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Synthesis and antimicrobial activities of novel 6-(1,3-thiazol-4-yl)-1,3-benzoxazol-2(3*H*)-one derivatives

Abstract: Synthesis, characterization and investigation of antimicrobial activities of seven new 6-(1,3-thiazol-4-yl)-1,3-benzoxazol-2(3*H*)-ones are presented. Their structures were determined using ^{1}H NMR, ^{13}C NMR and elemental analyses. The compounds possess some biological activity against Gram-positive bacteria, especially against *Micrococcus luteus* belonging to opportunistic pathogens, with an MIC of 31.25 μg/mL.

Keywords: antimicrobial drugs; benzoxazolones; DFT calculation; Gram-positive bacteria; thiazoles; thiosemicarbazones.

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Introduction

The observed increasing number of multidrug-resistant pathogens leads to the unprecedented interest in synthesis of new antimicrobial drugs [1]. Benzoxazolone derivatives are an important class of heterocyclic compounds widely studied in medicinal chemistry because they show a broad spectrum of activity against Gram-positive and Gram-negative bacteria and fungi [2–8], human immunodeficiency virus (HIV)-1 reverse transcriptase [9] and multidrug-resistant cancer cells [10, 11]. Various derivatives of benzoxazolone are commercially available, for example, benzolon **1** (myorelaxant), paraflex **2** (sedative anelgesic) and vinizene **3** (topical antiseptic) [12]. The benzoyl-benzoxazolone **4** (10194 CERM) and its sulfur bioisoster **5** (S-14080) have been studied in Phase II clinical trials as analgesic drugs (Figure 1) [12].

Another group of very important pharmacophores are thiazoles and their derivatives, which have been widely studied in medicinal chemistry because of their varied biological activities such as antibacterial [13–15], antifungal [16–18], anticancer [19] and antiproliferative activities [20]. This class of drugs prevents conversion of lanosterol into ergosterol, and accumulation of 14α-methyl sterols through inhibition of the fungal cytochrome P450 enzyme 14α-demethylase [21, 22].

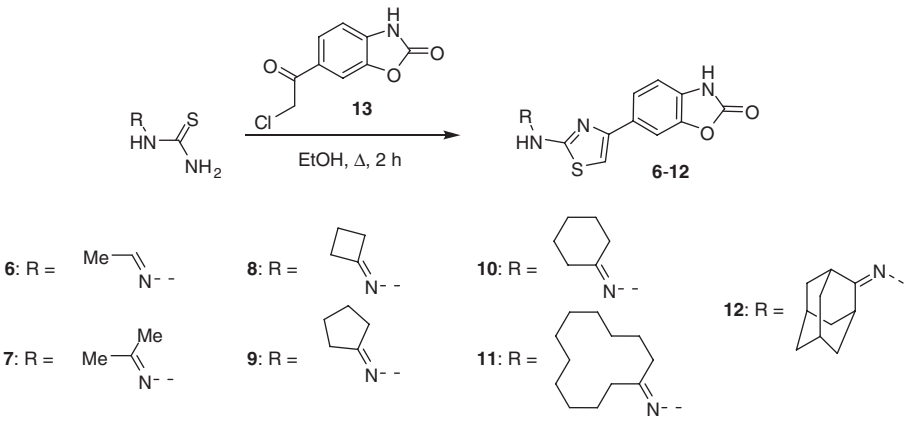
Considering the above suggestions and continuing our previous investigation on the synthesis and properties of biologically active heterocycles [23–26], it was decided to prepare a new series of biologically active agents containing both benzoxazolone and thiazole pharmacophores. These compounds were evaluated for their *in vitro* antibacterial and antifungal activities against a panel of reference strains of 25 microorganisms.

