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Short communication

Preparation and in vitro evaluation of benzylsulfanyl benzoxazole derivatives as potential antituberculosis agents

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ABSTRACT

A set of 2-benzylsulfanyl derivatives of benzoxazole was synthesized and evaluated for their in vitro antimycobacterial activity against *Mycobacterium tuberculosis,* non-tuberculous mycobacteria and multidrug-resistant *M. tuberculosis.* The activities were expressed as the minimum inhibitory concentration (MIC) in mmol/L. The substances showed similar activity against all tested strains. The lead compounds in the set, dinitro derivatives exhibited significant activity against both sensitive and resistant strains of *M. tuberculosis* and also against non-tuberculous mycobacteria. To facilitate drug design of benzoxazole as potential antituberculosis agent, we have explored the quantitative structure–activity relationship (QSAR). We demonstrated that lower lipophilicity has significant contribution to activity. Dinitrobenzylsulfanyl derivative of benzoxazole represents the promising small-molecule synthetic antimycobacterials.

1. Introduction

In 2006 almost 9.2 million new TB cases occurred, 80% of them in 22 countries and 1.7 million people died from TB. It is estimated that more than 2 billion people – one third of the world's population – are infected with TB bacilli, the microbes that cause TB. Drugs for treating tuberculosis (TB) have been available for over half a century, but the total number of deaths and TB cases is still rising due to population growth. TB annual incidence rates have peaked globally in 2003–2004, however, they are falling very slowly in all WHO regions, and stagnating in Eastern Europe. Five percentage of all TB cases have multidrug-resistant TB (MDR-TB) and in 2008 WHO ever recorded the highest rates of MDR-TB. Extensively drug-resistant TB (XDR-TB) cases have been confirmed in more than 45 countries. Already in 1993, the World Health Organization recognized this developing situation and declared TB "a global health emergency" [1–3]. In the last decade the number of TB research publications with a drug discovery focus increased [4,5], some of new compounds advanced into clinical trials and development [6].

Previously, we have reported a large number of alkylsulfanyl derivatives of pyridine [7,8], benzimidazole [9–11], and tetrazole [12] which showed promising antimycobacterial activity and detailed QSAR findings supported the hypothesis that an alkylsulfanyl group bound to an electron deficient carbon in various heterocycles was responsible for antimycobacterial activity [13]. In the course of our ongoing efforts to investigate benzylsulfanyl derivatives of benzazoles, we decided to replace the atom of nitrogen of the benzimidazole ring with the corresponding isosteric atom of oxygen. The activity of some benzylsulfanyl derivatives of benzoxazole was mentioned in our preliminary communication [14]. Herein, we report the synthesis of a large set of 2-benzylsulfanyl derivatives of benzoxazole, their antimycobacterial activity, as well as the quantitative structure–activity relationship.

2. Chemistry

The compounds were synthesized following the steps depicted in Scheme 1. The commercially available benzoxazole-2-thiol (**1**) serves as a convenient starting material in the syntheses and it is alkylated with benzyl halides (**2**). Thus, thiol **1** was converted to the corresponding sodium salt by dissolving in an ethanolic solution of sodium ethanolate, and the resulting salt was subjected to a nucleophilic substitution upon the addition of a benzyl halide **2**. The reaction was carried out in *N*,*N*-dimethylformamide (DMF) at

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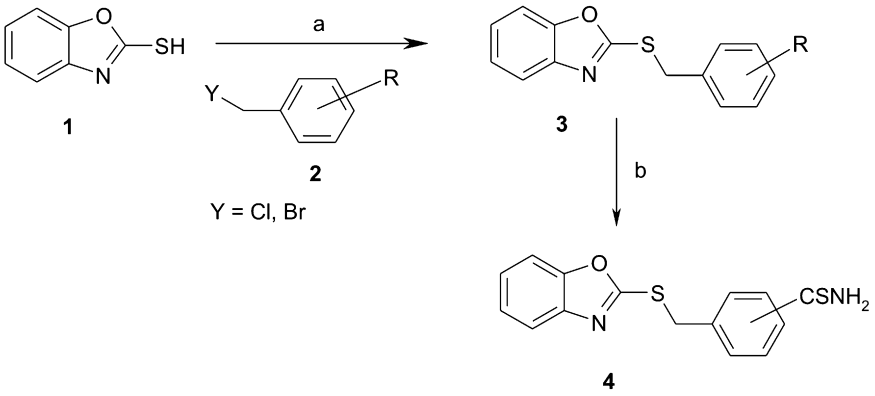

Scheme 1. Synthesis of 2-benzylsulfanylbenzoxazoles **3a–z, 3aa** and **4a** and **b.** Reagents and conditions: (a) Na, CH_3CH_2OH, DMF, rt, 2–6 h; (b) H_2S (g), Pyridine, TEA, rt, 3–4 h and 45 °C, 1 h.

room temperature, under anhydrous conditions. The reaction required 2–6 h to complete, depending on the alkylating agent and furnished products **3** in 53–97% yields. The benzylsulfanyl derivatives bearing the CN group **3z**, **3aa** were further converted into the corresponding carbothioamides **4a** and **4b** by the addition of hydrogen sulfide in pyridine/triethylamine solution.

The structures of the compounds were fully characterized by ^{1}H NMR, ^{13}C NMR, and IR spectral data, and their purity by elemental analysis. The ^{1}H NMR spectra of benzylsulfanyl derivatives have thetypical singlet of CH_2S group lying in the region 4.5–4.9 ppm and multiplet of aromatic part of molecules occurring in region between 7.0 and 8.0 ppm. The ^{13}C NMR signal of CH_2S group can be observed at 34.0–36.0 ppm. IR spectra of 2-benzylsulfanyl derivatives were also in agreement with the structures.

3. Biology

3.1. Antimycobacterial activity

In vitro antimycobacterial activity of the compounds was evaluated against *Mycobacterium tuberculosis* CNCTC My 331/88, *Mycobacterium kansasii* CNCTC My 235/80, *M. kansasii* 6509/96 and *Mycobacterium avium* CNCTC My 330/88 using the micromethod for the determination of the minimum inhibitory concentration (MIC). All strains were obtained from the Czech National Collection of Type Cultures (CNCTC), with the exception of *M. kansasii* 6509/96, which was a clinical isolate. The activities of the compounds were determined in the Šula semisynthetic medium (SEVAC, Prague). The compounds were added to the medium in dimethylsulfoxide solutions. The following concentrations were used: 1000, 500, 250, 125, 62, 32, 16, 8, 4 and 2 μmol/L. MICs were determined after incubation at 37 °C for 14 and 21 days, for *M. kansasii* for 7, 14, and 21 days. MIC was the lowest concentration of a substance, at which the inhibition of the growth of mycobacteria occurred. INH was used as a standard.

