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Synthesis of Modified 4*H*-1,2,4-Benzothiadiazine-1,1-dioxides and Determination of their Affinity and Selectivity for Different Types of K_{ATP} Channels

Stefan Lachenicht,[a] Andreas Fischer,[a] Claas Schmidt,[b] Marcus Winkler,[c] Achim Rood,[b] Horst Lemoine,[b] and Manfred Braun*[a]

Dedicated to Professor Johann Mulzer on the occasion of his 65th birthday

4*H*-1,2,4-Benzothiadiazine-1,1-dioxides with various substituents in positions 3, 5, and 7 were synthesized and tested as K_{ATP} channel agonists in artificial cell systems (CHO cells transfected with SUR1/Kir6.2, and HEK 293 transfected with SUR2B/Kir6.1) as model systems for insulin-secreting pancreatic β-cells and for smooth muscle cells, respectively. The effects of agonists were tested in intact cells using DiBAC4(3) [bis-(1,3-dibarbituric acid)trimethine oxanol] as a membrane potential dye, and the results compared with their binding affinity for the SUR2B-type K_{ATP} channels using the radioligand [^{3}H]P1075. Compounds with cycloalkyl and (cycloalkyl)methyl side chains in position 3 had higher affinities towards the SUR2B/Kir6.1 receptor compared with the parent compound diazoxide (**1a**). Compounds with bulky, nonpolar residues in position 3 exhibited remarkable selectivity for SUR2B-type K_{ATP} channels. The compound substituted with a bulky (1-adamantyl)methyl residue exhibited micromolar affinity and activity on SUR2B-type K_{ATP} channels without being able to activate the SUR1-type K_{ATP} channels.

Introduction

The so-called K_{ATP} channels are ATP-sensitive potassium channels that regulate the flux of potassium ions through the cell membrane and control the membrane potential. By regulating the potassium-ion permeability, they link the metabolic state of the cell to the excitability of the membrane. K_{ATP} channels consist of octamers of two membrane proteins: the sulfonylurea receptor (SUR) and the inward rectifying potassium channel (Kir). Four Kir (Kir6.x) and four SUR subunits associate to form an effective K_{ATP} channel. Kir is known to occur in two subtypes, Kir6.1 and Kir6.2, whereas the sulfonylurea receptors fall into three subtypes (SUR1, SUR2A, and SUR2B). K_{ATP} channels are incorporated in various cell types, such as insulin-secreting pancreatic β-cells (SUR1/Kir6.2), cardiac cells (SUR2A/Kir6.2) and smooth muscle cells (SUR2B/Kir6.1).[1–4] A series of drugs that target those cells by activating K_{ATP} channels, the so-called potassium-channel openers (PCOs or KCOs), have been discovered and developed during the last decade. Among them, diazoxide (**1a**), featuring the 4*H*-1,2,4-benzothiadiazine-1,1-dioxide skeleton, is an intensively studied compound.[5–8]

In a nonselective manner, diazoxide activates K_{ATP} channels of β-cells (SUR1/Kir6.2) and smooth muscle cells (SUR2B/Kir6.1), whereas it causes only a weak stimulation of heart cell K_{ATP} channels (SUR2A/Kir6.2). The drug is effective in the treatment of hyperinsulinemic obesity, but wider application is hampered by substantial side effects such as edema and headaches. As a consequence, a large variety of derivatives of diazoxide have been synthesized and studied as potential PCOs. Among them, compounds with the intact 4*H*-1,2,4-benzothiadiazine 1,1-dioxide skeleton (**2**)[9] and also pyridine (**3**) and thiophene (**4**) annulated analogues featuring a guanidine moiety were investigated (Figure 1).[9–12] The latter class of compounds attracted

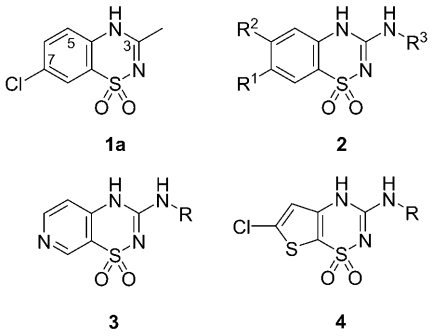


Figure 1. Potassium channel openers based on the thiadiazine-*S*,*S*-dioxide skeleton.

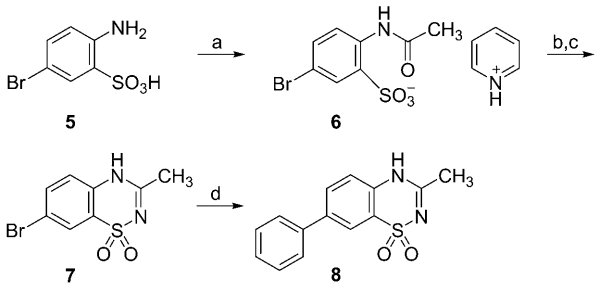
[a] *Dr. S. Lachenicht, A. Fischer, Prof. Dr. M. Braun*
Institute of Organic & Macromolecular Chemistry, University of Düsseldorf
Universitätsstr. 1, 40225 Düsseldorf (Germany)
Fax: (+49) 211-81-15079
E-mail: braunm@uni-duesseldorf.de

[b] *C. Schmidt, A. Rood, Prof. Dr. H. Lemoine*
Institute of Laser Medicine, Molecular Drug Research
University of Düsseldorf (Germany)

[c] *M. Winkler*
Institute of Pharmacology & Toxicology, Medical Faculty
University of Tübingen (Germany

attention because some of the thiophene annulated compounds exhibited nanomolar affinities for the SUR1 type K_{ATP} channels with significant lower effects on the SUR2B type. The reanalysis of these compounds on SUR2B type K_{ATP} channels, however, surprisingly revealed that compounds **4** with a 1-methylcycloalkyl residue (R) acted as agonists, whereas cycloalkyl derivatives missing the 1-methyl substituent turned out to be antagonists.[13,14]

For this reason we designed and synthesized various new derivatives of diazoxide (**1a**) and tested them for affinity and selectively in both types of K_{ATP} channels, focusing in our study on the influence of size and shape of the cycloalkyl side chain in the 3 position.

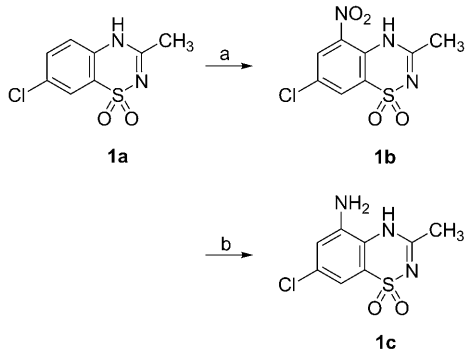


Scheme 2. Synthesis of 7-phenyl-benzothiadiazine-*S,S*-dioxide **8**. *Reagents and conditions*: a) Ac_2O, pyridine, RT, 84%; b) PCl_5, 58–60 °C; c) NH_3, H_2O, 0 °C, 54% (two steps); d) $PhB(OH)_2$; Cs_2CO_3, $Pd(dppf)_2Cl_2$, $MeOCH_2CH_2OMe$, H_2O, reflux, 50%.

Results and Discussion

Synthesis

Three series of diazoxide (**1a**) analogues were studied. In the first group of analogues, derivatives of **1a–c** with additional substituents in position 5 were synthesized. In a second class of compounds (**7** and **8**), different substituents were introduced in position 7. In a third series, the 3-methyl group of diazoxide was replaced by various substituents that differ in their steric and electronic properties. The corresponding compounds either featured the 7-chloro substituent (**13**) or were unsubstituted in this position (**12**). Thus, diazoxide (**1a**) was first derivatized to give 5-nitrodiazoxide (**1b**) and 5-aminodiazoxide (**1c**) according to known procedures,[15] as outlined in Scheme 1.



