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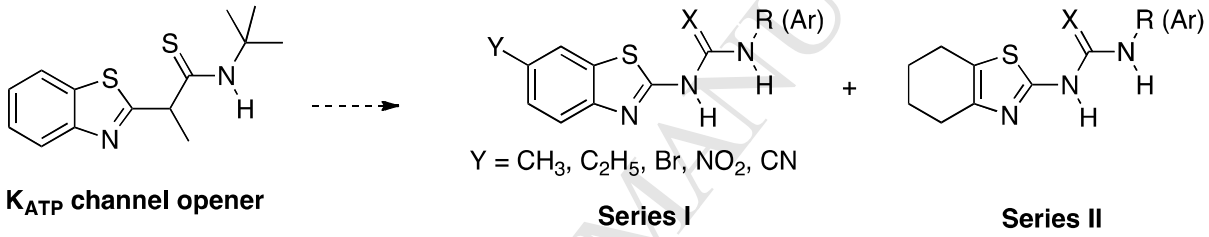
Graphical abstract



Synthesis, characterization and biological evaluation of benzothiazoles and tetrahydrobenzothiazoles bearing urea or thiourea moieties as vasorelaxants and inhibitors of the insulin releasing process

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Abstract

A series of 1,3-benzothiazoles (series **I**) and 4,5,6,7-tetrahydro-1,3-benzothiazoles (series **II**) bearing an urea or a thiourea moiety at the 2-position were synthesized and tested as myorelaxants and inhibitors of insulin secretion. Several compounds (i.e. **13u** and **13v**) from series **I** showed a marked myorelaxant activity. Benzothiazoles bearing a strong electron withdrawing group (NO_2, CN) at the 6-position and an alkyl group linked to the urea or the thiourea function at the 2-position were found to be the most potent compounds. The weak vasorelaxant activity of series **II** compounds evidenced the necessity of the presence of a complete aromatic heterocyclic system. The myorelaxant activity of some active compounds was reduced when measured on aorta rings precontracted by 80 mM KCl or by 30 mM KCl in the presence of 10 μM glibenclamide, suggesting the involvement of K_{ATP} channels in the vasorelaxant effect. Some compounds of series **I** tested on rat pancreatic islets provoked a marked inhibition of insulin secretion, among which **13a** exhibited a clear tissue selectivity for pancreatic β-cells.

Keywords: diazoxide; pinacidil; benzothiazole; benzothiadiazine; K_{ATP} channel; potassium channel openers.

1. Introduction

ATP-sensitive potassium channels, usually named K_{ATP} channels, are expressed in most excitable cells.[1,2] By tuning the membrane permeability to potassium ions as a function of intracellular ATP availability, they link membrane excitability to the metabolic state of the cell.[3,4] It is important to emphasize that K_{ATP} channels in different tissues display various biophysical and pharmacological properties. Such a diversity results from differences in the molecular composition of K_{ATP} channels. These channels are involved in the response to cerebral and cardiac ischemia, the regulation of insulin secretion by pancreatic β-cells, the control of vascular smooth muscle tone, the epithelial K^+ transport and the modulation of electrical activity in several types of neurons.[5-7] K_{ATP} channels are blocked by high concentrations of intracellular ATP and by several pharmacological substances, in particular by sulphonylurea drugs such as glibenclamide, which are used to treat non-insulin-dependent diabetes mellitus.[8] Conversely, K_{ATP} channels are activated by a variety of potassium channel openers (PCOs) such as cromakalim (**1**), pinacidil (**2**) and diazoxide (**3**) (Figure 1).[9]

The main challenge in the development of new K_{ATP} channel modulators as therapeutic agents is the discovery of compounds exhibiting the highest selectivity for a single K_{ATP} channel subtype. The reference PCO diazoxide is known to be non-selective; being equipotent on both vascular smooth muscle cells (vasorelaxant effect) and on pancreatic β-cells (inhibition of insulin release).

Chemical optimizations of diazoxide resulted in compounds such as BPDZ 44 (**4**), BPDZ 73 (**5**), BPDZ 216 (6) and NN414 (**7**), which potently and selectively activate the K_{ATP} channels of pancreatic β-cells.[10–13] Recent work reported the apparent high selectivity of some benzothiazole derivatives bearing a thioamide group (**8**) for the vascular smooth muscle K_{ATP} channels (Figure 1).[14] Moreover, the introduction of sulfonylurea moieties on the diazoxide skeleton at the 7-position (**9**) or at the 3-position (**10**) of the benzothiadiazine ring gave rise to a series of compounds expressing a vasodilatory activity similar to that of diazoxide.[15]

In order to develop new tissue selective K_{ATP} channel activators, we designed original compounds structurally related to compounds **8** and **10** (Figure 1). Starting from compound **8**, our strategy first involved the replacement of the carbon atom linked at the 2-position of the thiazole ring by a nitrogen atom providing urea and thiourea analogues (Figure 2). The second modification was the introduction of a substituent at the 6-position of the benzothiazole ring in order to better mimic benzothiadiazine dioxides

such as diazoxide or compounds **5**, **6**, **9** bearing a substituent at the 7-position. The substituent on the second nitrogen atom of the urea or thiourea moiety was selected as a short branched alkyl chain (as found in **8**), an alicyclic nucleus or a diversely substituted phenyl ring (figure 2, series I). A second series was obtained from the former by formal saturation of the benzene ring of benzothiazole, in order to highlight the role of the presence of an aromatic cycle. This led to tetrahydrobenzothiazole derivatives (Figure 2, series **II**).

The biological effects of the new compounds (series **I** and **II**) were characterized on rat pancreatic islets as well as on rat vascular smooth muscle in order to evaluate their potential inhibitory effects on insulin secretion and putative vasorelaxant activity. Further investigations were undertaken with a selection of compounds with the aim at determining the mechanism of action.

2. Chemistry

The diversely 6-substituted 2-aminobenzothiazoles **12** were generally obtained by the thiocyanation of the appropriate 4-substituted anilines (**11**), followed by ring closure in the presence of bromine according to the reaction of Hugerscorff. [16,17] The 6-substituted 2-aminobenzothiazoles **12** and commercially available alkyl/cycloalkyl/aryl isocyanates or isothiocyanates were refluxed in toluene to generate the urea and thiourea derivatives **13a-v** (Scheme 1**)**. The reaction rate and yields depended, firstly, on substituents introduced on the benzothiazole ring and, secondly, on types of isocyanates and isothiocyanates. We noticed that the reaction rate was greater when the substituent beared on the benzothiazole ring was an electron-donating group such as C_2H_5 or CH_3. The rate became very low when an electron-withdrawing group, such as NO_2, was introduced. Isocyanates are knowm to be more reactive than isothiocyanates due to the greater electronegativity of the oxygen atom compared to that of the sulfur atom.

The synthesis of 2-amino-4,5,6,7-tetrahydro-1,3-benzothiazole (**15**) was carried out according to the Hantzsch's method described by King and Hlavacek. [18,19] The solvent free reaction was performed by heating cyclohexanone (**14**) with thiourea in the presence of iodine. The reaction of **15** with appropriate isocyanates and isothiocyanates in dichloromethane successively provided the desired ureas and thioureas (**16a-h**) (Scheme 2). In contrast to the 2-amino-1,3-benzothiazoles **12**, 2-amino-4,5,6,7-tetrahydro-1.3-benzothiazole **15** readily reacted with isocyanates and isothiocyanates, clearly indicating that the aromaticity of the benzene cycle negatively influenced the reactivity of the 2-amino-1,3-benzothiazoles.

All newly synthesized benzothiazoles (**13a-v**) and tetrahydrobenzothiazoles (**16a-h**) were purified by crystallization and characterized by IR, ^{1}H NMR, ^{13}C NMR (APT) and elemental analyses.

3. Results and Discussion

The vasorelaxant activity of the newly synthesized compounds, 1,3-benzothiazoles **13a-v** and 4,5,6,7-tetrahydro-1,3-benzothiazoles **16a-h** (series **I** and **II,** respectively), was evaluated on isolated rat thoracic aorta rings precontracted with 30 mM KCl, by comparison with diazoxide and pinacidil. The effects of the drugs were expressed as the EC_{50} values, which corresponded to the drug concentrations giving 50% relaxation of the precontracted smooth muscle preparation. 1,3-Benzothiazoles **13a-v** (Table 1) exhibited, in most cases, a more pronounced myorelaxant activity than the 4,5,6,7-tetrahydrobenzothiazoles **16a-h** (Table 2), indicating that the presence of the aromatic ring was required for the biological activity. Compounds **13a**, **13d**, **13e, 13h-k**, **13m**, **13n**, **and 13q-s** were less active than diazoxide and pinacidil (EC_{50} > 35 μM), while compounds **13b**, **13f, 13l, 13o, 13p** and **13t** were equipotent or slightly more potent than diazoxide. Four compounds (**13c**, **13g**, **13u** and **13v**) were found to be clearly more active than diazoxide, especially **13u** and **13v** (EC_{50} equal to 2.78 and 2.11 μM, respectively). It can be observed that the preferred R group for the myorelaxant ativity was an alkyl chain, in particular a tert-butyl or a pentyl, while Y should be an electron-withdrawing group such as CN or NO_2. This observation could explain the weak activity of 4,5,6,7-tetrahydro-1,3-benzothiazoles **16a-h** (Table 2): the lack of aromatic cycle, which would fail to transmit the electron-withdrawing effect of the substituent at the 6-position to the 1,3-thiazole ring, seems to be required for a marked myorelaxant activity.