The most active compounds **3t** and **4a** were evaluated against four multidrug-resistant strains of *M. tuberculosis* using the micromethod for the determination of the minimum inhibitory concentration (MIC) under the same conditions as described above. The characterization of resistant strains of *M. tuberculosis* is following: *M. tuberculosis* 7357/98 resistant to isoniazid, rifampicin, ethambutol, streptomycin, ofloxacin, rifabutin, and ciprofloxacin; *M. tuberculosis* 4166/04 resistant to isoniazid, and streptomycin; *M. tuberculosis* 4977/03 resistant to isoniazid, rifampicin, ethambutol, streptomycin, ofloxacin, and rifabutin and *M. tuberculosis* 550/04 resistant to isoniazid, rifampicin, gentamicin, amikacin, and rifabutin.

3.2. Antiproliferative and cytotoxic effect

The target compounds were assayed against cell lines K-562 and L-929 for their antiproliferative effects and against HeLa for their cytotoxic effects. The cells were incubated with 10 concentrations of the test compounds [15].

Suspension cultures of K-562 in micro plates were analyzed by an electronic cell analyzer system CASY 1 (SCHÄRFE, Reutlingen, Germany) using an aperture of 150 μm. The software for data evaluation CASYSTAT (SCHÄRFE) offers fast graphical evaluation of the measurement parameters, e.g. as diagrams of cell diameter distributions, overlays of different curves, and cell volume distributions. The 0.2 mL content of each well in the micro plate was diluted 1:50 with CASYTON (NaCl: 7.93 g/L; Na_2EDTA: 0.38 g/L; KCl: 0.4 g/L; NaH_2PO_4 monohydrate: 0.22 g/L; NaH_2PO_4 dihydrate: 2.45 g/L; NaF: 0.3 g/L; SCHÄRFE). Every count/mL was automatically calculated from the arithmetic mean of three successive counts of 0.4 mL each. From the dose response curves the GI_{50} values (concentration which inhibited cell growth by 50%) were calculated with CASYSTAT. The GI_{50} value was defined as being where the concentration–response curve intersected the 50% line, determined by means of the cell counts/mL, compared to control.

The monolayers of the adherent L-929 and HeLa cells were fixed by glutaraldehyde and stained with a 0.05% solution of methylene blue for 15 min. After gently washing, the stain was eluted by 0.2 mL of 0.33 N HCl in the wells. The optical densities were measured at 630 nm in a DYNATECH MR 7000 micro plate reader. Comparisons of the different values were performed with Microsoft Excel.

4. Results and discussion

All prepared compounds were tested for antimycobacterial activities and the values of MICs are summarized in Table 1. In several cases (denoted >), the minimum inhibitory concentration (MIC) could not be determined due to the limited solubility of the compounds in the testing medium.

For the sake of comparison, the values of MICs of **1** and the standard isoniazide (INH) were also included. All of these compounds (**1**, **3**, **4**) displayed in vitro activity against all mycobacterial strains tested. The values of MICs are within a range of 2–500 μmol/L, most often between 32 and 62 μmol/L. By comparing their MIC values with those of INH, the compounds under study were less active against *M. tuberculosis* 331/88 and *M. kansasii* 6509/96. On the other hand, they possessed a more pronounced effect against *M. kansasii* My 235/80 and *M. avium* 330/88 than INH. In contrast to INH, the studied

Table 1
In vitro antimycobacterial activity of compounds **1**, **3a–z, 3aa** and **4a** and **b** expressed as MIC (μmol/L)

Compound	Strains									
	Mycobacterium tuberculosis My 331/88		*Mycobacterium kansasii My 235/80*			*Mycobacterium kansasii 6 509/96*			*Mycobacterium avium My 330/88*	
	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d	14 d	21 d
1	500	500	500	1000	1000	250	500	1000	250	500
3a	250	500	125	250	250	32	125	250	62	125
3b	250	>500	32	62	125	32	62	125	62	62
3c	62	125	32	32	62	32	32	62	32	32
3d	>250	>250	32	32	62	62	62	125	125	125
3e	250	500	32	62	125	62	62	125	62	62
3f	62	125	32	62	125	32	32	62	32	32
3g	125	250	32	32	62	32	32	62	62	62
3h	>62	>125	32	62	>62	32	62	125	32	62
3i	125	250	32	32	62	32	32	62	32	32
3j	>500	>500	62	62	125	62	62	125	32	62
3k	250	500	125	125	500	125	125	250	62	125
3l	62	125	32	32	62	32	32	62	32	62
3m	125	250	32	32	62	32	32	62	32	32
3n	>62	>125	32	32	>62	32	32	>32	>32	>62
3o	>62	125	32	>32	>32	32	32	>32	>32	>62
3p	>250	>250	125	125	>125	62	>62	>62	>125	>125
3q	62	125	32	32	62	32	32	62	62	62
3r	>125	>250	32	62	125	62	62	125	62	62
3s	125	125	62	125	125	32	62	125	62	125
3t	8	8	2	4	4	4	8	8	>16	>32
3u	8	8	4	4	4	4	8	8	>16	>32
3v	>62	>62	>32	>32	>32	32	>32	>62	62	125
3w	125	125	32	62	125	16	32	62	62	62
3x	62	125	32	32	62	32	32	62	32	32
3y	62	125	62	62	125	32	32	62	>125	>125
3z	>62	>62	32	32	>32	16	32	>32	62	62
3aa	125	125	32	62	125	32	62	62	62	62
4a	8	16	8	16	16	8	16	16	32	32
4b	8	16	8	32	32	8	32	32	62	62
INH	0.5	1	>250	>250	>250	2	2	4	>250	>250

compounds were comparably active against both strains of *M. kansasii*.

The starting benzoxazole-2-thiol (**1**) is characterized by the activity within a range of 250–1000 μmol/L. Comparisons of the MIC values for **1** to those of the benzylsulfanyl derivative (**3a**) indicate that the antimycobacterial activity is connected with the presence of the benzyl moiety at 2-position of the benzoxazole ring. It was according to our earlier hypothesis that an alkylsulfanyl group bound to an electron deficient carbon atom is responsible for antimycobacterial activity [16]. Further modifications of the benzyl moiety with various electron-accepting or electron-donor substituents improved the activity. It is important to point out that it is not affected by the electronic properties of the substituents, practically all compounds substituted on the benzyl moiety exhibited similar activity within the range 32–125 μmol/L. It is according to our previously presented group of 2-benzylsulfanylbenzimidazole derivatives [9,10] and similar 2-substituted 5,7-di-*tert*-butylbenzoxazoles [17], but it is on the contrary to what was observed for the previously synthesized 4-benzylsulfanyl derivatives of pyridine [13] where the electron-withdrawing substituents cause the increase of the activity. The incorporation of two nitro groups into the benzyl moiety led to the most active compounds **3t**, **3u** with the MICs values ranging from 2 to 32 μmol/L. The same conclusions were obtained in our previously synthesized benzylsulfanyl deriviatives of benzimidazoles [9,10]. Both compounds **3t** and **3u** exhibited the same activity against *M. tuberculosis* and non-tuberculous mycobacteria *M. kansasii*. Their activity against *M. kansasii* 235/80 (MIC = 2–4 μmol/L) exceeded the activity of INH (MIC_{INH} = >250 μmol/L), the activity against *M. kansasii* 6509/96 was 2-fold less than that of INH. The activity of INH against *M. tuberculosis* 331/88 (MIC = 8 μmol/L) was not reached (MIC_{INH} = 0.5–1 μmol/L). MICs against *M. avium* 330/88 could not be determined due to insolubility of compounds in the testing medium. The antimycobacterial activity of compounds **3t** and **3u** is probably connected with a nitro group. It is noteworthy that the many antibacterials that are used for treatment of anaerobic infections feature a nitro group (chloramphenicole, metronidazole) in their structure. In currently undergoing clinical trials there are two antituberculotics marked as PA-824 and OPC-67683 bearing also a nitro group [4,6,18,19]. The vast array of nitrated antimycobacterials reported that all of them probably being prodrugs to reduced into the corresponding active species acting on biochemical targets [20]. The study with PA-824 indicated that the metabolic activation of PA-824 by *M. tuberculosis* involves a nitro-reduction step and the resistant mutants had lost ability to carry out nitro-reduction [18]. A promising effect was also revealed for thioamide derivatives (**4a**, **4b**) although they did not reach the activity of dinitro derivatives (**3t**, **3u**). It is very likely that thioamide group is responsible for the antimycobacterial activity such as in the antituberculotics ethionamide. Ethionamide also is prodrug that is oxidized by EthA (a flavoprotein monooxygenase) to the sulfinic acid [4].

