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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Antimicrobial and anticancer effects of some 2-(substitutedsulfanyl)-*N*-(5-methyl-isoxazol-3-yl) acetamide derivatives

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Abstract In the present study, some isoxazole-(benz)azole derivatives (**3a–3j**) were synthesized by considering antimicrobial and anticancer potencies of azole compounds. Chemical structures of obtained compounds (**3a–3j**) were confirmed by the data of spectral and elemental analyses. Anticancer activity evaluation was performed at two steps. Initially, cytotoxic effects of the compounds (**3a–3j**) on HT-29 (colon carcinoma) and C-6 (melanoma) cell lines were determined by MTT assay. Secondly, the compounds **3g–3i**, which indicated significant cytotoxicity were selected and analysed for their inhibitory activity on DNA synthesis of carcinogenic cells. The compounds **3g** and **3h** showed notable DNA synthesis inhibition on both cancer cell lines. Antibacterial and antifungal activities of the synthesized compounds (**3a–3j**) were also examined. All of the compounds exhibited very poor antibacterial activity against gram negative and gram positive bacterial strains, whereas antifungal activity of the compounds **3a–3f** was equal to that of Ketoconazole.

Keywords Anticancer · Antibacterial · Antifungal · Isoxazole · Azole

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Introduction

Cancer is one of the leading causes of death worldwide. This crucial disease is a multistep process that involves the cumulation of consecutive mutations in oncogenes and suppressor genes that deregulate the cell cycle. It is estimated by WHO that there will be 12 million deaths in 2030 (Sarkar *et al.,* 2009; Villanueva, 2010; http://www.who.int/cancer/en/). Cancer therapy is based on surgery, radiation therapy, and chemotherapy, which, to date are not totally successful interferences. Therefore, anticancer drug design is a very active area in medicinal chemistry and over the past few decades extensive research has led to the development of many chemotherapeutic agents. However, none of these agents are able to eliminate the cancer completely (Cragg and Newman, 2005; Cragg *et al.,* 2009). The limitations of current anticancer drugs and rapid development of drug resistance (Sreedhar and Csermely, 2004; Pechan, 1991; McCubrey *et al.,* 2006) have highlighted the need for the discovery of new anticancer agents, preferably with novel mechanisms of action.

The aim of most cancer chemotherapeutic drugs currently in clinical use is to kill malignant tumor cells by inhibiting some of the mechanisms implied in cellular division (Thurston, 2007). Such chemotherapeutics can be classified in three major groups. The first group is alkylators, which react covalently with DNA bases. DNA strand breakers constitute the second group. They are reactive radicals that produce cleavage of the polynucleotide strands. The last group includes intercalators, which insert between the base pairs of the double helix and cause a significant change of DNA conformation. Intercalators bind to DNA by non-covalent interactions and constitute DNA–intercalator complex (Karikas *et al.,* 1998).

To identify new chemical entities for a more effective treatment of cancer, drug designers can follow many strategies, but the crucial decision is always the selection of a suitable starting point from the vast chemical space (Lloyd *et al.*, 2006). For instance, in DNA intercalators group, recognized forces that maintain the stability of the DNA–intercalator complex, even more than that of DNA alone, are van der Waals, hydrogen bonding, polarization, and hydrophobic forces (Waring and Bailly, 1994; Rehn and Pindur, 1996; Baginski *et al.,* 1997; Karikas *et al.,* 1998; Shui *et al.,* 2000; Neidle, 2008). The compounds, which bear heteroatoms such as nitrogen, increase the strength of the complex by forming hydrogen bonds with DNA. The force of interaction between compound and DNA usually correlates with the anticancer activity (Moore *et al.,* 1989; Pindur *et al.,* 1993; Zhigang *et al.,* 2005). Besides, when one or more heteroatoms exist on the chemical structure, intercalating chromophore possesses a polarized character and optimal interaction occurs (Neidle, 2008). Based on such reasons, it has been reported that the efficiency of the stacking interactions could be enhanced with the presence of polycondensed heterocyclic chromophore, which carries a side chain that protrude into one of the two DNA grooves (Geierstanger and Wemmer, 1995).