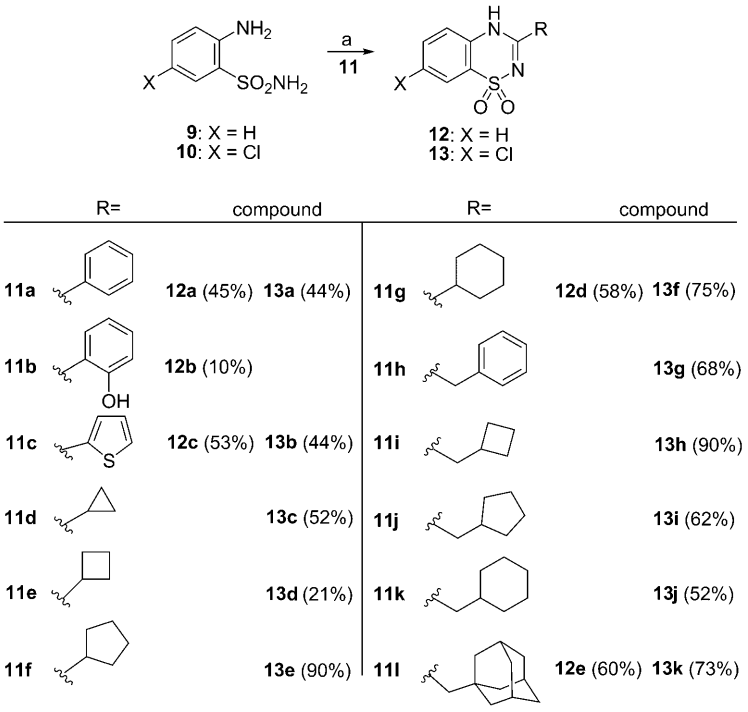
Scheme 1. Synthesis of 5-nitro- and 5-amino-diazoxide **1b** and **1c**.[15] *Reagents and conditions*: a) fumic HNO_3, 58%; b) Fe, NH_4Cl, MeOH, H_2O, 46%.

For the synthesis of 7-bromo-substituted benzothiadiazine-*S,S*-dioxide **7**, the cyclization of the pyridinium salt **6** turned out to be the most efficient method.[16] Thus, treatment of sulfonic acid **5**[17] with acetic anhydride in the presence of pyridine delivered the sulfonate salt **6**, as shown in Scheme 2. The subsequent reaction with phosphorus pentachloride followed by treatment of the resultant sulfonyl chloride with aqueous ammonia led to the formation of 7-bromo-benzothiadiazine-*S,S*-dioxide **7**[18] in 45% overall yield. While numerous derivatives of diazoxide with hetero substituents in positions 6 and 7 have been prepared, we were interested in whether the PCO activity would still be maintained in an aryl-substituted diazoxide. For this purpose, 7-bromo-benzothiadiazine-*S,S*-dioxide **7** was submitted to a Suzuki–Miyaura coupling[19] with phenyl boronic acid to give the phenyl-substituted diazoxide derivative **8** in 50% yield.

In the third series, benzothiadiazine-*S,S*-dioxide derivatives were prepared, wherein the 3-methyl group is replaced by various aryl, heteroaryl and cycloalkyl substituents that were attached directly or through a methylene spacer to the thiadiazine-*S,S*-dioxide skeleton. Following the protocol of Imai, Mochizuki and Kakimoto,[20] 2-aminobenzenesulfonamide (**9**) and 5-chloro-2-aminobenzene-sulfonamide (**10**) were condensed with carboxylic acids **11** in the presence of polyphosphoric acid trimethylsilylester (PPSE) to give the new benzothiadiazine-*S,S*-dioxides **12b**, **12e** and **13h–k** in addition to known compounds **12a**, **12c**, **12d**, and **13a–g**,[20,21] as shown in Scheme 3. The yields in the individual condensation reactions varied considerably. The particularly low yield of the phenolic compound **12b** may be due to undesired polymerization reactions. Generally, fair yields of the derivatives **12** and **13** were reached in the one-step protocol using commercially available starting materials **9**–**11**.

in vitro biological studies

To determine the envisaged activity and selectivity on different K_{ATP} channels, two artificial cell lines were used, CHO (SUR1/Kir6.2) and HEK 293 (SUR2B/Kir6.1) stably expressing the β-cell type and the smooth muscle type K_{ATP} channel, respectively. Using fluorescent membrane potential dyes, such as DiBAC4(3), it is possible to measure the drug-induced hyperpolarization by increasing the K^+ outward current that is the necessary prerequisite to reduce the Ca^{2+}-ion influx. This decrease, in turn, reduces insulin release by β-cells (SUR1/Kir6.2) and relaxation of smooth muscle (SUR2B/Kir6.1). The use of multichannel systems based on fluorescence techniques markedly facilitates testing of drug action because a manifold of cells of the same age cultivated in 12-well strips can be tested in parallel, thereby increasing the reliability of results with minimal experimental effort. All compounds were assayed for K_{ATP}



Scheme 3. Synthesis of benzothiadiazine-*S,S*-dioxides **12** and **13** modified in position 3. *Reagents and conditions*: a) RCO_2H (**11a–l**), PPSE, 160 °C, (isolated yields given in parentheses).

activity as shown in the representative experiment with the cyclopropyl derivative **13c** (Figure 2) demonstrating the exponential decline of the membrane potential with time induced by increasing concentrations of compound **13c**. The agonistic effects of the compounds were reversed upon addition of glibenclamide (not shown), a K_{ATP} channel inhibitor, confirming the involvement of K_{ATP} channels in the mechanism-of-action.

In both cell systems, diazoxide (**1a**) induced full agonistic effects as shown in Figure 3. The potency of compounds measured as the negative logarithm of that drug concentration that induces half maximum effect (pEC_{50}; $-\log$ [M]) was slightly higher (0.2–0.3 log units) in HEK 293 (SUR2B/Kir6.1) cells than in CHO (SUR1/Kir6.2) cells. This corresponds to previous studies[9] using more physiological models and demonstrating that diazoxide (**1a**) inhibits glucose-stimulated insulin release from rat islets and relaxes KCl-induced contractions of rat aortic rings with similar potency ($pEC_{50} = 4.7 \pm 0.1$).

In addition to the fluorescence measurements, the binding affinity of the parent compound **1a** and the investigated derivatives was measured in HEK 293 (SUR2B/Kir6.1) cells using the agonist radioligand [^{3}H]P1075.[22] The resulting pK_D values are in acceptable agreement with the pEC_{50} data for the membrane hyperpolarization assays (see Table 1) indicating that the binding of compounds to the SUR2B receptor of the K_{ATP} channel causes the membrane potential effects. The selectivity of compounds for SUR1- and SUR2B-type K_{ATP} channels was determined by functional methods (i.e. membrane potential measurements, see Figures 2 and 3) because an agonist radioligand for the SUR1-

Table 1. Comparison of dissociation constants and pEC_{50} values for membrane hyperpolarization of the compounds.