The antimycobacterial activities against multidrug-resistant strains of *M. tuberculosis* are summed up in Table 2. MIC values for **3t** and **4a** are within a range of 2–4 μmol/L, and 8–32 μmol/L, respectively. In comparison to the sensitive strain of *M. tuberculosis* compound **3t** has been once or twice more active against multidrug-resistant strains, while compound **4a** exhibits the same or less efficacies. The level of activity against both sensitive and multidrug-resistant strains indicates that these compounds may target biochemical mechanisms different from the one mutated in the resistant strains.

Table 2
In vitro antituberculotic activity against MDR *M. tuberculosis* (MIC expressed in μmol/L)

Compound	Strains							
	Mycobacterium tuberculosis 7357/98[a]		*Mycobacterium tuberculosis 4166/04*[b]		*Mycobacterium tuberculosis 4977/03*[c]		*Mycobacterium tuberculosis 550/04*[d]	
	14 d	21 d	14 d	21 d	14 d	21 d	14 d	21 d
3t	4	4	2	4	2	2	2	4
4a	32	32	8	16	32	32	8	16

[a] Resistant to isoniazid, rifampicin, ethambutol, streptomycin, ofloxacin, rifabutin, and ciprofloxacin.
[b] Resistant to isoniazid, and streptomycin.
[c] Resistant to isoniazid, rifampicin, ethambutol, streptomycin, ofloxacin, and rifabutin.
[d] Resistant to isoniazid, rifampicin, gentamicin, amikacin, and rifabutin.

Compounds **3t**, **3u** and **4a**, **4b** were assayed against cell lines K-562 and L-929 for their antiproliferative effects (GI_{50}: concentration which inhibited cell growth by 50%), and against HeLa cells for their cytotoxic effects (CC_{50}: cytotoxic concentration which contains a specific destructive action by 50%; used particularly in referring to the lysis of cells). The cells were incubated with 10 concentrations of the tested compounds. The values expressed as GI_{50} are ranging from 5.5 to >50 μg/mL and values of CC_{50} from 21 to 50 μg/mL. The lower cytotoxicity demonstrated the nitro derivatives. The most antimycobacterial compound **3t** exhibited the lowest cytotoxic effect (Table 3).

The formerly prepared compounds **3a**, **3b**, **3e**, **3h**, **and 3n** [21–24] were evaluated by Cranham for toxicity to red spider mite [21]. Of all tested compounds only **3b** was moderately active against the eggs and larvae of the glasshouse red spider mite.

A set of benzylsulfanyl derivatives of benzoxazole was analyzed by quantitative structure–activity relationship techniques. Physicochemical and quantum-chemical parameters taken into consideration were log *P* for the hydrophobic effects, σ (Hammet constant) and energy of HOMO, LUMO orbitals (ε_{HOMO}, ε_{LUMO}) as the electronic influences, MR (molar refractivity) for the steric interactions and their values are summarized in Table 4. The biological activities are expressed as a log 1/MIC (MIC values in mol/L). Multiple linear regression analysis which involves finding the best fit of dependent variable (antimycobacterial activity) to a linear combination of independent variables (descriptors) is used by the least squares method. Regression analyses were carried out for each strain separately, however, the statistically significant equations were obtained for *M. tuberculosis*.

Mycobacteria are surrounded by a thick and waxy cell wall; hence, efficient drugs have a reasonable lipophilicity in order to penetrate this barrier. Newly prepared compounds **3** and **4** are characterized by a calculated log *P* within range from 3.5 to 5.6. The most active compounds **3t**, **3u**, **4a**, and **4b** possess values log *P* 3.8 and 3.5, respectively (see Table 4). An antituberculous drug should be taken orally, and, according to the "Lipinski rule of five", a compound with good oral bioavailability generally has log $P < 5$ [25]. The limit of log *P* values by benzylsulfanyl derivatives was not exceeded.

The QSAR analysis reveals that the parameter molar refractivity (MR) is the most significant for the potency of studied compounds (Eq. (1)). Parameter MR has connection with steric interactions of compound with biomacromolecules. An enlargement of MR of molecule resulted in better activity. Extending Eq. (1) with log *P* (Eq. (2)) lead to the deterioration of statistic parameters ($F = 29.89$). The value of regression coefficient for log *P* indicates that the antituberculotic activity increases with diminishing lipophilicity. The lowest log *P* exhibited the most active derivatives **4** and **3t**. Eqs. (3) and (4) show a small contribution of electronic parameters to activity. That is in accordance with our previous finding that it is not affected by the electronic properties of the substituents. Practically all compounds substituted with whatever substituent on the benzyl moiety exhibited similar activity. More significant correlations are obtained by replacing Hammet constant with energies of HOMO, LUMO orbitals (Eqs. (4) and (5)). HOMO and LUMO orbitals represent electron properties of the whole molecule and express the ability of molecule to be subjected to oxidation (energy of HOMO orbitals) or reduction (energie of LUMO orbitals). As mentioned above the activity of some compounds requires activation by reduction/oxidation processes.

$$\log(1/C) = 0.08(\pm 0.01)\mathrm{MR} - 1.92(\pm 0.91) \quad r = 0.85;\ r^2 = 0.72;\ s = 0.29;\ F = 44.94;\ n = 20 \qquad (1)$$

Table 3
Antiproliferative and cytotoxic effect expressed in μg/mL

Compound	L-929 GI_{50}	K-562 GI_{50}	HeLa CC_{50}
3t	22.4	9.2	>50
3u	>50	10.4	45.1
4a	23.8	9.4	23.6
4b	17.3	5.5	21.6

Table 4
Physicochemical and quantum-chemical parameter values of compounds **3** and **4**