In order to verify whether the vasorelaxant activity was related to smooth muscle cell K_{ATP} channel activation, additional experiments were performed using the active compounds **13c**, **13f**, **13g**, **13u** and **13v**. These investigations included: i) two experimental conditions into which the vasodilatory activity of K_{ATP} channel activators is known to be suppressed or markedly decreased [20,21,25] [i.e. depolarization of rat aorta rings by either 30 mM K^+ in the continuous presence of the specific K_{ATP} channel blocker glibenclamide (10 μM) or depolarization by 80 mM K^+], and ii) experimental conditions allowing to determine the involvement of endothelial cells in the compounds effects (i.e. cell depolarization by 30 mM K^+ on rat aorta rings with or without endothelium).

As shown in Table 3, the presence of glibenclamide in the bathing medium provoked a substantial increase in the EC_{50} value of the selected compounds (about 2 fold for **13c** and **13g**, about 6 fold for **13u**, and about 9 fold for **13v**), suggesting the involvement, at least in part, of K_{ATP} channels in the vasodilatory activity of these molecules. Under identical experimental conditions (Table 3), the EC_{50} value for diazoxide and pinacidil, two known K_{ATP} channel activators, were increased by more than 4 fold and 20 fold; respectively.

The same effects were observed with aorta rings precontracted by 80 mM KCl. The EC_{50} values were increased about 2 fold for **13c** and **13f**, about 3 fold for **13g**, about 5 fold for **13u**, and about 7 fold for **13v**, while those of diazoxide and pinacidil were increased by more than 4 fold and 230 fold; respectively.

Such findings indicated that the vasodilatory action of the selected compounds was not exclusively mediated by K_{ATP} channels activation.

The myorelaxant effects of the selected compounds were not significantly different in the presence or absence of endothelium, indicating that the site of action was located on the vascular smooth muscle cells and not on the endothelial cells (Table 3).

In the second set of pharmacological investigations, five newly synthesized compounds (**13a, 13c, 13e, 13f, 13g**) were selected and tested (at 1, 10 and 50 µM) as inhibitors of insulin secretion from glucose-stimulated (16.7 mM glucose) pancreatic β-cells (Table 4). Diazoxide, pinacidil and BPDZ 44 (**4**) were used as reference compounds.

The results clearly showed that all selected products provoked a marked inhibition of insulin secretion at 50 µM, an effect more pronounced than that induced by pinacidil or diazoxide. At 10 µM, **13g** and **13a** still exhibited a strong inhibitory activity on insulin secretion. At 1µM, the effect of **13a** was again more pronounced than that of diazoxide, pinacidil or BPDZ 44 (**4**).

When the effects of the tested compounds were compared on vascular and pancreatic cells (Table 4), it was observed that **13c**, **13e**, **13f** and **13g** did not disclose any marked tissue-selectivity. Compound **13a**, which clearly showed a preference for pancreatic β-cells, expressed a tissue-selectivity roughly similar to that of BPDZ 44 (**4**). It could also be noted that **13a** was the only tested compound with an aromatic R group, while the other molecules were bearing an alkyl chain (**13c**, **13f** and **13g**) or a benzyl group (**13e**).

As previously observed for the antihypertensive drug diazoxide, the lack of obvious vascular smooth muscle tissue selectivity requires to pay attention to the risk of developing hyperglycemia when the compounds are administered *in vivo*. Compound **13a**, with a clear selectivity for the pancreatic tissue, might be used in pathologies characterized by the presence of a hyperinsulinism.

4. Conclusion

Starting from the structural modulation of compound **8**, a benzothiazole recently described as K_{ATP} channel opener [14], two series of compounds were prepared: series I (**13)** and II (**16**). Some molecules from series I (**13b**, **13c**, **13f**, **13g**, **13l**, **13o**, **13p**, **13t**, and **13u-13v)** exhibited a myorelaxant activity on 30 mM KCl-precontracted rat aorta rings, especially **13u** and **13v** which were found to be more potent than diazoxide. The myorelaxant activity of **13c**, **13f**, **13g**, **13u** and **13v** was reduced by the presence of glibenclamide (10 µM) in the experimental medium or when the contraction was induced by 80 mM KCl. Such observations suggested an involvement, at least in part, of K_{ATP} channels in the vasodilatory activity of these molecules. Further investigations also indicated that the site of action was mainly located on the vascular smooth muscle cells rather than on the endothelial cells. Five selected compounds (**13a, 13c, 13e, 13f** and **13g**) also provoked a marked inhibition of insulin secretion from rat pancreatic islets, in particular **13a** which exhibited a clear preference for pancreatic β-cells versus vascular smooth muscle cells.

By contrast, most compounds of series II (**16a-h**) did not show any substantial myorelaxant activity, indicating the potential role of an aromatic ring for inducing a vasorelaxant effect.

Further work should be conducted with new representatives of the two series in order to deepen the pharmacological profile of these compounds in terms of efficiency and tissue selectivity.

5. Experimental section

5.1. Relaxation of rat aorta rings

Female Wistar rats weighing approximately 180–220 g were anesthetized by intraperitoneal injection of 60 mg/kg pentobarbital, then sacrificed by exsanguination. The thoracic aorta was isolated and excess fat and connective tissue were removed. The vessels were cut into rings of about 2-3 mm length and mounted in an organ bath

containing 10 mL of Krebs' solution with the following composition (mM): NaCl 118, KCl 5.6, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11 and $CaCl_2$ 2.4. The tissue bath solution was maintained at 37 °C and bubbled with a 95% O_2 – 5% CO_2 mixture. The pH of the saturated solution was 7.4. Two stainless steel hooks were inserted into the aortic lumen; one was fixed while the other was connected to a force transducer connected to an analysis software. The aorta rings were equilibrated in the medium for 75 min and maintained under an optimal tension of 1.8–2.0 g. KCl (30 mM) was then added into the organ chamber to contract the rings. When the contraction reached a stable plateau (usually 15 min), the test compounds were added in the organ bath to assess their myorelaxant activity. Concentration–response curves were established by cumulative addition at concentrations of 1, 10, 30, 50 and 100 µM or, for the active compounds **13u** and **13v**, 0.1, 1, 5, 10, 30 and 100 µM. The same experiments were repeated for some potent compounds by using aorta rings into which the endothelium was removed. The endothelial removal was confirmed by the absence of vasodilator effect of acetylcholine (10 µM) added to vascular rings precontracted by 30 mM KCl. Contractile responses were calculated as the difference between resting tension and maximum tension developed in response to KCl stimulation. Data were expressed as mean ± S.E.M. from 4–6 (n) experiments. All experiments were performed in accordance with the ethical institutional guidelines.

5.2. Measurements of Insulin Release from Incubated Rat Pancreatic Islets

The method used for measuring insulin release from incubated pancreatic islets was previously described. [11,25] Experiments were performed with pancreatic islets isolated from adult fed Wistar rats. Groups of 10 islets, each derived from the same batch of islets, were preincubated for 30 min at 37°C in 1 mL of a physiological salt medium (in mM: NaCl 115, KCl 5, $CaCl_2$ 2.56, $MgCl_2$ 1, $NaHCO_3$ 24) supplemented with 2.8 mM glucose, 0.5% (w/v) dialysed albumin (fraction V, Sigma) and equilibrated against a mixture of O_2 (95%) and CO_2 (5%). The islets were then incubated at 37°C for 90 min in 1 mL of the same medium containing 16.7 mM glucose and, in addition, the reference or the required compound. The release of insulin was measured radioimmunologically using rat insulin as a standard.

Residual insulin secretion was expressed as a percentage of the value recorded in control experiments (100%); that is in the absence of drug and presence of 16.7 mM glucose.

Statistical analysis of the differences between the means of data was carried out through a t-Student test. The biological results are considered statistically different when $p < 0.05$.

5.3. Chemistry

Melting points were determined with a Büchi B-540 capillary apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a FTIR-8400 S, CE (SHIMADSU) spectrophotometer. The NMR spectra were recorded on a Bruker Avance (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR APT) instrument using DMSO-d_6 as the solvent with TMS as an internal standard; chemical shifts are reported in δ values (ppm) relative to that of internal standard TMS.

The abbreviations s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, br = broad and dd = doublet of doublet are used throughout. Elemental analyses (C, H, N, S) were realized on a Thermo Scientific Flash EA 1112-elemental analyzer and were within 0.4 % of the theoretical values. All reactions were routinely checked by TLC on silica gel (Merck 60 F_{254}).

5.3.1. General Method for the Synthesis of 1, 3-benzothiazoles (13)

5.3.1.1. 6-substituted 2-aminobenzothiazoles (12) [16,17]

The appropriate 4-substituted aniline (**11**; 20 mmol), glacial acetic acid (20 mL) and ammonium thiocyanate (40 mmol) were cooled in an ice bath and stirred for 10 min. A solution of bromine (20 mmol) in AcOH (20 mL) was then added over 20 min. The reaction mixture was stirred for 21 h at room temperature. The benzothiazole hydrobromide salt was filtered, washed with acetic acid and dried. It was subsequently dissolved in hot water and neutralized with aqueous ammonia solution (25%), filtered, washed with water and dried under vacuum, recrystallized with aqueous ethanol to obtain 6-substituted 1,3-benzothiazol-2-amine (**12**) in good yield.

5.3.1.2. 2-ureido (or thioureido)-susbstituted benzothiazoles

The solution of the appropriate 6-substituted 2-aminobenzothiazole (**12**) (1 mmol) in anhydrous toluene (10 mL) was supplemented with the selected isocyanate or isothiocyanate (1.1 mmol) and the reaction mixture was refluxed for 5 h. The solid product was washed with water and purified by recrystallization from aqueous ethanol, and air-dried to give the corresponding urea or thiourea compounds (**13a-v**).