Results and discussion

Chemistry

Initially, thiosemicarbazones were obtained by the reaction of appropriate ketones with thiosemicarbazide in absolute ethyl alcohol in the presence of catalytic amounts of acetic acid and under reflux. The desired benzoxazolone-thiazole conjugates **6–12** were synthesized via the Hantzsch condensation reaction of appropriate thiosemicarbazones with 6-(2-chloroacetyl)-benzo[*d*]oxazol-2(3*H*)-one (**13**) in absolute ethyl alcohol and under reflux with good yields (48–84%) and high chemical purity. The reaction pathway is summarized in Scheme 1.

The product **6** was obtained as a mixture of isomers *E*/*Z* in a 68:32 ratio; however, a careful separation using column chromatography gave pure, more stable (*E*)-isomer of compound **6**. Additionally, geometry optimization of **6** was carried out at the DFT B3LYP/6-311++G** level of theory using the Gaussian 09 code [27]. The resulting



Scheme 1 Synthesis of benzoxazolone-thiazole conjugates **6–12**.

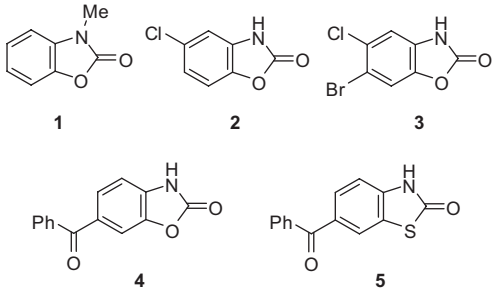
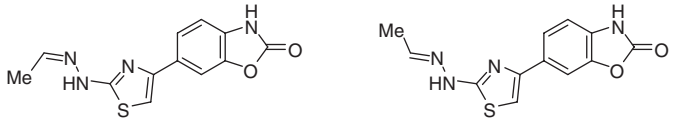


Figure 1 Commercial benzoxazolone derivatives **1–5**.

electronic energy of the (*Z*)-isomer was 0.87 kcal/mol higher than that of the (*E*)-isomer. The fractional population X of isomers *j* (*E*, *Z*) (Figure 2) was calculated using the Boltzmann's distribution [28] as:

$$X_j = \frac{\exp(-\Delta_j E/kT)}{\exp(-\Delta_E E/kT) + \exp(-\Delta_Z E/kT)}$$



Relative Δ*E* [kcal/mol]	0.87	0
Theoretical *E*/Z ratio	81	19
Experimental *E*/Z ratio	68	32

Figure 2 The fractional population *X* of isomers *j* (*E*, *Z*).

with $\Delta_j E$ denoting the relative electronic energy of isomer *j* (calculated with regard to the energy of the lower-energy isomer *E*, i.e., the relative energy is 0 and 0.87 kcal/mol for isomers *E* and *Z*, respectively), *k* being the Boltzmann constant, and *T* being the temperature (*T*=298.15 K) [29]. The *E*- and the *Z*-isomers were shown to constitute 81% and 19% of the population, respectively. This result is in a good qualitative agreement with the experimental one (68% and 32%, respectively). The structures and purity of all compounds (**6–12**) were confirmed by ^{1}H NMR, ^{13}C NMR, elemental analyses and by TLC on silica gel. The NMR spectral data were fully consistent with the assigned structures. ^{1}H NMR spectra of compounds show a singlet at δ 7.17–7.22 due to the thiazole-5H proton and a singlet at δ 10.80–11.95 indicating the presence of hydrazide NH proton, which confirms the conversion of substrates to the expected products. All compounds gave satisfactory elemental analysis. All reactions were repeated at least two times and are fully reproducible.

