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Synthesis, characterization, and antitumor activity of some novel S-functionalized benzo[*d*]thiazole-2-thiol derivatives; regioselective coupling to the –SH group

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Abstract: Several 2-substituted sulfanyl benzo[*d*]thiazoles were regioselectively synthesized by the reaction of benzo[*d*]thiazole-2-thiol (**1a**) with a variety of reagents under different basic conditions. Some 2-(2,3-disubstituted propyl sulfanyl)benzo[*d*]thiazoles were obtained from 2-(allylthio)benzo[*d*]thiazole, which was prepared by the allylation of **1a** with allyl bromide in the presence of sodium hydride in dry *N*,*N*-dimethylformamide. Reaction of **1a** with various pyrazolyl-quinoxaline derivatives was also investigated. Better yields and shorter reaction time were achieved for the synthesis of some derivatives by using ultrasound irradiation. The structural elucidation of all compounds was based on both analytical and spectroscopic data. The newly synthesized compounds were tested *in vitro* for their antitumor activity against Ehrlich ascites carcinoma (EAC) cells grown in albino mice. Doxorubicin was used as a standard antitumor antibiotic, and some compounds showed half maximal inhibitory concentration (IC_{50}) in the range 40–68 μg mL^{-1}.

Keywords: antitumor activity; benzo[*d*]thiazole-2-thiol; pyrazolyl-quinoxaline; ultrasound irradiation.

1 Introduction

In recent years, there have been interesting developments in the chemistry of benzo[*d*]thiazole derivatives because of their variable biological activities and wide spectrum of practical qualities. Benzo[*d*]thiazole-2-thiol (2-mercaptobenzothiazole, MBT) is a privileged bicyclic heteroatomic molecule known as an important vulcanization accelerator in the rubber industry [1]. It was also used as a corrosion inhibitor for copper [2, 3], and AA 2024-T3 aluminum alloys [4]. Recently, its role in promoting the performance of electroless nickel plating baths was investigated, and it was found to improve some physical properties such as corrosion resistance and mechanical strength [5]. Some benzo[*d*]thiazole-2-thiol derivatives are being widely used in leather-processing operations for controlling the growth of fungi and sulfate-reducing bacteria [6, 7] and also as intermediates in the synthesis of cyanine dyes modified by folic acid for use as breast cancer cell labels [8]. Also benzo[*d*]thiazole-2-thiol is an important scaffold known to be associated with several biological activities [9–12]. Moreover, its 2-substituted sulfanyl derivatives have shown many pharmacological and medicinal applications such as antimicrobial, anti-inflammatory, antinociceptive, antimycobacterial, anticonvulsant, antitumor, antitubercular, and analgesic acivity and as human cyclooxygenase inhibitors [13–21].

Ultrasound irradiation has been established as an important technique in organic synthesis [22–24]. In comparison with conventional energy sources, this method is more convenient for many organic reactions to improve the reaction rates, reaction times, product yields, and selectivity. However, a limited number of synthetic procedures has been reported for the S-alkylation of benzo[*d*]thiazole-2-thiol under ultrasound irradiation [25]. In continuation of our work on the synthesis of thiazole derivatives [26–28], we report here a highly regioselective synthesis of some novel biologically active 2-substituted sulfanyl benzo[*d*]thiazole derivatives under very mild basic conditions (Fig. 1). Some reactions were carried out under both conventional and ultrasound irradiation. Also, the antitumor activity of all compounds against EAC cells was investigated.

2 Results and discussion

The readily available benzo[*d*]thiazole-2-thiol (**1a**) [29] was used as a precursor for the synthesis of our target compounds. It has been reported that MBT could exist in