Entry	Compound	HEK 293 (SUR2B/Kir6.1) cells			CHO (SUR1/Kir6.2) cells		Selectivity[a]
		Radioligand binding[b]	Membrane potential effect[c]		Membrane potential effect[c]		
		pK_D[d]	pEC_{50}[d]	p	pEC_{50}[d]	p	Δ[e,d]
1	**1a**	4.97 ± 0.02	5.14 ± 0.02	1.31	4.72 ± 0.02	1.80	0.42 ± 0.03
2	**1b**	3.55[e] ± 0.05	3.80[e] ± 0.00	1.91	3.58[e] ± 0.16	0.81	0.23 ± 0.16
3	**1c**	3.69[e] ± 0.03	3.57[e] ± 0.10	1.09	3.36[e] ± 0.14	1.31	0.22 ± 0.17
4	**7**	5.01 ± 0.03	5.29 ± 0.02	1.35	4.88 ± 0.01	1.71	0.40 ± 0.02
5	**8**	4.50 ± 0.03	4.35 ± 0.02	1.37	3.79[e] ± 0.04	1.82	0.56 ± 0.04
6	**12a**	4.18 ± 0.02	4.00 ± 0.02	1.28	1.7 ± 0.7% with 100 μM	–	
7	**12b**	4.01 ± 0.02	4.06 ± 0.03	1.29	3.78[e] ± 0.03	1.32	0.27 ± 0.04
8	**12c**	3.95 ± 0.03	4.04 ± 0.03	1.31	6.8 ± 1.1% with 100 μM	–	
9	**13a**	5.17 ± 0.03	4.98 ± 0.03	1.35	4.34 ± 0.03	1.72	0.64 ± 0.05
10	**13b**	5.41 ± 0.02	5.13 ± 0.02	1.36	4.30 ± 0.02	1.80	0.83 ± 0.03
11	**13c**	5.65 ± 0.02	6.04 ± 0.03	1.18	5.47 ± 0.02	1.86	0.57 ± 0.04
12	**13d**	6.02 ± 0.02	6.22 ± 0.02	1.37	5.14 ± 0.02	1.39	1.08 ± 0.03
13	**13e**	6.23 ± 0.03	6.09 ± 0.02	1.42	5.39 ± 0.02	1.11	0.70 ± 0.03
14	**13f**	5.85 ± 0.02	5.65 ± 0.02	1.35	4.31 ± 0.06	0.93	1.34 ± 0.06
15	**12d**	4.81 ± 0.03	4.51 ± 0.03	1.13	3.56[d] ± 0.03	0.95	0.95 ± 0.13
16	**13g**	5.57 ± 0.03	5.55 ± 0.03	1.25	4.31 ± 0.01	1.76	1.24 ± 0.03
17	**13h**	5.86 ± 0.02	5.84 ± 0.02	1.29	5.09 ± 0.04	1.29	0.75 ± 0.05
18	**13i**	5.97 ± 0.03	5.66 ± 0.03	1.36	4.43 ± 0.02	0.89	1.23 ± 0.04
19	**13j**	5.94 ± 0.03	5.83 ± 0.03	1.17	4.32 ± 0.08	1.09	1.51 ± 0.08
20	**12e**	5.19 ± 0.03	4.95 ± 0.04	1.38	3.6 ± 1.4% with 50 μM	–	
21	**13k**	6.08 ± 0.04	5.90 ± 0.05	1.00	< 3.26[e]	1.05	> 2.64

[a] selectivity calculated as $\Delta = pEC_{50}(SUR2B/Kir6.1) - pEC_{50}(SUR1/Kir6.2)$. [b] pK_D values: ($-\log$ [M]) calculated by nonlinear regression according to Equation 1 (see Experimental Section). [c] pEC_{50} values: ($-\log$ [M]) calculated by nonlinear regression according to Equation 2 (see Experimental Section). [d] Values are given ± asymptotic standard deviation (ASD) estimated by nonlinear regression. [e] Extrapolated from concentrations > 100 μM.

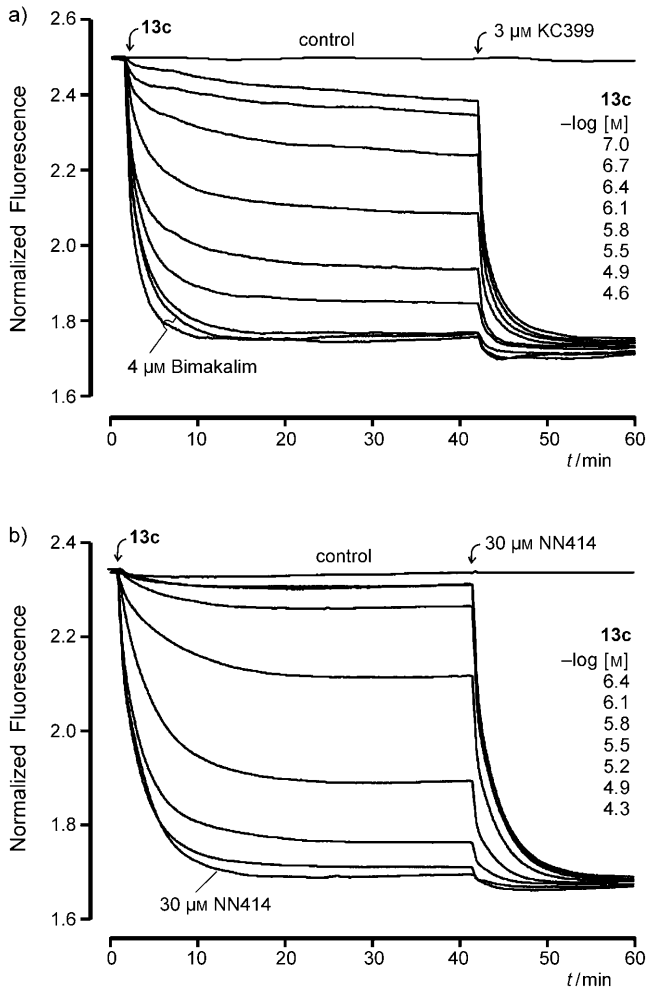


Figure 2. Hyperpolarizing effects of the cyclopropyl derivative **13c** on membrane potential responses in a) HEK 293 cells transfected with SUR2B/Kir6.1 and b) CHO cells transfected with SUR1/Kir6.2 assessed by the bis-oxonol dye DiBAC4(3). Representative experiments showing typical changes in fluorescence responses upon addition of increasing concentrations of **13c** indicated as decreasing (−log [M]) values on the right. Changes in DiBAC4(3) fluorescence (arbitrary units, data sampling at a frequency of 1 Hz) were normalized to maximum effects induced with 3 μM KC399 in HEK 293 (SUR2B/Kir6.1) cells and with 30 μM NNC414 in CHO (SUR1/Kir6.2) cells.

type is not available so far. Selectivity ratios ($\Delta \pm$ASD) were calculated as the difference between pEC_{50} values and are listed in Table 1.

The opposite electronic effects of substituents in position 5 of the diazoxide skeleton were studied in compounds **1b**, with the electron-withdrawing nitro group, and **1c**, featuring the electron-donating amino substituent. As shown in Table 1 (entries 2 and 3), both variations were deleterious for the activity of drugs in SUR2B cells as well as in SUR1 cells; pEC_{50} values for hyperpolarization decreased by more than an order of magnitude compared with the parent drug **1a** (entry 1).

