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# Synthesis of Benzofuran, Benzothiophene, and Benzothiazole-Based Thioamides and their Evaluation as $K_{ATP}$ Channel Openers

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Several series of benzofurans, benzothiophenes, and benzothiazoles, all featuring the thioamide group, were synthesized and tested as novel $K_{ATP}$ channel openers in artificial cell systems: CHO cells transfected with SUR1/Kir6.2, and HEK 293 cells transfected with SUR2B/Kir6.1; these served as model systems for insulin-secreting pancreatic β cells and smooth muscle cells, respectively. All compounds were investigated with respect to their binding affinity for the SUR2B-type $K_{ATP}$ channels using [$^3$H]P1075 as radioligand. Selected compounds were also tested as agonists in intact cells using $DiBAC_4(3)$ and DyeB (R7260) as membrane potential dyes. Remarkable affinity for SUR2B/Kir6.1 channels in the single-digit micromolar range was observed. In addition, benzothiazole-derived thioamides with sterically demanding, lipophilic substituents showed >100-fold selectivity in favor of SUR2B/Kir6.1. A one-carbon spacer between the heterocyclic skeleton and the thioamide moiety was observed to be crucial for affinity and selectivity. Two of the most potent and selective compounds were studied by crystal structure analyses.

## Introduction

ATP-sensitive potassium channels, usually called $K_{ATP}$ channels, are ubiquitously distributed and present in most excitable cells. By tuning the permeability of potassium ions, they link the metabolic state of the cell to the excitability of the membrane. In pancreatic β cells, they regulate insulin secretion. In cardiac cells, they decrease the duration of the action potential. They also control the vessel tone in smooth muscle cells and are responsible for the transmitter release in neurons. With regard to their structure, $K_{ATP}$ channels consist of octamers of two membrane proteins: the sulfonylurea receptor (SUR) and the inward rectifying potassium channel (Kir). To form an effective $K_{ATP}$ channel, four Kir (Kir6.x) channels associate with four SUR subunits. Kir falls into two subtypes: Kir6.1 and Kir6.2, whereas three different subtypes (SUR1, SUR2A, and SUR2B) of the sulfonylurea receptor are known. The individual SUR isoform is responsible for the tissue-specific properties of the $K_{ATP}$ channels: SUR1/Kir6.2 in pancreatic β cells, SUR2A/Kir6.2 in cardiac cells, and SUR2B/Kir6.1 in vascular smooth muscle cells. In view of the multitude of physiological functions, $K_{ATP}$ channels represent promising targets for various drugs. In the past, not only were antagonists like the anti-diabetic sulfonylureas developed, but agonists, the so-called potassium channel openers (PCOs or KCOs), were also studied intensively because they are regarded as tools to interfere with the excitability of various cells. Hypertension, asthma, urinary incontinence, and cardiac ischemia are only some of the potential indications for PCOs.[1]

Although a large number of compounds with various chemical structures have proven themselves as potent agonists of $K_{ATP}$ channels, most of the drugs rely on three skeletons

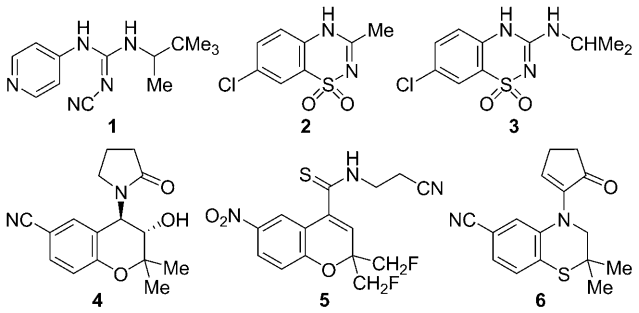


**Figure 1.** Structures of representative $K_{ATP}$ channel openers.

(Figure 1): 1. the cyanoguanidines such as pinacidil (**1**), 2. the thiadiazine-*S*,*S*-dioxides such as diazoxide (**2**) with its manifold analogues [BPDZ73 (**3**) may be considered a typical representa-

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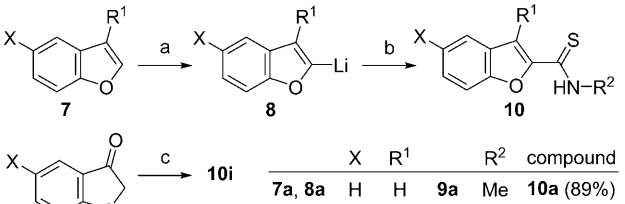
tive], and 3. the benzopyran and chromene family including *lev*-cromakalim (**4**), KC-399 (**5**), and the related benzothiazine **6** as representative drugs.[2]

Despite the impressive progress that has been made with the benzopyrans and benzothiazines in terms of affinity, the development of tissue-selective PCOs remains a challenge, and high affinity combined with selectivity toward the different $K_{ATP}$ channels composed of various combinations of SUR and Kir isoforms are highly desirable. In pursuing this goal, we were guided by the idea that heterocyclic skeletons, hitherto unused in $K_{ATP}$-active drugs, might be promising candidates for the development of new and selective PCOs. For this purpose we combined, for the first time, the thioamide pharmacophore[3] with the benzofuran, benzothiophene, and benzothiazole skeletons.

## Results and Discussion

### Synthesis

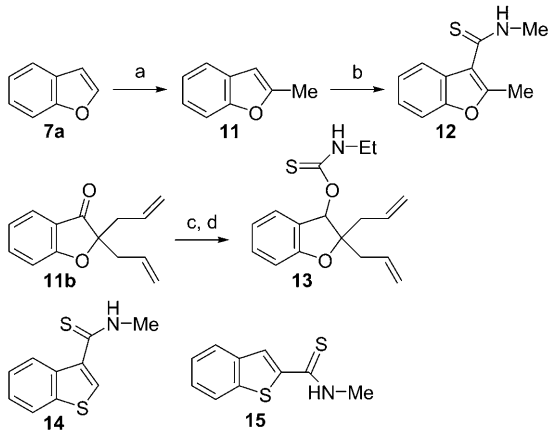
The heterocyclic skeletons of benzofuran, 3-coumaranone, benzothiophene, and benzothiazole were linked to the thioamide moiety that was introduced in all cases by using alkyl or aryl isothiocyanates as electrophilic reagents. In a first series (Scheme 1), we took advantage of the known acidity of benzofuran at carbon atom 2.[4] Thus, treatment of benzofuran **7a** with *n*-butyllithium generated 2-lithiobenzofuran **8a**, which was quenched with isothiocyanates **9a**–**e** to give the respective thioamides **10a**–**e**. In an analogous manner, thioamide **10h** was prepared from trifluoromethyl-substituted benzofuran **7c** via the lithiated intermediate **8c**. When 5-bromobenzofuran **7b** was used as the starting material, the deprotonation was performed with lithium diisopropylamide instead of *n*-butyllithium to avoid a bromine–lithium exchange. The relatively low yields of thioamides **10f** and **10g** resulting from lithiobenzofuran **8b** and isothiocyanates **9a** and **9b** was probably caused by incomplete deprotonation due to the weaker base and by the undesired addition of diisopropylamine to the isothiocyanates. 3-Benzofuranone **11** was treated with sodium hydride to generate the enolate prior to the reaction with ethyl isothiocyanate **9b** to give 3-hydroxybenzofuran-2-carbothioamide **10i**. It formed completely as the enol tautomer according to its spectroscopic data.




| | X | $R^1$ | | $R^2$ | compound |
|---|---|---|---|---|---|
| **7a**, **8a** | H | H | **9a** | Me | **10a** (89%) |
| **7a**, **8a** | H | H | **9b** | Et | **10b** (70%) |
| **7a**, **8a** | H | H | **9c** | *n*Bu | **10c** (99%) |
| **7a**, **8a** | H | H | **9d** | *t*Bu | **10d** (22%) |
| **7a**, **8a** | H | H | **9e** | Ph | **10e** (95%) |
| **7b**, **8b** | Br | H | **9a** | Me | **10f** (29%) |
| **7b**, **8b** | Br | H | **9b** | Et | **10g** (20%) |
| **7c**, **8c** | $CF_3$ | Me | **9b** | Et | **10h** (88%) |
| **11** | H | OH | **9b** | Et | **10i** (44%) |

**Scheme 1.** Synthesis of benzofuran-2-carbothioamides **10**. *Reagents and conditions*: a) *n*BuLi, THF, −78 °C, 1 h; b) $R^2N{=}C{=}S$ (**9**), −78 °C→RT, 16 h; c) NaH, THF, then **9b**, RT, 18 h.

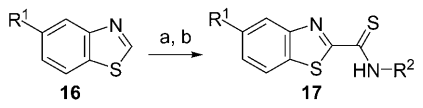
The position where the thioamide group is attached at the heterocyclic skeleton was expected to have a substantial influence on the affinity for $K_{ATP}$ channels. To prove this, benzofuran **12** carrying the thioamide residue at position 3 was prepared. The synthesis of **12** started with 2-methylbenzofuran **11**, itself available from **7a** by metallation and treatment with methyl iodide using a modified published procedure.[5] A Friedel–Crafts-type acylation[6] performed with methyl isothiocyanate **9a** permitted the introduction of the thioamide moiety at position 3 to give benzofuran **12** in a completely regioselective manner (Scheme 2). The substituted coumaranone **11b**[7] served us as starting material for the preparation of carbamate **13**, which was obtained by reduction with sodium borohydride and subsequent treatment with ethyl isothiocyanate **9b**. For reasons of comparison, the regioisomeric benzothiophene-derived thioamides **14** and **15**, prepared according to published procedures,[8] were also included in the evaluation as potential PCOs.




**Scheme 2.** Synthesis of benzofuran-derived thioamide **12** and carbamate **13**. Regioisomeric benzothiophenes **14** and **15**. *Reagents and conditions*: a) 1. *n*BuLi, THF, −78 °C, 1 h, 2. MeI, −78 °C→RT, 16 h, 68%; b) **9a**, $AlCl_3$, $MeNO_2$, RT, 24 h, 35%; c) $NaBH_4$ (12 equiv), EtOH, 0 °C→RT, 99%; d) *n*BuLi, **9b**, $Et_2O$, −78 °C→RT, 29%.

In two additional series, we determined whether a combination of the thioamide moiety with the benzothiazole skeleton would lead to compounds with activity at $K_{ATP}$ channels. Thus, benzothiazoles **16a** and **16b** were first coupled directly with isothiocyanates, taking advantage of the known acidity at position 2 of the ring skeleton.[9] The trifluoromethyl-substituted, hitherto unknown benzothiazole **16b** was obtained from 2-amino-4-(trifluoromethyl)thiophenol and formaldehyde in the presence of scandium trifluoromethanesulfonate in 72% yield.[10] The lithiation of benzothiazoles **16a** and **16b** at the 2-position was performed at −100 °C with *n*-butyllithium. The labile intermediates thus generated were quenched at the same temperature with isothiocyanates **9a**, **9b**, and **9e** to give the thioamides **17a**–**e** (Scheme 3).