1-(6-bromo-1,3-benzothiazol-2-yl)-3-(4-cyanophenyl)urea (13a) : 55 % yield ; mp > 300°C. IR (KBr) (ν_{max}/cm^{-1}) : 3238, 3083, 2219, 1714, 1596, 1529, 815. ^{1}H NMR (DMSO) (δ/ppm) : 7.55 (m, 2H, CHar), 7.73 (m, 2H, CHar), 7.78 (m, 2H, CHar), 8.20 (s, 1H, CHar), 9.66 (s, 1H, NH), 11.16 (s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : (CHar) : 118.35, 118.75, 124.23, 129.07, 133.35 ; (Cq) : 112.08, 114.89, 119.07, 126.81, 134.51, 142.63, 143.65, 160.84. Anal. ($C_{15}H_9BrN_4OS$) theoretical: 48.27% C, 2.43% H, 15.01% N, 8.59% S ; found: 48.38% C, 2.65% H, 15.13% N, 8.80% S.

1-(6-bromo-1,3-benzothiazol-2-yl)-3-isopropylurea (13b) : 50 % yield ; mp = 176-178°C. IR (KBr) (ν_{max}/cm^{-1}) : 3296, 3060, 2974, 1672, 1533, 1269, 806. ^{1}H NMR (DMSO) (δ/ppm) : 1.14 (d, J = 6.7 Hz, 6H, CH(CH_3)$_2$), 3.80 (m, 1H, CH(CH_3)$_2$), 6.62 (d, J = 6.9 Hz,1H, NH), 7.49 (dd, J = 8.5, 2.0 Hz, 1H, CHar), 7.54 (d, J = 8.5 Hz,1H, CHar), 8.13 (d, J = 1.7, 1H, CHar), 10.33 (s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : (CH_3)$_2$: 22.60 ; (CH) 41.55 ; (CHar) : 121.22, 123.82, 128.65 ; (Cq) : 114.32, 133.30, 148.18, 152.71, 160.29. Anal. ($C_{11}H_{12}BrN_3OS$) theoretical: 42.05% C, 3.85% H, 13.37% N, 10.21% S; found: 41.83% C, 3.84% H, 13.46% N, 10.40% S.

1-(6-bromo-1,3-benzothiazol-2-yl)-3-tert-butylurea (13c) : 40 % yield ; mp = 180-182°C. IR (KBr) (ν_{max}/cm^{-1}) : 3255, 3076, 2970, 1683, 1531, 1448, 1272, 810. ^{1}H NMR (DMSO) (δ/ppm) : 1.32 (s, 9H, (CH_3)$_3$), 6.62 (s, 1H, NH), 7.49 (dd, J = 8.2, 1.6 Hz, 1H, CHar), 7.53 (d, J = 8.2, 1H, CHar), 8.12 (d, J = 1.7 Hz, 1H, CHar), 10.36 (s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : (CH_3)$_3$: 28.65 ; (C(**C**H_3)$_3$) : 50.15 ; (CHar) : 121.20, 123.78, 128.63 ; (Cq) : 114.27, 133.66, 147.93, 152.04, 159.90. Anal. ($C_{12}H_{14}BrN_3OS$) theoretical: 43.91% C, 4.30% H, 12.80% N, 9.77% S; found: 43.84 % C, 4.26 % H, 13.08 % N, 10.07 % S.

***1-(6-bromo-1,3-benzothiazol-2-yl)-3-[2-chloro-4-(trifluoromethyl)]phenylurea (13d)*:** 48 % yield ; mp > 300°C**.** IR (KBr) (ν_{max}/cm^{-1}) : 3379, 3112, 2972, 1704, 1598, 1523, 1315, 1116, 808. ^{1}H NMR (DMSO) (δ/ppm) : 7.56 (dd, J = 8.6, 1.8 Hz, 1H, CHar), 7.64 (d, J = 8.5 Hz, 1H, CHar), 7.76 (d, J = 8.5 Hz, 1H, CHar), 7.95 (s, 1H, CHar), 8.23 (d, J = 1.4 Hz, 1H, CHar), 8.47 (d, J = 8.6 Hz, 1H, CHar), 9.43 (br s, 1H, NH), 11.55 (br s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : (CHar) : 120.92, 121.77, 124.05, 125.06, 126.48, 129.09 ; (Cq) :112.09, 115.12, 117.33, 122.39, 124.05, 124.31,

124.54 , 138.64, 151.10. Anal. ($C_{15}H_8BrClF_3N_3OS$) theoretical: 39.98% C, 1.79% H, 9.32% N, 7.12% S; found: 40.22% C, 1.87% H, 9.72% N, 6.95% S.

1-benzyl-3-(6-bromo-1,3-benzothiazol-2-yl)urea (13e) : 90 % yield ; mp = 222-224°C. IR (KBr) (ν_{max}/cm^{-1}) : 3375, 3070, 2958, 1685, 1560, 1452, 1271, 696. ^{1}H NMR (DMSO) (δ/ppm) : 4.38 (d, J = 5.8 Hz, 2H, C$\underline{H}_2$), 7.27 (t, J = 5.5 Hz, 1H, N$\underline{H}$), 7.30-7.38 (m, 5H, C$\underline{H}$ar,), 7.50 (dd, J = 8.5, 1.9 Hz, 1H, C$\underline{H}$ar), 7.55 (d, J = 8.5 Hz, 1H, C$\underline{H}$ar), 8.14 (s, 1H, C$\underline{H}$ar), 10.88 (s, 1H, N$\underline{H}$). ^{13}C NMR (DMSO) (δ/ppm) : (C$\underline{H}_2$) : 42.95 ; (C$\underline{H}$ar) : 121.27, 123.85, 126.98, 127.20, 128.40, 128.69 ; (C$\underline{q}$) : 114.40, 133.72, 139.35, 148.36, 153.79, 160.51. Anal. ($C_{15}H_{12}BrN_3OS$) theoretical: 49.73% C, 3.34% H, 11.60% N, 8.85% S ; found: 49.53% C, 3.48% H, 11.84% N, 8.80% S.

1-(6-nitro-1,3-benzothiazol-2-yl)-3-isopropylurea (13f) : 59 % yield ; mp = 252°C. IR (KBr) (ν_{max}/cm^{-1}) : 3305, 3109, 2977, 1679, 1550, 1446, 1330, 1274, 1124. ^{1}H NMR (DMSO) (δ/ppm) : 1.15 (d, J = 6.6 Hz, 6H, CH(C$\underline{H}_3$)$_2$), 3.84 (m, 1H, CH(C$\underline{H}_3$)$_2$), 6.70 (d, J = 6.5 Hz,1H, N$\underline{H}$), 7.74 (d, J = 8.9 Hz, 1H, C$\underline{H}$ar), 8.21 (dd, J = 8.9, 2.4 Hz,1H, C$\underline{H}$ar), 8.94 (d, J = 2.2 Hz,1H, C$\underline{H}$ar), 10.89 (s, 1H, N$\underline{H}$). ^{13}C NMR (DMSO) (δ/ppm) : (C$\underline{H}_3$)$_2$: 22.52 ; (C$\underline{(CH_3)_2}$) : 41.71 ; (C$\underline{H}$ar) : 118.48, 119.44, 121.61 ; (C$\underline{q}$) : 132.22, 142.22, 152.22, 154.34, 165.20. Anal. ($C_{11}H_{12}N_4O_3S$) theoretical: 47.13% C, 4.32% H, 19.99% N, 11.44% S; found: 47.01% C, 4.28% H, 19.60% N, 11.17% S.

1-tert-butyl-3-(6-nitro-1,3-benzothiazol-2-yl)urea (13g) : 65 % yield ; mp >300°C. IR (KBr) (ν_{max}/cm^{-1}) : 3402, 3255, 3105, 2979, 2364, 1686, 1517, 1340, 1278, 1203. ^{1}H NMR (DMSO) (δ/ppm) : 1.32 (s, 9H, (C$\underline{H}_3$)$_3$), 6.69 (br s, 1H, N$\underline{H}$), 7.74 (d, J = 8.9 Hz, 1H, C$\underline{H}$ar), 8.21 (dd, J = 8.9, 2.3 Hz, 1H, C$\underline{H}$ar), 8.93 (d, J = 2.1, 1H, C$\underline{H}$ar), 10.73 (br s, 1H, N$\underline{H}$). ^{13}C NMR (DMSO) (δ/ppm) : (C$\underline{H}_3$)$_3$: 28.66 ; (C$\underline{(\mathbf{C}H_3)_3}$) : 50.25 ; (C$\underline{H}$ar) : 118.77, 119.99, 121.34 ; (C$\underline{q}$) : 132.26, 141.72, 153.23, 155.64, 165.29. Anal. ($C_{12}H_{14}N_4O_3S$) theoretical: 48.42% C, 4.74% H, 18.91% N, 10.76% S; found: 48.30% C, 4.51% H, 18.99% N, 11.12% S.

