) with a plasma $t_{1/2}$ after oral administration ranging from 58 (**37**) to 250 min (**53**). In comparison, diazoxide was found to have an oral bioavailability of 154% with a plasma $t_{1/2}$ of 79 min after po administration. This is consistent with a reported plasma half-life of diazoxide of 1−2 h in rats.[62] The high oral bioavailability (f_{po}) above 100% most likely indicates hepatic recirculation. Despite the good oral bioavailability and the ability to potently inhibit insulin release upon iv administration, **50** was not able to reduce plasma insulin levels in obese Zucker rats after per os administration (data not shown).

To determine the pharmacodynamic properties of the compounds, **34** was given per os to rats (Figure 2). Compound **34** suppressed the mean plasma insulin levels both before (2.8 ± 2.8 vs 43.0 ± 3.5 pmol/L in vehicle-treated rats, $p < 0.01$) and after glucose challenge (66.1 ± 37.1 vs 268 ± 39.2 pmol/L in vehicle-treated rats, $p < 0.05$) when dosed 3 h before the latter (Figure 2A). Although the insulin secretion at 3 h after administration of **34** was suppressed by the potassium channel opener, the β-cells were able to respond to the glucose challenge. When dosed 6 h before glucose, no suppression of resting plasma insulin was seen but glucose-stimulated insulin was still reduced to 100.3 ± 23.5 pmol/L. No effect on resting or glucose-stimulated insulin was seen in rats dosed 12 h or more before challenge.

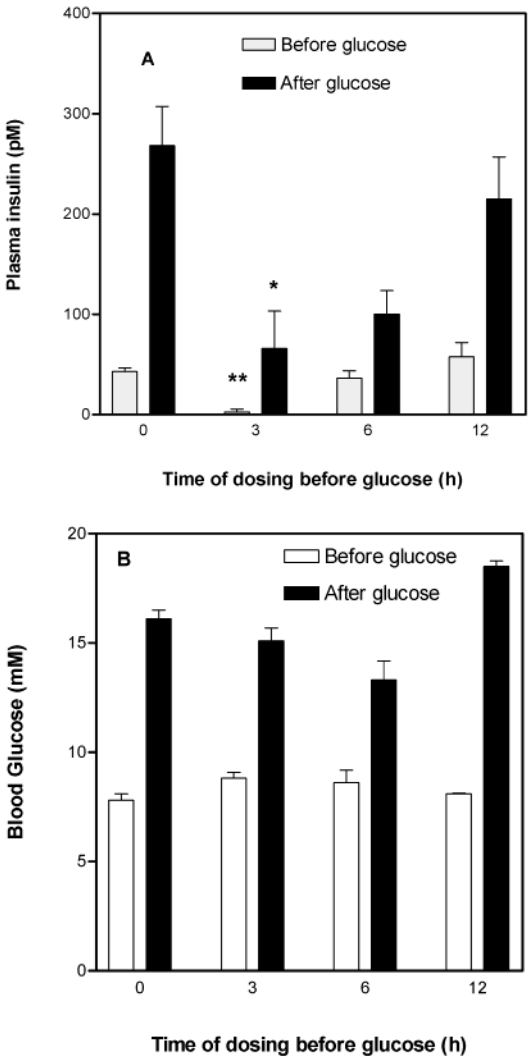


Figure 2. Plasma insulin (A) and blood glucose (B) levels in Sprague−Dawley rats pre- and postglucose challenge at different timepoints after per os administration of compound **34** (30 mg/kg). The values at $t = 0$ h indicate administration of vehicle. *$p < 0.01$ as compared to untreated $t = 0$, **$p < 0.05$ as compared to glucose challenged rats ($t = 0$) (ANOVA).

Figure 2B shows mean blood glucose values in the rats before and after glucose challenge. A slight but statistically insignificant blood glucose increase was seen before challenge at 3 and 6 h. In combination, Figure 2A,B suggests that **34** (30 mg/kg), after per oral administration to normal rats, reduces both resting and glucose-stimulated plasma insulin for up to 6 h without inducing resting hyperglycemia.

To measure effects on cardiovascular parameters in conscious animals, **54** (30 mg/kg po), **30**, **36**, **37**, and **53** (100 mg/kg po), **34** (100 and 300 mg/kg po), and diazoxide (100 and 300 mg/kg po) were given to female Sprague−Dawley rats. Blood pressure, heart rate, and body temperature were measured by telemetry for 4.5 (**30**, **36**, **37**, and **53**), 24 (**54**), or 48 h (**34** and diazoxide). Diazoxide induced a minor but significant and long-lasting increase in heart rate at 100 mg/kg and a pronounced effect on heart rate and mean arterial blood pressure at 300 mg/kg (Figure 3A). Compound **54** induced a potent depression of blood pressure and heart rate. Neither **30**, **36**, **37**, **53**, nor **34** (Figure 3B) had any significant effects on blood pressure, heart rate, and body temperature at the doses tested.

Conclusion

By using structural elements from the cyanoguanidine, pinacidil, and the thiadiazine 1,1-dioxide, diazoxide, a series of potent activators of ATP-regulated potassium channels have been synthesized. Selected 3-alkylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxides of the described series bind to the SUR1 component of the SUR1/Kir6.2 K_{ATP} channel and increase channel activity to inhibit glucose-stimulated insulin release. The activation of the K_{ATP} channel seems to be species-independent suggested by the fact that the compounds bind to and activate recombined human K_{ATP} channels and inhibit insulin release from rat islets and from mouse β-cell lines with approximately the same order of potency. Although previous reports have described compounds that are able to suppress insulin release through activation of ATP-regulated potassium channels,[28,29−31] the present compounds, e.g., **30**, **34**, **36**, **37**, **50**, and **53**, seem superior by showing good pharmacokinetic and pharmacodynamic properties. Compounds such as **34** (NNC 55-0118) and **53** (NN414) represent a new class of potassium channel openers highly potent and selective for the SUR1/Kir6.2 K_{ATP} channels both in vitro and in vivo. In addition, **34** is able to reduce plasma insulin levels after per oral administration to normal rats with minimal effect on cardiovascular parameters and therefore could be expected to possess less side effects than diazoxide. Compound **34** has been found to protect β-cells in vitro[32] and in vivo[32,33] and to prevent the development of type 2 diabetes in OLETF rats.[34] The present data strongly suggest that these effects could be mediated through activation of SUR1/Kir6.2 potassium channels. Of particular interest is also compound **54**, which is able to activate the SUR1/Kir6.2 K_{ATP} channels in the nanomolar range. Additional studies are needed for full exploration of the pharmacology and therapeutic potentials of these molecules.

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 parts per million (δ), and splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; sext, sextet; sept, septet; m, multiplet; and br, broad. The 70 eV EI solid mass spectra were recorded on a Finnigan MAT-TSQ 70 mass spectrometer. Liquid chromatography−mass spectrometry (LC−MS) analysis was performed on HP1100 MSD equipped with Waters Xterra MS C-18 × 3 mm column. IR spectra were recorded on a Perkin-Elmer 1600 Series Fourier transform infrared (FT-IR) spectrophotometer. Reactions were followed by thin-layer chromatography (TLC) performed on silica gel 60 F254 (Merck) or ALUGRAMSIL G/UV$_{254}$ (MACHEREY−NAGEL) TLC aluminum sheets. Elemental analyses (C, H, N) were performed by Novo Nordisk, Microanalytical Laboratory, Denmark, and were within ±0.4%.