Compound	σ	log *P*	MR (Å^3)	ε_{HOMO} (eV)	ε_{LUMO} (eV)
3a	0	3.872	69.716	−8.508	−0.490
3b	0.23	4.390	74.520	−8.594	−0.703
3c	0.37	4.390	74.520	−8.586	−0.661
3d	–	4.390	74.520	−8.509	−0.637
3e	0.06	4.012	69.932	−8.596	−0.696
3f	0.34	4.012	69.932	−8.599	−0.688
3g	–	4.012	69.932	−8.530	−0.649
3h	0.23	4.664	77.338	−8.612	−0.760
3i	0.39	4.664	77.338	−8.594	−0.680
3j	−0.17	4.339	74.757	−8.487	−0.479
3k	−0.07	4.339	74.757	−8.494	−0.463
3l	−0.27	3.619	76.179	−8.482	−0.445
3m	0.12	3.619	76.179	−8.488	−0.500
3n	0.78	3.826	77.040	−8.833	−1.506
3o	0.71	3.826	77.040	−8.777	−1.302
3p	–	3.826	77.040	−8.661	−1.301
3q	–	4.530	74.737	−8.511	−0.814
3r	0.60	4.908	79.325	−8.652	−0.857
3s	0.4	4.151	70.148	−8.684	−0.912
3t	1.42	3.779	84.365	−9.006	−2.024
3u	–	3.779	84.365	−8.951	−2.171
3v	–	3.965	77.257	−8.657	−1.487
3w	0.54	4.755	75.689	−8.721	−1.016
3x	0.43	4.755	75.689	−8.685	−0.879
3y	0.86	5.638	81.663	−8.848	−1.281
3z	0.66	3.908	75.453	−8.694	−1.030
3aa	0.56	3.908	75.453	−8.670	−0.855
4a	0.4	3.525	86.409	−8.620	−1.036
4b	–	3.525	86.409	−8.615	−0.847

$$\log(1/C) = 0.08(\pm0.01)\mathrm{MR} - 0.22(\pm0.13)\log P - 1.16(\pm1.17) \quad r = 0.85;\ r^2 = 0.72;\ s = 0.30;\ F = 22.89;\ n = 20 \tag{2}$$

$$\log(1/C) = 0.04(\pm0.02)\mathrm{MR} - 0.32(\pm0.12)\log P + 0.62(\pm0.20)\sigma - 1.64(\pm1.36) \quad r = 0.89;\ r^2 = 0.79;\ s = 0.25;\ F = 13.55;\ n = 20 \tag{3}$$

$$\log(1/C) = 0.06(\pm0.01)\mathrm{MR} - 0.32(\pm0.11)\log P - 1.51(\pm0.45)\ \mathrm{HOMO} - 12.0(\pm3.35) \quad r = 0.92;\ r^2 = 0.85;\ s = 0.24;\ F = 28.34;\ n = 20 \tag{4}$$

$$\log(1/C) = 0.05(\pm0.01)\mathrm{MR} - 0.26(\pm0.10)\log P - 0.53(\pm0.14)\ \mathrm{LUMO} + 0.68(\pm1.00) \quad r = 0.93;\ r^2 = 0.86;\ s = 0.22;\ F = 32.42;\ n = 20 \tag{5}$$

5. Conclusion

We reported a new set of 2-benzylsulfanylbenzoxazoles which exhibited antimycobacterial activity against *M. tuberculosis* and non-tuberculous mycobacteria tested. The activity is connected with a benzylsulfanyl moiety in position 2 and additional substituents on the phenyl ring increase its level. The introduction of two nitro groups or thioamide group on benzylsulfanyl moiety, regardless of position of substitution, increased the antimycobacterial activity. Thus, the substitutions with two nitro groups are the best substituents identified to date. The most active compounds **3t** exhibited the significant activity against sensitive strain and multidrug-resistant strains of *M. tuberculosis*. The QSAR study indicates that the lower value of lipophilicity and enlargement of molecule have significant contribution to antituberculous activity. The ratio between cytotoxicity and minimum inhibitory concentration for compound **3t** is low enough for further study.

6. Experimental protocols

6.1. Chemistry

The melting points were determined on a Kofler block and are uncorrected. Analytical samples were dried over P_4O_{10} at 78 °C or 61 °C and 2.4–2.6 kPa for 8–10 h. Elemental analyses were performed on CHNS–O CE instrument (FISONS EA 1110) and were within ±0.4% of the theoretical values. IR spectra were obtained on a Nicolet Impact 400 spectrometer in KBr pellets. NMR spectra were recorded in DMSO-d_6 and acetone-d_6 solutions at ambient temperature on a Varian Mercury-Vx BB 300 spectrometer operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Chemical shifts were recorded as δ values in ppm and were indirectly referred to tetramethylsilane (TMS). Coupling constants (J) are given in Hz. The reactions were monitored and the purity of the products was checked by TLC (Merck TLC plates silica gel 60 F_{254}, aluminum back) in acetone–light petroleum. The plates were visualised using UV light, iodine fumes and/or dipping in a solution of $Ce(SO_4)_2 \cdot 4H_2O$, $H_3Mo_{12}O_{40}P \cdot xH_2O$, H_2SO_4, H_2O and subsequent heating. Preparative thin layer chromatography was carried out on silica gel 60 F_{254} (0.015–0.040 mm, Merck). Silica gel 60 (0.015–0.040 mm, Merck) was used for column chromatography.

The following compounds had been described in the literature: **3a** (R = H) [21–24], **3b** (R = 4-Cl) [21,22], **3e** (R = 4-F) [21,22], **3h** (R = 4-Br) [21,22], **3n** (R = 4-NO_2) [21,22], **3t** (R = 3,5-$(NO_2)_2$) [14], **3z** (R = 4-CN) [14], and **4a** (R = 4-$CSNH_2$) [14].

6.1.1. General procedure for the synthesis of compounds 3

Benzoxazole-2-thiol (**1**) (0.75 g, 5 mmol) in dry DMF (8 mL) was added to a solution of sodium (0.12 g, 5 mmol) in dry ethanol (2.5 mL). After 10 min of stirring at ambient temperature and under atmosphere of argon, benzyl chloride (**2**) (5 mmol) was added in 2–3 portions, and the resultant suspension was stirred for 2–6 h. The reaction mixture was then poured onto crushed ice and left at 4 °C overnight. The solid was filtered off, washed with cold water (2 × 30 mL) and air-dried. The crude products were purified by preparative TLC or column chromatography using acetone–light petroleum (1:2, 1:3, 1:4, 1:5, 1:6), followed by crystallization from aqueous ethanol, toluene or ethylacetate to afford the title compounds as white or yellowish needles in 53–95% yields. The following compounds were prepared according this general procedure.

*6.1.1.1. 2-Benzylsulfanylbenzoxazole (**3a**).* Yield: 61%; mp: 48–49 °C (51–52.5 °C [21,22], 50 °C [23,24]); IR (KBr) ν_{max} (cm^{-1}) 3432, 3065, 3035 (C–H)$_{Ar}$, 1602, 1505,1453 (C=C$_v$)$_{Ar}$, 1471 (CH_2), 1238, 1212, 1129, 1096 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.61 (2H, s), 7.24–7.37 (5H, m), 7.46–7.52 (2H, m), 7.60–7.68 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) 35.4, 110.4, 118.5, 124.6, 124.9, 127.9, 128.8, 129.2, 136.8, 141.4, 151.5, 164.1.