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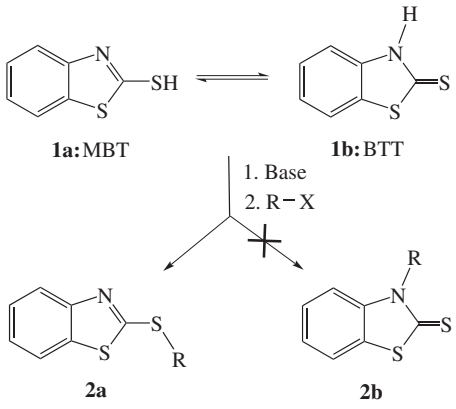
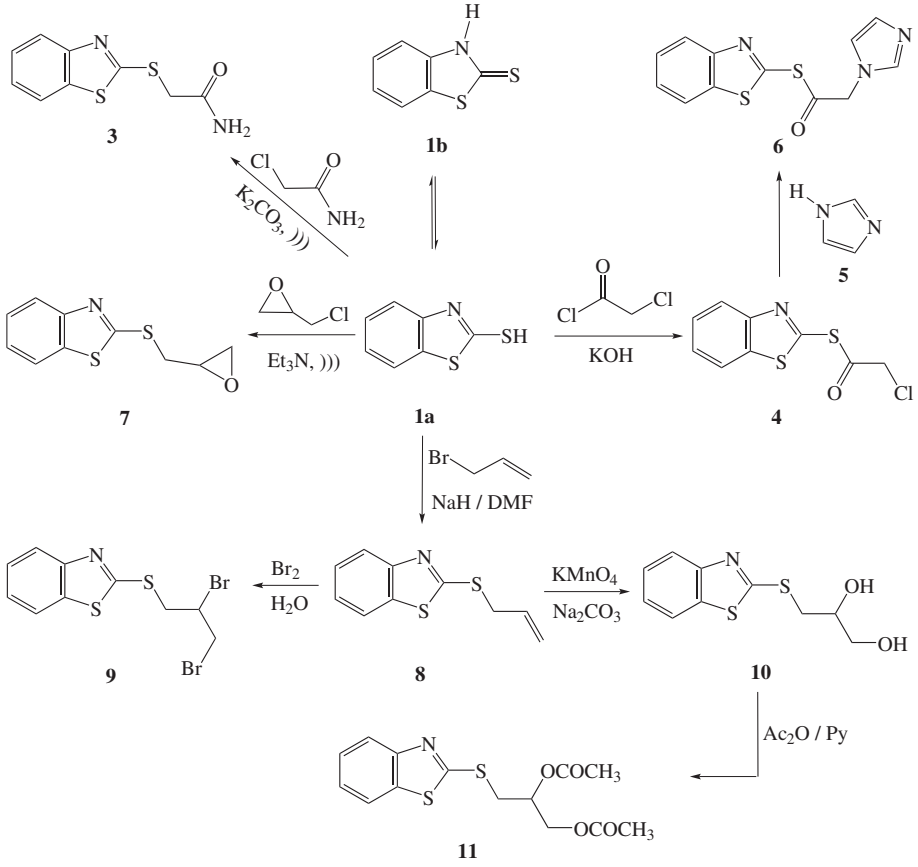


Fig. 1: Regioselective S-alkylation of 2-mercaptobenzothiazole.

different solvents as an equilibrium mixture of two tautomeric forms: 2-mercaptobenzothiazole (**1a**) (MBT, thio–enol tautomer), and benzothiazoline-2-thione (**1b**) (BTT, thione tautomer) [30]. However, the thio–enol tautomer **1a** is easily converted into its salt by means of a base, thus coupling reactions of the SH group in **1a** are favored in a fairly basic medium. In this work, we found that the reaction of MBT with various coupling reagents in basic medium afforded regioselectively the 2-substituted sulfanyl derivatives **2a** rather than the N-3 analogs **2b** (Fig. 1).

2-(Benzo[*d*]thiazol-2-ylthio)acetamide (**3**) was obtained in 78% yield by heating **1a** with 2-chloroacetamide in the presence of triethylamine as the base (Scheme 1). The reaction was completed in 8 h. When the reaction was conducted under ultrasound irradiation in the presence of a catalytic amount of anhydrous potassium carbonate, it required 25 min and the yield of **3** improved to 89%. The ^{1}H NMR spectrum of **3** showed a singlet peak and a broad singlet peak at $\delta = 4.14$ and 7.78 ppm, characteristic for the protons of S–CH_2 and NH_2 groups, respectively. The four aromatic protons appeared in two sets; the first was observed as two triplets at $\delta = 7.38$ and 7.47 ppm due to H-5 and H-6, while the second set consisted of two doublets in a lower field at $\delta = 7.83$ and 8.01 ppm, which were assigned to H-4 and H-7 as a consequence of their proximity to the nitrogen and sulfur atoms of the benzothiazole ring. Reaction of compound **1a** with 2-chloroacetyl chloride was carried out in the presence of a 5% aqueous solution of potassium hydroxide to yield *S*-benzo[*d*]thiazol-2-yl 2-chloroethane thioate (**4**) in good yield. *S*-Benzo[*d*]thiazol-2-yl 2-(1*H*-imidazol-1-yl)ethanethioate (**6**) was obtained in 83% yield by



Scheme 1: Regioselective coupling to the –SH group of benzo[*d*]thiazole-2-thiol (**1a**).