Microbiology

Potency was defined as follows: no bioactivity with MIC >1000 μg/mL, mild bioactivity with MIC in the

Table 1 Antibacterial activity data in MIC (MBC) (μg/mL) for benzoxazolone-thiazoles **6–12.**[a]

Species	Benzoxazolone-thiazoles							
	6	**7**	**8**	**9**	**10**	**11**	**12**	**CIP**
M. luteus ATCC 10240	250 (>1000)	1000 (>1000)	500 (>1000)	–	1000 (>1000)	31.25 (250)	31.25 (250)	0.976

[a]The standard antibiotic, ciprofloxacin (CIP) was used as a positive control.

range of 501–1000 μg/mL, moderate bioactivity with MIC in the range of 126–500 μg/mL, good bioactivity with MIC in the range of 26–125 μg/mL, strong bioactivity with MIC in the range of 10–25 μg/mL and very strong bioactivity with MIC <10 μg/mL. The antibacterial studies of compounds **6–8**, **10**–**12** against Gram-positive bacteria (staphylococci, streptococci, micrococci and *Bacillus* spp.) revealed some bioactivity against *Staphylococcus aureus* strains (i.e., *S. aureus* ATCC 25923, *S. aureus* ATCC 43300, *S. aureus* ATCC 6538, *S. aureus* NIL 1, *S. aureus* NIL 2) and *Staphylococcus epidermidis* ATCC 12228 with MIC values ranging from 250 to 1000 μg/mL. The MBC for the tested staphylococci was ≥1000 μg/mL. The compounds **6–8**, **10–12** show similar activity against *Bacillus subtilis* ATCC 6633 and *Bacillus cereus* ATCC 10876 with MIC between 500 and 1000 μg/mL and MBC ≥1000 μg/mL. Among obtained compounds, **6–8** and **10** are also active against *Micrococcus luteus* ATCC 10240 with MIC=250–1000 μg/mL and MBC ≥1000 μg/mL. In turn, **11** and **12** show good bioactivity with bacteriostatic effect against *M. luteus* ATCC 10240 with MIC=31.25 μg/mL, MBC=250 μg/mL and MBC/MIC=8 (Table 1).

Moreover, minimum concentrations of **7**, **8**, **11**, **12**, which inhibit the growth of streptococci range from 500 to 1000 μg/mL and MBC ≥1000 μg/mL.

Our results indicate that the tested compounds possess some activity against Gram-positive bacteria, especially against *M. luteus* belonging to the opportunistic pathogens. The tested compounds **6**–**12** have no influence on the growth of the reference strains of Gram-negative bacteria and of fungi belonging to *Candida* spp.

Conclusions

An efficient and economic method for the synthesis of benzoxazolone-thiazol conjugates was developed. The *in vitro* antimicrobial effects of the synthesized compounds on various pathogenic bacteria and fungi were evaluated. The tested compounds possess some biological activity against Gram-positive bacteria, especially against *M. luteus*, MIC 31.25 μg/mL belonging to opportunistic pathogens. Interestingly, compound **9** containing an odd number of carbon atoms in the ring is completely inactive. The bioactivity results provide good starting templates for further structural optimization of this type of derivative.

Experimental

Materials and methods

All experiments were carried out under air atmosphere. Reagents were generally the best quality commercial grade products and were used without further purification. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in DMSO-d_6 on a Bruker Ascend 400 multinuclear instrument. Melting points were determined in open glass capillaries and are uncorrected. Silica gel 60, E. Merck 230–400 mesh, was used for preparative column chromatography. Analytical TLC was performed using Macherey-Nagel Polygram Sil G/UV$_{254}$ 0.2 mm plates eluting with dichloromethane/methanol (9:1). 6-(2-Chloroacetyl)benzo[*d*]oxazol-2(*3H*)-one, thiosemicarbazide and appropriate ketones were commercial materials.

General procedure for the synthesis of compounds 6–12

A mixture of the appropriate carbonyl compound (20 mmol), thiosemicarbazide (20 mL) and a catalytic amount of acetic acid (1 mL) in absolute ethyl alcohol (20 mL) was magnetically stirred for 24 h and heated under reflux. A precipitate of the desired thiosemicarbazone was filtered, crystallized from a suitable solvent and dried. This thiosemicarbazone (1.0 mmol) was added to a stirred solution of 6-(2-chloroacetyl)benzo[*d*]oxazol-2(3*H*)-one (**13**, 0.212 g, 1.0 mmol) in absolute ethyl alcohol (15 mL), and the mixture was stirred under reflux for 2 h. After cooling to room temperature, a colorless solid of **6**–**12** began to separate. The product was filtered off, washed with ethyl alcohol, then suspended in water and the mixture was neutralized with a $NaHCO_3$ solution. The crude product was subjected to silica gel column chromatography (230–400 mesh) using dichloromethane/methanol (9:1) as an eluent. Chromatography afforded a pure (*E*)-**6** isomer.