Variations in benzothiadiazine-1,1-dioxides **1a** and **2** revealed that, as a rule, electron-withdrawing substituents in position 7 are favorable in comparison with electron-donating groups.[12] Bromo- and phenyl-substituted derivatives **7** and **8** were used to probe the question of whether a bulky substituent in the C-7 position would be compatible with sufficient activity on K_{ATP} channels. The substitution of chlorine by bromine led to only slightly higher activity of the bromo derivative **7** compared with the parent compound **1a** in both cell types (entry 4 vs. entry 1). In contrast to our results, where we observed similar potencies with compounds **1a** and **7**, de Tullio and co-workers reported a fivefold increase in potency (measured in a SUR2B test system) for diazoxide analogues **2** substituted with an isopropyl-amino side chain in position 3 when the 7-chloro was replaced by a 7-bromo substituent.[23]

As indicated by the data given in Table 1 (entry 5), the 7-phenyl-substituted analogue **8** loses activity by one order of magnitude compared with diazoxide (**1a**). Obviously, the phenyl substituent in C-7 position appears to substantially hinder receptor binding and activation due to steric demand or electronic properties.

In the third series, we first studied benzothiadiazine-1,1-dioxide derivatives missing the chlorine residue in position 7 (**12a–c**). It turned out that phenyl, 2-hydroxyphenyl and 2-thienyl substitution in position 3 caused a substantial loss of activity and affinity (entries 6–8) by one order of magnitude. In CHO (SUR1/Kir6.2) cells, the decrease in activity was even higher. Here, only the 2-hydroxyphenyl derivative **12b** caused a significant hyperpolarization, whereas relevant effects of the phenyl and the thienyl substituted derivatives **12a** and **12c** could not be detected. The comparison of the latter compounds with their 7-chloro-substituted analogous **13a** and **13b** (entries 9 and 10) revealed the relevance of this substituent; due to its presence, the affinity rose by more than one order of magnitude in HEK 293 cells (entry 6 vs. 9 and 8 vs. 10). A slightly, but substantially lower, enhancement was observed in the CHO cells.

The importance of C-7 substituents is discussed in the literature as an indirect influence arising through the increase or decrease in acidity of the N-4 proton by electron-withdrawing C-7 substituents (e.g. Cl, Br, NO_2) or electron-donating groups (e.g. NH_2, OMe), respectively.[24] By varying the diazoxide core substituted with cyclobutylamino in C-3, Peat and co-workers[24] could show that a chloro substituent at C-7 increased activity of the compound by tenfold in patches of oocytes transfected with SUR1/Kir6.2, whereas an amino group at C-7 decreased activity by tenfold as compared with the unsubstituted analogue. Similar findings were reported on the inhibition of insulin secretion from pancreatic β-cells by various alkylamino substituents of the diazoxide core, whereby a five- to tenfold increase in potency was observed by the introduction of chlorine in position 7.[12]

In view of this advantageous effect, we next investigated the effect of compounds **13c–f**, with cycloalkyl residues in position 3, with varying ring sizes (3–6). A remarkable increase in affinity and potency compared to the parent compound diazoxide (**1a**) was observed for all four derivatives (Table 1, entries 11–14, Figure 4). In HEK 293 (SUR2B/Kir6.1) cells, K_D and EC_{50} values reached the submicromolar concentration range (**13c–e**), thereby surpassing the corresponding affinities of **1a** by five- to tenfold. In CHO (SUR1/Kir6.2) cells, however, the increase in potency (pEC_{50}) was much smaller compared with

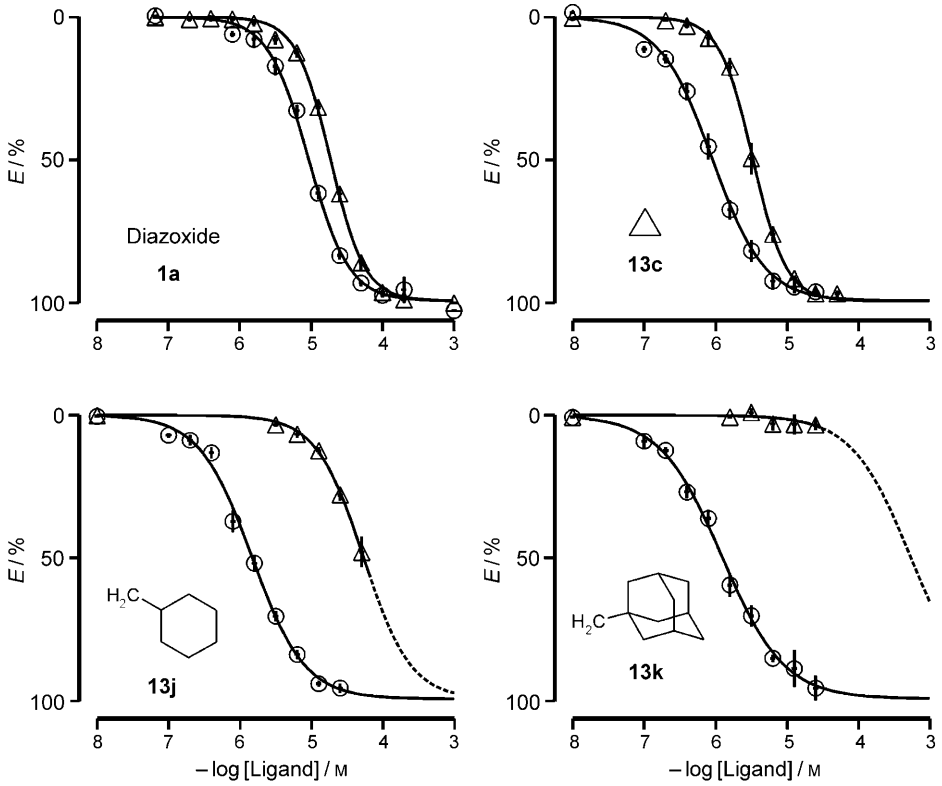


Figure 3. Comparison of the effects of diazoxide (**1a**) and its derivative on the membrane potential (*E*) of HEK 293 cells transfected with SUR2B/Kir6.1 (○) and on CHO cells transfected with SUR1/Kir6.2 (△). Membrane potential was measured by fluorescence using 5 μM DiBAC4(3). Whereas diazoxide **1a** and its cyclopropyl derivative **13c** act as full agonists on both K_{ATP} channels with moderate selectivities, the derivatives with bulkier side chains (cyclohexyl)methyl (**13j**) and (adamantly)methyl (**13k**) are highly selective for the SUR2B-type K_{ATP} channel. For compound **13k** no significant hyperpolarization could be measured in CHO (SUR1/Kir6.2) cells; the dashed line represents a hypothetical curve according to Equation 1 (see Experimental Section) indicating an upper limit for the effectiveness of **13k** on SUR1-type K_{ATP} channels. The drawings represent the residue attached in position 3 of the 4*H*-1,2,4-benzothiadiazine-1,1-dioxide skeleton.

1a. The effect is illustrated for the cyclopropyl derivative **13c** in Figure 2, depicting original recordings of membrane potential measurements, and in Figure 3 demonstrating concentration–effect curves. Compared to the smaller rings, the cyclohexyl derivative **13f** displayed a slightly lower potency in SUR2B-type K_{ATP} channels, but a clearly lower potency in SUR1-type K_{ATP} channels, thereby increasing selectivity for the SUR2B-type up to 1.3 log units (20-fold, entry 14). Compared with **13f**, the analogue missing the chlorine substituent at C-7 (**12d**) decreased in potency by approximately tenfold in both types of K_{ATP} channels, but retained the rather high selectivity for SUR2B-type channels (entry 15).