the nucleophilic substitution reaction of compound **4** with imidazole (**5**) in dry *N*,*N*-dimethylformamide. The signal due to the N–CH_2 protons in its ^{1}H NMR spectrum appeared as a singlet at $\delta = 3.89$ ppm, while one singlet and two doublet peaks at $\delta = 7.01$, 7.39, and 7.42 ppm were attributed to the imidazolyl protons. When compound **1a** was reacted with 2-(chloromethyl)oxirane in dry acetone and a catalytic amount of triethylamine under ultrasound irradiation for 5 min, it gave 2-((oxiran-2-ylmethyl)thio)benzo[*d*]thiazole (**7**) in 71% yield. Conventional synthesis of **7** required longer reaction time and gave only 49% yield. The ^{1}H NMR spectrum of compound **7** confirmed its structure, where the H-3′ and H-3″ of the oxirane ring appeared as a doublet of doublet at $\delta = 3.68$ and 3.71 ppm, while H-2′ was observed as a multiplet at $\delta = 4.71$–4.74 ppm, in addition to the S–CH_2 protons which resonated as a doublet at $\delta = 4.43$ ppm.

The allyl group is considered an excellent precursor for various chemical modifications, and thus 2-(allylthio)benzo[*d*]thiazole (**8**) was prepared by the reaction of **1a** with allyl bromide in the presence of sodium hydride in dry *N*,*N*-dimethylformamide. Reaction of **8** with bromine in water gave 2-((2,3-dibromopropyl)thio) benzo[*d*]thiazole (**9**). Its ^{1}H NMR spectrum showed two doublet of doublets at $\delta = 3.88$ and 4.11 ppm, another two doublet of doublets at $\delta = 4.99$, 5.06 ppm, and a multiplet at $\delta = 5.35$–5.38 ppm due to CH_2-S, CH_2-Br, and CH-Br groups, respectively. This indicated that the addition of bromine to the double bond had taken place. Hydroxylation of compound **8** with potassium permanganate in the presence of sodium carbonate gave the corresponding diol **10**. Subsequent acetylation of **10** with acetic anhydride in dry pyridine afforded 3-(benzo[*d*]thiazol-2-ylthio)propane-1,2-diyl diacetate (**11**) as a syrupy product. Its ^{1}H NMR spectrum showed two singlet peaks at $\delta = 1.95$ and 2.10 ppm for the two acetyl groups in addition to other signals, which confirmed its structure (Section 4).

The introduction of benzo[*d*]thiazole-2-thiol nucleus into pyrazolyl-quinoxalines **12** and **16** [31] is of interest from a biological point of view. Thus, a facile route to the conjugated pyrazolyl-quinoxaline linked to the benzo[*d*]thiazole ring was developed by a nucleophilic displacement of the chlorine atom in compound **12** by the sodium salt of **1a** under both conventional and ultrasound irradiation to give (3-(3-(benzo[*d*]thiazol-2-ylthio)quinoxalin-2-yl)-1-phenyl-1*H*-pyrazol-5-yl)methyl acetate (**13**). In comparison with the conventional heating method, the reaction time was reduced from 11 h to 30 min and the yield of the product increased from 49% to 63% when the reaction was achieved under ultrasound irradiation. Deacetylation of compound **13** with aqueous sodium hydroxide produced (3-(3-(benzo[*d*] thiazol-2-ylthio)quinoxalin-2-yl)-1-phenyl-1*H*-pyrazol-5-yl) methanol (**14**). Its IR spectrum indicated the absence of the OAc group found in its precursor, and instead showed a band at 3420.42 cm^{-1} for the O–H stretching vibration. When compound **14** was subjected to the reaction with phosphoryl chloride, the chloro derivative **15** was obtained in 80% yield. Unequivocal synthesis of **15** was achieved by treating the dichloro pyrazolyl-quinoxaline derivative **16** with **1a** in the presence of NaH in DMF. The reaction is highly regioselective, as the coupling of compound **16** with two equivalents of **1a** under a similar reaction conditions afforded exclusively the monosulfanyl derivative **15** (Scheme 2).