(*E*)-6-(2-(2-Ethylidenehydrazino)thiazol-4-yl)benzo[*d*]oxazol-2(3*H*)-one (6) Yield 0.16 g, (58%); R_f = 0.57; mp 250–253°C; ^{1}H NMR: δ 1.91 (d, 3H, CH_3, J = 5 Hz), 7.10 (d, 1H, CH, J = 8 Hz), 7.19 (s, 1H, CH), 7.40 (q, 1H, CH, J = 5 Hz), 7.65 (dd, 1H, CH, J_1 = 2 Hz, J_2 = 8 Hz), 7.70 (m, 1H, CH), 11.75 (bs, 1H, NH, D_2O exchangeable), 12.19 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 18.4, 102.4, 106.9, 109.8, 121.7, 126.9, 130.5, 143.7, 145.2, 154.5, 164.8, 168.6. Anal. Calcd for $C_{12}H_{10}N_4O_2S$: C, 52.54; H, 3.67; N, 20.43. Found: C, 52.48; H, 3.55; N, 20.62.

6-(2-[2-(Propan-2-ylidene)hydrazino]thiazol-4-yl)benzo[*d*]oxazol-2(3*H*)-one (7) Yield 0.24 g (84%); R_f = 0.26; mp 255–256°C; ^{1}H NMR: δ 1.95 (d, 3H, CH_3, J = 7 Hz), 7.11 (d, 1H, CH, J = 11 Hz), 7.21 (s, 1H, CH), 7.65 (dd, 1H, CH, J_1 = 2 Hz, J_2 = 8 Hz), 7.72 (m, 1H, CH), 10.90 (bs, 1H, NH, D_2O exchangeable), 11.77 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 18.1, 24.8, 102.6, 106.8, 109.9, 121.1, 127.3, 130.4, 143.8, 146.5, 153.1, 154.5, 169.7. Anal. Calcd for $C_{13}H_{12}N_4O_2S$: C, 54.15; H, 4.20; N, 19.43. Found: C, 54.23; H, 4.17; N, 19.48.

6-(2-[2-Cyclobutylidenehydrazino)thiazol-4-yl]benzo[*d*]oxazol-2(3*H*)-one (8) Yield 0.20 g; (67%); R_f = 0.86; mp 258–259°C; ^{1}H NMR: δ 1.87–1.99 (m, 2H, CH_2), 2.93 (q, 4H, $2CH_2$, J_1 = 7 Hz, J_2 = 14 Hz), 7.10 (d, 1H, CH, J = 11 Hz), 7.18 (s, 1H, CH), 7.65 (dd, 1H, CH, J_1 = 2 Hz, J_2 = 8 Hz), 7.72 (m, 1H, CH), 10.95 (bs, 1H, NH, D_2O exchangeable), 11.72 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 13.2, 33.4, 33.3, 102.2, 106.8, 109.8, 121.5, 128.6, 130.0, 143.7, 148.5, 154.5, 156.0, 169.2. Anal. Calcd for $C_{14}H_{12}N_4O_2S$: C, 55.99; H, 4.03; N, 18.65. Found: C, 56.06; H, 4.10; N, 18.71.