1-[2-chloro-4-(trifluoromethyl)]phenyl-3-(6-nitro-1,3-benzothiazol-2-yl)urea (13h) :
85 % yield ; mp >300°C. IR (KBr) (ν_{max}/cm^{-1}) : 3373, 3120, 2962, 1695, 1517, 1320, 744. ^{1}H NMR (DMSO) (δ/ppm) : 7.76 (d, J = 8.7 Hz, 1H, C$\underline{H}$ar), 7.84 (d, J = 8.9 Hz, 1H, C$\underline{H}$ar), 7.96 (s, 1H, C$\underline{H}$ar), 8.26 (dd, J = 8.9, 2.4 Hz, 1H, C$\underline{H}$ar), 8.48 (d, J = 8.7 Hz, 1H, C$\underline{H}$ar), 9.02 (d, J = 2.3 Hz, 1H, C$\underline{H}$ar), 9.23 (br s, 1H, N$\underline{H}$), 12.03 (br s, 1H,

NH). ^{13}C NMR (DMSO) (δ/ppm) : (CHar) : 118.76, 119.98, 121.04, 121.78, 125.05, 126.50 ; (Cq) : 117.77, 122.37, 124.54, 132.22, 134.76, 138.54, 142.59, 147.29, 159.43. Anal. ($C_{15}H_8ClF_3N_4O_3S$) theoretical: 43.23% C, 1.93% H, 13.44% N, 7.69% S; found: 43.27% C, 2.01% H, 13.50% N, 7.63% S.

1-(1,3-benzothiazol-2-yl)-3-[2-chloro-4-(trifluoromethyl)]phenylurea (13i) : 64 % yield ; mp = 162-163°C. IR (KBr) (ν_{max}/cm^{-1}) : 3373, 3311, 3110, 1726, 1595, 1541, 1307, 831. ^{1}H NMR (DMSO) (δ/ppm) : 7.63 (s, 4H, CHar), 7.70 (d, J = 8.8 Hz, 1H, CHar), 7.89 (s, 1H, CHar), 8.45 (d, J = 8.8 Hz, 1H, CHar), 8.69 (s, 1H, NH), 9.87 (s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : (CHar) : 119.73, 120.46, 124.89, 126.34, 132.76 ; (Cq) : 112.03, 115.67, 121.58, 123.05, 123.29, 139.47, 140.97, 151.60. Anal. ($C_{15}H_9ClF_3N_3OS$) theoretical: 48.46% C, 2.44% H, 11.30% N, 8.63% S; found: 48.48% C, 2.64% H, 11.37% N, 8.79% S.

1-(1,3-benzothiazol-2-yl)-3-isopropylurea (13j) *:* 50 % yield ; mp = 175°C. IR (KBr) (ν_{max}/cm^{-1}) : 3352, 3174, 3105, 2977, 1720, 1595, 1529, 1315, 1233, 837. ^{1}H NMR (DMSO) (δ/ppm) : 1.10 (d, J = 6.5 Hz, 6H, $(CH_3)_2$), 3.73 (m, 1H, CH), 6.13 (d, J = 7.5 Hz, 1H, NH), 7.53 (s, 2H, CHar), 7.61 (s, 2H, CHar), 9.14 (s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : $(CH_3)_2$: 22.83; (CH) : 40.03 ; (CHar) : 118.81, 119.72, 132.69, 133.03 ; (Cq) : 141.45, 142.74, 151.97, 154.03. Anal. ($C_{11}H_{13}N_3OS$) theoretical: 56.15% C, 5.57% H, 17.86% N, 13.63% S; found: 55.86% C, 5.35% H, 17.51% N, 14.07% S.

1-cyclohexyl-3-(6-methyl-1,3-benzothiazol-2-yl)thiourea (13k) : 35 % yield ; mp = 226-227°C. IR (KBr) (ν_{max}/cm^{-1}) : 3458, 3170, 3033, 2923, 2853, 1571, 1527, 1213. ^{1}H NMR (DMSO) (δ/ppm) : 1.31-1.95 (m, 10H, $(CH_2)_5$), 2.39 (s, 3H, CH_3), 4.16 (m, 1H, CH), 7.22 (d, J = 8.0 Hz, 1H, CHar), 7.56 (br s, 1H, CHar), 7.67 (s, 1H, CHar), 10.21 (s, 1H, NH), 11.60 (s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : (CH_3) : 20.93 ; (CH_2) : 23.81, 25.06, 31.19 ; (CH) : 52.47; (CHar) : 119.59, 121.32, 127.44 ; (Cq) : 112.91, 126.75, 133.18, 160.17, 168.89. Anal. ($C_{15}H_{19}N_3S_2$) theoretical: 58.98% C, 6.27% H, 13.76% N, 20.99% S; found: 58.66% C, 6.11% H, 13.91% N, 20.64% S.

1-(6-methyl-1,3-benzothiazol-2-yl)-3-isopropylurea (13l) : 44 % yield ; mp = 177-178°C. IR (KBr) (ν_{max}/cm^{-1}) : 3305, 3055, 2974, 1677, 1539, 1263. ^{1}H NMR (DMSO) (δ/ppm) : 1.13 (d, J = 6.6 Hz, 6H, $CH(CH_3)_2$) 2.37 (s, 3H, CH_3), 3.81 (m, 1H, $CH(CH_3)_2$), 6.61 (d, J = 6.9 Hz,1H, NH), 7.16 (dd, J = 8.2, 1.1 Hz, 1H, CHar), 7.48 (d, J

= 8.2 Hz, 1H, CHar), 7.65 (s, 1H, CHar), 10.32 (s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : ($\underline{C}H_3$) : 20.85, 22.66 ; ($\underline{C}H(CH_3)_2$) : 41.45 ; ($\underline{C}$Har) : 119.30, 121.00, 126.91 ; ($\underline{C}$q) : 131.49, 131.85, 147.10, 152.86, 158.80. Anal. ($C_{12}H_{15}N_3OS$) theoretical: 57.81% C, 6.06% H, 16.85% N, 12.86% S; found: 57.54% C, 6.04% H, 16.90% N, 12.59% S.

1-benzyl-3-(6-methyl-1,3-benzothiazol-2-yl)thiourea (13m) : 50 % yield ; mp = 230°C. IR (KBr) (ν_{max}/cm^{-1}) : 3419, 3188, 3040, 1562, 1527, 1444, 1271, 1207, 811. ^{1}H NMR (DMSO) (δ/ppm) : 2.38 (s, 3H, $C\underline{H}_3$), 4.85 (s, 2H, $C\underline{H}_2$), 7.20 (d, J = 8.1 Hz, 1H, CHar), 7.29 - 7.38 (m, 5H, CHar), 7.51 (m, 1H, CHar), 7.70 (m, 1H, CHar), 10.44 (s, 1H, NH), 11.84 (s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : ($\underline{C}H_3$) : 15.88 ; ($\underline{C}H_2$) : 28.08 ; ($\underline{C}$Har) : 127.18, 127.38, 127.46, 128.17, 128.46, 128.59; ($\underline{C}$q) : 132.91, 138.82, 148.79, 150.94, 159.71, 174.13. Anal. ($C_{16}H_{15}N_3S_2$) theoretical: 61.31% C, 4.82% H, 13.41% N, 20.46% S; found: 61.18% C, 4.80% H, 13.35% N, 20.22% S.

1-(3-nitrophenyl)-3-(6-ethyl-1,3-benzothiazol-2-yl)thiourea (13n) : 57 % yield ; mp = 204-205°C. IR (KBr) (ν_{max}/cm^{-1}) : 3180, 3026, 2964, 1647, 1521, 1338, 1186. ^{1}H NMR (DMSO) (δ/ppm) : 1.22 (t, J = 7.7 Hz, 3H, $C\underline{H}_3$), 2.70 (q, J = 7.6 Hz, 2H, $C\underline{H}_2$), 7.30 (d, J = 8.1 Hz, 1H, CHar), 7.41 (s, 1H, CHar), 7.62 (t, J = 8.0 Hz, 1H, CHar), 7.72 (s, 1H, CHar), 7.92 (m, 1H, CHar), 8.26 (m, 1H, CHar), 8.61 (m, 1H, CHar), 10.75 (s, 1H, NH), 13.19 (s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : ($\underline{C}H_3$) : 15.87 ; ($\underline{C}H_2$) : 28.05 ; ($\underline{C}$Har) : 112.73, 114.93, 117.69, 121.35, 124.63, 127.00, 129.66 ; ($\underline{C}$q) : 117.39, 129.45, 139.71, 141.03, 147.72, 169.66, 176.66. Anal. ($C_{16}H_{14}N_4O_2S_2$) theoretical: 53.61% C, 3.94% H, 15.63% N, 17.89% S; found: 53.37% C, 3.79% H, 15.63% N, 17.67% S.

1-(6-ethyl-1,3-benzothiazol-2-yl)-3-isopropylurea (13o) : 70 % yield ; mp = 137-138°C. IR (KBr) (ν_{max}/cm^{-1}) : 3309, 3058, 2964, 1674, 1544, 1265. ^{1}H NMR (DMSO) (δ/ppm) : 1.14 (d, J = 6.6 Hz, 6H, ($C\underline{H}_3$)$_2$), 1.21 (t, J = 7.6 Hz, 3H, $C\underline{H}_3$), 2.67 (q, J = 7.6 Hz, 2H, $C\underline{H}_2$), 3.81 (m, 1H, C$\underline{H}$), 6.61 (d, J = 6.9 Hz,1H, N$\underline{H}$), 7.19 (dd, J = 8.2, 1.6 Hz, 1H, CHar), 7.51 (d, J = 8.2 Hz,1H, CHar), 7.68 (s, 1H, CHar), 10.33 (s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : ($\underline{C}H_3$) : 15.97, 22.67 ; ($\underline{C}H_2$) : 28.05 ; ($\underline{C}(CH_3)_2$) : 40.78, ($\underline{C}$Har) : 119.38, 119.88, 125.82 ; ($\underline{C}$q) : 138.39, 141.42, 145.45, 147.28, 152.86. Anal. ($C_{13}H_{17}N_3OS$) theoretical: 59.29% C, 6.51% H, 15.96% N, 12.18% S; found: 59.17% C, 6.46% H, 15.78% N, 11.78% S.