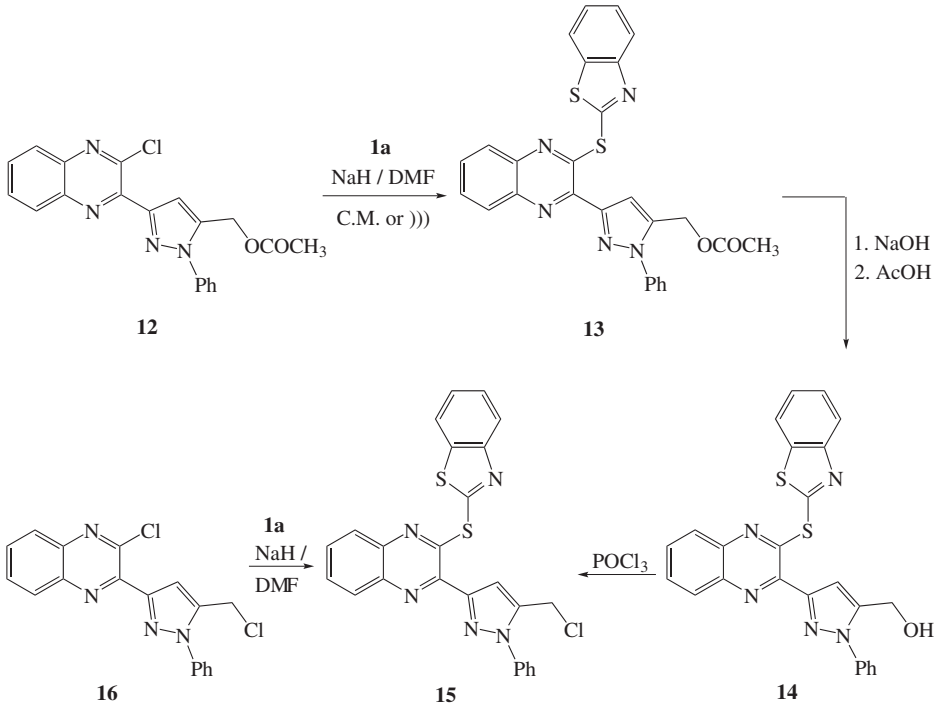
3 Antitumor activity

The antitumor efficacy of the synthesized compounds against Ehrlich ascites carcinoma (EAC) cell line was demonstrated and compared with doxorubicin as control. The effective dose was calculated as the half maximal inhibitory concentration (IC_{50} in μg mL^{-1}), which corresponds to the concentration of the compound resulting in 50% mortality in the total cell count (Table 1). The obtained results revealed that compounds **8**, **9**, and **15** displayed the highest activity against EAC cells with IC_{50} values 42, 41, and 40 μg mL^{-1}, respectively. On the other hand, compounds **3**, **6**, and **7** displayed moderate activity with IC_{50} values 68, 70, and 51 μg mL^{-1}, while **4**, **11**, **13**, and **14** showed no activity at all. It is clear that pyrazolyl-quinoxaline derivative **15** showed the lowest IC_{50} value, indicating the highest potency against the EAC cell line.

4 Experimental section

4.1 Materials and measurements

Commercially available solvents and reagents were purified according to standard procedures. Melting points were measured on a Mel-Temp apparatus (Dubuque, IA, USA) and are uncorrected. Thin-layer chromatography (TLC) was performed on aluminum silica gel 60 F_{254} (E-Merk). The spots were detected by iodine and UV light absorption. Fourier transform infrared (FT-IR) spectra were recorded on a Perkin Elmer SP 100 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker WM 400 and 600 MHz spectrometers. Chemical shifts (δ) are given in ppm relative to the signal for TMS as an internal standard, and coupling constants (J) are reported in hertz (Hz). The reactions that were carried out by ultrasound



Scheme 2: Synthesis of pyrazolyl-quinoxaline derivatives containing compound **1a**.

Table 1: *In vitro* antitumor activity of the synthesized compounds against Ehrlich ascites carcinoma (EAC) cells.

Compound	IC_{50} (μg mL^{-1})	Compound	IC_{50} (μg mL^{-1})
3	68	**11**	–
4	–	**13**	–
6	70	**14**	–
7	51	**15**	40
8	42	Doxorubicin	38
9	41		

irradiation was done using a Daihan (WiseClean, D-400H) ultrasonic bath. Microanalyses were performed on a Perkin Elmer elemental analyzer at the Faculty of Science, King Abdul Aziz University. Antitumor activity was performed in the Operations Development Section, Egyptian Petroleum Research Institute (EPRI), Cairo, Egypt.

4.2 2-(Benzo[*d*]thiazol-2-ylthio)acetamide (3)