6-(2-(2-Cyclopentylidenehydrazino)thiazol-4-yl)benzo[d]oxazol-2(3*H*)-one (9) Yield 0.19 g (60%); R_f = 0.72, mp 250°C (with decomp.); ^{1}H NMR: δ 1.70–1.75 (m, 2H, CH_2), 1.77–1.83 (m, 2H, CH_2), 2.37–2.43 (m, 4H, $2CH_2$), 7.12 (d, 1H, CH, J = 11 Hz), 7.22 (s, 1H, CH), 7.65 (dd, 1H, CH, J_1 = 2 Hz, J_2 = 8 Hz), 7.72 (m, 1H, CH), 10.95 (bs, 1H, NH, D_2O exchangeable), 11.82 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 24.5, 24.6, 29.2, 32.9, 102.5, 106.8, 109.9, 121.5, 127.2, 130.4, 143.8, 146.4, 154.5, 156.1, 169.4. Anal. Calcd for $C_{15}H_{14}N_4O_2S$: C, 57.31; H, 4.49; N, 17.82. Found: C, 57.25; H, 4.40; N, 17.93.

6-(2-(2-Cyclohexylidenehydrazino)thiazol-4-yl)benzo[d]oxazol-2(3*H*)-one (10) Yield 0.16 g (49%); R_f = 0.74; mp 248°C (with decomp.); ^{1}H NMR: δ 1.55–1.70 (m, 6H, $3CH_2$), 2.26 (t, 2H, CH_2, J = 7 Hz), 2.45 (t, 2H, CH_2, J = 6 Hz), 7.11 (d, 1H, CH, J = 11 Hz), 7.20 (s, 1H, CH), 7.65 (dd, 1H, CH, J_1 = 2 Hz, J_2 = 8 Hz), 7.68 (m, 1H, CH), 10.80 (bs, 1H, NH, D_2O exchangeable), 11.73 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 25.0, 25.6, 26.8, 27.8, 34.7, 102.7, 106.9, 109.9, 121.6, 126.3, 130.6, 143.8, 144.9, 154.4, 159.7, 169.8. Anal. Calcd for $C_{16}H_{16}N_4O_2S$: C, 58.52; H, 4.91; N, 17.06. Found: C, 58.60; H, 4.86; N, 17.15.

6-(2-(2-Cyclododecylidenehydrazino)thiazol-4-yl)benzo[d]oxazol-2(3*H*)-one (11) Yield 0.30 g (73%); R_f = 0.71; mp 220–223°C; ^{1}H NMR: δ 1.15–1.45 (m, 14H, $7CH_2$), 1.50–1.60 (m, 12H, CH_2), 1.65–1.75 (m, 2H, CH_2), 2.32 (t, 2H, CH_2, J = 6 Hz), 2.40 (t, 2H, CH_2, J = 6 Hz), 7.10 (d, 1H, CH, J = 11 Hz), 7.17 (s, 1H, CH), 7.65 (dd, 1H, CH, J_1 = 2 Hz, J_2 = 8 Hz), 7.71 (m, 1H, CH), 11.95 (bs, 1H, NH, D_2O exchangeable), 11.72 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 21.79, 22.33, 22.46, 22.62, 23.04, 23.14, 23.58, 25.58 (two signals), 28.63, 30.58, 102.36, 106.65, 109.8, 121.4, 128.9, 129.9, 143.8, 148.8, 154.5, 170.3. Anal. Calcd for $C_{22}H_{28}N_4O_2S$: C, 64.05; H, 6.84; N, 13.58. Found: C, 64.11; H, 6.82; N, 13.65.

6-(2-(2-Adamantylidenehydrazino)thiazol-4-yl)benzo[d]oxazol-2(3*H*)-one (12) Yield 0.16 g; (42%); R_f = 0.49; mp 241–244°C; ^{1}H NMR: δ 1.70–1.80 (m, 6H, $3CH_2$), 1.85–2.00 (m, 6H, $3CH_2$), 2.54 (m, 2H, CH_2), 3.37 (m, 2H, CH_2), 7.10 (d, 1H, CH, J = 11 Hz), 7.19 (s, 1H, CH), 7.65 (dd, 1H, CH, J_1 = 2 Hz, J_2 = 8 Hz), 7.71 (m, 1H, CH), 11.95 (bs, 1H, NH, D_2O, exchangeable), 11.74 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR: δ 18.6, 27.2 (three signals), 31.8, 35.8, 37.3 (two signals), 56.1, 102.6, 106.9, 109.9, 121.6, 126.7, 130.5, 143.7, 145.5, 154.5, 165.6, 169.9. Anal. Calcd for $C_{20}H_{20}N_4O_2S$: C, 63.14; H, 5.30; N, 14.73. Found: C, 63.19; H, 5.33; N, 14.81.