Ethyl 5-Chloro-2-sulfamoylthiophene-3-carboxylate (6). A solution of **5**[32,36] (60.0 g; 0.201 mol) in 700 mL of absolute EtOH saturated with HCl gas was heated at reflux with stirring for 17 h. The cooled solution was evaporated to dryness, and the residue was triturated with water and dried

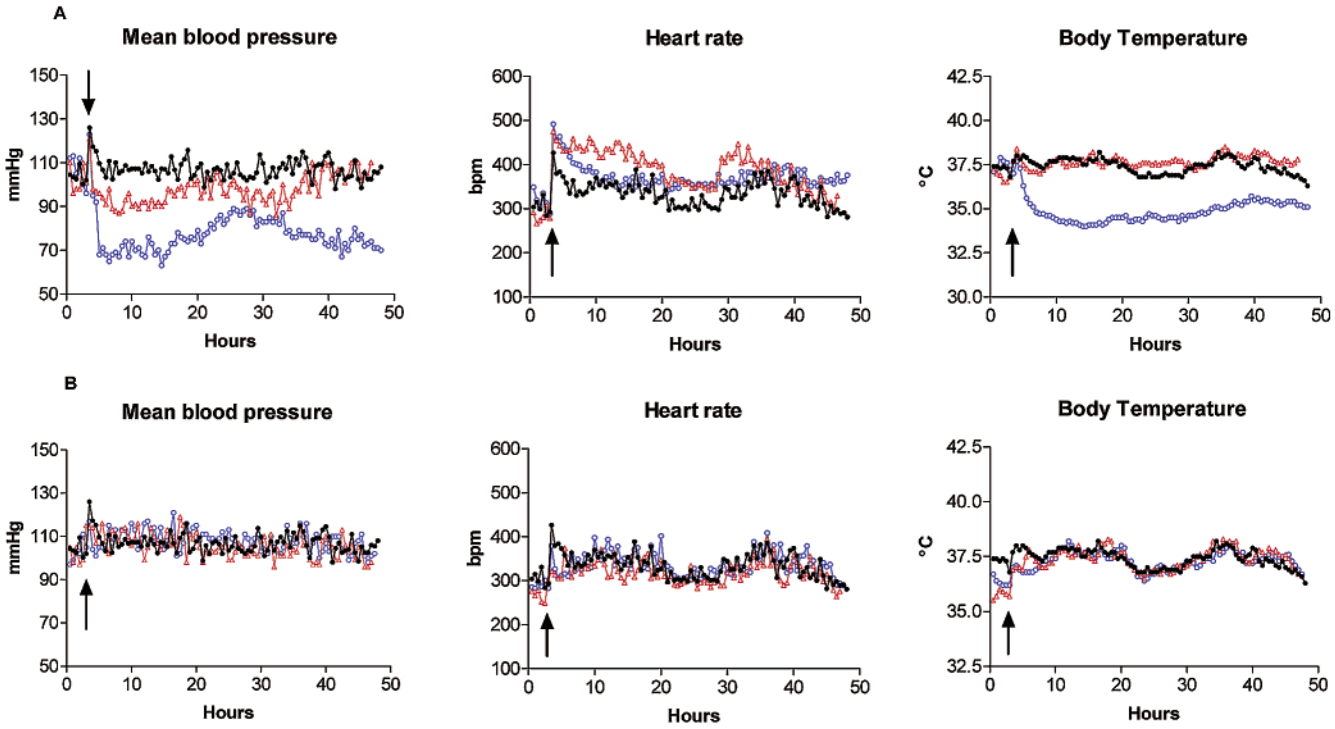


Figure 3. Mean values of mean arterial blood pressure, heart rate, and body temperature from vehicle-treated ($n = 4$) (●), diazoxide-treated ($n = 3$), or **34**-treated ($n = 3$) telemetered rats (100 (△) or 300 mg/kg (○)). Arrows indicate time of dosing. There was a decrease in mean blood pressure and an increase in heart rate at 100 and 300 mg/kg and a decrease in body temperature after 300 mg/kg diazoxide dosing (left panels of A). Compound **34** had no effect on mean blood pressure, heart rate, or body temperature (right panels of B).

to give 52.3 g (96%) of **6**; mp 113−114 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.31 (t, 3H), 4.32 (q, 2H), 7.55 (s, 1H), 7.77 (br s, 2H). LC-MS m/z 270/272 $(M + H)^+$. Anal. ($C_7H_8ClNO_4S_2$) C, H, N.

5-Chloro-3-(hydrazinocarbonyl)thiophene-2-sulfonamide (7). The ethyl ester **6** (50.0 g; 0.185 mol) was added in one portion to 98% hydrazine hydrate (50 mL) with stirring at ambient temperature. The reaction was slightly exothermic. The solution was stirred for 90 min and concentrated in vacuo. The residue was crystallized by trituration with 250 mL of water, and the mixture was acidified with 12.5 N HCl and stirred for 30 min at 0 °C. The product was isolated by filtration, washed with water, and dried to give 42.4 g (89%) of **7**; mp 158−160 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 4.7 (br s, 2H), 7.49 (s, 1H), 7.71 (br s, 2H), 9.94 (br s, 1H). LC-MS m/z 256/258 $(M + H)^+$. Anal. ($C_5H_6ClN_3O_3S_2$) C, H, N.

5-Chloro-2-sulfamoylthiophene-3-carbonyl Azide (8). A solution of sodium nitrite (3.0 g; 43.4 mmol) in 20 mL of water was added dropwise with stirring at 0 °C to a solution of **7** (10.0 g; 39.1 mmol) in 80 mL of 1 N HCl, and the mixture was stirred for 15 min. The precipitate was isolated by filtration, washed with water, and dried in vacuo over phosphorus pentoxide at room temperature to give 9.93 g (96%) of **8**. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 7.55 (s, 1H), 7.97 (br s, 2H). IR (KBr, cm $^{-1}$): 3276 (NH); 2155, 1211 (N_3); 1659 (C═O); 1349, 1173 (SO_2).

6-Chloro-2,3-dihydro-3-oxo-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (9). Carbonyl azide **8** (7.0 g; 26.2 mmol) was added in portions to 50 mL of refluxing dry toluene during 10 min. The mixture was stirred for 5 min and cooled on an ice bath. The precipitate was isolated by filtration, washed with toluene, and dried to give 5.90 g (94%) of **9**; mp 258−260 °C (decomposition with gas evolution). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 6.93 (s, 1H), ca. 8 (very br s, 1H), 11.98 (s, 1H). LC-MS m/z 239/241 $(M + H)^+$. Anal. ($C_5H_3ClN_2O_3S_2$) C, H, N.

3,6-Dichloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (10). Method A. A suspension of **9** (10.75 g, 0.045 mol) in phosphorus oxychloride (100 mL) was cooled on an ice bath, and dry pyridine (7.3 mL, 0.09 mol) was added dropwise at such a rate that the temperature did not exceed 10 °C. The mixture was then heated at 95−100 °C for 16 h and cooled to room temperature. A yellow byproduct was removed by filtration, and the filtrate was concentrated in vacuo. To the residue was added 200 g of ice, and the resulting mixture was stirred for 1 h and filtered. The isolated solid was dissolved in saturated aqueous $NaHCO_3$ (100 mL) and filtered to remove a small amount of insoluble material. After treatment with decolorizing charcoal, the filtrate was acidified with 4 N HCl and the precipitate that formed was filtered, washed with water, and dried to give 5.55 g (48%) of **10**; mp >240 °C (dec). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.08 (s, 1H), no NH signal was seen (hidden in a very broad H_2O peak). EI-MS m/z 256/258/260 (M^+). Anal. ($C_5H_2Cl_2N_2O_2S_2 \cdot 0.5H_2O$) C, H, N.

Method B. A mixture of **9** (1.60 g, 6.7 mmol), phosphorus oxychloride (7 mL), pyridine hydrochloride (2.55 g, 22 mmol), and 85% *ortho*-phosphoric acid (0.47 mL, 4.1 mmol) was heated at 100 °C for 15 h. The mixture was concentrated in vacuo at 50 °C, and the residual oil was carefully treated with ice water (20 mL) with stirring at 0 °C. The crude product was isolated by filtration, washed with water, and dissolved in saturated aqueous $NaHCO_3$ (25 mL). After treatment with decolorizing charcoal, the filtrate was acidified with 12.5 N HCl with stirring at 0 °C and the white precipitate that formed was filtered, washed with water, and dried to give 1.26 g (73%) of **10**.