*6.1.1.2. 2-(4-Chlorobenzylsulfanyl)benzoxazole (**3b**).* Yield: 92%; mp: 58–59 °C (61–62 °C [21,22]); IR (KBr) ν_{max} (cm^{-1}) 3432, 3060, 3058, 3022 (C–H)$_{Ar}$, 1599, 1500, 1492 (C=C$_v$)$_{Ar}$, 1471 (CH_2), 1238, 1216, 1133, 1097 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.60 (2H, s), 7.27–7.34 (2H, m), 7.35–7.42 (2H, m AA′BB′), 7.49–7.56 (2H, m AA′BB′), 7.59–7.68 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) 34.9, 110.5, 118.5, 124.6, 124.9, 128.8, 131.1, 132.5, 136.2, 141.4, 151.5, 163.9.

*6.1.1.3. 2-(3-Chlorobenzylsulfanyl)benzoxazole (**3c**).* Yield: 75%; mp: 32–34 °C; IR (KBr) ν_{max} (cm^{-1}) 3431, 3064 (C–H)$_{Ar}$, 1637, 1598, 1576, 1500, 1476 (CH_2), 1453, 1431 (C=C$_v$)$_{Ar}$, 1239, 1214, 1133 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.61 (2H, s), 7.27–7.40 (4H, m), 7.43–7.52 (1H, m), 7.57–7.69 (3H, m); ^{13}C NMR (75 MHz, DMSO-d_6) 34.9, 110.5, 118.6, 124.6, 124.9, 127.9, 127.9, 129.0, 130.7, 133.2, 139.6, 141.4, 151.5, 163.8.

*6.1.1.4. 2-(2-Chlorobenzylsulfanyl)benzoxazole (**3d**).* Yield: 77%; mp: 61–62 °C; IR (KBr) ν_{max} (cm^{-1}) 3432, 3054 (C–H)$_{Ar}$, 1599, 1500, 1453 (C=C$_v$)$_{Ar}$, 1471 (CH_2), 1237, 1215, 1156, 1132 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.69 (2H, s), 7.26–7.39 (4H, m), 7.45–7.53 (1H, m), 7.59–7.72 (3H, m); ^{13}C NMR (75 MHz, DMSO-d_6) 34.0, 110.5, 118.6, 124.7, 124.9, 127.7, 129.8, 130.1, 131.7, 133.6, 134.0, 141.4, 151.5, 163.6.

*6.1.1.5. 2-(4-Fluorobenzylsulfanyl)benzoxazole (**3e**).* Yield: 95%; mp: 44.5–45.5 °C (44–45 °C [21,22]); IR (KBr) ν_{max} (cm^{-1}) 3432, 3070, 3044 (C–H)$_{Ar}$, 1629, 1601, 1509, 1454, 1419 (C=C$_v$)$_{Ar}$, 1471 (CH_2), 1238, 1221, 1158, 1133, 1097 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.60 (2H, s), 7.50–7.58 (2H, m), 7.59–7.68 (2H, m), 7.11–7.20 (2H, m), 7.26–7.37 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) 34.9 (d, J = 0.8 Hz), 110.4, 115.6 (d, J = 21.4 Hz), 118.5, 124.7 (d, J = 22.6 Hz), 133.2 (d, J = 3.2 Hz), 131.3 (d, J = 8.3 Hz), 141.4, 151.5, 161.8 (d, J = 243.9 Hz), 164.0.

*6.1.1.6. 2-(3-Fluorobenzylsulfanyl)benzoxazole (**3f**).* Yield: 95%; mp: 34.5–36 °C; ν_{max} (cm^{-1}) 3425, 1617, 1590, 1501, 1453 (C=C$_v$)$_{Ar}$, 1471 (CH_2), 1238, 1132, 1097 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.62

(2H, s), 7.05–7.15 (1H, m), 7.26–7.42 (5H, m), 7.59–7.69 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) 35.0 (d, J = 2.0 Hz), 110.4, 114.6, 114.9, 116.0 (d, J = 21.8 Hz), 118.6, 124.7 (d, J = 21.2 Hz), 125.3 (d, J = 2.9 Hz), 130.7 (d, J = 8.6 Hz), 139.8, 139.9, 141.4, 151.5, 162.2 (d, J = 243.9 Hz), 163.9.

*6.1.1.7. 2-(2-Fluorobenzylsulfanyl)benzoxazole (**3g**).* Yield: 81%; mp: 36–38 °C; ν_{max} (cm^{-1}) 3432, 1636, 1617, 1604, 1587, 1471 (CH_2), 1493, 1453 (C=C$_v$)$_{Ar}$, 1237, 1132, 1096 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.64 (2H, s), 7.11–7.40 (2H, m), 7.27–7.39 (3H, m), 7.60–7.69 (2H, m overlapped), 7.58 (1H, dd, J_1 = 1.7 Hz, J_2 = 7.7 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) 29.5 (d, J = 3.2 Hz), 110.5, 115.7 (d, J = 20.9 Hz), 118.6, 123.6, 123.8, 124.8, 124.8, 124.8 (d, J = 18.3 Hz), 130.4 (d, J = 8.0 Hz), 131.6 (d, J = 3.7 Hz), 141.4, 151.5, 160.6 (d, J = 246.0 Hz), 163.5.

*6.1.1.8. 2-(4-Bromobenzylsulfanyl)benzoxazole (**3h**).* Yield: 80%; mp: 69–72 °C (70–72 °C [21,22]); IR (KBr) ν_{max} (cm^{-1}) 3432, 1636, 1454, 1492 (CH_2), 1432 (C=C$_v$)$_{Ar}$, 1238, 1135 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.58 (2H, s), 7.27–7.37 (2H, m), 7.43–7.49 (2H, m AA′BB′), 7.50–7.55 (2H, m AA′BB′), 7.60–7.68 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) 34.9, 110.4, 118.5, 121.1, 124.6, 124.9, 131.4, 131.7, 136.6, 141.4, 151.5, 163.9.

*6.1.1.9. 2-(3-Bromobenzylsulfanyl)benzoxazole (**3i**).* Yield: 70%; mp: 37–39 °C; ν_{max} (cm^{-1}) 3427, 1636, 1595, 1569, 1473 (CH_2), 1453, 1428 (C=C$_v$)$_{Ar}$, 1239, 1133, 1097 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.60 (2H, s), 7.26–7.36 (3H, m), 7.42–7.56 (2H, m), 7.58–7.69 (2H, m), 7.73 (1H, t, J = 1.6 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) 34.9, 110.4, 118.5, 121.8, 124.6, 124.9, 128.3, 130.7, 130.9, 131.9, 139.9, 141.4, 151.5, 163.8.