4.2.1 Method A

A solution of compound **1a** (2.0 g, 11.96 mmol) in dry acetone (50 mL) was treated with a solution of 2-chloroacetamide (1.12 g, 11.97 mmol) in a mixture of ethanol/acetone (1:10, 100 mL) and triethylamine (2.0 mL). The reaction mixture was heated under reflux for 8 h. The separated product was filtered off, dried, and recrystallized from ethanol to give compound **3** as colorless crystals. Yield: 78% (2.08 g), $R_f = 0.88$ (EtAc/MeOH, 1:1), m.p. 139–140°C. IR (KBr): $\nu = 3353.29$, 3211.44 (NH_2), 1677.87 (C=O), 1615.75 (C=C, C=N) cm^{-1}. ^{1}H NMR (600 MHz, [D_6]DMSO): $\delta = 4.14$ (s, 2 H, S–CH_2), 7.38 (t, $J = 7.2$ Hz, 1 H, Ar-H), 7.47 (t, $J = 7.2$ Hz, 1 H, Ar-H), 7.78 (bs, 2 H, D_2O-exchangeable, NH_2), 7.83 (d, $J = 7.8$ Hz, 1 H, Ar-H), 8.01 (d, $J = 7.8$ Hz, 1 H, Ar-H). ^{13}C NMR (150 MHz, [D_6]DMSO): $\delta = 36.75$ (S–CH_2), 120.92, 121.73, 124.34, 126.27, 134.57, 152.47 (Ar-C), 166.31 (C-2 of benzo[*d*]thiazole ring), 168.05 (C=O). $C_9H_8N_2OS_2$ (224.30): calcd. C 48.19, H 3.59, N 12.49; found C 48.27, H 3.88, N 12.32.

4.2.2 Method B

A solution of compound **1a** (2.0 g, 11.96 mmol) in dry acetone (50 mL) was treated with a solution of 2-chloroacetamide (1.12 g, 11.97 mmol) in a mixture of ethanol/acetone (1:10, 100 mL) and anhydrous potassium carbonate (1.98 g, 14.32 mmol). The reaction mixture was sonicated at a frequency of 40 kHz for 25 min at 40°C, cooled to room temperature, and poured into crushed ice (20 g). The separated solid was filtered, dried, and recrystallized from

ethanol to give a product (89% yield, 2.40 g) identical to that obtained from method A.

4.3 *S*-Benzo[*d*]thiazol-2-yl 2-chloroethanethioate (4)

A solution of compound **1a** (2.0 g, 11.96 mmol) in 5% aqueous solution of potassium hydroxide (45 mL) was treated with 2-chloroacetyl chloride (2.0 mL, 25.09 mmol). The reaction mixture was heated under reflux for 3 h. The product that separated out on cooling was filtered, dried, and recrystallized from ethanol to give colorless crystals. Yield: 69% (1.99 g), R_f = 0.74 (EtAc/MeOH, 1:1), m.p. 101–103°C (lit. [32] m.p. 97–98°C).

4.4 *S*-Benzo[*d*]thiazol-2-yl 2-(1*H*-imidazol-1-yl)ethanethioate (6)

A solution of compound **4** (0.53 g, 2.17 mmol) and imidazole (**5**) (0.15 g, 2.20 mmol) in dry *N*,*N*-dimethylformamide (10 mL) was stirred at room temperature for 10 h. Then, it was poured into cold water (10 mL) and the product was extracted with ethyl acetate (3×10 mL). The extract was dried over anhydrous sodium sulfate and then evaporated *in vacuo*. The isolated product was recrystallized from ethanol to give colorless crystals. Yield: 83% (0.50 g), R_f = 0.66 (EtAc/MeOH, 1:1), m.p. 290–292°C. IR (KBr): ν = 1652.0 (C=O), 1580.0 (C=C, C=N) cm^{-1}. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 3.89 (s, 2 H, N–CH$_2$), 7.01 (s, 1 H, CH of imidazole ring), 7.30 (t, J = 7.2 Hz, 1 H, Ar-H), 7.39 (d, J = 7.8 Hz, 1 H, CH of imidazole ring), 7.41 (t, J = 7.2 Hz, 1 H, Ar-H), 7.42 (d, J = 7.8 Hz, 1 H, CH of imidazole ring), 7.77 (d, J = 7.2 Hz, 1 H, Ar-H), 7.94 (d, J = 7.2 Hz, 1 H, Ar-H). $C_{12}H_9N_3OS_2$ (275.35): calcd. C 52.34, H 3.29, N 15.26; found C 52.16, H 3.59, N 15.72.

4.5 2-((Oxiran-2-ylmethyl)thio)benzo[*d*]thiazole (7)

4.5.1 Method A

A solution of compound **1a** (2.0 g, 11.96 mmol) in dry acetone (60 mL) was treated with anhydrous potassium carbonate (1.98 g, 14.3 mmol). The mixture was stirred at room temperature for 3 h, then 2-(chloromethyl)oxirane (1.0 mL, 12.75 mmol) was added, and the mixture was kept overnight with vigorous stirring. The solvent was evaporated *in vacuo* and the product was separated out as colorless syrup. Yield: 49% (1.30 g), R_f = 0.83 (*n*-hexane/EtAc, 3:1). IR (KBr): ν = 1625.0, 1580.0 (C=C, C=N), 1245.0 (C–O) cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 3.68, 3.71 (dd, J = 7.8, 7.8 Hz, 2 H, H-3′ and H-3″ of oxirane ring), 4.43 (d, J = 14.0 Hz, 2 H, S–CH$_2$), 4.71–4.74 (m, 1 H, H-2′ of oxirane ring), 7.73 (t, J = 6.9 Hz, 1 H, Ar-H), 7.80 (t, J = 6.9 Hz, 1 H, Ar-H), 8.13 (d, J = 8.3 Hz, 1 H, Ar-H), 8.32 (d, J = 8.1 Hz, 1 H, Ar-H). $C_{10}H_9NOS_2$ (223.31): calcd. C 53.78, H 4.06, N 6.27; found C 53.30, H 3.59, N 6.27.