Azoles and benzazoles, heterocyclic compounds with two or more heteroatoms, constitute a large group of organic substances exhibiting a wide range of biological activities (Rozhkova *et al.,* 2000). There are some important anticancer agents including these ring systems. For instance, imidazole analogs Dacarbazine and Tipifarnib (Fazeny-Dorner *et al.,* 2003; Perez-Ruixo *et al.,* 2006), benzimidazole derivatives Hoechst 33258, Hoechst 33342, and Hoechst 33377 (Harshman and Dervan, 1985; Finlay and Baguley, 1990; Satz *et al.,* 2001), benzoxazole compounds UK-1 and L-697.661 (Kumar *et al.,* 2002; Rida *et al.,* 2005), benzothiazole derivatives Phortress (NSC 710305) and NSC 703786 (Hose *et al.,* 2003; Brantley *et al.,* 2004), 1,2,4-triazole carrying compounds Vorozole, Letrozole ve Anastrazole (Al-Masoudi *et al.,* 2006; Sztanke *et al.,* 2008), tetrazole analog ZD9331 (Jackman *et al.,* 1997), and 1,3,4-thiadiazole compound NSC 4728 (Nelson *et al.,* 1977) are the anticancer agents. Besides, isoxazole is another pharmacophore in the azole family which has been subjected to anticancer agent development studies (Vekariya *et al.,* 2003; Lee *et al.,* 2009; Kamal *et al.,* 2010; Reddy *et al.,* 2010).

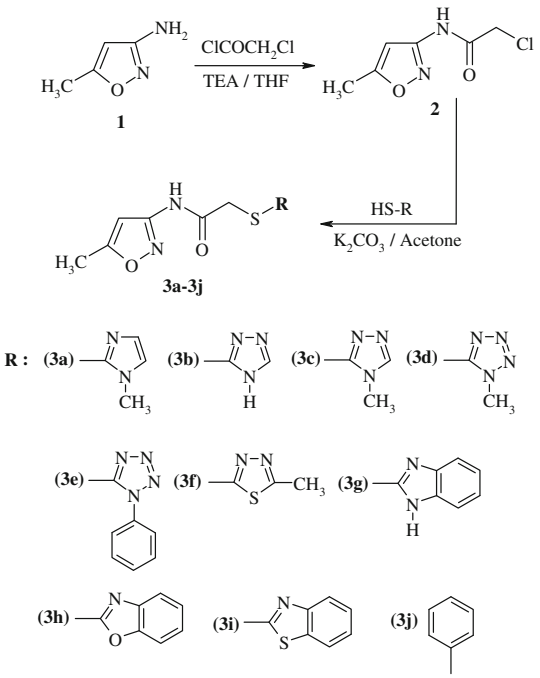
In addition to anticancer potential of azoles and benzazoles, there are many reports about antimicrobial activity of azole and benzazole ring systems carrying compounds (Kaplancikli *et al.,* 2004; Ozdemir *et al.,* 2010; Ozkay *et al.,* 2011). The basis of a special interest of researchers toward azoles has been imidazoles and triazoles, which constitute two important classes of antifungal agents (Ozkay *et al.,* 2011). Thus, medicinal chemists often carry out the synthesis studies including imidazole or triazole moieties or their bioisosters such as benzimidazole, benzoxazole, benzothiazole, tetrazole, and thiadiazole in order to discover new antimicrobial agents.

As a result, we designed some isoxazole-(benz)azole compounds in the light of chemotherapeutic potentials of azoles and benzazoles. Careful literature survey revealed that there has been no pharmacological observation including designed compounds. Hence, synthesis, anticancer, and antimicrobial evaluation of ten 2-(substitutedsulfanyl)-*N*-(5-methyl-isoxazol-3-yl) acetamide (**3a–3j**) derivatives are reported in the present study.