Thus, we wondered whether a substituent with a higher steric demand or flexibility would be able to enhance the desired selectivity. Indeed, 3-benzyl-substituted 7-chlorobenzothiadiazine-1,1-dioxide **13g**, compared with the 3-phenyl derivative **13a**, displayed a fourfold (0.6 log units) higher selectivity in favor of the SUR2B/Kir6.1 receptor, gaining a threefold (0.5 log unit) higher affinity and activity over the parent compound **1a** (Table 1, entry 16). The direct attachment of the phenyl ring in position 3 (**13a**) appears not to be favorable for receptor affinity and activity. The investigation of the (cycloalkyl)methyl derivatives **13h–j** revealed a clear correlation between ring size and selectivity (entries 17–19, Figure 4); in order of increasing selectivity: (cyclobutyl)methyl (**13h**), (cyclopentyl)methyl (**13i**) and (cyclohexyl)methyl (**13j**). This increase, however, is not caused by an increase in activity towards the SUR2B-type K_{ATP} channels, but a decrease in activity towards the SUR1-type K_{ATP} channels. The results of the membrane potential measurements for compound **13j** in the both cell lines clearly illustrate its high selectivity (Figure 3). In contrast to the SUR2B selectivity of the cycloalkyl derivatives (**13h–j**), cycloalkylamino derivatives (**2**) substituted with the same cycloalkyl residues were reported to act as SUR1-selective potassium channel openers, with a selectivity of approximately 30-fold.[12] However, when comparing the modified diazoxides **12** and **13** with agents **2** and **3**, the difference between the sulfonylamidine and the sulfonylguanidine moieties cannot be neglected. Predictions based on a pharmacophoric model[12] for the effects of alkyl and cycloalkylamino side chains do not seem to hold when these residues are attached to the diazoxide skeleton through a methylene group, as in derivatives **12** and **13**. Interestingly, in comparison with chloro-thieno derivatives substituted with 4-, 5- and 6-membered alkyl rings (**4**),[13,14] none of the new compounds **13h–j** exhibited any selectivity in favor of the SUR1-type or any antagonistic activity towards SUR2B-type K_{ATP} channels.

Guided by the concept that relates ring size to selectivity, we studied derivatives featuring the very bulky (1-adamantyl)-methyl residue in position 3 (**12e** and **13k**).[25] In spite of the high lipophilicity of these compounds, and the fact that K_{ATP} channel ligands have to pass the plasmalemmal membrane to reach their binding site at the SUR protein,[26] both adamantyl derivatives induced membrane potential responses, however, with significantly slower response kinetics. The half time of membrane hyperpolarization by adamantyl derivative **13k** (202 ± 9 sec) was much greater than that for the cyclopropyl derivative **13c** (38 ± 2 sec) at a concentration of 6 μM. Remarkably, compound **12e** was as active in HEK 293 cells as diazoxide **1a** even though it lacks the chlorine substituent in position 7 (Table 1, entry 20). As expected, 7-chloro substitution (**13k**) led to an increase in activity by an order of magnitude in HEK 293 cells over the parent compound **1a** (entry 21).

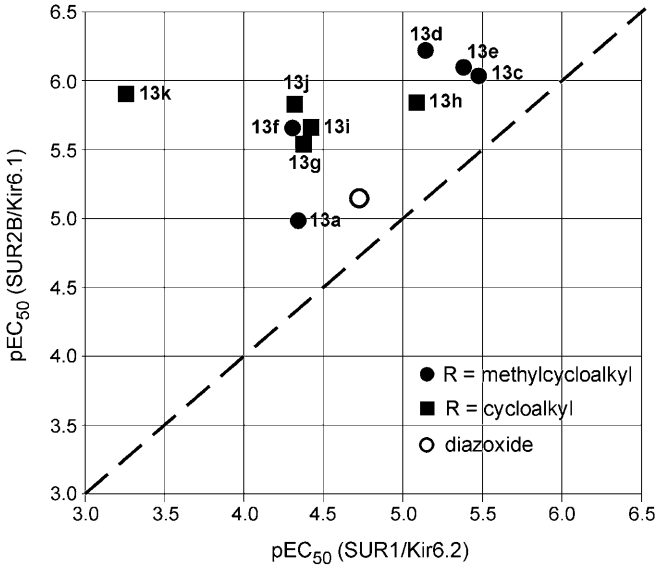


Figure 4. Correlation of pEC_{50} values of hyperpolarizing (agonistic) effects of the 7-chloro derivatives on membrane potential responses in CHO cells transfected with SUR1/Kir6.2 and in HEK 293 cells transfected with SUR2B/Kir6.1 assessed by the bis-oxonol dye DiBAC4(3). The effect of diazoxide is indicated (○). The highest selectivity was found with the (adamantyl)methyl derivative **13k**.

Compound **13k** was practically inactive in CHO (SUR1/Kir6.2) cells, the concentration–effect curve depicted in Figure 3 (-----) represents an upper limit of its hypothetical effectiveness in SUR1-type K_{ATP} channels. Thus, the selectivity in favor of the SUR2B/Kir6.1 receptor anticipated by incorporation of a bulky side chain can be estimated as more than 450-fold (2.64 log units, see Table 1 entry 21 and Figure 4). This result suggests a large globular subpocket in the binding site of the SUR2B receptor allowing lipophilic interaction. Conversely, the failure to detect any activity through activation of SUR1-type K_{ATP} channels indicates that large substituents are unfavorable for drug interaction with this receptor. For the first time, benzothiadiazine-*S,S*-dioxides were modified to give agents based on this skeleton that exhibit significant selectivity towards the SUR2B/Kir6.1 relative to the SUR1/Kir6.2 receptor. Previous research on the sulfonylurea receptors has shown that the binding domain of diazoxide, part of which is located at helices 8–11 of TMD 1,[3] is distinct from that of all other potassium channel openers, such as cyanoguanidines and benzopyrans. In this context, the new derivatives of diazoxide, with improved affinity and selectivity, could play an important role. Assuming they also bind to and act via the diazoxide-binding domain of SUR2-type K_{ATP} channels,[27] it may be possible to study the interaction of the cyanoguanidine and diazoxide-binding domain in the regulation of K_{ATP} channel activity. In addition, compounds that combine enhanced affinity with high selectivity, like **12** and **13**, may also give rise to a reanalysis of the cardioprotective action of diazoxide, which remains disputed.[28]

Conclusions

The introduction of different substituents in position 5 of the 4*H*-1,2,4-benzothiadiazine-1,1-dioxide skeleton, as well as a phenyl group in position 7, are deleterious to the activity of K_{ATP} channels in HEK 293 (SUR2B/Kir6.1) cells and CHO (SUR1/Kir6.2) cells. However, it was found that novel diazoxide analogues with small substituents (e.g. cyclopropyl) at C-3 of the heterocyclic core exhibit a significantly higher–up to tenfold–affinity for both types of K_{ATP} channels. Conversely, 4*H*-1,2,4-benzothiadiazine-1,1-dioxide derivatives with bulky nonpolar substituents in position 3 show an increased affinity and potency in HEK 293 (SUR2B/Kir6.1) cells, but an attenuated or even missing activation in CHO (SUR1/Kir6.2) cells. Pursuing this concept, the selectivity of compounds could be increased by more than 450-fold for SUR2B-type K_{ATP} channels when the globular hydrophobic (1-adamantyl)methyl residue, with its high steric demand, was attached in position 3 of 4*H*-1,2,4-benzothiadiazine-1,1-dioxide.

Experimental Section

Synthesis

Melting points were determined using a Büchi melting point apparatus 540 and are uncorrected. IR spectra were recorded on a Bruker Vector 22 spectrometer. Mass spectra were recorded on a Finnigan MAT 8200 spectrometer. TLC analysis was carried out on silica gel 60 F_{254} plates (Merck). Column chromatography was carried out using Macherey–Nagel silica gel 60 or Merck silica gel 60 (mesh size 0.04–0.063 mm). Elemental analysis was performed on a Perkin–Elmer CHN Analyzer 2400 at the Institute of Pharmaceutical Chemistry (University of Düsseldorf, Germany).