4.5.2 Method B

A solution of compound **1a** (2.0 g, 11.96 mmol) in dry acetone (60 mL) was treated with 2-(chloromethyl)oxirane (1.0 mL, 12.75 mmol) and triethylamine (2.0 mL). The reaction mixture was subjected to ultrasound irradiation at 40 kHz for 5 min, and the reaction mixture was processed as before to give a colorless syrup of compound **7** (71% yield, 1.89 g).

4.6 2-(Allylthio)benzo[*d*]thiazole (8)

A solution of compound **1a** (1.0 g, 5.98 mmol) in dry *N*,*N*-dimethylformamide (5.0 mL) was treated with sodium hydride (0.20 g, 8.33 mmol), and then it was heated under reflux for 1 h. The resulting mixture was cooled, allyl bromide (0.98 mL, 11.32 mmol) was added, and the mixture was stirred overnight at room temperature. It was poured into 20 mL of cold water, and the product was extracted with methylene chloride (3×15 mL). The methylene chloride extract was dried with anhydrous magnesium sulfate and concentrated under reduced pressure. The crude material obtained was distilled to give a colorless oil (0.73 g, 59% yield) [33].

4.7 2-((2,3-Dibromopropyl)thio)benzo[*d*]thiazole (9)

To a suspension of compound **8** (0.70 g, 3.38 mmol) in water (6.0 mL), bromine (1.0 mL) was added dropwise during 1 h with gentle stirring. The mixture was stirred for a further 3 h, and kept overnight at room temperature. The mixture was then diluted with 50 mL of boiling ethanol, and the separated product was filtered off, washed with water, dried, and recrystallized from ethanol to give pale yellow crystals. Yield: 76% (0.94 g), R_f = 0.56 (EtAc/MeOH, 1:1), m.p. 225–227°C. IR (KBr): ν = 1645.08 (C=C, C=N) cm^{-1}. ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 3.88 (dd, J = 7.2, 7.8 Hz, 1 H, S–CH$_2$), 4.11 (dd, J = 2.0, 2.4 Hz, 1 H, S–CH$_2$), 4.99 (dd, J = 6.0, 6.6 Hz, 1 H, CH$_2$–Br), 5.06 (dd, J = 3.6, 3.6 Hz, 1 H,

CH_2–Br), 5.35–5.38 (m, 1 H, CH–Br), 7.72 (t, J = 7.8 Hz, 1 H, Ar-H), 7.79 (t, J = 7.8 Hz, 1 H, Ar-H), 8.16 (d, J = 8.4 Hz, 1 H, Ar-H), 8.38 (d, J = 7.8 Hz, 1 H, Ar-H). ^{13}C NMR (150 MHz, [D_6]DMSO): δ = 34.70 (CH_2–Br), 36.98 (CH_2–S), 52.77 (CH–Br), 114.95, 124.11, 127.39, 127.94, 128.87, 141.24 (Ar-C), 174.36 (C-2 of benzo[*d*]thiazole ring). $C_{10}H_9Br_2NS_2$ (367.12): calcd. C 32.72, H 2.47, N 3.82; found C 32.30, H 2.80, N 3.73.

4.8 3-(Benzo[*d*]thiazol-2-ylthio)propane-1,2-diyl diacetate (11)