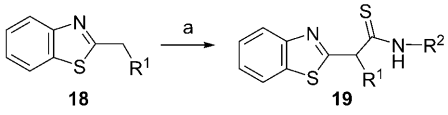



16 17

| | $R^1$ | | $R^2$ | compound |
|---|---|---|---|---|
| **16a** | H | **9a** | Me | **17a** (19%) |
| **16a** | H | **9b** | Et | **17b** (41%) |
| **16a** | H | **9e** | Ph | **17c** (31%) |
| **16b** | $CF_3$ | **9b** | Et | **17d** (48%) |
| **16b** | $CF_3$ | **9e** | Ph | **17e** (78%) |

**Scheme 3.** Synthesis of benzothiazole-2-carbothioamides **17**. *Reagents and conditions*: a) *n*BuLi, THF, −100 °C, 1 h; b) **9**, −75 °C→−50 °C, 20 min.

Finally, we envisaged incorporating a one-carbon spacer between the benzothiazole and the thioamide moieties. For this purpose, thiazoles **18**, carrying an alkyl side chain at position 2, were submitted to deprotonation[11] and subsequently quenched with isothiocyanates. Thus, thioamides **19a–d** were synthesized (Scheme 4). They contain either a methylene




18 19

| | $R^1$ | | $R^2$ | compound |
|---|---|---|---|---|
| **18a** | H | **9b** | Et | **19a** (16%) |
| **18b** | Me | **9b** | Et | **19b** (13%) |
| **18c** | Et | **9b** | Et | **19c** (9%) |
| **18b** | Me | **9d** | *t*Bu | **19d** (58%) |

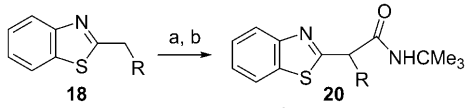
**Scheme 4.** Synthesis of "spacered" benzothiazole-derived thioamides **19**. *Reagents and conditions*: a) PhLi, $Et_2O$, −78 °C, 1 h; b) **9**, −78 °C→RT, overnight.

spacer (in **19a**) or a branched spacer (in **19b–d**). The latter compounds were prepared as racemic mixtures.

To evaluate the role of the carbothioamide in relation to the carboxylic amide, lithiated benzothiazoles **18a** and **18c** were allowed to react with *tert*-butylisocyanate to give the carboxylic amides **20a** and **20b**, respectively (Scheme 5).

### In vitro biological studies

In view of the fact that benzofurans, benzothiophenes, and benzothiazoles have not been recognized as $K_{ATP}$ channel openers, the binding affinity of all the compounds **10**, **12–15**, **17**, **19**, and **20** was measured in HEK 293 (SUR2B/Kir6.1) cells,




18 20

| | R | compound |
|---|---|---|
| **18a** | H | **20a** |
| **18b** | Me | **20b** |

**Scheme 5.** Synthesis of benzothiazole-derived amides **20**. *Reagents and conditions*: a) *n*BuLi, $Et_2O$, −78 °C, 1 h; b) $Me_3CNCO$, −78 °C→RT, overnight. **20a**: 36%, **20b**: 10%.

which stably express the arterial smooth-muscle-type $K_{ATP}$ channel. By using the agonist radioligand [$^3$H]P1075, the p$K_D$ values listed in Table 1 were determined. All the compounds investigated exhibited afinity; however, the afinity varied considerably, and the binding constants range from the three-digit to the single-digit micromolar level.

**Table 1.** Dissociation constants for compounds **10**, **12–15**, **17**, **19**, **20**, and **2** determined by radioligand binding at HEK 293 (SUR2B/Kir6.1) cells.

| Entry | Compd | p$K_D$[a] ± ASD[b] | Entry | Compd | p$K_D$[a] ± ASD[b] |
|---|---|---|---|---|---|
| 1 | **10a** | 3.82 ± 0.04 | 2 | **10b** | 4.23 ± 0.03 |
| 3 | **10c** | 4.39 ± 0.03 | 4 | **10d** | 4.38 ± 0.03 |
| 5 | **10e** | 3.60 ± 0.09 | 6 | **10f** | 4.34 ± 0.03 |
| 7 | **10g** | 4.44 ± 0.02 | 8 | **10h** | 3.99 ± 0.05 |
| 9 | **10i** | 4.08 ± 0.02 | 10 | **12** | 3.95 ± 0.04 |
| 11 | **14** | 4.18 ± 0.03 | 12 | **15** | 4.64 ± 0.03 |
| 13 | **17a** | 4.22 ± 0.06 | 14 | **17b** | 4.12 ± 0.03 |
| 15 | **17c** | < 3.30 | 16 | **17d** | 4.12 ± 0.04 |
| 17 | **17e** | 4.08 ± 0.05 | 18 | **19a** | 4.31 ± 0.03 |
| 19 | **19b** | 4.83 ± 0.03 | 20 | **19c** | 5.03 ± 0.03 |
| 21 | **19d** | 5.39 ± 0.03 | 22 | **20a** | 4.12 ± 0.02 |
| 23 | **20b** | 4.30 ± 0.03 | 24 | **2** | 4.97 ± 0.02 |
| 25 | **13** | 3.88 ± 0.04 | | | |

[a] p$K_D$ values (−log M) calculated by nonlinear regression according to Equation (1); see Experimental Section. [b] Asymptotic standard deviations estimated by nonlinear regression.

In a first series of benzofurans **10a–e** (Table 1 Entries 1–5), the influence of the substituent at the nitrogen atom of the 2-carbothioamide residue was tested. The affinity increases from methyl to ethyl (Entries 1 and 2) to *n*- or *tert*-butyl, the latter compounds **10c** and **10d** exhibiting very similar p$K_D$ values (Entries 3 and 4). These results suggest that lipophilic residues at the nitrogen atom are favorable with respect to affinity. On the other hand, a phenyl group at this position is deleterious (Entry 5). An additional bromine substituent at position 5 of the heterocyclic core leads to a slight enhancement in affinity (Entries 6 vs. 1, and 7 vs. 2). The electron-withdrawing trifluoromethyl group, which has been found to be advantageous in PCOs based on the benzopyran skeleton, did not lead to stronger binding in the case of benzofuran **10h**, which is equipped with that group at position 5. Compound **10i**, which features a hydroxy group in addition to the thioamide residue, did not provide higher affinity either.

To determine whether the position of the thioamide group influences binding at the SUR2B-type $K_{ATP}$ channel, furans **10a** and **12** (Table 1, Entry 1 vs. Entry 10) as well as the benzothiophenes **14** and **15** (Entry 11 vs. Entry 12) were compared. The marginal difference between the p$K_D$ values of compounds **10a** and **12** cannot be simply attributed to the substitution pattern of the thioamide residue, as benzofuran **12** differs from **10a** by an additional methyl substituent. Clearer insight comes from the regioisomeric benzothiophenes **14** and **15**: the attachment of the thioamide group at position 2 of the heterocyclic ring is more favorable than the 3-substitution pattern, and the p$K_D$ values differ by ~0.5 log units. On the other

hand, comparison of the activity of compounds **10a** and **15** (Entry 1 vs. Entry 12) clearly reveals that replacement of the oxygen atom in the five-membered ring with sulfur leads to an enhancement in affinity of 0.82 log units. This result suggests that an "enlarged" five-membered heterocyclic ring containing sulfur instead of oxygen might more effectively fill the space available in the receptor. Therefore, we designed and synthesized benzothiazole-derived thioamides, anticipating that the additional nitrogen atom would lead to an additional hydrophilic binding site at the receptor.

However, the affinity of neither benzothiazoles **17a** and **17b** nor derivatives **17d** and **17e**, with the electron-withdrawing trifluoromethyl substituent at position 5, surpassed that of benzothiophene **15** or of bromo-substituted benzofuran **10g** (Entry 12 vs. Entries 13, 14, 16, and 17). Here again, the *N*-phenyl unit diminished the affinity substantially (Entry 15).

Thus we turned to benzothiazoles containing a spacer carbon atom between the heterocyclic ring and the thioamide moiety, guided by the idea that this pattern might provide more flexibility in the pharmacophoric units thus permitting a better fit and enhanced affinity at the SUR2B receptor. Whereas compound **19a**, with a methylene spacer, showed a p$K_D$ value of 4.31 (Entry 18), benzothiazoles **19b** and **19c**, featuring a branched spacer, led to a substantial increase in affinity (Entries 19 and 20). Finally, the combination of a branched spacer with a bulky *tert*-butyl residue at the nitrogen atom provided the highest affinity in the single-digit micromolar range (Entry 21). The low affinity of the carboxylic amides **20a** and **20b** clearly reveals the role of the carbothioamide group as a true pharmacophore. Thus, the p$K_D$ values of compounds **19d** and **20b**, with a substitution of sulfur by oxygen and otherwise identical skeleton, differ by more than one log unit (Entry 21 vs. 23). For reasons of comparison, the p$K_D$ value of the drug diazoxide (**2**) is also included (Entry 24).[12] Finally, carbamate **13** represents a swap of the lipophilic and hydrophilic regions relative to the benzofurans **10**; this dramatic modification is deleterious to the affinity (Entry 25).

In a recent study on modified diazoxides, we had observed that sterically demanding lipophilic residues at the heterocyclic ring caused not only enhanced affinity but also remarkable selectivity between the SUR2B/Kir6.1 and the SUR1/Kir6.2 receptors.[12] Therefore, we were also interested to determine the selectivity of the current compounds. However, with no agonist radioligands for SUR1-type $K_{ATP}$ channels yet available which would permit the determination of $K_D$ values for SUR1-agonists, the selectivity of compounds for different channel types must be measured by functional methods. The measurement of agonist-induced membrane hyperpolarization is an adequate method to discriminate between agonistic and antagonistic effects and to determine $pEC_{50}$ values in CHO (SUR1/Kir6.2) and HEK 293 (SUR2B/Kir6.1) cells.[13] Membrane hyperpolarization by increasing the outward $K^+$ current is a necessary prerequisite to decrease the influx of $Ca^{2+}$ ions, which, in turn, decreases the insulin release of the β cell (SUR1/Kir6.2) and the relaxation of smooth muscle (SUR2B/Kir6.1). Membrane potential can be measured by fluorescence using fluorescent dyes such as $DiBAC_4(3)$ and DyeB (R7260) which makes it possible to test a manifold of cells of the same age cultivated in 12-well strips, thereby increasing the reliability of results with decreasing experimental effort. The reliability of membrane hyperpolarization for the detection of agonistic versus antagonistic effects has been highlighted.[14]

Several compounds that showed significant affinity according to the data obtained from radioligand binding (Table 1) were assayed for $K_{ATP}$ activity by means of the membrane polarization measurements, the results of which are listed in Table 2. For reasons of comparison, the corresponding data for diazoxide (**2**) are also included. The representative experiment with thioamide **19d** displayed in Figure 2b demonstrates the exponential decline of the membrane potential with time, induced by increasing concentrations of **19d** in HEK 293 (SUR2B/Kir6.1) cells. The agonistic effects of compounds were reversed upon addition of glibenclamide (not shown), a $K_{ATP}$ channel inhibitor, confirming a $K_{ATP}$ mechanism. Remarkably, benzothiazole **19d** (at 25 μM) failed to induce membrane hyperpolarization in SUR1-type $K_{ATP}$ channels (Figure 2a), whereas it was effective in hyperpolarizing membrane potential in the SUR2B-type channels.