1-tert-butyl-3-(6-ethyl-1,3-benzothiazol-2-yl)urea (13p) : 85 % yield ; mp = 164-166°C. IR (KBr) (ν_{max}/cm^{-1}) : 3411, 3238, 3097, 2964, 1681, 1546, 1272. ^{1}H NMR (DMSO) (δ/ppm) : 1.21 (t, J = 7.6 Hz, 3H, C<u>H</u>$_3$), 1.32 (s, 9H, (C<u>H</u>$_3$)$_3$), 2.67 (q, J = 7.6 Hz, 2H, C<u>H</u>$_2$), 6.64 (s, 1H, N<u>H</u>), 7.19 (dd, J = 8.2, 1.6 Hz, 1H, C<u>H</u>ar), 7.50 (d, J = 8.2 Hz, 1H, C<u>H</u>ar), 7.67 (s, 1H, C<u>H</u>ar), 10.19 (s, 1H, N<u>H</u>). ^{13}C NMR (DMSO) (δ/ppm) : (<u>C</u>H$_3$) : 15.96, 28.38 ; (<u>C</u>H$_2$) : 28.04 ; (<u>C</u>(CH$_3$)$_3$) : 50.03 ; (<u>C</u>Har) : 119.34, 119.85, 125.80 ; (<u>C</u>q) : 131.46, 138.34, 147.22, 152.54, 158.72. Anal. ($C_{14}H_{19}N_3OS$) theoretical: 60.62% C, 6.90% H, 15.15% N, 11.56% S; found: 60.72% C, 6.75% H, 15.15% N, 11.50% S.

1-benzyl-3-(6-ethyl-1,3-benzothiazol-2-yl)urea (13q) : 70 % yield ; mp = 183-185°C. IR (KBr) (ν_{max}/cm^{-1}) : 3340, 3053, 2966, 1706, 1533, 1258. ^{1}H NMR (DMSO) (δ/ppm) : 1.21 (t, J = 7.6 Hz, 3H, C<u>H</u>$_3$), 2.68 (q, J = 7.6 Hz, 2H, C<u>H</u>$_2$), 4.38 (d, J = 5.9 Hz, 2H, C<u>H</u>$_2$-ar), 7.20 (dd, J = 8.2, 1.2 Hz, 1H, C<u>H</u>ar), 7.24-7.34 (m, 5H, CHar), 7.37 (m,1H, N<u>H</u>), 7.53 (d, J = 8.2 Hz, 1H, C<u>H</u>ar), 7.69 (s, 1H, C<u>H</u>ar), 10.69 (s, 1H, N<u>H</u>). ^{13}C NMR (DMSO) (δ/ppm) : (<u>C</u>H$_3$) : 15.98 ; (<u>C</u>H$_2$) : 28.05 ; (CH$_2$-Ar) : 42.98, (<u>C</u>Har) : 119.42, 119.90, 125.87, 126.94, 127.19, 128.39, (<u>C</u>q) : 133.60, 138.48, 139.38, 144.59, 153.34, 155.72. Anal. ($C_{17}H_{17}N_3SO$) theoretical: 65.57% C, 5.50% H, 13.49% N, 10.30% S ; found: 65.37% C, 5.52% H, 13.65% N, 10.09% S.

1-[2-chloro-4-(trifluoromethyl)]phenyl-3-(6-ethyl-1,3-benzothiazol-2-yl)urea (13r) : 50 % yield ; mp > 300°C. IR (KBr) (ν_{max}/cm^{-1}) : 3384, 3134, 2970, 1701, 1599, 1535, 1327, 1271, 1116, 752. ^{1}H NMR (DMSO) (δ/ppm) : 1.23 (t, J = 7.7 Hz, 3H, C<u>H</u>$_3$), 2.71 (q, J = 7.6 Hz, 2H, C<u>H</u>$_2$), 7.27 (dd, J = 8.3, 1.6 Hz, 1H, C<u>H</u>ar), 7.61 (d, J = 8.2 Hz, 1H, C<u>H</u>ar), 7.75 (dd, J = 8.8, 1.6 Hz, 1H, C<u>H</u>ar), 7.78 (s, 1H, C<u>H</u>ar), 7.94 (d, J = 1.5 Hz, 1H, C<u>H</u>ar), 8.50 (d, J = 8.7 Hz, 1H, C<u>H</u>ar), 9.43 (s, 1H, N<u>H</u>), 11.55 (s, 1H, N<u>H</u>). ^{13}C NMR (DMSO) (δ/ppm) : (<u>C</u>H$_3$) : 15.92 ; (<u>C</u>H$_2$) : 28.08 ; (<u>C</u>Har) : 120.16, 120.80, 125.00, 125.03, 126.27, 126.48 ; (<u>C</u>q) :122.30, 123.57, 123.83, 124.09, 124.58, 138.86, 139.19, 143.62, 152.03. Anal. ($C_{17}H_{13}ClF_3N_3OS$) theoretical: 51.07% C, 3.28% H, 10.51% N, 8.02% S; found: 50.76% C, 3.30% H, 10.56% N, 7.98% S.

1-benzyl-3-(6-ethyl-1,3-benzothiazol-2-yl)thiourea (13s) : 25 % yield ; mp > 300°C. IR (KBr) (ν_{max}/cm^{-1}) : 3398, 3174, 3046, 1568, 1529, 1464, 1276, 1220, 689. ^{1}H NMR (DMSO) (δ/ppm) : 1.21 (t, J = 7.6 Hz, 3H, C<u>H</u>$_3$), 2.68 (q, 2H, C<u>H</u>$_2$), 4.86 (s, 2H, C<u>H</u>$_2$), 7.23 (d, J = 8.3 Hz, 1H, C<u>H</u>ar), 7.29-7.38 (m, 5H, C<u>H</u>ar), 7.53 (m, 1H, C<u>H</u>ar), 7.73 (s,

1H, CHar), 10.45 (s, 1H, NH), 11.85 (s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : ($\underline{C}H_3$) : 15.88 ; ($\underline{C}H_2$) : 28.08, 47.61 ; ($\underline{C}$Har) : 126.27, 126.36, 127.07, 127.37, 128.20, 128.45 ; ($\underline{C}$q) : 136.31, 139.59, 143.97, 153.03, 168.37, 170.75. Anal. ($C_{17}H_{17}N_3S_2$) theoretical: 62.35% C, 5.23% H, 12.83% N, 19.58% S; found: 62.07% C, 5.27% H, 12.75% N, 18.98% S.

1-(6-cyano-1,3-benzothiazol-2-yl)-3-pentylurea (13t) : 61 % yield ; mp = 320-322 °C. IR (KBr) (ν_{max}/cm^{-1}) : 3430, 2960, 2225, 1670, 1600, 1550, 1280. ^{1}H NMR (DMSO) (δ/ppm) : 0.88 (t, 3H, C$\underline{H}_3$), 1.31 (m, 4H, (C$\underline{H}_2$)$_2$), 1.49 (m , 2H, C$\underline{H}_2$), 3.17 (q, J = 6.0 Hz, 2H, C$\underline{H}_2$-N), 6.79 (s, 1H, NH), 7.75 (dd, J = 8.39, 1.43 Hz, 1H, CHar), 7.82 (d, J = 8.41 Hz, 1H, CHar), 8.46 (s, 1H, CHar), 11.01 (s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : ($\underline{C}H_3$) : 13.92 ; ($\underline{C}H_2$) : 21.78, 28.44, 28.95 ; ($\underline{C}$Har) : 120.14, 126.37, 129.43 ; ($\underline{C}$q) : 104.17, 119.35, 132.26, 142.39, 152.49, 163.64. Anal. ($C_{14}H_{16}N_4OS$) theoretical: 55.61% C, 4.67% H, 18.53% N, 10.61% S; found: 55.63% C, 4.43% H, 18.49% N, 10.73% S.

1-tert-butyl-3-(6-cyano-1,3-benzothiazol-2-yl)urea (13u) : 56 % yield ; mp =390-392°C. IR (KBr) (ν_{max}/cm^{-1}) **:** 3420, 2960, 2225, 1700, 1645, 1540, 1260. ^{1}H NMR (DMSO) (δ/ppm) : 1.33 (s, 9H, (C$\underline{H}_3$)$_3$), 6.67 (s, 1H, NH), 7.72 (d, J = 8.43 Hz, 1H, CHar), 7.75 (dd, J = 8.43, 1.54 Hz, 1H, CHar), 8.45 (d, J = 1 Hz, 1H, CHar), 10.63 (s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : ($\underline{C}H_3$) : 28.58 ; ($\underline{C}$(**C**H$_3$)$_3$) : 50.30 ; ($\underline{C}$Har) : 120.17, 126.37, 129.44 ; ($\underline{C}$qar) : 104.15, 119.35, 131.95, 139.99, 151.95, 163.24. Anal. ($C_{14}H_{16}N_4OS$) theoretical: 53.41% C, 4.86% H, 19.16% N, 10.97% S; found: 53.42% C, 4.92% H, 19.36% N, 11.39% S.