In vitro antimicrobial assay

Compounds **6–12** were screened *in vitro* for antibacterial and antifungal activities using the broth microdilution method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) [30] and Clinical and Laboratory Standards Institute guidelines [31] against a panel of reference strains of 25 microorganisms, including Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* NIL1, *Staphylococcus aureus* NIL 2, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus pyogenes* ATCC 19615, *Streptococcus pneumoniae* ATCC 49619, *Streptococcus mutans* ATCC 25175, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876, *Micrococcus luteus* ATCC 10240), Gram-negative bacteria (*Escherichia coli* ATCC 3521, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 12453, *Bordetella bronchiseptica* ATCC 4617, *Salmonella typhimurium* ATCC 14028, *Enterobacter cloacae* NIL, *Pseudomonas aeruginosa* ATCC 9027, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* NIL) and fungi belonging to yeasts (*Candida albicans* ATCC 2091, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019). These microorganisms were obtained from the American Type Culture Collection (ATCC), routinely used for the evaluation of antimicrobials, and from the National Medicines Institute in Warsaw (NIL). All microbial cultures were first subcultured on nutrient agar or Sabouraud agar at 35°C for 18–24 h or 30°C for 24–48 h for bacteria and fungi, respectively.

The surface of Mueller-Hinton agar or Mueller-Hinton agar with sheep blood (for bacteria) and RPMI 1640 with MOPS (for fungi) were inoculated with the suspensions of bacterial or fungal species. Microbial suspensions were prepared in sterile saline (0.85% NaCl) with an optical density of McFarland standard scale 0.5 – approximately 1.5×10^8 CFU/mL for bacteria and 0.5 McFarland standard scale – approximately 5×10^5 CFU/mL for fungi. Samples containing examined compounds **6–12** were dissolved in DMSO and were dropped into the wells on the above-mentioned agar media. The agar plates were preincubated at room temperature for 1 h, and then they were incubated at 37°C for 24 h and 30°C for 48 h for bacteria and fungi, respectively. The well containing DMSO without any compound was used as the negative control and ciprofloxacin or fluconazole (Sigma) as positive controls.

Subsequently, minimal inhibitory concentration (MIC) of the compounds **6–12** was examined by the microdilution broth method, using their 2-fold dilutions in Mueller-Hinton broth or RPMI 1640 broth with MOPS prepared in 96-well polystyrene plates. Final concentrations of the compounds ranged from 1000 to 0.488 μg/mL. Microbial suspensions were prepared in sterile saline (0.85% NaCl) with an optical density of 0.5 McFarland standard. The bacterial or fungal suspension was added per each well containing broth and various concentrations of the examined compounds. After incubation, the MIC was assessed spectrophotometrically as the lowest concentration of the samples showing complete bacterial or fungal growth inhibition. Appropriate DMSO, growth and sterile controls were carried out. The medium with no tested substances was used as the control.

The MBC (minimal bactericidal concentration) or MFC (minimal fungicidal concentration) are defined as the lowest concentration of the compounds that is required to kill a particular bacterial or fungal species. MBC or MFC was determined by removing the culture using MIC determinations from each well

and spotting onto appropriate agar medium. After incubation, the lowest compound concentration with no visible growth observed was assessed as a bactericidal/fungicidal concentration. All experiments were repeated three times and representative data are presented [32].

The MBC/MIC or MFC/MIC ratios were calculated to determine the bactericidal/fungicidal (MBC/MIC ≤4, MFC/MIC ≤4) or bacteriostatic/fungistatic (MBC/MIC >4, MFC/MIC >4) effect of the tested compounds.

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