6-Chloro-3-fluoro-4H-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (11). A mixture of compound **10** (2.57 g, 10 mmol) and anhydrous cesium fluoride (6.09 g, 40 mmol) in dry DMSO (10 mL) was stirred for 18 h at 150 °C in a sealed flask. The cooled mixture was diluted with water (30 mL) and stirred for 5 min before collection of the precipitate. The beige solid was washed with water and dried to give 2.12 g (88%) of crude **11**, which was used without further purification. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.09 (s, 1H), 7.34 (br s, 1H). EI-MS: m/z 240/242 (M^+).

3-Amino-*N*-(*tert*-butyl)-5-chlorothiophene-2-sulfonamide (13). A solution of **12**[36] (38.1 g, 0.15 mol) in dry THF (300 mL) was cooled to −70 °C, and *n*-BuLi (1.6 N in hexane,

190 mL) was added, maintaining the temperature < −65 °C. After this was added, the mixture was allowed to warm to −20 °C and stirred at this temperature for 30 min. A solution of *p*-toluenesulfonyl azide (34 g, 0.17 mol) in dry THF (100 mL) was added, maintaining the temperature at −20 °C, and the cooling bath was removed. After the mixture had reached room temperature, water (100 mL) was added. The organic phase was isolated, and the aqueous phase was extracted with toluene (2 × 50 mL). To the combined organic phases was added hexadecyltributylphosphonium bromide (7.62 g, 15 mmol) followed by the dropwise addition of a solution of sodium borohydride (6.58 g, 0.174 mol) in water (20 mL) with stirring and cooling to room temperature. The mixture was stirred overnight at room temperature, and water (100 mL) was added. The organic phase was isolated, washed with water (2 × 100 mL), dried, and evaporated to dryness. The oily residue was dissolved in EtOAc (150 mL) and washed with 1 N NaOH (6 × 100 mL). The organic phase was dried with Na_2SO_4 and evaporated to afford a quantitative yield (40.6 g) of crude **13** as an oily substance, which was used without further purification in the next step. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.16 (s, 9H), 6.05 (br s, 2H), 6.59 (s, 1H), 7.40 (br s, 1H).

3-Amino-5-chlorothiophene-2-sulfonamide Hydrochloride (14). Compound **13** (40.4 g) was heated with stirring at 50−60 °C in 12.5 N HCl (200 mL) for 2.5 h. The crude product was isolated from the cooled mixture by filtration, dried, and triturated with ether (60 mL) to give 17.8 g (48%) of the hydrochloride salt **14**; mp >165 °C (dec). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 6.64 (s, 1H), 6.84 (very br s, 5H). LC-MS *m*/*z* 213/215 $(M + H)^+$. Anal. ($C_4H_5ClN_2O_2S_2$·HCl 0.5H_2O) C, H, N.

Methyl 5-Chloro-2-sulfamoylthien-3-ylcarbamate (18a). A mixture of carboxylic acid **5**[36] (100.0 g; 0.336 mol) in triethyl orthoformate (700 mL, 4.2 mol) was refluxed overnight. The cooled mixture was concentrated at reduced pressure to a volume of about 150 mL. After it was stirred at 0 °C, the resulting crystalline precipitate was isolated by filtration and dried to give 88.8 g (81%) of ethyl 2-(*tert*-butylamino)sulfonyl-5-chloro-3-thiophenecarboxylate (**16)** as a white solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.20 (s, 9H), 1.30 (t, 3H), 4.32 (q, 2H), 7.47 (br s, 1H), 7.53 (s, 1H).

A solution of crude **16** (10 g, 30 mmol) in NMP (7.5 mL) was treated with hydrazine monohydrate (3 mL, 61.8 mmol) at 20−30 °C under external cooling and stirred overnight at room temperature. Methyl *tert*-butyl ether (25 mL) and water (30 mL) were added to the mixture with stirring, and the pH was adjusted to about 2 with 6 N HCl. After it was stirred for 30 min, the organic phase was isolated and evaporated under reduced pressure to give the crude *N*-(*tert*-butyl)-5-chloro-3-hydrazinocarbonylthiophene-2-sulfonamide (**17),** which was used in the next step without further purification.

A solution of crude **17** (10 g, ∼32 mmol) in MeOH (16 mL) was added dropwise simultaneously with isoamyl nitrite (9 mL, 67 mmol) to a stirred refluxing mixture of MeOH (100 mL) and trifluoroacetic acid (0.4 mL). The mixture was refluxed for an additional hour and then cooled and concentrated at reduced pressure. The resulting residue was hydrolyzed by heating at reflux in a mixture of MeOH (9 mL) and 12.5 N HCl (9 mL) for about 6 h during which about 3 mL of solvent was distilled off. After a further amount of MeOH was added (6 mL), the hot mixture was treated with decolorizing charcoal, filtered, and cooled. The resulting precipitate was isolated by filtration, washed with a small amount of aqueous EtOH, and recrystallized from MeOH to give 1.5 g (18%) of the desired methyl carbamate **18a** as pale yellow crystals; mp 178−180 °C. ^{1}H NMR (DMSO-d_6): δ: 3.73 (s, 3H), 7.58 (s, 1H), 7.85 (s, 2H), 8.89 (s, 1H). Anal. ($C_6H_6ClN_2O_4S_2$) C, H, N.

Ethyl 5-Chloro-2-sulfamoylthien-3-ylcarbamate (18b). Solid carbonyl azide **8** (2.67 g; 10 mmol) was added in portions to absolute EtOH (50 mL) with stirring and gentle reflux on an oil bath. The resulting solution was refluxed for 4 h, cooled to room temperature, and concentrated under reduced pressure. The residue was triturated with water (100 mL), and the resulting white precipitate was isolated by filtration, washed with water, and dried to give 2.15 g (75%) of pure **18b**; mp 132−134 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.26 (t, 3H), 4.18 (q, 2H), 7.60 (s, 1H), 7.86 (br s, 2H), 8.85 (br s, 1H). Anal. ($C_7H_9ClN_2O_4S_2$) C, H, N.

3-Amino-6-chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (27). A suspension of compound **10** (10.0 g, 38.9 mmol) in ammonia (35 mL of 25% solution in water) was stirred for 16 h at 100 °C in a sealed flask. The mixture was cooled, concentrated in vacuo, and filtered. The solid was washed with water, dissolved in 2 N NaOH (30 mL), and treated with decolorizing charcoal. The filtered solution was stirred at 50 °C, 4 N HCl was slowly added until the mixture was slightly acidic, and the resulting suspension was stirred at 50 °C for 30 min and then at room temperature overnight. The precipitate was isolated by filtration, washed with water, and dried in vacuo at 50 °C overnight to give 9.39 g (94%) of **27** as a white solid (monohydrate). Anal. ($C_5H_4ClN_3O_2S_2$·1H_2O) C, H, N. Recrystallization from EtOH followed by drying in vacuo afforded the anhydrous product; mp >350 °C (dec). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.02 (s, 1H), 7.10 (br s, 2H), 11.18 (s, 1H). LC-MS *m*/*z* 238/240 $(M + H)^+$. Anal. ($C_5H_4ClN_3O_2S_2$) C, H, N.

6-Chloro-3-methylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (28). A solution of compound **10** (514 mg, 2 mmol) and methylamine (0.8 mL of 33% solution in EtOH, 6.4 mmol) in absolute EtOH (4 mL) was stirred for 3 days at 55 °C in a sealed flask. The cooled mixture was concentrated in vacuo, and the residue was treated with water and acidified to pH <2 with 1 N HCl. Stirring was continued until the product crystallized, and the solid was isolated by filtration, washed with water, and dried to give 406 mg (80%) of almost pure **28**. An analytical sample was prepared by crystallization from AcOH/EtOH with decolorizing charcoal followed by precipitation from an alkaline solution with 1 N HCl and drying in vacuo at 40 °C; mp >325 °C (dec). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.75 (d, 3H), 7.02 (s, 1H), 7.23 (br s, 1H), 11.2 (br s, 1H). EI-MS *m*/*z* 251/253 (M^+). Anal. ($C_6H_6ClN_3O_2S_2$) C, H, N.