1-(6-nitro-1,3-benzothiazol-2-yl)-3-pentylurea (13v) : 58 % yield ; mp = 195-197 °C. IR (KBr) (ν_{max}/cm^{-1}) : 3440, 2920, 1680, 1600, 1510, 1340, 1275. ^{1}H NMR (DMSO) (δ/ppm) : 0.89 (t, 3H, C$\underline{H}_3$), 1.30 (m, 4H, (C$\underline{H}_2$)$_2$), 1.48 (m, 2H, C$\underline{H}_2$), 3.17 (q, J = 6.3 Hz, 2H, C$\underline{H}_2$-N), 6.81 (s, 1H, NH) 7.76 (d, J = 8.41 Hz, 1H, CHar), 8.23 (dd, J = 8.39, 1.43 Hz, 1H, CHar), 8.95 (d, J = 1.02 Hz, 1H, CHar). 11.13 (s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : ($\underline{C}H_3$) : 13.92 ; ($\underline{C}H_2$) : 21.79, 28.44, 28.94 ; ($\underline{C}$Har) : 118.51, 119.48, 121.63 ; ($\underline{C}$q) : 132.23, 142.21, 153.46, 161.22, 165.29. Anal. ($C_{13}H_{16}N_4O_3S$) theoretical: 50.64% C, 5.23% H, 18.17% N, 10.40% S; found: 50.48% C, 4.96% H, 17.95% N, 10.18% S.

5.3.2. General Method for the Synthesis of 4,5,6,7-tetrahydrobenzothiazoles (16)

5.3.2.1. 2-amino-4,5,6,7-tetrahydrobenzothiazoles (15) [18,19]

Cyclohexanone (2.07 mL, 20 mmol) was added to a mixture of thiourea (3.04 g, 40 mmol) and iodine (5.07 g, 20 mmol) and heated without solvent at 110°C for 12 hrs. The reaction mixture was then cooled to 20°C and quenched in water (20 mL) and extracted by diethylether. The organic layer was washed with saturated sodium bicarbonate solution and then filtered to remove insoluble solids (2-amino-4,5,6,7-tetrahydrobenzothiazole hydroiodide salt).

The 2-amino-4,5,6,7-tetrahydrobenzothiazole hydroiodide salt was solubilized in saturated sodium carbonate solution and heated, then the aqueous solution was extracted with dichloromethane, dried over $MgSO_4$ and evaporated. The residue was precipitated in diethylether to lead to 2-amino-4,5,6,7-tetrahydrobenzothiazole (**15**).

5.3.2.2. 2-ureido (or thioureido)-4,5,6,7-tetrahydrobenzothiazoles (16a-h)

The solution of (**15**) (1 mmol) in anhydrous dichloromethane (10 mL) was supplemented with the appropriate isocyanate or isothiocyanate (1.1 mmol) and refluxed for 5 h. The solid product was washed with water and purified by re-crystallization from aqueous ethanol, and air-dried to give the corresponding urea or thiourea compounds (**16a-h**).

1-(3-nitrophenyl)-3-(4,5,6,7-tetrahydro-1,3-benzothiazol-2-yl)thiourea (16a) : 70 % yield ; mp = 203°C. IR (KBr) (ν_{max}/cm^{-1}) : 3473, 3182, 3029, 2939, 1585, 1521, 1342, 1197, 686. ^{1}H NMR (DMSO) (δ/ppm) : 1.79 (br s, 4H, C$\underline{H}_2$), 2.54 (br s, 4H, C$\underline{H}_2$), 7.55 (t, J = 8.2 Hz, 1H, C$\underline{H}$ar), 7.83 (d, J = 7.9 Hz, 1H, C$\underline{H}$ar), 8.28 (d, J = 7.6 Hz, 1H, C$\underline{H}$ar), 8.58 (s, 1H, C$\underline{H}$ar), 10.34 (br s, 1H, N$\underline{H}$), 12.73 (br s, 1H, N$\underline{H}$). ^{13}C NMR (DMSO) (δ/ppm) : (C$\underline{H}_2$) : 21.60, 22.28, 22,43 ; (C$\underline{H}$ar) : 115.15, 116.66, 127.17, 129.51 ; (C$\underline{q}$) : 135.58, 141.43, 147.77, 159.50, 166.17, 183.83. Anal. ($C_{14}H_{14}N_4O_2S_2$) theoretical: 50.28% C, 4.22% H, 16.75% N, 19.18% S; found: 50.53% C, 4.36% H, 16.55% N, 18.91% S.

1-isopropyl-3-(4,5,6,7-tetrahydro-1,3-benzothiazol-2-yl)urea (16b) : 60 % yield ; mp = 92-94°C. IR (KBr) (ν_{max}/cm^{-1}) : 3294, 3070, 2927, 1679, 1569, 1234. ^{1}H NMR (DMSO) (δ/ppm) : 1.09 (d, J = 6.6 Hz, 6H, (C$\underline{H}_3$)$_2$), 1.73 (br s, 4H, C$\underline{H}_2$), 2.47 (br s, 2H, C$\underline{H}_2$), 2.56 (br s, 2H, C$\underline{H}_2$), 3.75 (m, 1H, C$\underline{H}$), 6.41 (d, J = 7.3 Hz, 1H, N$\underline{H}$), 9.83 (br s, 1H,

NH). ^{13}C NMR (DMSO) (δ/ppm) : (CH$_3$) : 22.76 ; (CH$_2$) : 22.04, 22.63, 22.98, 25.94 ; (CH) : 41.23 ; (Cq) : 141.45, 142.74 , 151.97, 167.83. Anal. ($C_{11}H_{17}N_3OS$) theoretical: 55.20% C, 7.16% H, 17.56% N, 13.39% S; found: 55.55% C, 7.21% H, 17.30% N, 12.99% S.

1-isopropyl-3-(4,5,6,7-tetrahydro-1,3-benzothiazol-2-yl)thiourea (16c) : 50 % yield ; mp = 103-105°C. IR (KBr) (ν_{max}/cm^{-1}) : 3326, 3224, 2926, 1670, 1560, 1442, 1232, 675. ^{1}H NMR (DMSO) (δ/ppm) : 1.10 (d, J = 6.5 Hz, 6H, (CH$_3$)$_2$), 1.73 (br s, 4H, CH$_2$), 2.47 (br s, 2H, CH$_2$), 2.56 (br s, 2H, CH$_2$), 3.75 (m, 1H, CH), 6.41 (d, J = 6.7 Hz, 1H, NH), 9.82 (br s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : (CH$_3$) : 22.76 ; (CH$_2$) : 22.04, 22.63, 22.98, 25.94 ; (CH) : 41.23 ; (Cq) : 143.45, 152.97, 156.76, 167.92. Anal. ($C_{11}H_{17}N_3S_2$) theoretical: 51.73% C, 6.71% H, 16.45% N, 25.11% S; found: 52.12% C, 7.01% H, 16.52% N, 24.11% S.

1-tert-butyl-3-(4,5,6,7-tetrahydro-1,3-benzothiazol-2-yl)urea (16d) : 63 % yield ; mp = 178 -180°C. IR (KBr) (ν_{max}/cm^{-1}) : 3375, 3068, 2939, 1701, 1676, 1556, 1261. ^{1}H NMR (DMSO) (δ/ppm) : 1.28 (s, 9H, (CH$_3$)$_3$), 1.73 (br s, 4H, CH$_2$), 2.46 (br s, 2H, CH$_2$), 2.56 (br s, 2H, CH$_2$), 6.47 (br s, 1H, NH), 9.70 (br s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : (CH$_2$) : 22.19, 22.64, 22.98, 26.00 ; (CH$_3$) : 28.79 ; (CH(CH$_3$)$_3$) : 49.75 ; (Cq) : 119.08, 143.39, 152.66, 156.64. Anal. ($C_{12}H_{19}N_3OS$) theoretical: 56.89% C, 7.56% H, 16.58% N, 12.66% S; found: 56.53% C, 7.40% H, 16.33% N, 12.63% S.

1-(4-cyanophenyl)-3-(4,5,6,7-tetrahydro-1,3-benzothiazol-2-yl)urea (16e): 70% yield ; mp = 247-248°C. IR (KBr) (ν_{max}/cm^{-1}) : 3371, 3109, 2935, 2216, 1706, 1647, 1581, 1523, 1434, 1307, 1220. ^{1}H NMR (DMSO) (δ/ppm) : 1.76 (br s, 4H, CH$_2$), 2.59 (br s, 4H, CH$_2$), 7.69 (d, J = 8.0 Hz, 2H, CHar), 7.73 (d, 2H, CHar), 9.53 (br s, 1H, NH), 10.80 (br s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : (CH$_2$) : 22.24, 22.32, 22.78, 25.06 ; (CHar) : 118.25, 133.22 ; (Cq) : 103.64, 118.60, 133.31, 142.11, 143.32, 143.76, 159.76. Anal. ($C_{15}H_{14}N_4OS$) theoretical: 60.38% C, 4.74% H, 18.78% N, 10.74% S; found: 60.26% C, 4.69% H, 18.80% N, 10.83% S.

1-(4-cyanophenyl)-3-(4,5,6,7-tetrahydro-1,3-benzothiazol-2-yl)thiourea (16f) : 65 % yield ; mp = 206°C. IR (KBr) (ν_{max}/cm^{-1}) : 3423, 3201, 3024, 2941, 2223, 1604, 1529, 1442, 1372, 1308, 1173. ^{1}H NMR (DMSO) (δ/ppm) : 1.78 (br s, 4H, CH$_2$), 2.53 (s, 4H, CH$_2$), 7.69 (d, J = 7.9 Hz, 2H, CHar), 8.03 (d, J = 8.2 Hz, 2H, CHar), 10.31 (s, 1H, NH),

12.92 (s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : (CH$_2$) : 21.51, 22.12, 22.25, 22.36 ; (CHar) : 120.17, 132.68 ; (Cq) : 99.49, 103.13, 117.07, 119.50, 144.42, 155.02, 182.51. Anal. ($C_{15}H_{14}N_4S_2$) theoretical: 57.30% C, 4.49% H, 17.82% N, 20.40% S; found: 57.18% C, 4.43% H, 18.02% N, 20.08% S.