*6.1.1.10. 2-(4-Methylbenzylsulfanyl)benzoxazole (**3j**).* Yield: 95%; mp: 52–54 °C; ν_{max} (cm^{-1}) 3448, 3095, 3081, 3046, 3023 (C–H)$_{Ar}$, 2970, 1395 (CH_3), 2919, 1470 (CH_2), 1774, 1636, 1617, 1516, 1503, 1453 (C=C$_v$)$_{Ar}$, 1236, 1134, 1095 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 2.24 (3H, s), 4.56 (2H, s), 7.08–7.16 (2H, m AA′BB′ overlapped), 7.26–7.40 (2H, m overlapped), 7.34–7.40 (2H, m AA′BB′ overlapped), 7.58–7.68 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) 20.9, 35.6, 110.4, 118.5, 124.5, 124.8, 129.1, 129.4, 133.6, 137.2, 141.5, 151.4, 164.1.

*6.1.1.11. 2-(3-Methylbenzylsulfanyl)benzoxazole (**3k**).* Yield: 93%; oil, solidified by 5 °C; ν_{max} (cm^{-1}) 3423, 3051, 3025 (C–H)$_{Ar}$, 2862, 1378 (CH_3), 2919, 1470 (CH_2), 1774, 1607, 1591 (C=C$_v$)$_{Ar}$, 1236, 1180, 1095 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 2.26 (3H, s), 4.57 (2H, s), 7.08 (1H, bd, J = 7.4 Hz), 7.25–7.40 (4H, m), 7.21 (1H, t, J = 7.4 Hz), 7.58–7.71 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) 21.1, 35.7, 110.4, 118.5, 124.5, 124.9, 126.3, 128.6, 128.7, 129.8, 136.5, 138.0, 141.4, 151.4, 164.1.

*6.1.1.12. 2-(4-Methoxybenzylsulfanyl)benzoxazole (**3l**).* Yield: 79%; mp: 55–58 °C; ν_{max} (cm^{-1}) 2935, 2841, 1470 (CH_2), 1610, 1582, 1513, 1497, 1453, 1441 (C=C$_v$)$_{Ar}$, 1243, 1182, 1131 (Ar–H), 1214, 1095 (C–O Ar–O–CH_3); ^{1}H NMR (300 MHz, DMSO-d_6) 3.71 (3H, s), 4.56 (2H, s), 6.84–6.92 (2H, m AA′BB′), 7.26–7.37 (2H, m), 7.38–7.45 (2H, m AA′BB′), 7.58–7.69 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) 35.4, 55.3, 110.4, 114.2, 114.2, 118.5, 124.5, 124.8, 130.5, 141.5, 151.4, 159.0, 164.2.

*6.1.1.13. 2-(3-Methoxybenzylsulfanyl)benzoxazole (**3m**).* Yield: 70%; mp: 37–41 °C; ν_{max} (cm^{-1}) 2939, 2836, 1470 (CH_2), 1636, 1601, 1585, 1453, 1438 (C=C$_v$)$_{Ar}$, 1239, 1153, 1096 (Ar–H), 1214, 1096 (C–O Ar–O–CH_3); ^{1}H NMR (300 MHz, DMSO-d_6) 3.71 (3H, s), 4.58 (2H, s), 6.84 (1H, bd, J_1 = 2.5 Hz J_2 = 8.2 Hz), 7.03–7.10 (2H, m), 7.26–7.37 (2H, m), 7.24 (1H, t, J = 7.8 Hz), 7.61–7.69 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) 35.7, 55.2, 110.4, 113.4, 114.8, 118.5, 121.3, 124.6, 124.9, 129.9, 138.3, 141.4, 151.5, 159.5, 164.1.

*6.1.1.14. 2-(4-Nitrobenzylsulfanyl)benzoxazole (**3n**).* Yield: 94%; mp: 109–112 °C (114–115 °C [21,22]); ν_{max} (cm^{-1}) 3432, 3080 (C–H)$_{Ar}$, 1648, 1608, 1601 (C=C$_v$)$_{Ar}$, 1518, 1347 (NO_2), 1472 (CH_2), 1455, 1432 (C=C$_v$)$_{Ar}$, 1238, 1135, 1099 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.88 (2H, s), 7.26–7.36 (2H, m AA′BB′) 7.51–7.66 (1H, m overlapped), 7.51–7.60 (2H, m AA′BB′ overlapped), 7.71 (1H, td, J_1 = 1.4 Hz, J_2 = 7.6 Hz), 7.85 (1H, dd, J_1 = 1.1 Hz, J_2 = 7.7 Hz), 8.06 (1H, dd, J_1 = 1.1 Hz, J_2 = 8.2 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) 34.8, 110.5, 118.6, 123.9, 124.7, 124.9, 130.4, 141.3, 145.3, 147.0, 151.6, 163.6.

*6.1.1.15. 2-(3-Nitrobenzylsulfanyl)benzoxazole (**3o**).* Yield: 77%; mp: 79–81 °C; ν_{max} (cm^{-1}) 3428, 3098, 3083 (C–H)$_{Ar}$, 1638, 1617, 1505 (C=C$_v$)$_{Ar}$, 1533, 1349 (NO_2), 1471 (CH_2), 1453, 1408 (C=C$_v$)$_{Ar}$, 1239, 1133, 1098 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.74 (2H, s), 7.27–7.37 (2H, m), 7.59–7.66 (3H, m), 7.98 (1H, bd, J = 7.7 Hz), 8.12 (1H, dm, J = 8.2 Hz), 8.43 (1H, bt, J = 1.8 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) 34.6, 110.5, 118.6, 122.8, 124.0, 124.7, 124.9, 130.3, 135.9, 139.8, 141.3, 147.9, 151.5, 163.7.

*6.1.1.16. 2-(2-Nitrobenzylsulfanyl)benzoxazole (**3p**).* Yield: 81%; mp: 65–67 °C; ν_{max} (cm^{-1}) 3432, 1636, 1676 (C=C$_v$)$_{Ar}$, 1525, 1336 (NO_2), 1471 (CH_2), 1453, 1414 (C=C$_v$)$_{Ar}$, 1238, 1133, 1097 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.88 (2H, s), 7.26–7.36 (2H, m), 7.52–7.66 (3H, m), 7.71 (1H, td, J_1 = 1.4 Hz, J_2 = 7.6 Hz), 7.85 (1H, dd, J_1 = 1.1 Hz, J_2 = 7.7 Hz), 8.06 (1H, dd, J_1 = 1.1 Hz, J_2 = 8.0 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) 33.1, 110.5, 118.6, 124.6, 124.9, 125.3, 129.7, 132.4, 132.7, 134.3, 141.2, 148.4, 151.6, 163.8.

*6.1.1.17. 2-(2-Fluoro-6-chlorobenzylsulfanyl)benzoxazole (**3q**).* Yield: 61%; mp: 41–43 °C; ν_{max} (cm^{-1}) 3427, 1636, 1600, 1501, 1453, 1429 (C=C$_v$)$_{Ar}$, 1240, 1133, 1097 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.74 (2H, d, J = 1.7 Hz), 7.60–7.70 (2H, m), 7.23–7.47 (5H, m); ^{13}C NMR (75 MHz, DMSO-d_6) 27.8, 110.5, 115.0 (d, J = 22.0 Hz), 118.7, 121.9 (d, J = 18.0 Hz), 124.9 (d, J = 8.9 Hz), 126.0 (d, J = 3.4 Hz), 131.2 (d, J = 9.7 Hz), 134.9, 141.4, 151.6, 161.0 (d, J = 250.2 Hz), 162.8.