7-Bromo-3-methyl-4*H*-1λ^6,2,4-benzothiadiazine-1,1-dione (7): A mixture of **5** (1.88 g, 7.46 mmol), pyridine (1.81 mL, 1.77 g, 22.4 mmol), and Ac_2O (3.52 mL, 3.8 g, 37.3 mmol) was stirred in a flask fitted with a drying tube ($CaCl_2$). After stirring overnight at RT, Et_2O (30 mL) was added, and stirring was continued for 30 min. A white precipitate formed and was isolated by filtration, washed with a small amount of Et_2O and air dried. The solid material was dissolved in MeOH (5–10 mL) and added dropwise to Et_2O (250 mL) to give pyridinium salt **6** as a colorless solid (2.35 g, 84%): ^{1}H NMR (500 MHz, CD_3OD): $\delta = 2.20$ (s, 3H, CH_3), 7.54 (dd, $J =$ 8.83 Hz, $J = 2.52$ Hz, 1H, 4-H, anion), 7.94 (d, $J = 2.20$ Hz, 1H, 6-H, anion), 8.12 (t, $J = 7.25$ Hz, 2H, 3-H, 5-H, pyridinium cation), 8.22 (d, $J = 8.83$ Hz, 1H, 3-H, anion), 8.67 (tt, $J = 7.8$ Hz, $J = 1.58$ Hz, 1H, 4-H, pyridinium cation), 8.88 ppm (d, $J = 5.36$ Hz, 2H, 2-H, 6-H, pyridinium cation). ^{13}C NMR (125 MHz, CD_3OD): $\delta = 26.17$ (CH_3), 117.93 (C-5, anion), 125.86 (C-3, anion), 130.16 (C-6, anion), 132.44 (C-2, pyridinium cation), 136.08 (C-2, anion), 138.08 (C-1, anion), 144.35 (C-1, pyridinium cation), 172.47 ppm (C=O). A 50 mL flask was preheated in an oil bath at 60 °C. A mixture of **6** (3.34 g, 9.35 mmol) and PCl_5 (2.70 g, 12.95 mmol) were poured into the flask and stirred at 58–60 °C for 150 min. The flask was cooled in an ice bath and an ice-cold 25% aq NH_3 (60 mL) was added. The mixture was stirred at RT overnight, filtered and the filtrate was neutralized by the addition of HCl (2 N). The precipitate was filtered and the residue was resuspended in MeOH. After filtration, the filtrate was concentrated in vacuo. The residue was dissolved in aq NaOH (0.1 N) and filtered. The filtrate was acidified to pH 6.7 with HCl (2 N). The resultant precipitate was filtered, washed with a small amount of deionized

water and dried in vacuo to give **7** as a colorless crystalline solid (1.41 g, 54%): mp 335.8 °C, Lit.[18b] 333.5–335 °C. ^{1}H NMR (500 MHz, CD_3OD): $\delta = 2.24$ (s, 3H, CH_3), 4.51 (s, 1H, NH), 7.10 (d, $J = 8.83$ Hz, 1H, 5-H), 7.68 (dd, $J = 8.82$ Hz, $J = 2.20$ Hz, 1H, 6-H), 7.85 ppm (d, J 2.20 Hz, 1H, 8-H). MS (EI, 70 eV): *m/z* (%): 276 (70) $[M+1]^+$, 274 (66) $[M]^+$, 235 (99) $[C_6H_4BrNO_2S]$, 233 (100) $[C_6H_4BrNO_2S]$. Anal. calcd for $C_8H_7BrN_2O_2S$: C 34.92, H 2.56, N 10.18, found: C 34.84, H 2.50, N 9.98.

3-Methyl-7-phenyl-4*H*-1λ^6,2,4-benzothiadiazine-1,1-dioxide (8): A 100 mL two-necked flask, equipped with a magnetic stirrer, a reflux condenser, and a connection to a combined nitrogen/vacuum line, was charged with **7** (0.1 g, 0.36 mmol), phenylboronic acid (0.044 g, 0.36 mmol), Cs_2CO_3 (0.293 g, 0.9 mmol) and dichloro-[1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloromethane adduct (0.0145 g, 0.018 mmol). The flask was closed with a septum; the air in the flask was replaced by N_2. A degassed solution of 1,2-dimethoxyethane (12 mL) and water (4 mL) was injected by syringe and the mixture was refluxed for 15 h. After addition of water (40 mL), the mixture was filtered through celite and the residue was washed with aq NaOH (0.1 N, 30 mL). The combined filtrates were acidified with dilute HCl. The crystalline material that precipitated was collected and air dried to give **8** as a pale yellowish solid (0.050 g, 50%): mp 331.3 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 2.32$ (s, 3H, CH_3), 2.51 (s, 1H, NH), 7.41 (m, 2H, 5-H and 4-phenyl H), 7.50 (t, $J = 7.7$ Hz, 2H, 3,5-phenyl H), 7.71 (d, $J = 7.9$ Hz, 2H, 2,6-phenyl H), 7.97 (d, $J = 1.58$ Hz, 1H, 8-H), 8.00 ppm (dd, $J = 8.51$ Hz, $J = 1.58$ Hz, 1H, 6-H). ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 22.90$ (CH_3), 118.49 (C-5), 120.93 (C-8), 121.82 (C-8′), 126.87 (phenyl C-4), 128.53 (phenyl C-3), 129.69 (phenyl C-2), 131.79 (C-6), 134.76 (C-7), 138.27 (phenyl C-1), 138.44 (C-4′), 157.73 ppm (C-3). MS (EI, 70 eV): *m/z* (%): 273 (23) $[M+1]^+$, 272 (61) $[M]^+$, 167 (100). Anal. calcd for $C_{14}H_{12}N_2O_2S$: C 61.75, H 4.44, N 10.29, found: C 61.60, H 4.42, N 10.08.

*General procedure for the preparation of benzothiadiazine-2,2-dioxides **12** and **13***: In a 50 mL one-necked flask equipped with a magnetic stirrer and a reflux condenser, polyphosphoric acid trimethylsilyl ester (PPSE; 7 mL) was heated in an oil bath to 160 °C (bath temperature). 2-Aminobenzenesulfonamide (**9**) (0.860 g, 5.0 mmol) or 2-amino-5-chlorosulfonamide (**10**) (1.033 g, 5.0 mmol) and carboxylic acid **11** (5.0 mmol) were added and the mixture was stirred at the same temperature for 3 h. Thereafter, the mixture was poured cautiously into ice water (300 mL) and stirred at RT for 2 h. In the course of this, a precipitate formed that was filtered and recrystallized from 90% aq EtOH. The following compounds were obtained:

3-(2-Hydroxyphenyl)-4*H*-1λ^6,2,4-benzothiadiazine-1,1-dione (12b): Prepared from **9** (5.0 mmol) and 2-hydroxybenzoic acid (**11b**) (5.0 mmol): Yield: 0.14 g (10%): mp 285.7 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 2.51$ (s, 2H, NH, OH), 7.00 (t, $J = 7.09$ Hz, 1H, 7-H), 7.05 (d, $J = 8.20$ Hz, 1H, 5-H), 7.46 (t, $J = 7.09$ Hz, 1H, 6-H), 7.47–7.53 (m, 2H, 8-H and 6′-H), 7.70 (dq, $J_d = 1.10$ Hz, $J_q = 7.72$ Hz, 2H, 3′-H, 5′-H), 7.85 ppm (dd, $J = 8.0$ Hz, $J = 1.10$ Hz, 1H, 4′-H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 117.1$ (C-2′), 118.6 (C-6′), 118.9 (C-4), 119.9 (C-7), 121.6 (C-4′), 123.6 (C-3′), 127.1 (C-8), 130.5 (C-5′), 133.6 (C-8), 134.0 (C-6), 124.9 (C-1′), 156.7 ppm (C-3); MS (EI, 70 eV): *m/z* (%); 275 (16) $[M+1]^+$, 274 (100) $[M]^+$, 156 (26), 155 (60), 120 (13), 92 (34), 91 (77). Anal. calcd for $C_{13}H_{10}N_2O_3S$: C 56.92, H 3.67, N 10.21, found: C 56.72, H 3.45, N 9.89.