6-Chloro-3-ethylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (29). Phosphorus pentoxide (2.84 g, 20 mmol), *N*,*N*-dimethylcyclohexylamine (3 mL, 20 mmol), and ethylamine hydrochloride (1.63 g, 20 mmol) were carefully mixed and heated with stirring on an oil bath at 180 °C for 20 min. To the homogeneous mass was added the oxo compound **9** (1.2 g, 5 mmol), and the mixture was stirred at 180 °C for 5 h. After it was cooled to about 100 °C, water (150 mL) was carefully added and the mixture was stirred for 1 h at room temperature. The resulting mixture was extracted with EtOAc (3 × 100 mL), and the organic phase was washed with saturated aqueous $NaHCO_3$, dried, and evaporated to dryness. The residue was recrystallized from EtOAc/MeOH to give 282 mg (21%) of pure **29**; mp 271−274 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.11 (t, 3H), 3.22 (quint, 2H), 7.04 (s, 1H), 7.3 (br s, 1H), 11.1 (br s, 1H). EI-MS *m*/*z* 265/267 (M^+). Anal. ($C_7H_8ClN_3O_2S_2$) C, H, N.

6-Chloro-3-propylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (30). A solution of **10** (15 g, 58.3 mmol) and propylamine (40 mL, 0.49 mol) in propan-2-ol (50 mL) was stirred for 20 h at 85 °C in a sealed flask. The cooled mixture was concentrated in vacuo, the residue was treated with water (150 mL) and acidified with 2 N HCl, and stirring was continued until the product crystallized. The solid was isolated by filtration, washed with water, and then dissolved in 1 N NaOH (400 mL) by gentle heating. After treatment with decolorizing charcoal and filtration, the filtrate was cooled and acidified with 6 N HCl. The resulting precipitate was isolated by filtration, washed with water, and recrystallized from EtOH and dried to give 10.72 g (66%) of pure **30** as a white solid; mp 259−260 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.89 (t, 3H), 1.52 (sext, 2H), 3.15 (q, 2H), 7.05 (s, 1H), 7.29 (br s, 1H), 10.95 (br s, 1H). EI-MS *m*/*z* 279/281 (M^+). Anal. ($C_8H_{10}ClN_3O_2S_2$) C, H, N.

3-Butylamino-6-chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (31). Potassium *tert*-butoxide (0.494 g, 4.4

mmol) was added to a solution of compound **14** (0.5 g, 2.0 mmol) in dry dimethyl formamide (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 butyl isothiocyanate (0.29 mL, 2.4 mmol). The mixture was stirred at 0 °C for an additional 0.5 h, the cooling bath was removed, and stirring was continued at room temperature for 30 min. The mixture was concentrated in vacuo at <50 °C, and 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 to give 0.26 g (39%) of *N*-(3-amino-5-chlorothien-2-yl)sulfonyl-*N*′-butylthiourea **15a** (R = $(CH_2)_3CH_3$); mp 115−117 °C (dec). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 0.85 (t, 3H), 1.22 (sext, 2H), 1.48 (quint, 2H), 3.47 (q, 2H), 6.48 (br s, 2H), 6.64 (s, 1H), 8.28 (br t 1H), 11.25 (br s, 1H).

Phosgene (0.44 mL of a 20% solution in toluene, 0.85 mmol) was added dropwise to a solution of **15a** (252 mg, 0.77 mmol) and dry triethylamine (0.214 mL, 1.54 mmol) in dry THF (4 mL) with stirring at 0 °C. The mixture was stirred for 75 min at 0 °C and concentrated in vacuo. The residue was triturated with water, and the precipitate was isolated by filtration, washed with water, and crystallized from EtOAc to give 162 mg (71%) of **31**; mp 218−219 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 0.90 (t, 3H), 1.35 (sext, 2H), 1.50 (quint, 2H), 3.18 (q, 2H), 7.02 (s, 1H), 7.25 (br s, 1H), 10.95 (br s, 1H). EI-MS *m*/*z* 293/295 (M^+). Anal. ($C_9H_{12}ClN_3O_2S_2$) C, H, N.

6-Chloro-3-hexylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (32). Potassium *tert*-butoxide (0.99 g, 8.8 mmol) was added to a solution of compound **14** (1.0 g, 4.0 mmol) in dry DMF (10 mL) with stirring on an ice bath. The resulting mixture was stirred at 0 °C for 10 min before the dropwise addition of hexyl isothiocyanate (0.69 mL, 4.8 mmol). The mixture was stirred at 0 °C for an additional 1 h, the cooling bath was removed, and stirring was continued at room temperature for 30 min. The mixture was concentrated in vacuo, and the residue was taken up in water (50 mL), treated with decolorizing charcoal, and filtered. The filtrate was acidified with AcOH to pH 6 and stirred at 0 °C until the product solidified. The solid was isolated by filtration and dried in vacuo at 40 °C to give 1.02 g (72%) of *N*-(3-amino-5-chlorothien-2-yl)sulfonyl-*N*′-hexylthiourea **15b** (R = $(CH_2)_5CH_3$). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 0.86 (distorted t, 3H), 1.22 (m, 6H), 1.49 (m, 2H), 3.45 (q, 2H), 6.50 (br s, 1H), 6.65 (s, 1H), 8.29 (br t, 1H) 11.3 (br s, 1H).

Phosgene (1.5 mL of a 20% solution in toluene, 3.09 mmol) was added dropwise to a solution of **15b** (1.0 g, 2.81 mmol) and dry triethylamine (0.78 mL, 5.6 mmol) in dry THF (8 mL) with stirring at 0 °C. The mixture was stirred for 1 h at 0 °C and concentrated in vacuo. The residue was triturated with water (50 mL), and the resulting sticky precipitate was isolated by decantation, washed with water, and crystallized from EtOAc to give 302 mg (33%) of **32**; mp 207−209 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 0.87 (distorted t, 3H), 1.28 (m, 6H), 1.51 (m, 2H), 3.17 (q, 2H), 7.05 (s, 1H), 7.30 (br s, 1H), 11.01 (s, 1H). EI-MS *m*/*z* 321/323 (M^+). Anal. ($C_{11}H_{16}ClN_3O_2S_2$) C, H, N.

6-Chloro-3-octylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (33). The title compound was prepared via intermediate **15c** (R = $(CH_2)_7CH_3$) as described for compound **31** using **14** (1.0 g, 4.0 mmol) and octyl isothiocyanate (0.94 mL, 4.8 mmol); yield 0.55 g (39%); mp 202−205 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 0.85 (t, 3H), 1.28 (m, 10H), 1.50 (m, 2H), 3.18 (q, 2H), 7.03 (s, 1H), 7.25 (br s, 1H), 10.2 (br s, 1H). EI-MS *m*/*z* 349/351 (M^+). Anal. ($C_{13}H_{20}ClN_3O_2S_2$) C, H, N.

6-Chloro-3-isopropylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (34). Route A. The title compound **34** was prepared as described for compound **29** using the oxo compound **9** (1.2 g, 5 mmol) and isopropylamine hydrochloride (1.91 g, 20 mmol); yield 150 mg (11%); mp 281−283 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.15 (d, 6H), 3.88 (m, 1H), 7.08 (s, 1H), 7.20 (br d, 1H), 10.74 (br d, 1H). EI-MS *m*/*z* 279/281 (M^+). Anal. ($C_8H_{10}ClN_3O_2S_2$) C, H, N.