Most of the compounds were characterized by a two- to threefold (i.e., 0.3–0.5 log units) higher functional potency ($EC_{50}$) than binding affinity measured as p$K_D$ (Table 1), reflecting the fact that in artificial cell lines that overexpress $K_{ATP}$ channels, only part of the functional $K_{ATP}$ channels are necessary for membrane hyperpolarization, whereas all of the binding sites equally contribute to radioligand binding. In all cases, the membrane potentials were determined using $DiBAC_4(3)$. In addition, measurements with benzothiazoles **19b**–**d** were also performed with DyeB, an alternative membrane potential dye, which turned out to be less sensitive to quenching effects.[15]

**Table 2.** Membrane hyperpolarization ($pEC_{50}$ values and selectivity) at HEK 293 and CHO cells.

| Entry | Compd | $pEC_{50}$[a] | | Selectivity [Δ][b] |
|---|---|---|---|---|
| | | HEK 293 (SUR2B/Kir6.1) | CHO (SUR1/Kir6.2) | |
| 1 | **2** | 5.14 ± 0.02 | 4.72 ± 0.02 | 0.42 |
| 2 | **15** | 4.80 ± 0.03 | 4.62 ± 0.02 | 0.18 |
| 3 | **10d** | 4.72 ± 0.04 | 3.82 ± 0.03 | 0.90 |
| 4 | **19b** | 5.07 ± 0.02 (4.93 ± 0.02)[c] | 5.4 ± 0.06 %[d] | > 1.4 |
| 5 | **19c** | 5.08 ± 0.03 (5.14 ± 0.03)[c] | 6.3 ± 1.1 %[e] | > 1.3 |
| 6 | **19d** | 5.41 ± 0.03 (5.45 ± 0.03)[c] | 2.5 ± 1.5 %[f] | > 2.0 |
| 7 | **20b** | 3.97 ± 0.02[c,g] | 7.8 ± 1.0 %[h] | –[i] |

[a] Membrane potentials determined with $DiBAC_4(3)$; $pEC_{50}$ values (−log M) ± ASD calculated by nonlinear regression according to Equation (2); see Experimental Section. [b] Selectivity calculated as $\Delta = pEC_{50(SUR2B/Kir6.1)} - pEC_{50(SUR1/Kir6.2)}$. [c] Membrane potential determined with DyeB. [d] $pEC_{50} = 3.45 \pm 0.09$ at 25 μM (extrapolated). [e] $pEC_{50} = 3.69 \pm 0.03$ at 25 μM (extrapolated). [f] $pEC_{50} = 3.30 \pm 0.02$ at 25 μM (extrapolated). [g] Extrapolated. [h] $pEC_{50} = 3.5 \pm 0.1$ at 25 μM (extrapolated). [i] Δ value not given because both $pEC_{50}$ values are extrapolated.

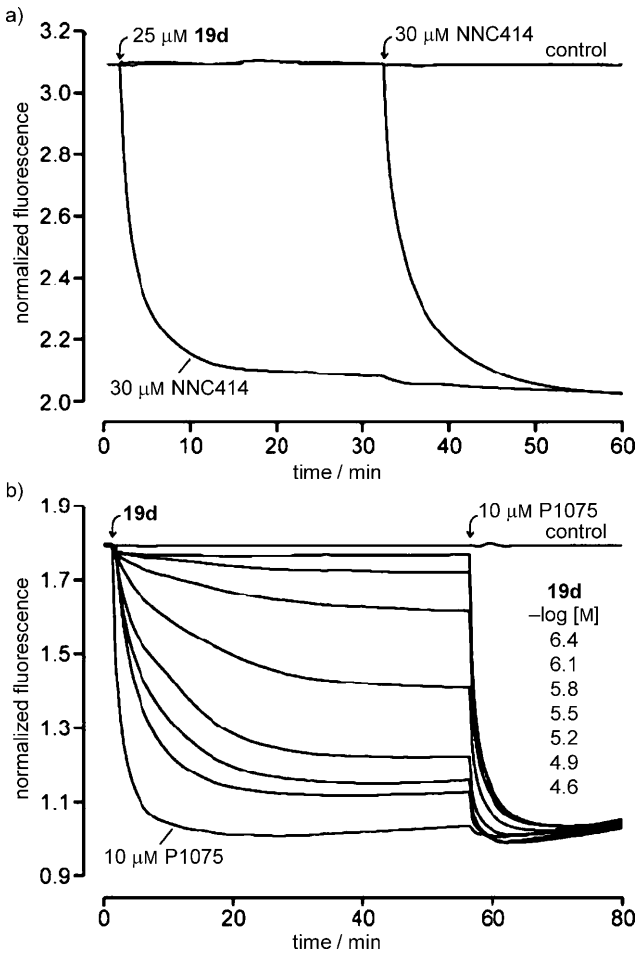



**Figure 2.** Representative experiments demonstrating the measurement of membrane hyperpolarization in a) CHO cells transfected with SUR1/Kir6.2 and b) HEK 293 cells transfected with SUR2B/Kir6.1 with thioamide **19d**. Membrane potential was measured by fluorescence using the bis-oxonol dye $DiBAC_4(3)$. Maximum effects were induced with the thienothiadiazine derivative NNC414 and with the cyanoguanidine P1075 in SUR1- and SUR2B-type channels, respectively. Changes in $DiBAC_4(3)$ fluorescence (arbitrary units, data sampling at a frequency of 1 Hz) were normalized to maximum effects. Note that 25 μM **19d** in panel a) coincides with its −log value of 4.6 in panel b).

In comparing the drug diazoxide (**2**) with benzothiophene **15** regarding affinity and selectivity toward the two receptors, SUR2B/Kir6.1 and SUR1/Kir6.2, it is evident that **15** is inferior in both respects to diazoxide (Table 2, Entries 1 and 2). Although replacement of sulfur with oxygen in the five-membered heterocycles does not improve affinity (Table 2, Entry 2 vs. 3), the membrane potential measurement of benzofuran **10d** reveals a substantial increase in selectivity in favor of the SUR2B-type $K_{ATP}$ channels (Table 2, Entry 3). This effect may be caused by the sterically demanding, lipophilic *tert*-butyl residue at the thioamide moiety of the benzofuran **10d**. Another favorable effect came from the combination of a spacer carbon atom between the heterocyclic skeleton and the thioamide residue and a lipophilic group at the thioamide nitrogen atom. These structural features were realized in benzothiazoles **19b–d** (Table 2, Entries 4–6). Thus, the optimum result was obtained with the "spacered", *tert*-butyl-substituted compound **19d**, which not only displays the highest affinity in the series but also the highest selectivity. Its affinity for the SUR2B/Kir6.1 receptor of HEK 293 cells surpasses that for the CHO (SUR1/Kir6.2) cells by more than two orders of magnitude. In a sense, this result parallels our recent finding that the introduction of bulky, lipophilic residues in the side chain of diazoxide leads to a remarkable enhancement in selectivity in favor of the SUR2B receptor. These results are illustrated for selected compounds, benzothiophene **15**, benzofuran **10d**, and benzothiazole **19d** in comparison with diazoxide (**2**) in Figure 3, which displays the measurement of the membrane potential of HEK 293 (SUR2B/Kir6.1) and CHO (SUR1/Kir6.2) cells. In particular, it shows the remarkable selectivity of thioamide **19d**. Here again, the crucial role played by the carbothioamide pharmacophore is illustrated by the marginal membrane polarization of the carboxylic amide **20b** that differs from thioamide **19d** only in the exchange of sulfur for oxygen (Entry 7). Comparison of the membrane hyperpolarization with HEK 293 and CHO cells also reveals that replacement of the carbothioamide group with a carboxylic amide is not only deleterious to affinity but also provides only marginal selectivity (Entry 7 vs. 6).

As the most potent compounds **19b–d** are used as racemic mixtures, the question of differences in biological activity between individual enantiomers might arise. However, due to facile enolization, racemization under physiological conditions is expected to occur. This was proven by keeping compound **19d** in a neutral solution of DMSO and $D_2O$ at room temperature for one day: a complete deuterium exchange occurring at the stereogenic carbon center (in addition to the carbothioamide nitrogen atom) under these conditions clearly proves enolization and, in an indirect manner, the configurational instability at the stereogenic center.

Crystal structure analyses were obtained for two of the most potent compounds, **19b** and **19d**. They are shown in Figure 4. The hydrogen-bond donor (NH of the thioamide group) and concomitant hydrogen-bond acceptor (the thiazole nitrogen atom) lead to a dimeric arrangement in the unit cell. Thus, the crystal structures, in combination with the structure–activity relationship, reveal three features that might be important for the affinity and selectivity as $K_{ATP}$ channel openers: 1) the hydrogen-bonding domain, 2) a region of high electron density arising from the lone pairs at the sulfur atoms, and 3) the lipophilic side chain at the thioamide moiety. A model based thereupon is illustrated in Figure 5 for thioamide **19d**. Work intended to evaluate and verify the three-binding-sites model through various heterocyclic skeletons and ring sizes is in progress.

## Conclusion

In summary, this is the first demonstration that the heterocyclic skeletons of benzofuran, benzothiophene, and benzothiazole combined with a thioamide moiety provide compounds with substantial activity as $K_{ATP}$ channel openers. With regard to affinity, the best results were obtained if the benzothiazole heterocycle is linked to the thioamide group through a branched

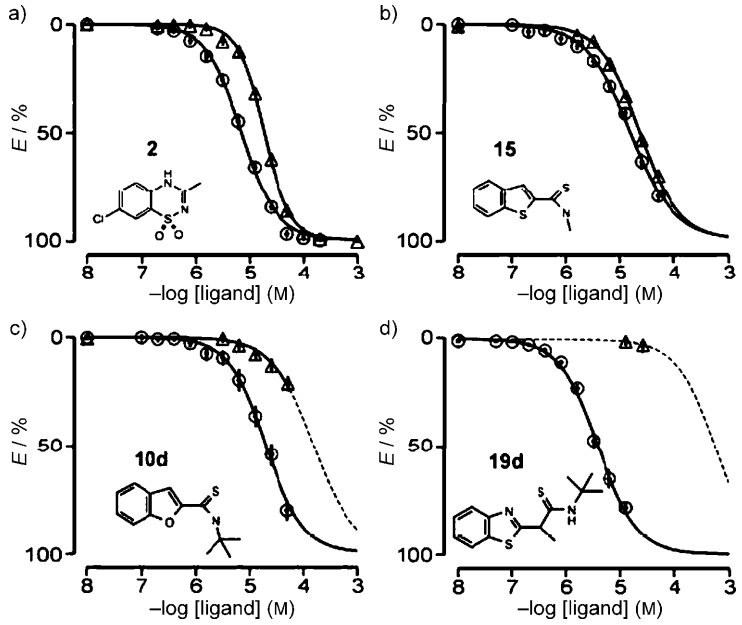



**Figure 3.** Comparison of the effects of a) diazoxide (**2**), b) benzothiophene **15**, c) benzofuran **10d**, and d) benzothiazole **19d** on the membrane potential (*E*) of HEK 293 cells transfected with SUR2B/Kir6.1 (○) and on CHO cells transfected with SUR1/Kir6.2 (△). For compound **19d** no significant hyperpolarization could be measured in CHO (SUR1/Kir6.2) cells; the dashed line represents a hypothetical curve according to Eq. (1), indicating an upper limit for the effectiveness of **19d** on SUR1-type $K_{ATP}$ channels.