3-(Tricyclo[3.3.1.1^{3,7}]dec-1-ylmethyl)-4*H*-1λ^6,2,4-benzothiadiazine-1,1-dione (12e): Prepared from **9** (2.5 mmol) and **11l** (2.5 mmol); yield: 0.517 g (60%): mp 351.1 °C; ^{1}H NMR (500 MHz, CD_3OD): $\delta = 1.69$–1.82 (m, 12H, CH_2), 2.00 (broad s, 3H, adamantyl CH), 2.32 (s, 2H, CH_2), 7.33 (d, $J = 8.20$ Hz, 1H, 5-H), 7.49 (t, $J = 7.57$ Hz, 1H, 7-H), 7.68 (t, $J = 7.72$ Hz, 1H, 6-H), 7.87 ppm (d, $J = 7.88$ Hz, 1H, 8-H); ^{13}C NMR (100 MHz, CD_3OD/[D$_6$]DMSO): $\delta = 28.5$ (adamantyl CH), 34.2 (adamantyl CH_2), 36.5 (adamantyl CH_2), 49.5 (adamantyl quart. C), 49.5 (C-1′), 117.4 (C-5), 121.3 (C-8), 123.6 (C-7), 126.4 (C-8), 133.1 (C-6), 135.2 (C-4), 158.2 ppm (C-3); MS (EI, 70 eV): *m/z* (%): 330 (7) $[M]^+$, 265 (12), 135 (100), 91 (31). Anal. calcd for $C_{18}H_{22}N_2O_2S$: C 65.42, H 6.71, N 8.48, found: C 65.14, H 6.64, N 8.35.

7-Chloro-3-(cyclobutylmethyl)-4*H*-1λ^6,2,4-benzothiadiazine-1,1-dione (13h): Prepared from **10** (5.0 mmol) and **11i** (5.0 mmol); yield: 1.28 g (90%): mp 252 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta =$ 1.71–2.10 (m, 6H, cyclobutyl), 2.64 (d, $J = 7.64$ Hz, 2H, CH_2), 2.60–2.77 (m, 1H, cyclobutyl, 1-H), 7.35 (d, $J = 8.83$ Hz, 1H, 5-H), 7.72 (dd, $J = 8.82$ Hz, $J = 2.31$ Hz, 1H, 6-H), 7.82 (d, $J = 2.31$ Hz, 1H, 8.H), 12.11 ppm (s, 1H, NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 18.5$ (cyclobutyl C-3), 27.8 (cyclobutyl C-2, C-4), 33.3 (cyclobutyl C-1), 42.1 (CH_2), 120.1 (C-5), 122.6 (C-8), 123.1 (C-7), 129.9 (C-8′), 133.6 (C-6), 134.4 (C-4′), 159.7 ppm (C-3); MS (EI, 70 eV): *m/z* (%): 284 (10) $[M]^+$, 256 (6), 230 (100), 211 (4), 191 (7), 125 (22). Anal. calcd for $C_{12}H_{13}ClN_2O_2S$: C 50.61, H 4.60, N 9.84, found: C 49.70, H 4.81, N 9.05.

7-Chloro-3-(cyclopentylmethyl)-4*H*-1λ^6,2,4-benzothiadiazine-1,1-dione (13i): Prepared from **10** (5.0 mmol) and **11j** (5.0 mmol); yield: 0.92 g (62%): mp 278 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta =$ 1.18–1.80 (m, 8H, cyclopentyl), 2.23–2.30 (m, 1H, cyclopentyl 1-H) 2.53 (d, $J = 7.56$ Hz, 2H, CH_2), 7.36 (d, $J = 8.83$ Hz, 1H, 5-H), 7.72 (dd, $J = 8.83$ Hz, $J = 2.37$ Hz, 1H, 6-H), 7.83 (d, $J = 2.33$ Hz, 1H, 8-H), 12.13 ppm (s, 1H, NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 24.3$ (cyclopentyl C-3, C-4), 31.5 (cyclopentyl C-2, C-5), 37.5 (cyclopentyl C-1), 41.0 (CH_2), 119.7 (C-5), 122.1 (C-8), 122.7 (C-7), 129.5 (C-8′), 133.1 (C-6), 134.0 (C-4′), 160.1 ppm (C-3); MS (EI, 70 eV): *m/z* (%): 298 (3) $[M]^+$, 257 (2), 230 (100), 205 (2), 190 (7), 126 (18). Anal. calcd for $C_{13}H_{15}ClN_2O_2S$: C 52.26, H 5.06, N 9.38, found: C 52.11, H 5.12, N 9.21.

7-Chloro-3-(cyclohexylmethyl)-4*H*-1λ^6,2,4-benzothiadiazine-1,1-dione (13j): Prepared from **10** (5.0 mmol) and **11k** (5.0 mmol); yield: 0.81 g (52%): mp 279 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta =$ 1.06–1.14 (m, 2H, cyclohexyl), 1.10–1.25 (m, 4H, cyclohexyl), 1.64–1.72 (m, 4H, cyclohexyl), 1.77–1.87 (m, 1H, cyclohexyl 1-H), 2.41 (d, $J = 7.26$ Hz, 2H, CH_2), 7.36 (d, $J = 8.81$ Hz, 1H, 5-H), 7.73 (dd, $J = 8.79$ Hz, $J = 1.76$ Hz, 1H, 6-H), 7.84 (d, $J = 1.86$ Hz, 1H, 8-H), 12.12 ppm (s, 1H, NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 25.2$ (cyclohexyl C-3, C-5), 25.4 (cyclohexyl C-4), 31.9 (cyclohexyl C-1), 35.6 (cyclohexyl C-2, C-6), 42.5 (CH_2), 119.5 (C-5), 121.9 (C-8), 122.5 (C-7), 129.4 (C-8′), 133.0 (C-6), 133.7 (C-4′), 159.5 ppm (C-3); MS (EI, 70 eV): *m/z* (%): 312 (5) $[M]^+$, 230 (100), 190 (4), 125 (8), 98 (9). Anal. calcd for $C_{14}H_{17}ClN_2O_2S$: C 53.75, H 5.48, N 8.96, found: C 52.73, H 5.47, N, 8.87.