Route B. A solution of **10** (3.0 g, 11.67 mmol) in isopropylamine (20 mL) was stirred in a Teflon-lined stainless steel pressure bomb at 100 °C for 30 h. Excess amine was evaporated under reduced pressure, and the residue was stirred in water (50 mL) followed by the addition of 4 N HCl to pH 2. The mixture was stirred on an ice bath for 1 h and the precipitate was filtered off, washed with water, and recrystallized from EtOAc/MeOH to give 2.42 g (74%) of pure **34** as a white crystalline product.

Route D. The title compound **34** was prepared via intermediate **15f** (R = $CH(CH_3)_2$ as described for compound **31** using **14** (0.5 g, 2 mmol) and isopropyl isothiocyanate (0.26 mL, 2.4 mmol); yield 0.41 g (74%).

Route E. K_2CO_3 (0.67 g, 4.9 mmol) was added to a solution of the ethyl carbamate **18b** (1.14 g, 4.0 mmol) in dry acetone (10 mL) with stirring at room temperature. Then, isopropyl isothiocyanate (0.51 mL, 4.8 mmol) was added dropwise and the resulting mixture was stirred at gentle reflux for 5 h. The cooled mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in water (25 mL), acidified with 4 N HCl to pH 2, and stirred at 0 °C until the product solidified. The solid was isolated by filtration, washed with water, and dried in vacuo to give 1.35 g (87%) of *N*-{5-chloro-3-[(ethoxycarbonyl)amino]thien-2-yl}-sulfonyl-*N*′-isopropylthiourea **20** (R = $CH(CH_3)_2$). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.12 (d, 6H), 1.25 (t, 3H), 4.2 (q + m, 3H), 7.67 (s, 1H), 8.49 (br d, 1H), 9.0 (s, 1H).

Phosgene (0.57 mL of a 20% solution in toluene, 1.1 mmol) was added dropwise to a solution of **20** (386 mg, 1 mmol) and dry triethylamine (0.28 mL, 2 mmol) in dry THF (5 mL) with stirring at 0 °C. The mixture was stirred at this temperature for 40 min and concentrated in vacuo. The residue was triturated with water (5 mL), and the resulting sticky precipitate was isolated by decantation, washed with water, and crystallized from EtOH/Et_2O/petroleum ether to give 35 mg (10%) of pure ethyl 6-chloro-3-isopropylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine-4-carboxylate 1,1-dioxide **24** (R = $CH(CH_3)_2$); mp 130−131 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.20 (d, 6H), 1.30 (t, 3H), 3.98 (m, 1H), 4.33 (q, 2H), 7.57 (s, 1H), 8.90 (br d, 1H). An additional crop of **24** (275 mg, 78%) was obtained as a sticky solid by concentration of the organic mother liquor and was used without further purification.

Crude **24** (275 mg, 0.78 mmol) was refluxed in 2 N NaOH (2 mL, 4 mmol) for 17 h. The mixture was cooled to room temperature, and 4 N HCl was added dropwise to pH 2 (gas evolution). The precipitate was isolated by filtration, washed with water, and crystallized from EtOAc/MeOH to give 116 mg (53%) of pure **34**.

(*RS*)-3-*sec*-Butylamino-6-chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (35). A mixture of **10** (0.6 g, 2.33 mmol), *sec*-butylamine (0.25 mL, 2.43 mmol), and K_2CO_3 (0.5 g, 3.62 mmol) in propan-2-ol (2 mL) was stirred at 100 °C for 16 h in a sealed flask. The cooled mixture was concentrated in vacuo, the residue was treated with water (20 mL) and acidified with 1 N HCl, and stirring was continued until the product crystallized. The solid was isolated by filtration, washed with water, and then dissolved in 1 N NaOH (50 mL) by gentle heating. After treatment with decolorizing charcoal and filtration, the filtrate was cooled and acidified with 6 N HCl. The resulting precipitate was isolated by filtration, washed with water, and dried to give 0.42 g (61%) of **35** as a white solid; mp 224−226 °C (EtOH). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 0.88 (t, 3H), 1.12 (d, 3H), 1.48 (m, 2H), 3.69 (m, 1H), 7.07 (s, 1H), 7.14 (br s, 1H), 10.70 (br s, 1H). EI-MS *m*/*z* 293/295 (M^+). Anal. ($C_9H_{12}ClN_3O_2S_2$) C, H, N.

(*R*)-3-*sec*-Butylamino-6-chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (36). The title compound was prepared as described for compound **35** using **10** (10 g, 38.9 mmol) and (*R*)-(−)-*sec*-butylamine (5 mL, 48.8 mmol) by heating at 85 °C overnight; yield 7.22 g (63%); mp 224−225 °C (EtOH). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 0.88 (t, 3H), 1.11 (d, 3H), 1.48 (m, 2H), 3.69 (m, 1H), 7.07 (s, 1H), 7.14 (br s, 1H), 10.75 (br s, 1H). EI-MS *m*/*z* 293/295 (M^+). Anal. ($C_9H_{12}ClN_3O_2S_2$) C, H, N.

(*S*)-3-*sec*-Butylamino-6-chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (37). The title compound was prepared as described for compound **35** using **10** (10 g, 38.9 mmol) and (*S*)-(+)-*sec*-butylamine (4.8 mL, 46.8 mmol) by heating at 120 °C overnight; yield 5.37 g (47%); mp 223−224 °C (EtOH). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.88 (t, 3H), 1.12 (d, 3H), 1.48 (m, 2H), 3.69 (m, 1H), 7.07 (s, 1H), 7.14 (br s, 1H), 10.75 (br s, 1H). EI-MS *m*/*z* 293/295 (M^+). Anal. ($C_9H_{12}ClN_3O_2S_2$) C, H, N.

3-*tert*-Butylamino-6-chloro-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (38). The title compound was prepared as described for compound **28** using **10** (5.0 g, 19.4 mmol) and *tert*-butylamine (20 mL, 0.19 mol) except that the mixture was stirred for 20 h at 125 °C; yield 2.91 g (52%); mp >364 °C (dec) (MeOH). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.37 (s, 9H), 6.79 (br s, 1H), 7.11 (s, 1H), 10.55 (br s, 1H). EI-MS *m*/*z* 293/295 (M^+). Anal. ($C_9H_{12}ClN_3O_2S_2$) C, H, N.

6-Chloro-3-(1,1-dimethylpropylamino)-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (39). The title compound was prepared as described for compound **28** using **10** (5.0 g, 19.4 mmol) and 1,1-dimethylpropylamine (10 mL, 85.7 mmol) except that the mixture was stirred for 30 h at 125 °C; yield 3.38 g (56%); mp 359−360 °C (dec) (MeOH). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.82 (t, 3H), 1.31 (s, 6H), 1.73 (q, 2H), 6.67 (br s, 1H), 7.12 (s, 1H), 10.57 (br s, 1H). EI-MS *m*/*z* 307/309 (M^+). Anal. ($C_{10}H_{14}ClN_3O_2S_2$) C, H, N.

(*RS*)-6-Chloro-3-(1-methylbutyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (40). The title compound was prepared as described for compound **35** using **10** (3 g, 11.7 mmol) and 1-methylbutylamine (2.75 mL, 23.3 mmol) by heating at 120 °C overnight; yield 2.22 g (62%); mp 195−196 °C (EtOH). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 0.90 (t, 3H), 1.13 (d, 3H), 1.20−1.54 (m, 4H), 3.79 (m, 1H), 7.07 (s, 1H), 7.12 (br s, 1H), 10.74 (br s, 1H). EI-MS *m*/*z* 307/309 (M^+). Anal. ($C_{10}H_{14}ClN_3O_2S_2$) C, H, N.