*6.1.1.18. 2-(3,4-Dichlorobenzylsulfanyl)benzoxazole (**3r**).* Yield: 57%; mp: 63.5–66 °C; ν_{max} (cm^{-1}) 3432, 3081, 3053, 3047 (C–H)$_{Ar}$, 1630, 1501, 1471 (CH_2), 1455, 1408 (C=C$_v$)$_{Ar}$, 1238, 1134, 1099 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.59 (2H, s), 7.26–7.36 (2H, m), 7.50 (1H, dd, J_1 = 1.9 Hz, J_2 = 8.4 Hz), 7.60–7.67 (2H, m), 7.58 (1H, d, J = 8.2 Hz), 7.78 (1H, d, J = 1.9 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) 34.3, 110.4, 118.6, 124.6, 124.9, 129.5, 130.5, 130.9, 131.2, 131.2, 138.5, 141.3, 151.5, 163.7.

*6.1.1.19. 2-(3,4-Difluorobenzylsulfanyl)benzoxazole (**3s**).* Yield: 71%; mp: 52–53 °C; ν_{max} (cm^{-1}) 3432, 3069 (C–H)$_{Ar}$, 1636, 1610, 1518, 1501, 1454, 1411 (C=C$_v$)$_{Ar}$, 1471 (CH_2), 1238, 1134, 1097 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.59 (2H, s), 7.27–7.44 (4H, m), 7.55–7.68 (3H, m); ^{13}C NMR (75 MHz, DMSO-d_6) 34.5 (d, J = 1.4 Hz), 110.5, 117.8 (d, J = 17.7 Hz), 118.3 (d, J = 17.1 Hz), 118.6, 124.6, 124.9, 126.2 (dd, J_1 = 6.7 Hz, J_2 = 3.6 Hz), 134.9 (dd, J_1 = 6.3 Hz, J_2 = 3.7 Hz), 141.3, 149.1 (dd, J_1 = 245.8 Hz, J_2 = 14.8 Hz), 149.3 (dd, J_1 = 245.6 Hz, J_2 = 14.6 Hz), 151.5, 163.8.

*6.1.1.20. 2-(3,5-Dinitrobenzylsulfanyl)benzoxazole (**3t**).* Yield: 62%; mp: 96–106 °C (96–106 °C [14]); ν_{max} (cm^{-1}) 3042, 3107, 3086 (C–H)$_{Ar}$, 1627, 1596, 1541, 1345 (NO_2), 1470 (CH_2), 1454 (C=C$_v$)$_{Ar}$, 1236, 1134, 1094 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.85 (2H, s), 7.26–7.37 (2H, m), 7.58–7.66 (2H, m), 8.69 (1H, t, J = 2.2 Hz), 8.87 (2H, d, J = 2.2 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) 34.0, 110.5, 118.0, 118.5, 124.7, 125.0, 129.9, 141.2, 142.2, 148.1, 151.6, 163.5.

*6.1.1.21. 2-(2,4-Dinitrobenzylsulfanyl)benzoxazole (**3u**).* Yield: 58%; mp: 97.5–102 °C; ν_{max} (cm^{-1}) 3443, 3095, 3080, 3055, 3044 (C–H)$_{Ar}$, 1763, 1609 (C=C$_v$)$_{Ar}$, 1540, 1346 (NO_2), 1471 (CH_2), 1453, 1421

($C{=}C_v)_{Ar}$, 1235, 1135, 1096 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.98 (2H, s), 7.23–7.37 (2H, m), 7.55–7.65 (2H, m), 8.15 (1H, d, $J = 8.5$ Hz), 8.52 (1H, dd, $J_1 = 2.3$ Hz, $J_2 = 8.7$ Hz), 8.73 (1H, d, $J = 2.2$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6) 32.4, 110.6, 118.6, 120.5, 124.8, 125.0, 128.1, 134.3, 139.3, 141.1, 147.1, 148.6, 151.7, 163.3.

*6.1.1.22. 2-(2-Fluoro-6-nitrobenzylsulfanyl)benzoxazole (**3v**).* Yield: 53%; mp: 89–93 °C; ν_{max} (cm^{-1}) 3447, 3100, 3052 (C–H)$_{Ar}$, 1618 ($C{=}C_v)_{Ar}$, 1533, 1365 (NO_2), 1464 (CH_2), 1453, 1411 ($C{=}C_v)_{Ar}$, 1239, 1135, 1096 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.89 (2H, d, $J = 1.4$ Hz), 7.27–7.37 (2H, m), 7.54–7.74 (4H, m), 7.90 (1H, dt, $J_1 = 1.4$ Hz, $J_2 = 8.0$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6) 25.9, 110.5, 118.6, 120.3 (d, $J = 18.3$ Hz), 121.4 (d, $J = 20.0$ Hz), 124.8, 124.9, 130.8 (d, $J = 9.7$ Hz), 141.2, 149.8, 151.6, 160.7 (d, $J = 250.5$ Hz), 163.1.

*6.1.1.23. 2-(4-Trifluoromethylbenzylsulfanyl)benzoxazole (**3w**).* Yield: 63%; mp: 48–54 °C; ν_{max} (cm^{-1}) 1617, 1499 ($C{=}C_v)_{Ar}$, 1455, 1429, 1414 ($C{=}C_v)_{Ar}$, 1332, 1172, 1133 (CF_3), 1238, 1071 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.70 (2H, s), 7.30–7.35 (2H, m), 7.62–7.67 (2H, m), 7.68–7.77 (4H, m); ^{13}C NMR (75 MHz, DMSO-d_6) 34.9, 110.5, 118.6, 124.4 (q, $J = 272.1$ Hz), 125.6, 125.7 (d, $J = 3.8$ Hz), 128.3 (q, $J = 32.2$ Hz), 130.0, 141.4, 142.1, 142.2, 151.5, 163.8.

*6.1.1.24. 2-(3-Trifluoromethylbenzylsulfanyl)benzoxazole (**3x**).* Yield: 55%; mp: 32–36 °C; ν_{max} (cm^{-1}) 3070 (C–H)$_{Ar}$, 1773, 1664, 1505, 1471 (CH_2), 1454 ($C{=}C_v)_{Ar}$, 1332, 1167, 1132 (CF_3), 1239, 1096 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.69 (2H, s), 7.01–7.14 (1H, m), 7.23–7.37 (2H, m), 7.50–7.73 (3H, m), 7.83 (1H, bd, $J = 7.4$ Hz), 7.90 (1H, bs); ^{13}C NMR (75 MHz, DMSO-d_6) 34.9, 109.9, 110.4, 118.5, 122.0, 123.9, 124.3 (q, $J = 272.4$ Hz), 124.5 (d, $J = 3.8$ Hz), 124.9, 128.7, 129.4 (q, $J = 31.5$ Hz), 133.4, 138.8, 139.1, 141.3, 143.5, 151.5, 163.8.