7-Chloro-3-(tricyclo[3.3.1.1.^{3,7}]dec-1-ylmethyl)-4*H*-1,2,4-benzothia-diazine-1,1-dione (13k): Prepared from **10** (0.970 g, 5.0 mmol) and **11l** (1.03 g, 5.0 mmol); yield: 1.32 g (73%): mp 349.0 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 1.53$–1.73 (m, 12H, adamantyl CH_2), 1.94 (broad s, 3H, adamantyl CH), 2.27 (s, 2H, CH_2), 7.38 (d, $J = 8.82$ Hz, 1H, 5-H), 7.73 (dd, $J = 8.82$ Hz, $J = 2.20$ Hz, 1H, 6-H), 7.84 ppm (d, $J = 2.2$ Hz, 1H, 8-H); ^{13}C NMR (100 MHz, CD_3OD/[D$_6$]DMSO): $\delta = 28.4$ (adamantyl CH), 34.4 (adamantyl CH_2), 36.6 (adamantyl CH_2), 42.2 (adamantyl quart. C), 49.5 (C-1′), 120.2 (C-5), 122.4 (C-8), 123.1 (C-7), 130.0 (C-8′), 133.6 (C-6), 134.2 (C-4′), 158.4 ppm (C-3); MS (EI, 70 eV): *m/z* (%): 364 (40) $[M]^+$, 299 (9), 231

(10), 203 (6), 135 (100). Anal. calcd for $C_{18}H_{21}ClN_2O_2S$: C 59.25, H 5.80, N 7.68, found: C 59.29, H 5.78, N 7.59.

Pharmacological methods

Cell culture: HEK 293 (human embryonic kidney) cells stably expressing murine SUR2B and Kir6.1[29] were cultured at 37 °C in an humidified atmosphere of 93% air and 7% CO_2 in α-MEM (modified Eagles medium, Invitrogen) with glucose supplemented with 10% FCS (fetal calf serum) and 0.15 mg mL^{-1} Zeocin and 0.3 mg mL^{-1} G418 as selection medium. Cells were seeded out in poly-L-lysine-coated 12-well strips (96-well format) at a density of 60 000 cells/well and cultured for 3 d in 200 μL/well before the day of the experiment. CHO (Chinese hamster ovary) cells stably expressing human SUR1 and Kir6.2 were cultured under identical condition using G418 as selection marker, and culturing cells only for 1.5 d in 12-well strips before testing.

[^{3}H]P1075 Binding in HEK 293 (SUR2B/Kir6.1) cells: Cells were cultured in 96-well plates for 3 d and radiolabeled with 2 nM [^{3}H]P1075 (*N*-cyano-*N*′-(1,1-dimethyl-[2,2,3,3-^{3}H]propyl)-*N*″-(3-pyridinyl)guanidine[22]) with a specific activity of 108 Ci mmol^{-1} in the absence and presence of increasing concentrations of test compounds in 100 μL HBSS (Hanks' balanced salt solution) containing NaCl (137 mM), KCl (5.4 mM), $MgSO_4$ (1.2 mM), Na_2HPO_4 (0.34 mM), $NaHCO_3$ (4.2 mM), KH_2PO_4 (0.44 mM), HEPES (20 mM), glucose (5.5 mM) and $CaCl_2$ (1.3 mM) for 45 min at 37 °C. Radioligand binding was analyzed in cell monolayers adhered to the bottom of 96-well plates.[30] The binding observed in the presence of 1 μM P1075 was regarded as nonspecific. To determine the quantity of receptor-bound radioligand, the reaction was quickly stopped by suction of the incubation medium followed by five successive washings with cold HBSS. Bound radioligand was transferred to microscintillation vials after incubation of the cells with 1 M NaOH for 30 min at RT. After addition of 1.2 mL scintillation fluid (Ultima Gold, Packard, USA) the vials were vigorously shaken for 1 h and counted in a scintillation counter (Packard 1500) with 50% efficiency.

Membrane potential assays with DiBAC4(3): Confluent monolayers cultivated in 12-well strips (96-well format) were rinsed twice with 200 μL assay buffer (120 mM NaCl, 2.0 mM KCl, 1.0 mM $MgCl_2$, 2.0 mM $CaCl_2$, 5 mM glucose) supplemented with 20 mM HEPES (*N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulphonic acid) at pH 7.4 and 30 °C before 200 μL assay buffer supplemented with 5 μM DiBAC4(3) was added.[31,32] Labeling of cells cultured in 12-well strips was continuously observed in 12-channel fluorescence detectors at 30 °C until the distribution of DiBAC4(3) across the plasma membrane reached equilibrium (60–90 min). Test compounds were added in a volume comprising 10% of total buffer volume and gently mixed resulting in a time-dependent reduction of fluorescence indicating hyperpolarization of the membrane potential. Maximum hyperpolarization was induced with 30 μM NNC414[33] in CHO (SUR1/Kir6.2) cells and with 3 μM KC399[34] in HEK 293 (SUR2B/Kir6.1) cells.

Fluorescence was excited with blue light (488 nm), which was reflected by a dichroidic beam splitter (505 nm) mounted at 45° to illuminate adhered cells from the bottom of the 12-well strips. Excitation was performed with short light pulses of 30 msec to minimize photobleaching of the dye. Emitted light again passed the dichroidic beam splitter and a long pass emission filter (OG515, Schott, Mainz, Germany) before it was detected by low noise silicone photodiodes (OSD15-5T, Centronic, Croydon, UK). After a more than 100 000-fold amplification of the photocurrent using a two-stage amplifier (operational amplifiers OPA111 and OPA121, Texas Instruments, USA), fluorescent signals were digitized and sent (RS232, 38400 baud) to a personal computer (Pentium, 1 GHz) where the fluorescence data was continuously registered, displayed and saved for subsequent analysis. As all electronic components were chosen to be smaller than 9 mm (i.e. 96-well format) each of the 12 channels could be equipped with independent excitation and detection units, so that fluorescence data could be continuously monitored with high data sampling rate (1 Hz).[35]

Data analysis

Competition binding: Experiments were performed in the presence of the radioligand ([^{3}H]P1075; [L*]) and increasing concentrations of competing ligands [L]. Displacement curves were analyzed by nonlinear regression as reported[36] according to Equation (1):

$$B_s([L]) = B_0 \cdot \frac{[L]}{[L]+K_D \cdot (1+[L^*]/K_L^*)} \quad (1)$$

where B_0 and B_s([L]) represent the specific binding of L* to K_{ATP} channels in the absence and presence of L, K_L^* denotes the dissociation constant of the radioligand L*, and K_D is the equilibrium dissociation constant of the test compound L. Experimental data were analyzed after transformation of data to obtain homoscedasticity,[37] which resulted in reliable estimates of parameters (B_0, B_{ns} and pK_D) and asymptotic standard deviations (ASD). Data were fitted to the hyperbola defined by nonlinear regression using the SAS software package STAT. Data points in the figures are means ± SEM (standard error of the mean).

Membrane potential assays: Equilibrium effects of ligand(L)-induced changes in fluorescence induced with diazoxide and its derivatives were analyzed according to Equation (2):

$$E\,(\%) = \frac{[L]^P}{[L]^P + EC_{50}{}^P} \cdot 100 \quad (2)$$

Concentration–effect curves were fitted by nonlinear regression analysis according to Lemoine.[37] Exponents p indicate the Hill-coefficient correcting for the steepness of curves.

Drugs and materials

The following materials were purchased from commercial sources: DiBAC4(3) (Molecular Probes), glibenclamide (Sigma, Deisenhofen, Germany), [^{3}H]P1075 (Pharmacia Biotech). The following materials were received as gifts: P 1075 (from Leo, Ballerup, Denmark), bimakalim (from Merck, Darmstadt, Germany), NNC414 (from Dr. J. B. Hansen, Novo Nordisk Research, Denmark). KCOs (PCOs) were dissolved in DMSO and further diluted with incubation buffer to give a final concentration of <0.1% (membrane potential assay) or <1% (radioligand binding assay).

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Keywords: cyclization · drug design · fluorescent probes · ion channels · structure–activity relationships

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