1-[4-chloro-3-(trifluoromethyl)]phenyl-3-(4,5,6,7-tetrahydro-1,3-benzothiazol-2-yl) urea (16g) : 70 % yield ; mp = 196-197°C. IR (KBr) (ν_{max}/cm^{-1}) : 3413, 3101, 2950, 1699, 1605, 1416, 1264, 1134, 738. ^{1}H NMR (DMSO) (δ/ppm) : 1.77 (m, 4H, CH$_2$), 2.58 (m, 4H, CH$_2$), 7.62 (d, J = 8.5 Hz, 1H, CHar), 7.75 (d, J = 8.5 Hz, 1H, CHar), 8.14 (s, 1H, CHar), 9.48 (s, 1H, NH), 10.85 (s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : (CH$_2$) : 22.22, 22.25, 22;74 ; (CHar) : 116.86, 123.18, 131.95 ; (Cq) : 119.52, 121.61, 122.52, 125.85, 126.50, 126.74, 139.06, 150.96. Anal. ($C_{15}H_{13}ClF_3N_3OS$) theoretical: 47.94% C, 3.49% H, 11.18% N, 8.53% S; found: 48.17% C, 3.60% H, 11.18% N, 8.88% S.

1-(4-chlorophenyl)-3-(4,5,6,7-tetrahydro-1,3-benzothiazol-2-yl)urea (16h): 70 % yield ; mp = 265°C. IR (KBr) (ν_{max}/cm^{-1}) : 3357, 3294, 3074, 2935, 1674, 1552, 1296, 821. ^{1}H NMR (DMSO) (δ/ppm) : 1.76 (m, 4H, CH$_2$), 2.60 (m, 4H, CH$_2$), 7.34 (d, J = 7.6 Hz, 2H, CHar), 7.51 (d, J = 7.9 Hz, 2H, CHar), 9.14 (s, 1H, NH), 10.38 (s, 1H, NH). ^{13}C NMR (DMSO) (δ/ppm) : (CH$_2$) : 22.22, 22.45, 22.85 ; (CHar) : 119.93, 128.66 ; (Cq) : 114.53, 125.41, 125.88, 138.00, 138.52, 152.30. Anal. ($C_{14}H_{14}ClN_3OS$) theoretical: 54.63% C, 4.58% H, 13.65% N, 10.42% S; found: 54.31% C, 4.65% H, 13.22% N, 9.90% S.

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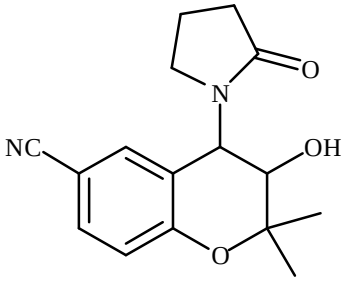
Legends for figures

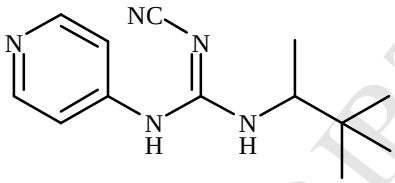
Figure 1: Chemical structure of reference potassium channel openers.

Figure 2 : Modulation of compound **8** leading to benzothiazoles and tetrahydrobenzothiazoles.

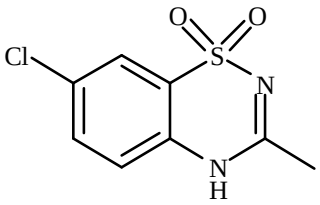
Figures



Cromakalim (**1**)



Pinacidil (**2**)



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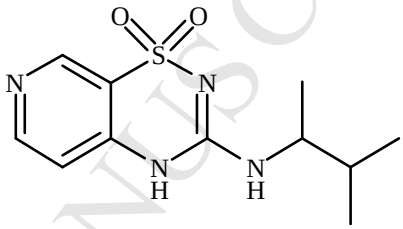
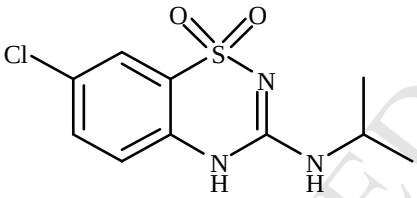
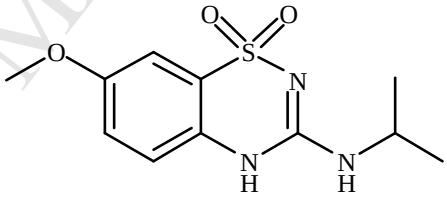


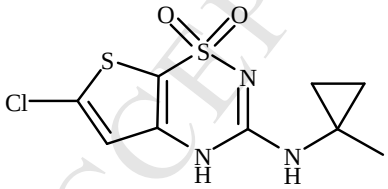
BPDZ 44 (**4**)



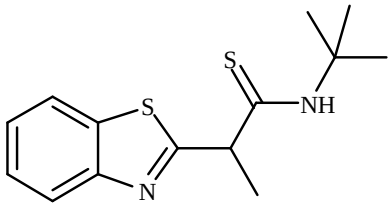
BPDZ 73 (**5**)



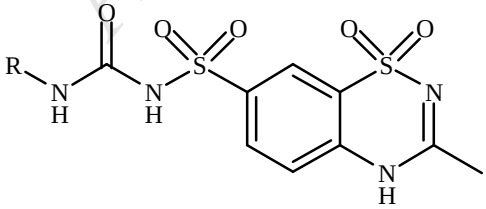
BPDZ 216 (**6**)



NN414 (**7**)



(**8**)



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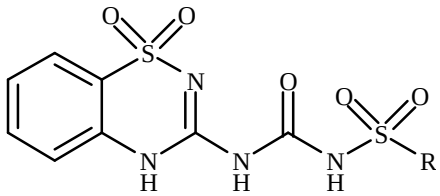


(**10**)

Figure 1

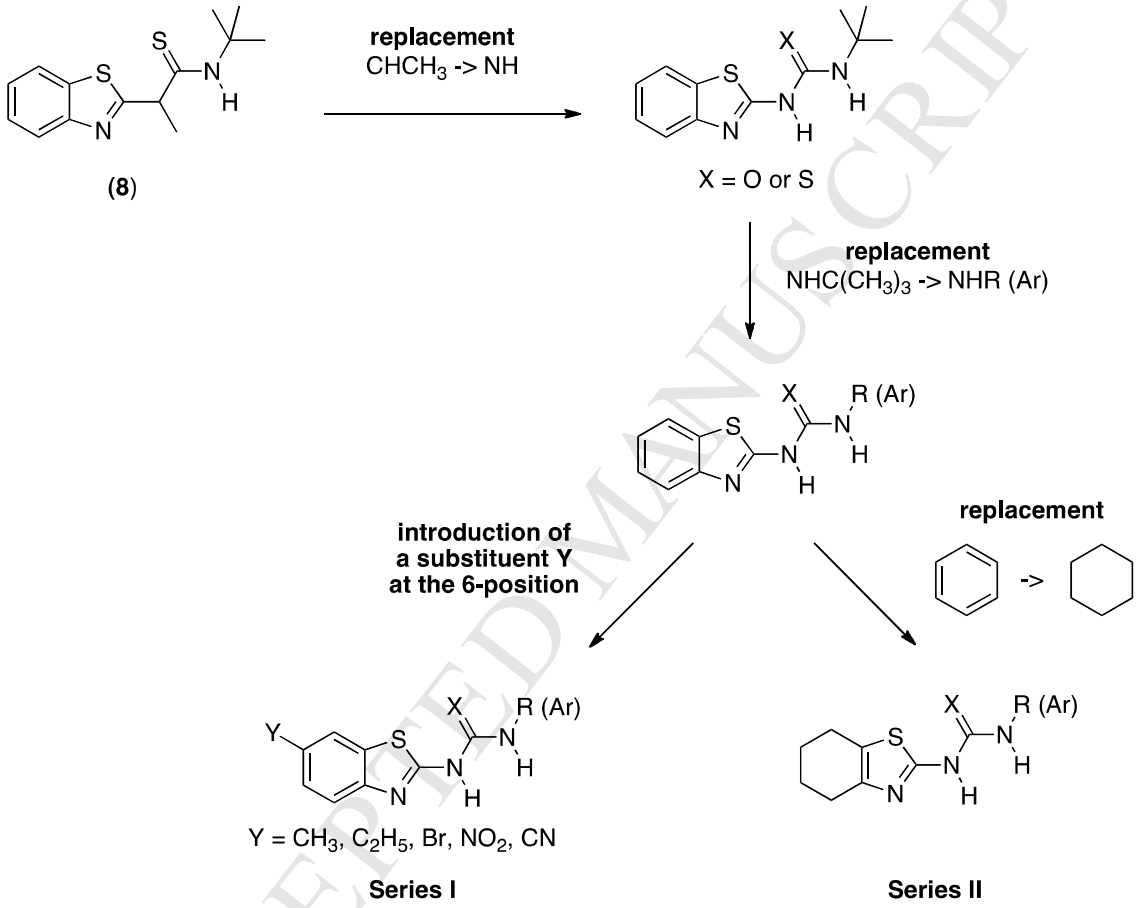
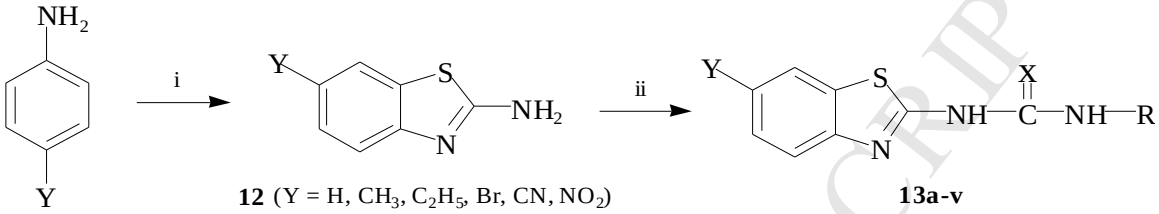