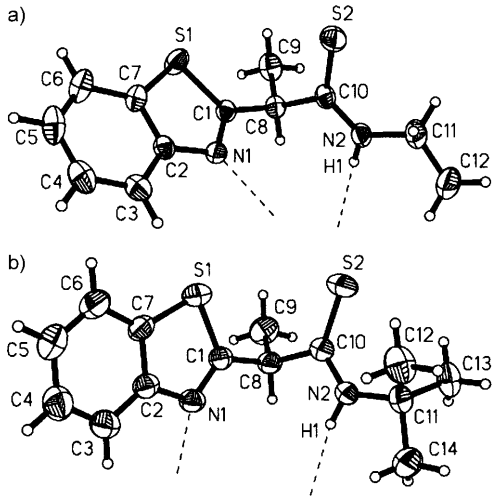



**Figure 4.** Diagrams of the molecular structures of a) **19b** and b) **19d**; displacement ellipsoids are drawn at the 50% probability level, radii of H atoms are chosen arbitrarily, and only H atoms involved in hydrogen bonding are labeled. Selected geometric parameters, **19b** [**19d**] bond lengths {in Å}: S1–C7 1.721(2) [1.736(3)], S1–C1 1.736(2) [1.731(2)], S2–C10 1.658(2) [1.658(2)], N1–C1 1.291(3) [1.303(2)], N1–C2 1.395(3) [1.396(3)], N2–C10 1.320(3) [1.317(3)], N2–C11 1.464(3) [1.493(3)], C1–C8 1.511(3) [1.510(3)], C2–C3 1.387(3) [1.403(3)], C2–C7 1.395(3) [1.387(3)], C3–C4 1.371(4) [1.373(4)], C4–C5 1.381(4) [1.383(4)], C5–C6 1.370(4) [1.368(4)], C6–C7 1.400(3) [1.393(3)], C8–C9 1.524(3) [1.530(3)], C8–C10 1.537(3) [1.549(3)], C11–C12 1.481(4) [1.526(3)], C11–C13 [1.530(3)], C11–C14 [1.519(3)], N2–H1 0.85(2) [0.89(2)], H1···N1′ 2.16(2) [2.24(3)]; bond angles {in °}: N2–H1···N1′ 178(2) [162(2)], S1–C1–C8–C9 −14.7(3) [−56.8(2)].

one-carbon spacer. The concept of introducing sterically demanding, lipophilic residues turned out to be fruitful for high

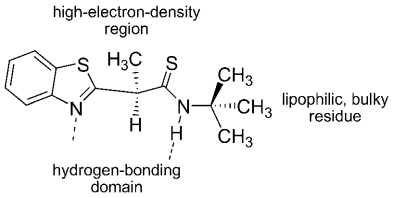



**Figure 5.** Three-binding-sites model of the PCO **19d**.

selectivity. They clearly prevent benzothiazoles from binding to the SUR1 receptor, so that the selectivity for SUR2B-type $K_{ATP}$ channels is >100-fold higher. This selectivity distinguishes the benz-annulated compounds also developed from the established drug diazoxide (**2**) that is in clinical use.

## Experimental Section

### *Syntheses*

Melting points (uncorrected) were determined with a Büchi melting point apparatus 540. IR spectra: Bruker Vector 22. NMR spectra ($\delta$ values in ppm): Bruker AM-200-SY and AM-500. Mass spectra: Finnigan MAT 8200. TLC: Silica gel 60 $F_{254}$ (Merck). Column chromatography: Macherey–Nagel Kieselgel 60 and Merck Kieselgel 60, mesh size 0.04–0.063 mm. Elemental analyses were carried out with a Perkin–Elmer CHN Analyzer 2400 at the Institute of Pharmaceutical Chemistry (University of Düsseldorf). All reactions involving organometallic compounds were carried out under an atmosphere of anhydrous $N_2$. THF and $Et_2O$ were predried with KOH and distilled under $N_2$ from sodium/benzophenone. They were taken from the distillation flask, which was closed by a septum, with syringes or cannulae. Reactions at temperatures <0 °C were monitored by a thermocouple connected to a resistance thermometer (Ebro).

*General procedure for the preparation of benzofuran-2-carbothioamides **10a–e***: A 100 mL two-necked flask was equipped with a magnetic stirrer, a connection to a combined $N_2$/vacuum line and closed with a septum. The air in the flask was replaced by $N_2$, and benzofuran **7a** or **7c** (20.0 mmol), dissolved in 15 mL dry THF, was injected by syringe. While stirring, the mixture was cooled to −78 °C in a dry-ice/acetone bath. A 1.6 M solution of *n*-butyllithium in hexane (12.5 mL, 20.0 mmol) was injected slowly. After stirring for 1 h at the same temperature, the corresponding isothiocyanate **9a–e**, dissolved in 2 mL dry THF, was added by syringe. The solution was allowed to reach room temperature while stirring overnight. After the addition of pentane (50 mL), the mixture was filtered and the filtrate was concentrated in a rotary evaporator. The oily residue was purified by column chromatography.

**According to this procedure, the following compounds were obtained:**

***N*-Methylbenzofuran-2-carbothioamide (10a)** from **7a** (20.0 mmol) and methyl isothiocyanate (**9a**) (20.0 mmol): Yield: 3.4 g (89%); mp: 123.6 °C; $R_f$ = 0.5 (pentane/EtOAc 1:1); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 3.32 (d, $J$ = 4.73 Hz, 3H, $CH_3$), 7.22 (d, $J$ = 7.88 Hz, 1H, 6-H), 7.33–7.40 (m, 2H, 4-H and 5-H), 7.59 (d, $J$ = 7.88 Hz, 1H, 7-H), 7.66 (s, 1H, 3-H), 8.27 (br s, 1H, NH); $^{13}$C NMR

(125 MHz, $CDCl_3$): $\delta = 32.5$ ($CH_3$), 112.1 (C4), 113.8 (C3), 123.2 (C7), 124.3 (C6), 127.9 (C3′), 128.5 (C5), 153.0 (C7′), 154.9 (C2), 184.5 (C=S); IR (KBr): $\tilde{\nu} = 3324.7$, 2362.2, 1578.6, 1526.3, 1474.2, 1444.3, 1363.4, 1291.7, 1161.5, 1121.5, 1047.2, 977.7, 939.4, 886.0, 831.3, 781.2, 751.3, 691.6, 612.8, 502.4 $cm^{-1}$; MS (EI, 70 eV): *m/z* (%) = 192 ($[M+1]^+$, 13), 191 ($[M]^+$, 100), 162 (24), 161 (32), 158 (18), 150 (35), 131 (18), 121 (12), 118 (35), 90 (11), 89 (24), 63 (9); Anal. calcd for $C_{10}H_9NOS$ (191.25): C 62.80, H 4.74, N 7.32, found: C 62.72, H 4.62, N 7.11.

***N*-Ethylbenzofuran-2-carbothioamide (10b)** from **7a** (10.0 mmol) and ethyl isothiocyanate (**9b**) (10.0 mmol): Yield: 1.9 g (93%); mp: 272.7 °C; $R_f = 0.5$ ($CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 1.35$ (t, $J$ = 7.4 Hz, 3H, $CH_3$), 3.84 (dq, $J = 5.9$ Hz, $J = 7.0$ Hz, 2H, $CH_2$), 7.22 (t, $J = 7.7$ Hz, 1H, 6-H), 7.36 (t, $J = 8.0$ Hz, 1H, 5-H), 7.40 (d, $J = 8.5$ Hz, 1H, 4-H), 7.59 (d, $J = 7.9$ Hz, 1H, 7-H), 7.66 (s, 1H, 3-H), 8.13 (s (br), 1H, NH); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta = 13.9$ ($CH_3$), 40.6 ($CH_2$), 112.1 (C4), 114.0 (C3), 123.2 (C7), 124.3 (C6), 127.9 (C3′), 128.5 (C5), 152.9 (C7′), 154.9 (C2), 183.3 (C=S); MS (EI, 70 eV): *m/z* (%) = 206 ($[M+1]^+$, 17), 205 ($[M]^+$, 100), 204 (27), 172 (12), 161 (37), 144 (42), 118 (33), 89 (22), 44 (14); Anal. calcd for $C_{11}H_{11}NOS$ (205.28): C 64.39, H 5.36, N 6.82, found: C 64.15, H 5.48, N 6.93.

***N*-*n*-Butylbenzofuran-2-carbothioamide (10c)** from **7a** (4.64 mmol) and *n*-butyl isothiocyanate (**9c**) (4.64 mmol): Yield: 1.07 g (99%); mp: 49.2 °C; $R_f = 0.6$ ($CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 0.92$ (t, $J = 7.4$ Hz, 3H, $CH_3$), 1.41 (sext, $J = 7.6$ Hz, 2H, $CH_2$-$CH_3$), 1.70 (quint, $J = 7.4$ Hz, 2H, $CH_2$-$CH_2$-$CH_3$) 3.79 (q, $J$ = 6.5 Hz, 2H, -NH-$CH_2$), 7.20 (t, $J = 7.4$ Hz, 1H, 6-H), 7.34 (t, $J = 7.4$ Hz, 1H, 5-H), 7.39 (d, $J = 8.5$ Hz, 1H, 4-H), 7.57 (d, $J = 7.9$ Hz, 1H, 7-H), 7.64 (s, 1H, 3-H), 8.15 (br s, 1H, NH); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 14.2 ($CH_3$), 20.7 ($CH_2$-$CH_3$), 30.7 ($CH_2$-$CH_2$-$CH_3$), 45.5 (NH-$CH_2$), 112.1 (C4), 114.0 (C3), 123.1 (C7), 124.2 (C6), 127.8 (C3′), 128.5 (C5), 153.0 (C7′), 154.9 (C2), 183.4 (C=S); MS (EI, 70 eV): *m/z* (%) = 234 ($[M+1]^+$, 14), 233 ($[M]^+$, 85), 200 (51), 191 (52), 162 (24), 161 (100), 150 (27), 144 (65), 118 (10), 89 (21); Anal. calcd for $C_{13}H_{15}NOS$ (233.33): C 66.92, H 6.48, N 6.00, found: C 66.72, H 6.69, N 6.00.