A suspension of compound **8** (1.10 g, 5.31 mmol) in a solution of sodium carbonate (2.0 g, 18.86 mmol) in water (10 mL) was stirred at room temperature for 10 min. An alkaline solution of potassium permanganate was then added dropwise until the color of $KMnO_4$ persisted. The mixture was stirred for a further 3 h, then kept overnight at room temperature, and the product was extracted with CH_2Cl_2 (4 × 10 mL). The extract was evaporated *in vacuo* till dryness, and the separated residue of diol **10** (0.55 g, 2.28 mmol) was dissolved in dry pyridine (10 mL), followed by adding 10 mL of acetic anhydride with occasional stirring. The mixture was kept at 18–20°C overnight and then poured into crushed ice (20 g). The product was extracted with CH_2Cl_2 (4 × 10 mL) and the solvent was evaporated under reduced pressure to give a colorless oil. Yield: 69% (0.51 g), R_f = 0.75 (EtAc/MeOH, 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.94, 2.10 (2s, 6 H, 2 COCH_3), 3.97 (d, J = 6.76 Hz, 2 H, S–CH_2), 5.17–5.39 (m, 2 H, CH_2OCOCH_3), 5.96–6.06 (m, 1 H, CHOCOCH_3), 7.27 (t, J = 7.2 Hz, 1 H, Ar-H), 7.38 (t, J = 7.2 Hz, 1 H, Ar-H), 7.74 (d, J = 7.5 Hz, 1 H, Ar-H), 7.85 (d, J = 8.1 Hz, 1 H, Ar-H). $C_{14}H_{15}NO_4S_2$ (325.40): calcd. C 51.67, H 4.65, N 4.30; found C 51.50, H 4.92, N 3.88.

4.9 (3-(3-(Benzo[*d*]thiazol-2-ylthio)quinoxalin-2-yl)-1-phenyl-1*H*-pyrazol-5-yl)methyl acetate (13)

4.9.1 Method A

To a stirred solution of compound **1a** (1.0 g, 5.98 mmol) in dry DMF (10 mL), NaH (0.30 g, 12.5 mmol) was added. After the evolution of H_2 gas was almost complete, the mixture was heated under reflux for 1 h. A solution of compound **12** (2.27 g, 5.99 mmol) in a mixture of EtOH and DMF (2:1, 90 mL) was added, and the reaction mixture was heated under reflux for an additional 10 h, until the reaction was complete. The reaction progress was monitored by TLC using EtAc/MeOH (1:1). Upon cooling the reaction mixture, a white solid separated out. It was filtered off and recrystallized from EtOH to give colorless crystals. Yield: 49% (1.47 g), R_f = 0.89 (EtAc/MeOH, 1:1), m.p. 208–211°C. IR (KBr): ν = 1730.63 (C=O) cm^{-1}. ^{1}H NMR (600 MHz, [D_6]DMSO): δ = 2.06 (s, 3 H, CH_3CO), 5.24 (s, 2 H, CH_2OCOCH$_3$), 7.31–8.19 (m, 14 H, Ar-H + pyrazole-H). ^{13}C NMR (150 MHz, [D_6]DMSO): δ = 20.38 (OCOCH_3), 56.10 (CH_2OCOCH$_3$), 109.63, 110.95, 112.34, 115.11, 123.42–147.76, 154.0 (Ar-C), 169.80 (OCOCH$_3$). $C_{27}H_{19}N_5O_2S_2$ (509.60): calcd. C 63.64, H 3.76, N 13.74; found C 64.06, H 3.68, N 14.24.

4.9.2 Method B

To a solution of compound **1a** (1.0 g, 5.98 mmol) in dry DMF (10 mL), NaH (0.30 g, 12.5 mmol) was added. Then, a solution of **12** (2.27 g, 5.99 mmol) in a mixture of EtOH and DMF (2:1, 90 mL) was added. The reaction mixture was sonicated at 40 kHz for 30 min at 45°C. The reaction mixture was processed as before to give a product identical to that obtained from method A (63% yield, 1.9 g).

4.10 (3-(3-(Benzo[*d*]thiazol-2-ylthio)quinoxalin-2-yl)-1-phenyl-1*H*-pyrazol-5-yl) methanol (14)

A solution of compound **13** (0.60 g, 1.18 mmol) in absolute EtOH (25 mL) was treated with an aqueous solution of NaOH (0.50 M, 30 mL) and the mixture was heated under reflux for 9 h. The resulting solution was cooled and neutralized with glacial acetic acid. The product was filtered off, washed with water, and recrystallized from EtOH to give colorless crystals. Yield: 58% (0.32 g), R_f = 0.35 (*n*-hexane/EtAc, 3:1), m.p. 244–246°C. IR (KBr): ν = 3420.42 (O–H) cm^{-1}. ^{1}H NMR (600 MHz, [D_6]DMSO): δ = 4.55 (s, 1 H, D_2O-exchangeable, O–H), 5.60 (s, 2 H, CH_2OH), 7.31–7.84 (m, 14 H, Ar-H + pyrazole-H). ^{13}C NMR (150 MHz, [D_6]DMSO): δ = 56.08 (CH_2OH), 111.31, 115.24–131.96, 132.02, 139.39, 143.88, 146.69, 149.01, 154.13 (Ar-C). $C_{25}H_{17}N_5OS_2$ (467.57): calcd. C 64.22, H 3.66, N 14.98; found C 64.32, H 3.95, N 15.09.