*6.1.1.25. 2-[3,5-Bis(trifluoromethyl)benzylsulfanyl]benzoxazole (**3y**).* Yield: 54%; mp: 47–48.5 °C; ν_{max} (cm^{-1}) 1623, 1507, 1472 (CH_2), 1467, 1454 ($C{=}C_v)_{Ar}$, 1280, 1173, 1129 (CF_3), 1239, 1097 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.76 (2H, s), 7.26–7.36 (2H, m), 7.58–7.63 (2H, m), 7.99 (1H, bs), 8.27 (2H, bs); ^{13}C NMR (75 MHz, DMSO-d_6) 34.3, 121.5, 121.6, 123.4 (q, $J = 270.0$ Hz), 130.4, 130.4 (q, $J = 32.8$ Hz), 141.1, 141.1, 141.2, 141.2, 151.5, 163.5.

*6.1.1.26. 2-(4-Cyanobenzylsulfanyl)benzoxazole (**3z**).* Yield: 80%; mp: 102–106 °C (102–106 °C [14]); ν_{max} (cm^{-1}) 3048 (C–H)$_{Ar}$, 2227 (C≡N), 1636, 1604, 1509, 1470 (CH_2), 1453, 1419 ($C{=}C_v)_{Ar}$, 1242, 1139, 1098 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.67 (2H, s), 7.26–7.36 (2H, m), 7.59–7.67 (2H, m), 7.68–7.73 (2H, m AA′BB′), 7.77–7.82 (2H, m AA′BB′); ^{13}C NMR (75 MHz, DMSO-d_6) 35.1, 110.5, 110.6, 118.6, 118.9, 124.7, 124.9, 130.2, 132.7, 141.3, 151.6, 163.7.

*6.1.1.27. 2-(3-Cyanobenzylsulfanyl)benzoxazole (**3aa**).* Yield: 72%; mp: 66–79 °C; ν_{max} (cm^{-1}) 3078, 3057 (C–H)$_{Ar}$, 2228 (C≡N), 1608, 1600, 1508, 1471 (CH_2), 1453, 1431 ($C{=}C_v)_{Ar}$, 1242, 1139, 1098 (Ar–H); ^{1}H NMR (300 MHz, DMSO-d_6) 4.65 (2H, s), 7.25–7.38 (2H, m), 7.54 (1H, t, $J = 7.8$ Hz), 7.59–7.68 (2H, m), 7.74 (1H, dt, $J_1 = 1.4$ Hz, $J_2 = 8.0$ Hz), 7.86 (1H, dt, $J_1 = 1.5$ Hz, $J_2 = 8.0$ Hz), 7.97 (1H, bt, $J = 1.5$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6) 34.7, 110.4, 111.6, 118.5, 118.7, 124.6, 124.9, 130.0, 131.6, 132.8, 134.1, 139.0, 141.3, 151.5, 163.7.

*6.1.2. General procedure for the synthesis of compounds **4***

Dry hydrogen sulfide was passed through the solution of a cyano compound (**3z**, **3aa**) (0.5 g, 2 mmol) dissolved in a mixture of dry pyridine (7 mL) and triethylamine (0.7 mL). The reaction mixture was maintained at room temperature for 3–4 h and then heated to 45 °C for an additional hour. After cooling, the mixture was poured onto crushed ice with intensive stirring, the precipitated product was filtered off, washed with cold water and air-dried. Preparative TLC chromatography in acetone–light petroleum (1:1 or 1:2) and crystallization from aqueous ethanol gave the products as yellow needles in 36–53% yields. The following compounds were prepared according this general procedure.

*6.1.2.1. 4-(2-Benzoxazolylsulfanylmethyl)benzothioamide (**4a**).* Yield: 53%; mp: 155–160 °C (143–160 °C [14]); ν_{max} (cm^{-1}) 3424, 3386, 3289 (NH), 3166 (C–H)$_{Ar}$, 2925, 2853, 1470 (CH_2), 1621, 1607, 1575, 1493, 1452, 1438 ($C{=}C_v)_{Ar}$, 1230, 1133, 1098 (Ar–H), 1190 (C=S –NH–C=S); ^{1}H NMR (300 MHz, acetone-d_6) 4.67 (2H, bs), 7.27–7.38 (2H, m), 7.50–7.66 (2H, m overlapped), 7.58–7.66 (2H, m AA′BB′ overlapped), 7.92–7.98 (2H, m AA′BB′), 8.86 (1H, bs), 9.04 (1H, bs); ^{13}C NMR (75 MHz, acetone-d_6) 36.1, 110.8, 119.2, 125.0, 125.3, 128.4, 129.5, 140.0, 141.3, 142.6, 152.7, 164.8, 202.1.

*6.1.2.2. 3-(2-Benzoxazolylsulfanylmethyl)benzothioamide (**4b**).* Yield: 36%; mp: 163–167 °C; ν_{max} (cm^{-1}) 3425, 3242 (NH), 3081 (C–H)$_{Ar}$, 2925, 1471 (CH_2), 1664, 1601, 1489, 1454, 1438, 1414 ($C{=}C_v)_{Ar}$, 1231, 1140, 1099 (Ar–H); ^{1}H NMR (300 MHz, acetone-d_6) 4.68 (2H, bs), 7.26–7.47 (2H, m overlapped), 7.39 (1H, t overlapped, $J = 7.7$ Hz), 7.50–7.67 (2H, m), 7.71 (1H, bd, $J = 7.7$ Hz), 8.19 (1H, bt, $J = 1.7$ Hz), 8.92 (1H, bs), 9.07 (1H, bs); ^{13}C NMR (75 MHz, acetone-d_6) 36.3, 110.8, 119.2, 125.0, 125.3, 127.1, 129.1, 129.2, 132.6, 137.8, 141.1, 142.7, 152.7, 161.8, 202.3.

6.2. QSAR study

Quantum-chemical calculations were run on a PC computer using software HyperChem Suite for Windows (release 5.1) The semi-empirical AM1 (Austin Model 1) method was used for all molecular modelling calculations. The most stable conformations of the molecules were found by the molecular dynamics, by simulated heating to 7000 K followed by the Monte Carlo method (293 K, 500 steps). Calculations of log*P* values were carried out on the software HyperChem Suite for Windows (release 5.1), the module ChemPlus 1.6 using atomic parameters derived by Ghose et al. [26] and Viswanadhan et al. [27]. The MR was estimated by the same method as log *P*. Atomic contributions for these calculations were published by Ghose and Crippen [28] and Viswanadhan et al. [27] Parameters σ were taken from the table given by Kuchař and Rejholec [29].

Correlation and regression analyses of the QSAR study were run on a PC computer using the Microsoft Excel program. In the equations, the figures in the parentheses are the standard errors of the regression coefficients, n is the number of compounds, r is the multiple correlation coefficient, r^2 is the determination coefficient, F is the significance test (F-test) and s is the standard error of estimate. F-test values are for all equations statistically significant at the 1% level of probability.

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