***N*-*tert*-Butylbenzofuran-2-carbothioamide (10d)** from **7a** (4.64 mmol) and *tert*-butyl isothiocyanate (**9d**) (4.64 mmol): Yield: 241 mg (22%); mp: 80.3 °C; $R_f = 0.6$ ($CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 1.26$ (s, 9H, $CH_3$), 7.19 (t, $J = 7.4$ Hz, 1H, 6-H), 7.33 (t, $J$ = 8.0 Hz, 1H, 5-H), 7.38 (d, $J = 8.5$ Hz, 1H, 4-H), 7.56 (d, $J = 7.6$ Hz, 1H, 7-H), 7.62 (s, 1H, 3-H), 8.04 (br s, 1H, NH); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta = 28.4$ (-$CH_3$), 56.3 (-$C(CH_3)_3$), 112.1 (C4), 113.3 (C3), 123.0 (C7), 124.2 (C6), 127.6 (C3′), 128.7 (C5), 153.9 (C7′), 154.7 (C2), 182.3 (C=S); MS (EI, 70 eV): *m/z* (%) = 234 ($[M+1]^+$, 15), 233 ($[M]^+$, 86), 232 (41), 177 (33), 161 (23), 150 (30), 145 (13), 144 (100), 143 (11), 89 (13); Anal. calcd for $C_{13}H_{15}NOS$ (233.33): C 66.92, H 6.48, N 6.00, found: C 66.89, H 6.56, N 5.90.

***N*-Phenylbenzofuran-2-carbothioamide (10e)** from **7a** (10.1 mmol) and phenyl isothiocyanate (**9e**) (10.1 mmol): Yield: 2.42 g (95%); mp: 152.6 °C; $R_f = 0.8$ ($CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.21–7.26 (m, 2H, phenyl *o*-H), 7.36–7.42 (m, 3H, phenyl *m*,*p*-H), 7.45 (d, $J = 8.5$ Hz, 1H, 4-H), 7.61 (d, $J = 7.9$ Hz, 1H, 7-H), 7.75 (s, 1H, 3-H), 7.83 (d, $J = 8.2$ Hz, 2H, 5-H and 6-H), 9.68 (br s, 1H, NH); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta = 112.2$ (C4), 115.1 (C3), 123.2 (C7), 124.0 (C6), 124.5 (C3′), 127.5 (phenyl C-2), 128.2 (phenyl C-4), 128.7 (C5), 129.4 (phenyl C-3), 138.4 (phenyl C-1), 153.5 (C7′), 154.8 (C2), 181.4 (C=S); MS (EI, 70 eV): *m/z* (%) = 254 ($[M+1]^+$, 20), 253 ($[M]^+$, 97), 252 (93), 220 (42), 161 (100), 144 (15), 110 (15), 101 (11), 89 (15); Anal. calcd for $C_{15}H_{11}NOS$ (253.32): C 71.12, H 4.38, N 5.53, found: C 70.39, H 4.46, N 5.54.

***N*-Ethyl-3-methyl-5-trifluoromethylbenzofuran-2-carbothioamide (10h)** from **7c** (1.37 mmol) and ethyl isothiocyanate (**9b**) (1.37 mmol): Yield: 0.35 g (88%); mp: 109.6 °C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 1.33$ (t, $J = 7.3$ Hz, 3H, $CH_2$-$CH_3$), 2.73 (s, 3H, 3-$CH_3$), 3.80 (dq, $J = 5.9$ Hz, $J = 7.1$ Hz, 2H, $CH_2$), 7.45 (d, $J = 8.8$ Hz, 1H, 6-H), 7.61 (d, $J = 8.2$ Hz, 1H, 7-H), 7.84 (s, 1H, 4-H), 8.81 (br s, 1H, NH); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta = 10.5$ (3-$CH_3$), 12.4 ($CH_2$-$CH_3$), 38.6 ($CH_2$), 110.9 (C4), 117.9 (C3), 118.0 (C7), 123.1 (C6), 123.5 (C3′), 129.6 ($CF_3$), 145.8 (C7′), 151.8 (C2), 182.1 (C=S); MS (EI, 70 eV): *m/z* (%) = 288 ($[M+1]^+$, 16), 287 ($[M]^+$, 100), 286 (16), 268 (6), 255 (7), 254 (43), 244 (14), 243 (30), 242 (13), 231 (9), 229 (5), 227 (20), 226 (73), 225 (9), 224 (6), 200 (7), 199 (19), 170 (5), 169 (6), 159 (7), 151 (15), 44 (7).

*General procedure for the preparation of 5-bromobenzofuran-2-carbothioamides **10f,g***: A 100 mL two-necked flask was equipped with a magnetic stirrer, a connection to a combined $N_2$/vacuum line and closed with a septum. The air in the flask was replaced by $N_2$, and diisopropylamine (0.22 mL, 0.159 g, 1.58 mmol) and dry THF (10 mL) were injected by syringes. After cooling to −78 °C by means of a dry-ice/acetone bath, a 1.6 M solution of *n*-butyllithium in hexane (0.99 mL, 1.58 mmol) was injected and stirring was continued for 30 min at 0 °C. Thereafter, the mixture was cooled to −15 °C. A solution of 5-bromobenzofuran **7b** (0.300 g, 1.52 mmol) and THF (5 mL) generated under $N_2$, was added slowly by syringe. Stirring was continued for 2 h at the same temperature. Then, isothiocyanate **9a** or **9b** (1.2 mmol), dissolved in 2 mL dry THF, was added, and stirring was continued at −15 °C for 2 h. The mixture was poured into $H_2O$ and extracted with $CHCl_3$ (3×150 mL). The combined organic layers were dried with $Na_2SO_4$ and concentrated in a rotary evaporator. The oily residue was purified by column chromatography.

**According to this procedure, the following compounds were obtained:**

**5-Bromo-*N*-methylbenzofuran-2-carbothioamide (10f)** from **7b** (1.58 mmol) and methyl isothiocyanate (**9a**) (1.58 mmol): Yield: 120 mg (29%); mp: 170.4 °C; $R_f = 0.4$ ($CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 3.39$ (d, $J = 4.7$ Hz, 3H, $CH_3$), 7.34 (d, $J = 8.8$ Hz, 1H, 7-H), 7.52 (dd, $J = 2.2$ Hz, $J = 8.8$ Hz, 1H, 6-H), 7.66 (d, $J = 0.6$ Hz, 1H, 3-H), 7.80 (d, $J = 1.9$ Hz, 1H, 4-H), 8.32 (br s, 1H, NH); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta = 32.6$ ($CH_3$), 112.8 (C3), 113.6 (C5), 117.4 (C7), 125.6 (C4), 130.3 (C3′), 130.8 (C6), 153.5 (C7′), 153.8 (C2), 184.0 (C=S); MS (EI, 70 eV): *m/z* (%) = 272 ($[M+1]^+$, 12), 271 ($[M]^+$, 86), 270 (25), 269 (100), 242 (12), 241 (11), 240 (14), 239 (19), 238 (15), 236 (16), 230 (51), 229 (10), 228 (41), 227 (14), 211 (9), 209 (10), 198 (23), 196 (27), 149 (15), 121 (13), 95 (15), 89 (22), 88 (15), 87 (14), 74 (13), 44 (12); Anal. calcd for $C_{10}H_8BrNOS$ (270.15): C 44.46, H 2.98, N 5.18, found: C 44.11, H 2.35, N 4.72.

**5-Bromo-*N*-ethylbenzofuran-2-carbothioamide (10g)** from **7b** (1.35 mmol) and ethyl isothiocyanate (**9b**) (1.35 mmol): Yield: 78 mg (20%); $R_f = 0.5$ ($CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 1.34$ (t, $J = 7.4$ Hz, 3H, $CH_3$), 3.82 (dq, $J = 5.5$ Hz, $J = 7.3$ Hz, 2H, $CH_2$), 7.28 (d, $J = 8.8$ Hz, 1H, 7-H), 7.44 (dd, $J = 2.2$ Hz, $J = 8.8$ Hz, 1H, 6-H), 7.59 (s, 1H, 3-H), 7.72 (d, $J = 1.9$ Hz, 1H, 4-H), 8.11 (br s, 1H, NH); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta = 13.7$ ($CH_3$), 31.3 ($CH_2$), 112.8 (C3), 113.6 (C5), 117.4 (C7), 125.6 (C4), 130.3 (C3′), 130.8 (C6), 153.5 (C7′), 153.8 (C2), 184.0 (C=S); MS (EI, 70 eV): *m/z* (%) = 286 ($[M+1]^+$, 14), 285 ($[M]^+$, 99), 284 (34), 283 (100), 282 (23), 252 (13), 250 (15), 242 (35), 241 (24), 240 (35), 239 (23), 225 (12), 224 (36), 223 (17), 222 (35), 198 (29), 196 (30), 143 (11), 89 (11), 44 (27).

**3-Hydroxy-*N*-ethylbenzofuran-2-carbothioamide (10i)**: A 100 mL two-necked flask, equipped with a magnetic stirrer and a connection to a combined $N_2$/vacuum line, was charged with NaH (60% dispersion paraffin, 1 g, 25 mmol). The flask was closed with a septum and the air in the flask was replaced by $N_2$. Dry THF (20 mL) was added. Under stirring in an ice bath, a solution of **11** (1.0 g, 7.46 mmol) in dry THF (15 mL) was added by syringe. After stirring at room temperature for 30 min, ethyl isothiocyanate **9b** (0.65 g, 7.46 mmol) was injected. After stirring at room temperature for 18 h, cold brine (5 mL) and 2 N HCl (20 mL) were added. The mixture was extracted with $CHCl_3$ (3×150 mL). The combined organic layers were washed with 50 mL portions of dilute NaOH and 1 N HCl and deionized $H_2O$ and dried with $Na_2SO_4$. The solvent was removed in a rotary evaporator and the residue was exposed to oil-pump vacuum for 12 h to give pale-yellow, oily **10i**. Yield: 1.6 g (97%); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.31 (t, $J$ = 7.3 Hz, 3H, $CH_3$), 3.76 (dq, $J$ = 6.1 Hz, $J$ = 7.1 Hz, 2H, $CH_2$), 7.21 (t, $J$ = 7.7 Hz, 1H, 5-H), 7.28 (d, $J$ = 8.2 Hz, 1H, 6-H), 7.41 (t, $J$ = 8.0 Hz, 1H, 4-H), 7.49 (br s, 1H, NH), 11.16 (s, 1H, OH); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 14.2 ($CH_3$), 38.8 ($CH_2$), 112.2 (C7), 121.4 (C4), 122.1 (C5), 123.6 (C6), 130.0 (C3′), 130.9 (C2), 151.0 (C3), 151.4 (C7′), 180.8 (C=S); MS (EI, 70 eV): $m/z$ (%) = 222 ([$M$+1]$^+$, 13), 221 ([$M$]$^+$, 100), 188 (27), 177 (11), 176 (21), 160 (62), 159 (15), 121 (18); Anal. calcd for $C_{10}H_8BrNOS$ (221.28): C 59.71, H 5.01, N 6.33, found: C 59.84, H 4.95, N 6.36.