4.11 2-((3-(5-(Chloromethyl)-1-phenyl-1*H*-pyrazol-3-yl)quinoxalin-2-yl)thio) benzo[*d*]thiazole (15)

4.11.1 Method A

A suspension of compound **14** (0.20 g, 0.43 mmol) in phosphoryl chloride (7.0 mL) was heated on a boiling water

bath for 7 h, then cooled to 20°C, treated with ice-cold water (50 mL), and basified with aqueous K_2CO_3 to pH 9. The product was collected by filtration and recrystallized from EtOH as colorless crystals. Yield: 80% (0.16 g), $R_f = 0.76$ (EtAc/MeOH, 1:1), m.p. 136–138°C. IR (KBr): $\nu = 1635.03$ (C=C, C=N) cm^{-1}. ^1H NMR (600 MHz, [D_6]DMSO): $\delta = 4.90$ (s, 2 H, CH_2–Cl), 7.29–8.31 (m, 14 H, Ar-H + pyrazole-H). ^{13}C NMR (150 MHz, [D_6]DMSO): $\delta = 27.48$ (CH_2–Cl), 110.27, 134.85, 121.30–131.60, 138.78–140.25, 144.67, 147.76, 148.90, 152.37, 164.85 (Ar-C). $C_{25}H_{16}ClN_5S_2$ (486.01): calcd. C 61.78, H 3.32, N 14.41; found C 61.95, H 3.64, N 14.52.

4.11.2 Method B

A solution of compound **1a** (0.17 g, 1.02 mmol) in dry DMF (3.0 mL) was treated with NaH (0.05 g, 2.1 mmol) and heated under reflux for 1 h. Then, a solution of compound **16** (0.18 g, 0.51 mmol) in absolute EtOH (25 mL) was added. The reaction mixture was heated under reflux for an additional 7 h. The product that separated out on cooling was filtered and recrystallized from EtOH to give colorless crystals. Yield: 84% (0.21 g), m.p. 134–136°C. IR, ^1H NMR and ^{13}C NMR spectra were found to be superimposable with those of the product from method A.

4.12 Antitumor testing

Ehrlich cells were derived from the ascetic fluid from diseased mouse purchased from the National Cancer Institute (NCI), Cairo, Egypt. These cells were maintained by weekly intraperitoneal transplantation of 2.5×10^6 cells in female Swiss albino mice. The tumor is characterized by a moderately rapid growth, which leads to the death of the mice in about 20 days due to distal metastasis. EAC is of mammary origin, as spontaneous breast cancer served as the original tumor from which the ascites variant was obtained [34]. Ascetic fluid was withdrawn under aseptic conditions (ultraviolet laminar flow system) from the peritoneal cavity of tumor-bearing mice by needle aspiration after 7 days of EAC cells inoculation. To adjust the number of EAC cells, tumor cells obtained were diluted several times with normal saline. EAC viable cells were counted by the trypan blue exclusion method [35], where 10 µL trypan blue (0.05%) was mixed with 10 µL of the cell suspension. Within 5 min, the mixture was spread onto hemocytometer, covered with a cover slip, and then the cells were examined under a microscope. Dead cells were blue-stained but viable cells were not. The cell suspension was adjusted to contain 2.5×10^6 viable cells per mL. EAC cells, RPMI 1640 medium (Sigma Chemical Co.), and the tested compounds in DMSO were added in sterile test tubes. The cells were incubated for 1 and 24 h at 37°C under a constant overlay of 5% CO_2. EAC viable cells were counted by trypan blue exclusion using a hemocytometer as mentioned above. The fraction of surviving cells was calculated as T/C, where T and C represent the number of viable cells in unit volume and the number of total (viable dead) cells in the same unit volume, respectively.

5 Supporting information

Copies of ^1H NMR and ^{13}C NMR spectra of the synthesized compounds are given as Supporting Information available online (https://doi.org/10.1515/znb-2018-0078).

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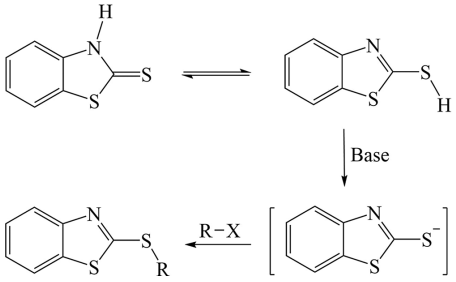
Graphical synopsis

El Sayed Ramadan, Hamida M. Abdel Hamid, Sawsan A. Noureddin and Khadija O. Badahdah

Synthesis, characterization, and antitumor activity of some novel S-functionalized benzo[*d*]thiazole-2-thiol derivatives; regioselective coupling to the –SH group



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