(*RS*)-6-Chloro-3-(1-methylhexyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (41). The title compound was prepared as described for compound **31** using **14** (0.5 g, 2.0 mmol) and 2-isothiocyanatoheptane (0.38 g, 2.4 mmol), except that the intermediate *N*-(3-amino-5-chlorothien-2-yl)-sulfonyl-*N*′-(1-methylhexyl)thiourea **15g** (R = (*RS*)-CH-$(CH_3)(CH_2)_4CH_3$) was isolated by extraction with CH_2Cl_2 (3 × 15 mL) before the cyclization to **41**; yield 180 mg (27%); mp 179−181 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 0.86 (distorted t, 3H), 1.13 (d, 3H), 1.18−1.55 (m, 8H), 3.75 (m, 1H), 7.06 (s, 1H), 7.13 (br s, 1H), 10.74 (s, 1H). EI-MS *m*/*z* 335/337 (M^+). Anal. ($C_{12}H_{18}ClN_3O_2S_2$) C, H, N.

(*RS*)-6-Chloro-3-(1,5-dimethylhexyl)amino-4*H*-thieno-[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (42). The title compound was prepared as described for compound **31** using **14** (1.0 g, 4.0 mmol) and 2-isothiocyanato-6-methylheptane (0.75 g, 4.3 mmol), except that the intermediate *N*-(3-amino-5-chlorothien-2-yl)sulfonyl-*N*′-(1,5-dimethylhexyl)thiourea **15i** (R = (*RS*)-$CH(CH_3)(CH_2)_3CH(CH_3)_2$) was isolated as an oil by extraction with Et_2O (3 × 30 mL) and purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 20:1) before the final cyclization step to **42**; yield 133 mg (10%); mp 197−199 °C (EtOAc). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.85 (d, 6H), 1.12 (d, 3H), 1.1−1.6 (m, 7H), 3.77 (m, 1H), 7.07 (s, 1H), 7.12 (br s, 1H), 10.72 (br s, 1H). LC-MS *m*/*z* 350/352 (M^+). Anal. ($C_{13}H_{20}ClN_3O_2S_2$) C, H, N.

(*RS*)-6-Chloro-3-(1,2-dimethylpropyl)amino-4*H*-thieno-[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (43). A solution of **10** (0.5 g, 1.94 mmol) in 1,2-dimethylpropylamine (5 mL) was stirred for 48 h at 100 °C in a sealed flask. The cooled mixture was concentrated under reduced pressure, and the residue was stirred with water (20 mL) followed by adjustment to pH 2 with 4 N HCl. The initially formed sticky mass crystallized by stirring the mixture for about 2 h at 0 °C. The precipitate was isolated by filtration, washed with water, and recrystallized from EtOAc/MeOH followed by drying in vacuo at 60 °C overnight to give 0.43 g (72%) of pure **43**; mp 216.5−218 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 0.90 (d, 6H), 1.08 (d, 3H), 1.75 (m, 1H), 3.65 (m, 1H), 7.11 (br s, 2H), 10.68 (s, 1H). EI-MS *m*/*z* 307/309 (M^+). Anal. ($C_{10}H_{14}ClN_3O_2S_2$) C, H, N.

Isolation of Optically Active Compounds. The enantiomers of **43** (**44** and **45**) were separated by high-performance liquid chromatography (HPLC) using Chiralpak AS with a mobile phase consisting of heptane/EtOH (50:50) at a flow rate of 0.7 mL/min at ambient temperature. A UV detector set to 225 and 254 nm was used, and the first enantiomer to elute was **44**. Chiral purity was determined to be 100% ee for **44** and 99.2% ee for **45**. The absolute configuration of the enantiomers was determined by chiral HPLC analysis of **43** as compared with an authentic sample of **45** prepared from (*R*)-1,2-dimethylpropylamine.

(*S*)-6-Chloro-3-(1,2-dimethylpropyl)amino-4*H*-thieno-[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (44). mp 212−214 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.90 (d, 6H), 1.08 (d, 3H), 1.75 (m, 1H), 3.65 (m, 1H), 7.11 (br s, 2H), 10.68 (s, 1H). Anal. ($C_{10}H_{14}ClN_3O_2S_2$) C, H, N.

(*R*)-6-Chloro-3-(1,2-dimethylpropyl)amino-4*H*-thieno-[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (45). mp 218−219 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.90 (d, 6H), 1.08 (d, 3H), 1.75 (m, 1H), 3.65 (m, 1H), 7.11 (br s, 2H), 10.68 (s, 1H). Anal. ($C_{10}H_{14}ClN_3O_2S_2$) C, H, N.

6-Chloro-3-(2-ethylbutylamino)-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (46). The title compound was prepared as described for compound **28** using **10** (514 mg, 2.0 mmol) and 2-ethylbutylamine (0.41 mL, 4.0 mmol) except that the mixture was stirred at 120 °C; yield 0.24 g (37%); mp 258−259 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.88 (t, 6H), 1.29 (quint, 4H), 1.46 (sept, 1H), 3.11 (t, 2H), 7.08 (s, 1H), 7.17 (br, 1H), 10.86 (br s, 1H). EI-MS *m*/*z* 321/323 (M^+). Anal. ($C_{11}H_{16}ClN_3O_2S_2$) C, H, N.

(*RS*)-6-Chloro-3-(2-methylbutyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (47). The title compound was prepared as described for compound **28** using **10** (9.0 g, 35 mmol) and 2-methylbutylamine (8.5 mL, 72 mmol), except that the mixture was stirred at 120 °C overnight; yield 7.15 g (66%); mp 240−241 °C (EtOH). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.86 (d, 3H), 0.87 (t, 3H), 1.13 (m, 1H), 1.39 (m, 1H), 1.62 (m, 1H), 3.08 (m, 2H), 7.08 (s, 1H), 7.25 (br, 1H), 10.90 (br s, 1H). EI-MS *m*/*z* 307/309 (M^+). Anal. ($C_{10}H_{14}ClN_3O_2S_2$) C, H, N.

(*RS*)-6-Chloro-3-(1,2,2-trimethylpropyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (48). The title compound was prepared as described for compound **28** using **10** (200 mg, 0.78 mmol) and 1,2,2-trimethylpropylamine (0.50 mL, 3.78 mmol), except that the mixture was stirred at 110 °C for 17 h; yield 137 mg (55%); mp 294−295 °C (EtOAc). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.90 (s, 9H), 1.06(d, 3H), 3.68 (m, 1H), 6.90 (br s, 1H), 7.12 (s, 1H), 10.65 (s, 1H). EI-MS *m*/*z* 321/323 (M^+). Anal. ($C_{11}H_{16}ClN_3O_2S_2$) C, H, N.

6-Chloro-3-cyclopropylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (49). The title compound was prepared as described for compound **35** by heating a mixture of **10** (3.0 g, 11.7 mmol), cyclopropylamine (1.62 mL, 23.3 mmol), and K_2CO_3 (3.2 g, 23.3 mmol) in propan-2-ol (20 mL) for 18 h at 80 °C in a sealed flask to give 2.17 g (67%) of **49** as a beige solid; mp 272−273 °C (EtOAc/MeOH). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.52−0.61 (m, 2H), 0.75−0.82 (m, 2H), 2.54−2.65 (m, 1H), 7.11 (s, 1H), 7.89 (br s, 1H), 10.95 (br s, 1H). EI-MS *m*/*z* 277/279 (M^+). Anal. ($C_8H_8ClN_3O_2S_2$) C, H, N.

6-Chloro-3-cyclobutylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (50). The title compound was prepared as described for compound **28** using **10** (3.0 g, 11.7 mmol) and cyclobutylamine (5 g, 70 mmol) except that the mixture was stirred for 20 h at 100 °C; yield 2.79 g (82%); mp 313−314 °C (dec) (EtOH/water). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.58−1.75 (m, 2H), 1.89−2.05 (m, 2H), 2.19−2.30 (m, 2H), 4.16 (m, 1H), 7.06 (s, 1H), 7.62 (br s, 1H), 10.83 (br s, 1H). EI-MS *m*/*z* 291/293 (M^+). Anal. ($C_9H_{10}ClN_3O_2S_2$) C, H, N.