**(*O*-2,2-Diallyl-2,3-dihydrobenzofuran-3-yl)ethylcarbamothioate (13)**: A 100 mL flask was charged with **11b** (0.9 g, 4.2 mmol), which was dissolved in dry EtOH (25 mL). Within 1 h, $NaBH_4$ (1.91 g, 50.4 mmol) was added in small portions at 0 °C. The mixture was hydrolyzed with 20 mL $H_2O$ and extracted with $Et_2O$ (3×75 mL). The combined organic layers were dried with $Na_2SO_4$ and the solvent was removed in a rotary evaporator to obtain the corresponding alcohol. Yield: 0.81 g (89%); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 2.30–2.71 (m, 4H, $CH_2$), 4.98–5.20 (m, 4H, =$CH_2$), 5.23 (d, $J$ = 17.3 Hz, 1H, C*H*-OH), 5.72–6.14 (m, 2H, allyl-CH), 6.80–6.84 (m, 1H, 7-H), 6.89–6.94 (m, 1H, 5-H), 7.22–7.28 (m, 1H, 4-H), 7.34–7.39 (m, 1H, 6-H); MS (EI, 70 eV): $m/z$ (%) = 216 ([$M$]$^+$, 90), 174 (41), 147 (34), 133 (100), 121 (39), 91 (50), 77 (40).

A 100 mL two-necked flask was equipped with a magnetic stirrer and a connection to a combined $N_2$/vacuum line. The flask was closed with a septum and the air in the flask was replaced by $N_2$. The crude alcohol (0.4 g, 1.85 mmol) was added and dissolved in dry $Et_2O$ (25 mL). The resulting solution was cooled to −78 °C by means of a dry-ice/acetone bath. Under stirring at this temperature, a 1.6 M solution of *n*-butyllithium in hexane (1.4 mL, 2.22 mmol) was added by syringe and stirring was continued for 1 h. Then **9b** (0.16 g, 1.85 mmol) was injected. The solution was allowed to reach room temperature under stirring overnight. The mixture was hydrolyzed with 20 mL $H_2O$ and extracted with $Et_2O$ (3×75 mL). The combined organic layers were dried with $Na_2SO_4$ and the solvent was removed in a rotary evaporator. The residue was purified by column chromatography. Yield: 0.16 g (29%); $R_f$ = 0.56 ($CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.31 (t, $J$ = 7.3 Hz, 3H, $CH_3$), 3.76 (dq, $J$ = 6.1 Hz, $J$ = 7.1 Hz, 2H, $CH_2$), 7.21 (t, $J$ = 7.7 Hz, 1H, 5-H), 7.28 (d, $J$ = 8.2 Hz, 1H, 6-H), 7.41 (t, $J$ = 8.0 Hz, 1H, 4-H), 7.49 (br s, 1H, NH), 11.16 (s, 1H, OH); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 14.2 ($CH_3$), 38.8 ($CH_2$), 112.2 (C7), 121.4 (C4), 122.1 (C5), 123.6 (C6), 130.0 (C3′), 130.9 (C2), 151.0 (C3), 151.4 (C7′), 180.8 (C=S); MS (EI, 70 eV): $m/z$ (%) = 303 ([$M$]$^+$, 4), 262 (12), 199 (32), 191 (52), 163 (11), 158 (100), 129 (45); Anal. calcd for $C_{17}H_{21}NO_2S$ (303.42): C 67.29, H 6.98, N 4.62, found: C 67.28, H 7.22, N 4.69.

*General procedure for the preparation of benzothiazole-2-carbothioamides **17a–e**:* A 100 mL two-necked flask was equipped with a magnetic stirrer, a connection to a combined $N_2$/vacuum line and closed with a septum. The air in the flask was replaced by $N_2$, and benzothiazole **16a** or **16b** (9.1 mmol), dissolved in 15 mL of dry THF, was injected by syringe. By means of a liquid $N_2$/pentane bath, the solution was cooled to −100 °C, and a 1.6 M solution of *n*-butyllithium in hexane (5.7 mL, 9.1 mmol) was added at such a rate that the temperature, monitored by an electronic inlet thermometer, did not exceed −90 °C. After warming to −75 °C under stirring, isothiocyanates **9a**, **9b**, or **9e** (9.1 mmol) were injected. Stirring was continued at the same temperature for 20 min. $H_2O$ (20 mL) was then added and the mixture was extracted with $CHCl_3$ (3×150 mL). The combined organic layers were dried with $Na_2SO_4$ and the solvent was removed in a rotary evaporator. The residue was submitted to column chromatography or recrystallization from EtOH (**17d**).

**According to this procedure, the following compounds were obtained:**

***N*-Methyl-1,3-benzothiazole-2-carbothioamide (17a)** from **15a** (9.1 mmol) and methyl isothiocyanate (**9a**) (9.1 mmol): Yield: 366 mg (19%); mp: 120.2 °C; $R_f$ = 0.8 ($CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 3.33 (d, $J$ = 5.0 Hz, 3H, $CH_3$), 7.39 (t, $J$ = 7.4 Hz, 1H, 6-H), 7.46 (t, $J$ = 8.0 Hz, 1H, 5-H), 7.85 (d, $J$ = 8.2 Hz, 1H, 7-H), 7.95 (d, $J$ = 8.2 Hz, 1H, 4-H), 9.28 (br s, 1H, NH); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 33.0 ($CH_3$), 122.4 (C7), 124.9 (C4), 126.9 (C6), 127.4 (C5), 139.3 (C7′), 153.5 (C3′), 168.8 (C2), 186.1 (C=S); MS (EI, 70 eV): $m/z$ (%) = 210 ([$M$+2]$^+$, 6), 209 ([$M$+1]$^+$, 9), 208 ([$M$]$^+$, 67), 181 (9), 180 (14), 179 (100), 178 (13), 175 (7), 167 (8), 135 (24), 134 (5), 108 (11), 102 (6), 74 (5); Anal. calcd for $C_9H_8N_2S_2$ (208.3): C 51.89, H 3.87, N 13.45, found: C 51.92, H 3.70, N 13.23.

***N*-Ethyl-1,3-benzothiazole-2-carbothioamide (17b)** from **15a** (9.1 mmol) and ethyl isothiocyanate (**9b**) (9.1 mmol): Yield: 0.83 g (41%); mp: 84.1 °C; $R_f$ = 0.8 ($CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.35 (t, $J$ = 7.3 Hz, 3H, $CH_3$), 3.80 (dt, $J$ = 7.2 Hz, $J$ = 13.0 Hz, 2H, $CH_2$), 7.37 (t, $J$ = 7.7 Hz, 1H, 6-H), 7.44 (t, $J$ = 8.0 Hz, 1H, 5-H), 7.83 (d, $J$ = 8.2 Hz, 1H, 7-H), 7.94 (d, $J$ = 8.2 Hz, 1H, 4-H), 9.17 (br s, 1H, NH); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 13.7 ($CH_3$), 41.2 ($CH_2$), 122.4 (C7), 125.0 (C4), 126.9 (C6), 127.3 (C5), 139.4 (C7′), 153.5 (C3′), 169.1 (C2), 184.8 (C=S); MS (EI, 70 eV): $m/z$ (%) = 222 ([$M$]$^+$, 55), 181 (9), 180 (13), 179 (100), 178 (16), 161 (12), 135 (21), 108 (7), 102 (6); Anal. calcd for $C_{10}H_{10}N_2S_2$ (222.33): C 54.02, H 4.53, N 12.60, found: C 54.12, H 4.56, N 12.42.

***N*-Phenyl-1,3-benzothiazole-2-carbothioamide (17c)** from **15a** (9.1 mmol) and phenyl isothiocyanate (**9e**) (9.1 mmol): Yield: 0.76 g (45%); mp: 156.2 °C; $R_f$ = 0.8 ($CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.23 (tt, $J$ = 0.5 Hz, $J$ = 7.4 Hz, 1H, phenyl 4-H), 7.37–7.42 (m, 3H, phenyl 2-H and 3-H), 7.47 (dt, $J$ = 1.3 Hz, $J$ = 7.7 Hz, 1H, phenyl 3-H), 7.85 (d, $J$ = 7.6 Hz, 1H, 7-H), 7.99 (dd, $J$ = 7.9 Hz, $J$ = 12.3 Hz, 3H, 4-H, 5-H and 6-H), 10.92 (br s, 1H, NH); MS (EI, 70 eV): $m/z$ (%) = 271 ([$M$+1]$^+$, 21), 270 ([$M$]$^+$, 78), 269 (100), 238 (10), 237 (55), 167 (22), 161 (15), 135 (23), 109 (12), 108 (11), 77 (16).

***N*-Ethyl-5-(trifluoromethyl)benzothiazole-2-carbothioamide (17d)** from **15b** (0.49 mmol) and ethyl isothiocyanate (**9b**) (0.49 mmol): Yield: 68 mg (48%); mp: 104.0 °C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.45 (t, $J$ = 7.3 Hz, 3H, $CH_3$), 3.89 (dq, $J$ = 5.7 Hz, $J$ = 7.3 Hz, 2H, $CH_2$), 7.67–7.71 (m, 1H, 6-H), 8.02–8.06 (m, 1H, 7-H), 8.29–8.31 (m, 1H, 5-H), 9.21 (br s, 1H, NH); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 13.2 ($CH_3$), 40.9 ($CH_2$), 121.8 ($CF_3$), 121.8 (C6), 121.9 (C4), 122.7 (C7), 129.7 (C5), 142.1 (C7′), 152.7 (C3′), 170.8 (C2), 183.7 (C=S); MS (EI, 70 eV): $m/z$ (%) = 290 ([$M$]$^+$, 64), 271 (3), 247 (100), 229

(16), 203 (14), 170 (5), 157 (5), 44 (17); Anal. calcd for $C_{11}H_9F_3N_2S_2$ (290.33): C 45.51, H 3.12, N 9.65, found: C 45.97, H 3.08, N 9.26.