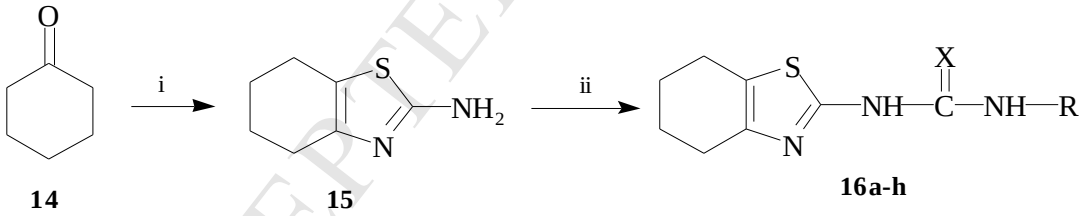
Figure 2

Schemes



11 **12** (Y = H, CH_3, C_2H_5, Br, CN, NO_2) **13a-v**

Scheme1. Reagents and conditions: (i) NH_4SCN, Br_2, CH_3COOH; (ii) RNCX, toluene.

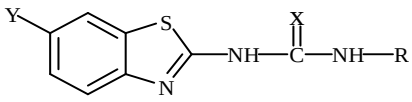


14 **15** **16a-h**

Scheme 2. Reagents and conditions: (i) 1) thiourea, I_2, 110°C, 2) Na_2CO_3, H_2O; (ii) RNCX, CH_2Cl_2.

Tables

Table 1. Effects of benzothiazoles **13a–v** on the contractile activity of K^+-depolarized rat aorta rings.

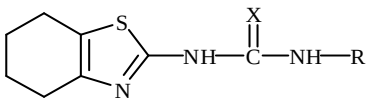


13a-v

Compound	Y	X	R	EC_{50} (μM)[a] (mean ± SEM (n))
13a	Br	O	4-CN-phenyl	> 100
13b	Br	O	isopropyl	22.79 ± 4.42 (5)
13c	Br	O	tert-butyl	13.03 ± 2.35 (5)
13d	Br	O	2-Cl,4-CF_3-phenyl	>100
13e	Br	O	benzyl	64.20 ± 7.39 (4)
13f	NO_2	O	isopropyl	16.16 ± 4.77 (6)
13g	NO_2	O	tert-butyl	10.76 ± 2.52 (6)
13h	NO_2	O	2-Cl,4-CF_3-phenyl	54.32 ± 8.18 (4)
13i	H	O	2-Cl,4-CF_3-phenyl	> 100
13j	H	O	isopropyl	35.49 ± 4.93 (4)
13k	CH_3	S	cyclohexyl	52.06 ± 8.35 (4)
13l	CH_3	O	isopropyl	18.47 ± 3.37 (6)
13m	CH_3	S	benzyl	>100
13n	C_2H_5	S	3-NO_2-phenyl	>100
13o	C_2H_5	O	isopropyl	24.40 ± 1.15 (6)
13p	C_2H_5	O	tert-butyl	25.27 ± 0.96 (5)
13q	C_2H_5	O	benzyl	>100
13r	C_2H_5	O	2-Cl,4-CF_3-phenyl	> 100
13s	C_2H_5	S	benzyl	> 100
13t	CN	O	pentyl	14.76 ± 6.47 (5)
13u	CN	O	tert-butyl	2.78 ± 0.94 (6)
13v	NO_2	O	pentyl	2.11 ± 1.12 (5)
Diazoxide	---	---	---	23.68 ± 3.31 (6)
Pinacidil	---	---	---	0.39 ± 0.07 (4)

[a] EC_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings. Results are expressed as mean ± sem. Numbers in parentheses refer to the number (n) of individual experiments performed in each group.

Table 2. Effects of tetrahydrobenzothiazoles **16a-h** on the contractile activity of K^+-depolarized rat aorta rings.



16a-h

Compound	X	R	EC_{50} (μM) [a] (mean ± SEM (n))
16a	S	3-NO_2-phenyl	> 100
16b	O	isopropyl	47.63 ± 7.62 (6)
16c	S	isopropyl	> 100
16d	O	tert-butyl	75.24 ± 4.34 (5)
16e	O	4-CN-phenyl	> 100
16f	S	4-CN-phenyl	> 100
16g	O	4-Cl,3-CF_3-phenyl	> 100
16h	O	4-Cl-phenyl	> 100
Diazoxide	---	---	23.68 ± 3.31 (6)
Pinacidil	---	---	0.39 ± 0.07 (4)

[a] EC_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings. Results are expressed as mean ± sem. Numbers in parentheses refer to the number (n) of individual experiments performed in each group.

Table 3. Myorelaxant effect of selected compounds on 30 mM KCl-precontracted rat aorta rings in the absence or presence of 10 µM glibenclamide, as well as on 80 mM KCl-precontracted rat aorta rings.

Compound	**rat aorta rings 30 mM KCl EC_{50} (µM) [a] (mean ± SEM (n))**			**rat aorta rings 80 mM KCl EC_{50} (*µ*M) [a] mean ± SEM (*n*)**
	presence of endothelium	absence of endothelium	10 µM glibenclamide [b]	
13c	13.03 ± 2.35 (5)	12.12 ± 2.10 (4)	23.30 ± 5.14 (4)	26.87 ± 4.13 (4)
13f	16.16 ± 4.77 (6)	17.82 ± 2.88 (4)	nd	36.39 ± 2.14 (5)
13g	10.76 ± 2.52 (6)	14.79 ± 5.09 (5)	19.46 ± 2.82 (5)	31.00 ± 4.15 (5)
13u	2.78 ± 0.94 (6)	4.66 ± 1.27 (5)	15.61 ± 3.62 (4)	15.02 ± 5.49 (4)
13v	2.11 ± 1.12 (5)	4.75 ± 1.04 (4)	19.57 ± 4.42 (3)	14.19 ± 3.38 (5)
Diazoxide	23.68 ± 3.31 (6)	23.17 ± 3.57 (4)	>100	>100
Pinacidil	0.39 ± 0.07 (4)	nd	8.02 ± 2.50 (5)	92.06 ± 17.06 (5)

[a] EC_{50} is the drug concentration (µM) giving 50% relaxation of the 30 or 80 mM KCl-induced contraction (mean ± s.e.m.). Numbers in parentheses refer to the number (n) of individual experiments performed in each group. [b] EC_{50} values obtained when glibenclamide (10 µM) was added to the bath medium.

Table 4. Effects of **13a**, **13c**, **13e**, **13f** and **13g** on insulin secretion from rat pancreatic islets and on contractile activity of K^+-depolarized rat aorta rings.

Compound	Rat pancreatic β-cells % RIS [a] (mean ± SEM (n)) 50 µM	10 µM	1 µM	Myorelaxant Activity EC_{50} (μM) [b] Mean ± SEM (*n*)
13a	10.68 ± 1.16 (20)	11.03 ± 0.98 (15)	60.50± 2.27 (24)	> 100
13c	15.89 ± 0.83 (22)	77.97 ± 3.65 (15)	nd	13.05 ± 4.40 (5)
13e	17.85 ± 1.11 (21)	82.97 ± 5.40 (24)	nd	81.91 ± 7.39 (4)
13f	8.52 ± 0.69 (19)	62.03 ± 2.16 (23)	nd	15.70 ± 3.00 (6)
13g	11.77 ± 0.55 (22)	30.98 ± 2.06 (23)	83.52 ±5.87 (15)	11.09 ± 1.69 (6)
Diazoxide	26.7 ± 1.6 (16) [c]	73.9 ± 4.4 (16) [c]	87.5 ± 5.0 (15) [c]	23.68 ± 3.31 (6)
Pinacidil	88.8 ± 4.5 (13) [c]	92.1 ± 3.9 (13) [c]	97.7 ± 6.7 (19) [c]	0.39 ± 0.07 (4)
BPDZ 44	8.6 ± 0.9 (23) [d]	13.7 ± 1.2 (23) [d]	82.9 ± 3.6 (22)	154.4 ± 14.5 (8) [d]

[a] % RIS: percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose. Effects of the drugs at 1, 10 and 50 µM. [b] EC_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings. Numbers in parentheses refer to the number (n) of individual experiments performed in each group. Published results : [c] ref 23, [d] ref 24.

Synthesis, characterization and biological evaluation of benzothiazoles and tetrahydrobenzothiazoles bearing urea or thiourea moieties as vasorelaxants and inhibitors of the insulin releasing process

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Highlights

A series of 1,3-benzothiazoles and 4,5,6,7-tetrahydro-1,3-benzothiazoles were synthesized.

Several 1,3-benzothiazole derivatives showed a myorelaxant activity higher than diazoxide.

Their myorelaxant activity was explained, at least in part, by the opening of K_{ATP} channels.

Some 1,3-benzothiazole derivatives also provoked a marked inhibition of insulin secretion.