6-Chloro-3-cyclopentylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (51). The title compound was prepared as described for compound **31** using **14** (2.0 g, 8.0 mmol) and cyclopentyl isothiocyanate (1.22 g, 9.6 mmol); yield 1.35

g (57%); mp 299−300 °C (EtOH). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.40−1.70 (m, 6H), 1.90 (m, 2H), 3.95 (sext, 1H), 7.05 (s, 1H), 7.3 (br, 1H), 10.70 (br s, 1H). EI-MS *m*/*z* 305/307 (M^+). Anal. ($C_{10}H_{12}ClN_3O_2S_2$) C, H, N.

6-Chloro-3-cyclohexylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (52). The title compound was prepared as described for compound **31** using **14** (1.0 g, 4.0 mmol) and cyclohexyl isothiocyanate (0.656 mL, 4.8 mmol); yield 0.65 g (51%); mp 282−284 °C (EtOH/MeOH). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.1−1.9 (m, 10H), 3.55 (m, 1H), 7.08 (s, 1H), 7.19 (br, 1H), 10.73 (br s, 1H). EI-MS *m*/*z* 321/319 (M^+). Anal. ($C_{11}H_{14}ClN_3O_2S_2$) C, H, N.

6-Chloro-3-(1-methylcyclopropyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (53). Route B. A solution of **10** (386 mg, 1.5 mmol) in 1-methylcyclopropylamine[63](1.0 g, 14 mmol) was stirred for 24 h at 85 °C in a sealed flask. The cooled solution was concentrated in vacuo, and the residue was stirred with EtOAc (1−2 mL) and filtered. The white precipitate was stirred in 4 N HCl (5 mL) for 2 h and then filtered off and chromatographed on silica gel with ethyl acetate to give 112 mg (26%) of pure **53**; mp 251−252 °C dec (MeOH/water). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.65−0.79 (m, 4H), 1.36 (s, 3H), 7.11 (s, 1H), 7.82 (br s, 1H), 10.78 (br s, 1H). EI-MS *m*/*z* 291/293 (M^+). Anal. ($C_9H_{10}ClN_3O_2S_2$) C, H, N.

Route C. A solution of the fluoro compound **11** (145 mg, 0.6 mmol), 1-methylcyclopropylamine hydrochloride[64] (78 mg, 0.72 mmol), and triethylamine (0.184 mL, 1.32 mmol) in ethanol (0.5 mL) was stirred for 21 h at 60 °C in a sealed flask. The cooled mixture was concentrated in vacuo, and the residue was triturated with water (3 mL) followed by adjustment to pH <2 with 4 N HCl. The crude sticky product was dissolved in 1 N NaOH (4 mL), treated with decolorizing charcoal, and filtered. The filtrate was acidified with 4 N HCl, and the resulting precipitate was isolated by filtration, washed with water, and recrystallized from AcOH to give 95 mg (54%) of **53**.

6-Chloro-3-(1-methylcyclobutyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (54). A mixture of the fluoro compound **11** (1.3 g, 5.3 mmol), 1-methylcyclobutylamine hydrochloride[65] (1.0 g, 8.1 mmol), and triethylamine (2.5 mL, 18.1 mmol) in EtOH (10 mL) was stirred for 16 h at 50 °C and then for 5 h at 70 °C in a sealed flask. The cooled mixture was concentrated in vacuo, and the residue was triturated with water (25 mL) followed by adjustment to pH <2 with 1 N HCl. The crude product was isolated by filtration, recrystallized from acetic acid, and finally purified by chromatography (C18; 20−60% acetonitrile + 0.01% TFA) to give 363 mg (22%) of **54**; mp 294−296 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.47 (s, 3H), 1.75−1.88 (m, 2H), 1.94−2.05 (m, 2H), 2.18−2.31 (m, 2H), 7.07 (s, 1H), 7.33 (br s, 1H), 10.67 (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-(1-methylcyclopentyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (55). A mixture of **11** (0.60 g, 2.5 mmol), 1-methylcyclopentylamine hydrochloride[66,67] (0.5 g, 3.7 mmol), and triethylamine (1.03 mL, 7.4 mmol) in EtOH (2.5 mL) was stirred for 16 h at 50 °C and then for 24 h at 65 °C in a sealed flask. The cooled mixture was concentrated in vacuo, and the residue was triturated with water followed by adjustment to pH <2 with 1 N HCl. The crude product was isolated by filtration, dried, and recrystallized from AcOH to give 208 mg (26%) of **55**; mp >300 °C (dec). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.43 (s, 1H), 1.53−1.72 (m, 6H), 1.92−2.10 (m, 2H), 6.91 (br s, 1H), 7.10 (s, 1H), 10.52 (br s, 1H). LC-MS *m*/*z* 320/322 $(M + 1)^+$. Anal. ($C_{11}H_{14}ClN_3O_2S_2$) C, H, N.

6-Chloro-3-(1-methylcyclohexyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (56). A mixture of **11** (0.5 g, 2.08 mmol), 1-methylcyclohexylamine hydrochloride[66,67] (373 mg, 2.49 mmol), and triethylamine (0.58 mL, 4.16 mmol) in EtOH (3 mL) was stirred for 20 h at 50 °C and then for 22 h at 100 °C in a sealed flask. The cooled mixture was concentrated in vacuo, and the residue was triturated with water followed by adjustment to pH <2 with 4 N HCl. The crude product was isolated by filtration and dissolved in 1 N NaOH followed by treatment with decolorizing charcoal. After it was filtered, the solution was acidified to pH <2 with 4 N HCl and the precipitate was filtered off and purified by chromatography (dichloromethane/MeOH (19:1)). Recrystallization from EtOH afforded 55 mg (8%) of pure **56**; mp 218−219 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.18−1.54 (m, 11H), 1.97−2.12 (m, 2H), 6.55 (br s, 1H), 7.12 (s, 1H), 10.60 (br s, 1H). LC-MS *m*/*z* 334/336 $(M + 1)^+$. Anal. ($C_{12}H_{16}ClN_3O_2S_2$) C, H, N.

6-Chloro-3-(1-ethylcyclobutyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-Dioxide (57). A mixture of **10** (1.02 g, 3.95 mmol), freshly activated KF (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 the fluoro compound **11**. The mixture was allowed to cool to room temperature and then reacted directly with 1-ethylcyclobutylamine hydrochloride (0.8 g, 5.93 mmol) (prepared from 1-ethylcyclobutanecarboxylic acid[68] analogously to 1-methylcyclopropylamine hydrochloride described by Kirmse[64]) and triethylamine (1.65 mL, 11.9 mmol) for 3 days at 75 °C in the sealed flask. The cooled mixture was poured into water, acidified to pH <2 with 1 N HCl, and extracted with ethyl acetate. The organic phase was dried with Na_2SO_4, evaporated to dryness, and recrystallized from AcOH to give 214 mg (17%) of pure **57**; mp 244−246 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.79 (t, 3H), 1.70−1.93 (m, 4H), 1.96−2.08 (m, 2H), 2.13−2.25 (m, 2H), 7.09 (s, 1H), 7.24 (br s, 1H), 10.57 (br s, 1H). LC-MS *m*/*z* 320/322 $(M+1)^+$. Anal. ($C_{11}H_{14}ClN_3O_2S_2$) C, H, N.

LogP−p*K*$_a$ Measurements. Potentiometric titration experiments were conducted on a GLpK_a instrument from Sirius Analytical Instruments Ltd. The determination of the ionization constant of the compound was performed in the following way: approximately 3 mg of the compound was weighed into a small glass vial and placed in a temperature-controlled sample holder. A 10 mL amount of cosolvent (80% MeOH) was added, and the pH was adjusted to approximately 2. Three titrations were performed in the range of pH 2 to 10.5 on the same sample, adding 3.00 mL of 0.15 M KCl before the second titration and 3.00 mL of 0.15 M KCl before the third. For each titration, a p$_s K_a$ value was measured by refining of the data. The pK_a value at 0% cosolvent was determined by using a Yasuda−Shedlovsky extrapolation procedure on the p$_s K_a$ values determined for the three measured difference curves.