***N*-Phenyl-5-(trifluoromethyl)benzothiazole-2-carbothioamide (17e)** from **15b** (0.49 mmol) and phenyl isothiocyanate (**9e**) (0.49 mmol): Yield: 130 mg (78%); mp: 160.0 °C; $R_f$ = 0.74 ($CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.32–7.36 (m, 1H, 7.67–7.71 (m, 1H, phenyl *p*-H), 7.47–7.52 (m, 2H, phenyl *o*-H), 7.69–7.73 (m, 1H, 6-H), 8.04–8.08 (m, 1H, 7-H), 8.04–8.08 (m, 2H, phenyl *m*-H), 8.36–8.38 (m, 1H, 4-H), 10.97 (br s, 1H, NH); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 122.0 (-$CF_3$), 122.0 (C6), 122.2 (C4), 122.7 (C7), 127.2 (phenyl *o*-C), 129.2 (phenyl *m*-C), 129.6 (C5), 129.8 (phenyl *p*-C), 137.9 (NH-C), 142.7 (C7′), 152.5 (C3′), 172.2 (C2), 180.5 (C=S); MS (EI, 70 eV): *m/z* (%) = 338 ([*M*]$^+$, 66), 337 (100), 305 (38), 235 (15), 77 (25).

*General procedure for the preparation of benzothiazole-2-carbothioamides **19a–d** and carboxylic amides **20a,b**:* A 100 mL two-necked flask was equipped with a magnetic stirrer, a connection to a combined $N_2$/vacuum line and closed with a septum. Benzothiazole **18a**, **18b** or **18c** (10.0 mmol) was inserted and the air in the flask was replaced by $N_2$. By means of a dry-ice/acetone bath, the solution was cooled to −78 °C, and a 1.6 M solution of *n*-butyllithium in hexane (6.6 mL, 10.5 mmol) was added at such a rate that the temperature, monitored by an electronic inlet thermometer, did not exceed −75 °C. After stirring for one hour at −78 °C, isothiocyanate **9b** or **9d** (10.0 mmol) or *tert*-butylisocyanate was injected. The reaction mixture was stirred overnight and allowed to warm up to room temperature. Then, $H_2O$ (20 mL) and saturated ammonium chloride solution (20 mL) was added and the mixture was extracted with $CHCl_3$ (4×75 mL). The combined organic layers were dried with $Na_2SO_4$ and the solvent was removed in a rotary evaporator. The residue was submitted to column chromatography or recrystallization.

**2-(Benzothiazol-2-yl)-*N*-ethylethanethioamide (19a)** from **18a** (1.49 g, 10.0 mmol) and ethyl isothiocyanate (**9b**) (0.88 g, 10.0 mmol): Yield: 0.39 g (16%); mp: 109.8 °C; $R_f$ = 0.22 (*n*-hexane/EtOAc, 2:1), $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.30 (t, *J* = 7.3 Hz, 3H, $CH_3$), 3.72 (dq, *J* = 5.4 Hz, *J* = 7.3 Hz, 2H, $CH_2$-$CH_3$), 4.57 (s, 2H, $CH_2CS$), 7.40–7.45 (m, 1H, 6-H), 7.49–7.54 (m, 1H, 5-H), 7.87–7.91 (m, 1H, 7-H), 7.99–8.03 (m, 1H, 4-H), 9.53 (br s, 1H, NH); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 12.9 ($CH_3$), 41.3 ($CH_2$-$CH_3$), 48.8 ($CH_2$-CS) 121.8 (C4), 122.6 (C7), 125.6 (C6), 126.5 (C5), 134.5 (C7′), 152.4 (C3′), 165.6 (C2), 194.7 (C=S); MS (EI, 70 eV): *m/z* (%) = 236 ([*M*]$^+$, 33), 203 (21), 175 (7), 149 (100), 101 (9); Anal. calcd for $C_{11}H_{12}N_2S_2$ (236.36): C 55.90, H 5.12, N 11.85, found: C 55.91, H 5.28, N 11.64.

**2-(Benzothiazol-2-yl)-*N*-ethylpropanethioamide (19b)** from **18b** (1.63 g, 10.0 mmol) and ethyl isothiocyanate (**9b**) (0.88 g, 10.0 mmol): Yield: 0.34 g (14%); mp: 96.9 °C; $R_f$ = 0.23 (*n*-hexane/EtOAc 1:1); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.27 (t, *J* = 8.1 Hz, 1H, $CH_2$-$CH_3$), 1.86 (d, *J* = 7.2 Hz, 3H, CH-$CH_3$), 3.61–3.76 (m, 2H, $CH_2$-$CH_3$), 4.62 (q, *J* = 7.2 Hz, 1H, CH), 7.41 (ddd, *J* = 1.1 Hz, *J* = 7.5 Hz, *J* = 7.8 Hz, 1H, 6-H), 7.50 (ddd, *J* = 1.2 Hz, *J* = 7.3 Hz, *J* = 8.3 Hz, 1H, 5-H), 7.88 (d, *J* = 7.8 Hz, 1H, 7-H), 8.00 (d, *J* = 8.1 Hz, 1H, 4-H), 9.26 (s, 1H, NH); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 12.9 ($CH_2$-$CH_3$), 23.6 (CH-$CH_3$), 41.3 ($CH_2$-$CH_3$), 53.5 (CH) 121.8 (C4), 122.7 (C7), 125.4 (C6), 126.3 (C5), 134.4 (C7′), 152.7 (C3′), 171.7 (C2), 201.1 (C=S); MS (EI, 70 eV): *m/z* (%) = 250 ([*M*]$^+$, 50), 217 (78), 205 (7), 162 (100), 136 (15), 109 (32); Anal. calcd for $C_{12}H_{14}N_2S_2$ (250.38): C 57.56, H 5.64, N 11.19, found: C 57.76, H 5.58, N 11.14.

**2-(Benzothiazol-2-yl)-*N*-ethylbutanethioamide (19c)** from **18c** (1.77 g, 10.0 mmol) and ethyl isothiocyanate (**9b**) (0.88 g, 10.0 mmol): Yield: 0.24 g (9%); mp: 129.8 °C; $R_f$ = 0.43 ($CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 0.97 (t, *J* = 7.4 Hz, 3H, CH-$CH_2$-$CH_3$), 1.27 (t, *J* = 7.3 Hz, 3H, $NCH_2$-$CH_3$), 2.20–2.39 (m, 2H, CH-$CH_2$-$CH_3$), 3.61–3.76 (m, 2H, $NCH_2$-$CH_3$), 4.46 (dd, *J* = 6.0 Hz, *J* = 8.3 Hz, 1H, CH), 7.41 (t, *J* = 7.6 Hz, 1H, 6-H), 7.50 (t, *J* = 7.7 Hz, 1H, 5-H), 7.88 (d, *J* = 8.0 Hz, 1H, 7-H), 8.00 (d, *J* = 8.2 Hz, 1H, 4-H), 9.32 (br s, 1H, NH); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 11.7 (CH-$CH_2$-$CH_3$) 13.3 ($NCH_2$-$CH_3$), 31.7 (CH-$CH_2$-$CH_3$), 41.2 ($NCH_2$-$CH_3$), 61.0 (CH) 121.7 (C4), 122.6 (C7), 125.3 (C6), 126.3 (C5), 134.5 (C7′), 152.7 (C3′), 170.6 (C2), 200.1 (C=S); MS (EI, 70 eV): *m/z* (%) = 264 ([*M*]$^+$, 45), 231 (19), 203 (14), 192 (18), 176 (100), 162 (91), 148 (12), 109 (17); Anal. calcd for $C_{13}H_{16}N_2S_2$ (264.41): C 59.05, H 6.10, N 10.59, found: C 59.09, H 6.12, N 10.58.

**2-(Benzothiazol-2-yl)-*N*-*tert*-butylpropanethioamide (19d)** from **18d** (0.67 g, 4.1 mmol) and *tert*-butylisothiocyanate (**9d**) (0.52 g, 4.1 mmol): Yield: 0.66 g (58%); mp: 124.7 °C; $R_f$ = 0.64 (EtOAc); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.54 (s, 9H, $C(CH_3)_3$), 1.82 (d, *J* = 7.2 Hz, 3H, CH-$CH_3$), 4.49 (q, *J* = 7.2 Hz, 1H, CH), 7.39–7.43 (m, 1H, 6-H), 7.48–7.52 (m, 1H, 5-H), 7.87–7.90 (m, 1H, 7-H), 7.97–8.00 (m, 1H, 4-H), 9.21 (br s, 1H, NH); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 23.9 (CH-$CH_3$) 27.3 ($C(CH_3)_3$), 55.7 ($C(CH_3)_3$), 56.0 (CH), 121.8 (C4) 122.6 (C7), 125.3 (C6), 126.3 (C5), 134.4 (C7′), 152.7 (C3′), 172.1 (C2), 199.8 (C=S); MS (EI, 70 eV): *m/z* (%) = 278 ([*M*]$^+$, 22), 189 (13), 163 (100), 109 (15), 57 (20); Anal. calcd for $C_{14}H_{18}N_2S_2$ (278.44): C 60.39, H 6.52, N 10.06, found: C 60.90, H 6.36, N 9.58.

**2-(Benzothiazol-2-yl)-*N*-*tert*-butylacetamide (20a)** from **18a** (1.49 g, 10.0 mmol) and *tert*-butylisocyanate (0.99 g, 10.0 mmol): Yield: 0.89 g (36%); mp: 154.6 °C; $R_f$ = 0.04 ($CHCl_3$), $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.36 (s, 9H, $C(CH_3)_3$), 3.99 (s, 2H, $CH_2$), 6.90 (br s, 1H, NH), 7.41 (t, *J* = 7.6 Hz, 1H, 6-H), 7.50 (t, *J* = 7.6 Hz, 1H, 5-H), 7.90 (d, *J* = 7.9 Hz, 1H, 7-H), 7.99 (d, *J* = 8.2 Hz, 1H, 4-H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 28.6 ($C(CH_3)$), 42.1 ($CH_2$), 51.6 ($C(CH_3)$) 121.6 (C4), 122.5 (C7), 125.3 (C6), 126.3 (C5), 135.1 (C7′), 152.5 (C3′), 165.5 (C2), 165.6 (C=O); MS (EI, 70 eV): *m/z* (%) = 248 ([*M*]$^+$, 5), 233 (2), 176 (3), 149 (100), 108 (5), 57 (15); Anal. calcd for $C_{13}H_{16}N_2OS$ (248.34): C 62.87, H 6.49, N 11.28, found: C 62.72, H 6.74, N 11.08.

**2-(Benzothiazol-2-yl)-*N*-*tert*-butylpropanamide (20b)** from **18b** (1.63 g, 10.0 mmol) and *tert*-butylisocyanate (0.99 g, 10.0 mmol): Yield: 0.27 g (10%); mp: 135.2 °C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.32 (s, 9H, $C(CH_3)_3$), 1.70 (d, *J* = 7.2 Hz, 3H, $CH_3$), 4.00 (q, *J* = 7.2 Hz, 1H, CH), 6.57 (br s, 1H, NH), 7.39 (t, *J* = 7.2 Hz, 1H, 6-H), 7.48 (t, *J* = 7.2 Hz, 1H, 5-H), 7.88 (d, *J* = 8.0 Hz, 1H, 7-H), 7.98 (d, *J* = 8.1 Hz, 1H, 4-H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 18.7 ($CH_3$), 28.5 ($C(CH_3)$), 46.9 (CH), 51.4 ($C(CH_3)$) 121.7 (C4), 122.6 (C7), 125.1 (C6), 126.1 (C5), 134.9 (C7′), 152.7 (C3′), 169.4 (C2), 172.1 (C=O); MS (EI, 70 eV): *m/z* (%) = 190 ([*M*]$^+$, 2), 163 (100), 135 (2), 109 (12), 57 (16); Anal. calcd for $C_{14}H_{18}N_2OS$ (262.37): C 64.09, H 6.91, N 10.68, found: C 63.91, H 6.87, N 10.73.

**5-(Trifluoromethyl)benzothiazole (15b):** A 100 mL two-necked flask was equipped with a magnetic stirrer, a connection to a combined $N_2$/vacuum line and closed with a septum. The air in the flask was replaced by $N_2$, and 2-amino-4-(trifluoromethyl)thiophenol (1.9 g, 2.5 mmol), aqueous formaldehyde (37% solution, 0.42 g, 14 mmol), scandium(III) triflate (0.35 g, 0.70 mmol) and dry THF (40 mL) were injected. The mixture was stirred for three days at room temperature under through-flow of compressed air. After this period EtOAc (300 mL) was added and the organic layer was washed with an aqueous solution of $K_2CO_3$ (5%, 40 mL) and brine (40 mL). The combined organic extracts were dried with $Na_2SO_4$ and the solvent was removed in a rotary evaporator. The residue was submitted to column chromatography. Yield: 0.36 g (72%);

$R_f = 0.47$ ($CHCl_3$); MS (EI, 70 eV): $m/z$ (%) = 203 ($[M]^+$, 100), 184 (18), 176 (12), 157 (21), 132 (13).

### Crystal structure determinations

Crystals of compounds **19b** and **19d** suitable for X-ray study were investigated with a Stoe Imaging Plate Diffraction System using graphite-monochromatized $Mo_{K\alpha}$ radiation at $T = 291$ K. Unit cell parameters were determined by least-squares refinements on the positions of 8000 and 2156 reflections in the range $2.8^\circ < \theta < 25.8^\circ$ and $2.0^\circ < \theta < 25.2^\circ$, respectively. In the case of **19b** the monoclinic lattice and systematic extinctions are consistent with space group types *Cc* and *C*2/*c*, in the case of **19d** the anorthic lattice is consistent with space group types *P*1 and $P\bar{1}$. *C*2/*c* and $P\bar{1}$ proved to be the correct space groups in the course of structure refinements. The structures were solved by direct methods,[16] and the positions of all the H atoms were found via $\Delta F$ syntheses. Refinements[17] by full-matrix least-squares calculations on $F^2$ converged to the indicators above. Anisotropic displacement parameters were refined for all atoms heavier than hydrogen. Positional parameters and isotropic displacement parameters were refined for the H atoms involved in hydrogen bonding. Idealized bond lengths and angles were used for the $CH_3$, $CH_2$, and CH groups; the riding model was applied for their H atoms. In addition, the H atoms of the $CH_3$ groups were allowed to rotate around the neighboring C–C bonds. Isotropic displacement parameters of the H atoms were kept equal to 150, 120, 120, and 120% of the equivalent isotropic displacement parameters of the parent primary, secondary, tertiary, and "aromatic" carbon atoms, respectively. Crystal data and further details: (**19b**) $C_{12}H_{14}N_2S_2$, $M_r = 250.39\ g\,mol^{-1}$, monoclinic, *C*2/*c*, $a = 22.8724(14)$ Å, $b = 7.2817(4)$ Å, $c = 16.4463(9)$ Å, $\beta = 102.283(7)^\circ$, $V = 2676.4(3)$ Å$^3$, $Z = 8$, $D_{calcd} = 1.243\ Mg\,m^{-3}$, $\mu = 0.374\ mm^{-1}$, $F(000) = 1056$, crystal size $0.38 \times 0.30 \times 0.15\ mm^3$, $Mo_{K\alpha}$ ($\lambda = 0.71073$ Å), $2.79 < \theta < 25.00^\circ$, total of 16958 reflections, 2237 independent reflections [$R_{int} = 0.034$], completeness to $\theta = 25.00^\circ$: 94.7%, Full-matrix least-squares refinement on $F^2$, 2237 data, 151 parameters, GOF on $F^2 = 1.106$, $R_1 = 0.036$, $wR_2 = 0.075$ [$I > 2\sigma(I)$], $R_1 = 0.051$, $wR_2 = 0.077$ (all data), $\Delta\rho_{max}/\Delta\rho_{min}$: $0.20/-0.16\ e\,Å^{-3}$; (**19d**) $C_{14}H_{18}N_2S_2$, $M_r = 278.44\ g\,mol^{-1}$, triclinic, $P\bar{1}$, $a = 7.9491(15)$ Å, $b = 9.6764(17)$ Å, $c = 9.9800(17)$ Å, $\alpha = 95.40(2)^\circ$, $\beta = 97.55(2)^\circ$, $\gamma = 92.96(2)^\circ$, $V = 756.0(2)$ Å$^3$, $Z = 2$, $D_{calcd} = 1.223\ Mg\,m^{-3}$, $\mu = 0.337\ mm^{-1}$, $F(000) = 296$, crystal size $0.23 \times 0.17 \times 0.12\ mm^3$, $Mo_{K\alpha}$ ($\lambda = 0.71073$ Å), $2.07 < \theta < 25.00^\circ$, total of 10018 reflections, 2237 independent reflections [$R_{int} = 0.073$], completeness to $\theta = 25.00^\circ$: 97.0%, Full-matrix least-squares refinement on $F^2$, 2237 data, 171 parameters, GOF on $F^2 = 0.996$, $R_1 = 0.039$, $wR_2 = 0.068$ [$I > 2\sigma(I)$], $R_1 = 0.080$, $wR_2 = 0.073$ (all data), $\Delta\rho_{max}/\Delta\rho_{min}$: $0.19/-0.19\ e\,Å^{-3}$.

CCDC 768915 (**19b**) and CCDC 768916 (**19d**) contain the supplementary crystallographic data (excluding structure factors) for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

### Pharmacological methods

**Cell culture:** HEK 293 (human embryonic kidney) cells stably expressing murine SUR2B and Kir6.1[18] were cultured at 37 °C in a humidified atmosphere of 93% air and 7% $CO_2$ in α-MEM (modified Eagle's medium, Invitrogen) with glucose supplemented with 10% 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 60000 cells per well and cultured for 3 days in volumes of 200 μL per well before testing. Chinese hamster ovary (CHO) cells stably expressing human SUR1 and Kir6.2 were cultured under identical conditions as HEK 293 cells using G418 as selection marker and culturing cells only for 1.5 days in 12-well strips before testing.

**Membrane potential assays with $DiBAC_4(3)$:** 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-ethanesulfonic acid)], pH 7.4) at 30 °C before 200 μL assay buffer supplemented with 5 μM $DiBAC_4(3)$ was added.[19] Labeling of cells cultured in 12-well strips was continuously observed in 12-channel fluorescence detectors at 30 °C until the distribution of $DiBAC_4(3)$ across the plasma membrane reached equilibrium (60–90 min). Test compounds were added in a volume comprising 1/10 total buffer volume and gently mixed resulting in a time-dependent decrease in fluorescence indicating hyperpolarization of the membrane potential. Maximum hyperpolarization was induced with 30 μM NNC414[20] in CHO (SUR1/Kir6.2) cells and with 3 μM KC399[2h,i] or with 10 μM P1075 in HEK 293 (SUR2B/Kir6.1) cells.

Fluorescence was effected with blue light ($\lambda = 488$ nm) excitation, which was reflected by a dichroidic beam splitter (505 nm) mounted at 45° to illuminate adherent cells from the bottom of the 12-well strips. Excitation was performed with short light pulses of 30 ms 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 >100000-fold amplification of the photocurrent with 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). Computer software was programmed to allow continuous registry of fluorescence data, their display on a conventional screen, and to file data 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 at a high data sampling rate (1 Hz).[21]

**Membrane potential assays with DyeB:** DyeB is the membrane potential dye R7260 from Molecular Probes manufactured for use in high-throughput screening devices.[13] Confluent monolayers cultivated in 12-well strips (96-well format) were incubated with 0.125 mg mL$^{-1}$ DyeB. Fluorescence was effected with green light ($\lambda = 505$ nm) excitation. Emitted fluorescence light was filtered with a long-pass filter of 530 nm (OG530, Schott, Mainz, Germany). All other procedures were as described above for the use of $DiBAC_4(3)$.

### 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[22] according to Equation (1):

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

in which $B_0$ 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 dis-

sociation constant of the test compound L. Experimental data were analyzed after transformation of data to obtain homoscedasticity[21] 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.

**Membrane potential assays:** Equilibrium effects of ligand (L)-induced changes in fluorescence induced with $K_{ATP}$ channel openers were analyzed according to Equation (2):

$$I\,(\%) = 100 \times [L]^p/\{[L]^p + EC_{50}{}^p\} \quad (2)$$

Concentration–effect curves were fitted by nonlinear regression analysis according to Lemoine.[23] Exponents p indicate the Hill coefficient correcting for the steepness of curves.

**Drugs and materials:** $DiBAC_4(3)$ was from Molecular Probes (Invitrogen, Karlsruhe, Germany), DyeB (R7260) was from Molecular Devices (1311 Orleans Drive, Sunnyvale, CA, USA), glibenclamide was from Sigma (Deisenhofen, Germany), and [$^3$H]P1075 was from Pharmacia Biotech. P1075 was a gift from Leo Pharma (Ballerup, Denmark), NNC414 was from Dr. J. B. Hansen (Novo Nordisk Research, 2760, Måløv, Denmark). PCOs were dissolved in DMSO and further diluted with incubation buffer (final concentration in the membrane potential assays: $<0.1\,\%$, in radioligand binding: $<1\,\%$).

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[15] When *N*-phenyl-substituted thioamides (such as **10e**, **17c**, and **17e**) were submitted to the membrane potential assay using $DiBAC_4(3)$, a limitation of the method became relevant, inasmuch as the apparent affinities in the membrane potential measurement were substantially higher than those obtained from the radioligand experiments, so that $pEC_{50}$ values surpassed the $pK_D$ values by ~3 log units. It seems that these compounds interfere directly with the fluorescence mechanism of $DiBAC_4(3)$. Therefore, the experiments were repeated with wild-type HEK 293 cells that are expected to be resistant to PCO action. As the relevant compounds markedly inhibited $DiBAC_4(3)$ fluorescence in the wild-type cells, the apparent high affinity must be interpreted as an artifact. The problem was overcome by using the fluorescent dye DyeB.

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