The determination of the octanol/water distribution coefficient, logP, of the compound was performed in the following way: approximately 3 mg of the compound was weighed into a small glass vial and placed in the temperature-controlled sample holder. A 10.0 mL amount of 0.15 M KCl and 0.1 mL of octanol were added. The pH was adjusted to 10.5 and titrated to pH 2.0. Three titrations were performed on the same sample, adding 1.00 mL of octanol before the second titration and 10.00 mL before the third. Prior to data refinement, the measured value for the aqueous pK_a had to be entered into the GLpK_a software. For each titration, the shift in the pK_a value was measured. The three refined data sets were combined in a multiset, and the octanol/water distribution coefficient was extracted according to the possible partitioning equations. The Moriguchi LogP (mLogP) was calculated using commercial available software.[44]

Effects on Membrane Potential in *β*TC3 Cells. Measurement of $DiBAC_4(3)$ fluorescence was made using the procedure described by Lebrun et al.[30] For each compound and concentration (0.01, 0.1, 1, 10, and 100 μMol/L), a total of four individual wells was used to construct the data sets underlying the values in Tables 2−4. Briefly, data were normalized to the initial value for each well and expressed as 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 *β*TC6 Cells. βTC6 cells[69] were cultured at 5×10^4 cells/microtiter well in DMEM + 10% fetal calf serum (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, $2H_2O$, 2.5; HEPES, 10) supplemented with 0.1% bovine serum albumin (BSA) and incubated for 60 min in this buffer. All wells were aspirated, and the cells were incubated for 3 h with NN buffer, 22 mM glucose, 0.1 mM IBMX, and serial dilutions of the compounds. A reference compound 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 enzyme-linked immunosorbent assay (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, and 11 mM glucose. The islets were handpicked and placed at 10 islets/microtiter well and cultured overnight in DMEM, 10% FCS, and 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 Smooth Muscle In Vitro. (a) Aorta and Bladder. Female Wistar rats weighing approximately 150−200 g were killed by cervical dislocation, and the thoracic aorta or bladder body was removed. Aorta and bladder were cut into rings of 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 or 0.5 g (for aorta and bladder, respectively) 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; and 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 either by 0.3 μM phenylephrine or 10 μM bethanechol, in aorta and bladder, respectively. All test compounds were freshly dissolved in DMSO. Potency of a compound (EC_{50}), with regard to producing smooth muscle relaxation, was defined, as the concentration required obtaining 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). Data generated in bladder smooth muscle were corrected for time decay of the bethanechol-induced contractile response.

(b) Mesenteric Resistance Arteries. Adult male Wistar rats (aged 12−16 weeks) were killed by a sharp blow on the head and cervical dislocation. The mesentery was removed and immersed in cold (4 °C) Krebs Ringer solution. Third to fourth order side branches were dissected from the superior mesenteric artery. Arteries were chemically sympathectomized by incubation in 6-hydroxydopamine.[70] Vessels were then mounted in a wire myograph at 37 °C, in Krebs Ringer solution ($CaCl_2$, 2.5; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; NaCl, 118.5; $NaHCO_3$, 25.0; and glucose, 5.5, all in mM) and continuously aerated with 95% O_2/5% CO_2. Arteries were normalized[71] to find their individual optimal lumen diameter for isometric force development. For all test compounds, cumulative concentration response curves were constructed in vessels precontracted with 10 μM phenylephrine. Data were analyzed as described above.

[^{3}H]Glibenclamide Binding. HEK293 cells stably expressing SUR1/Kir6.2 were grown in DMEM with 1% penicillin/streptomycin, 0.6 mg/mL G418, and 10% FCS. Cells were washed with phosphate-buffered saline (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 and 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): [^{3}H]glibenclamide (1 nM, New England Nuclear, NET1024), test compound, and assay buffer (30 mM HEPES, 2 mM $MgCl_2$, 2 mM Na_2-ATP, pH 7.4) to a final volume of 250 μL was mixed. 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 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, California, U.S.A.

***β*-Cell Selectivity 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.[72] 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) coupled to a pressure transducer (Statham P23XC) and a pressure coupler (HSE 561, Hugo Sachs Elektronik Kg, Germany) was introduced into the carotid artery to measure blood pressure (MAP), which was recorded continuously and fed to a plotter (Linearcorder Mark VII, WR 3310, Hugo Sachs Elektronik Kg, Germany) and a computer and analyzed offline. 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, body weight about 180 g, rats 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, rats were given 0.5 mg/kg of **34**, **39**, **50**, or diazoxide and 0.89 mg/kg of **30**, **36**, **38**, or **54**. For oral administration, 1.89 mg/kg of **30**, **36**, **37**, or **53** and 2.0 mg/kg of **34, 39, 50**, or diazoxide were used. Dosage vehicle for the compounds was potassium phosphate buffer 0.1 M, pH 8.0, except for **34** for which vehicle was PEG 300, 20−30% in water and for diazoxide the vehicle was PEG 300, 40%, and Cremophor, 10%, in water. The plasma concentrations of dosed compounds were determined by an LC/MS/MS method. The concentration−time data sets were analyzed by noncompartmental pharmacokinetic methods based on statistical moments theory.

In Vivo Effect of Orally Administered 34 on Pancreatic Function in Glucose-Challenged Rats. Groups of male Sprague−Dawley rats, body weight about 360 g, were fasted overnight and dosed orally with **34** (30 mg/kg, $n = 3$/group) 3−12 h prior to experimentation or with vehicle at 3 h before ($n = 6$). Vehicle consisted of 1:1:4:4 (v/v) glycerol/water/0.5% gelatine solution/10% methylhydroxypropyl-cellulose E3, and a dose volume of 2 mL/kg was used. Before administration of a glucose challenge, rats were anaesthetized with Hypnorm and Dormicum.[72] A 200 μL blood sample was taken from the retro-orbital plexus into chilled, heparinized tubes for deter-

mination of plasma glucose (EBIO Plus autoanalyzer; Eppendorff, Germany) and plasma insulin by ELISA as above. Glucose was injected at four different sc sites at a load of 2 g/6 mL/kg, and a second blood sample was collected 15 min later. Statistical analysis was done using one way analysis of variance (ANOVA) with Bonferroni's post hoc test.

In Vivo Measurement of Cardiovascular Parameters in Telemetered Sprague−Dawley Rats. Female 12−18 month Mol:SPRD rats (M&B Ltd., Ll.) weighing 250−350 g were used. For insertion of the telemetry probe, which was done at least 2 months before the start of the experiments, each rat was anaesthetized with Isofluran. The abdomen of the rat was opened with a 3−5 cm long incision. A catheter was inserted in the aorta (1.5 cm), and the catheter was sealed to the aorta by the use of surgical glue. Hereafter, the telemetry probe (TL11M2-C50-PXT, Data Sciences International, St. Paul, MN) was placed in the abdomen. Between and during the study, the rats were pair-housed (only one rat with sensor) in type III cages with aspen bedding. The rats were fed Altromin 1324 (Brogaarden, Gentofte, Denmark) and water ad libitum. Lights were on from 6:00 am to 6:00 pm with no twilight periods, and room air was changed 8−15 times per hour.

Data acquisition was performed by using PONEMAH and cardiovascular parameters, and body temperature was followed continuously throughout the experiment. Mean values (every 30 min) were calculated.

Supporting Information Available: Assay method and table of $^{86}Rb^+$ efflux data for compounds **27**−**33** and diazoxide. Synthetic procedures and table of biological data for additional compounds **58**−**67**. This material is available free of charge via the Internet at http://pubs.acs.org.

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