Results and discussion

Chemistry

In the present work, the reaction sequence outlined in Scheme 1 was followed for the synthesis of 2-(substitutedsulfanyl)-*N*-(5-methyl-isoxazol-3-yl)acetamide derivatives (**3a–3j**). Initially, 3-amino-5-methyl-isoxazole in THF was acetylated with chloroacetyl chloride to afford 2-chloro-*N*-(5-methyl-isoxazol-3-yl) acetamide (**1**) as a starting compound. Then the compound **1** in acetone was reacted with appropriate (benz)azolethiol derivative in the



Scheme 1 Reaction sequence for the synthesis of target compounds (**3a–3j**)

presence of K_2CO_3 to give the final products (**3a–3j**). The chemical structures of the compounds (**3a–3j**) were confirmed by IR, ^{1}H NMR, and mass spectral data and elemental analyses.

In the IR spectra, some significant stretching absorption of amide group were determined at 3,314–3,388 cm^{-1} for N–H and at 1,666–1,678 cm^{-1} for C=O groups. Stretching absorption of C=N and C=C bands were observed at 1,408–1,611 cm^{-1}. Disappearance of stretching absorption for S–H bond at about 2,550 cm^{-1} was commented as an evidence for target compounds. In the ^{1}H NMR spectra, entire aromatic and aliphatic protons were recorded at estimated areas. A singlet was recorded at 2.35–2.37 ppm for CH_3 group on the 5th position of isoxazole ring. Acetamide ($NHCOCH_2$) group gave peaks at 4.44–4.49 ppm and 10.65–10.72 ppm as a singlet, belonging to $–CH_2$ and N–H protons, respectively. Aromatic proton on the 4th position of isoxazole ring was observed at 6.61–6.64 ppm as a singlet. The other aromatic protons, which vary according to side groups, were recorded at the area of 7.19–8.63 ppm. The mass spectra (Es-Ms) of compounds showed [M+1] peaks, in agreement with their molecular formula. All compounds gave satisfactory elemental analysis results.

Cytotoxicity

In the research of new anticancer agents, the most common screening methods employ cytotoxicity tests against a panel of cancer cell lines. These assays include high throughput screening, which represent compounds with the highest cytotoxic effect (Popiolkiewicz *et al.,* 2005). MTT, based on the capability of metabolically active cells to convert the pale yellow MTT dye to a spectrophotometrically quantifiable blue formazan product, is one of the most preferred cytotoxicity tests (Varache-Lembège *et al.,* 2008).

In the MTT test, HT-29 and C-6 cell lines were incubated with six various concentrations (0.01, 0.05, 0.1, 0.5, 1, and 5 μM) of compounds **3a–3j**. After the completion of incubation period (24 h), cytotoxic activity of the compounds was examined and IC_{50} values were calculated. Anticancer agent Cisplatin was used as a positive control. Results are presented in Table 1. The compounds **3g–3i** which carry *1H*-benzimidazol-2-thiol, benzoxazol-2-thiol, and benzothiazol-2-thiol substructures as variable groups showed higher cytotoxicity than the compounds **3a–3h** and **3j**. Cytotoxicity for compound **3j**, which carries phenyl side group instead of azole or benzazole substructure, could not be determined. Cytotoxic effect of **3g** was very close to that of Cisplatin against both cell lines. Such results suggest that hydrophobic forces have substantial effect on cytotoxic activity. Due to fused benzene ring to azole moiety, lipophilicities of the compounds **3g–3i** were higher than those of **3a–3h**. Thus, compounds **3g–3i** were selected for analysis of DNA synthesis assay because of their significant cytotoxic activity.

Table 1 IC_{50} values of the compounds **3a–3j** against HT-29 and C-6 cell lines

Compound	IC_{50} (μM)	
	HT-29	C6
3a	1.13	1.41
3b	1.13	3.19
3c	1.02	1.47
3d	nd	1.21
3e	0.74	0.64
3f	0.67	0.58
3g[a]	0.09	0.10
3h[a]	0.22	0.44
3i[a]	0.40	0.21
3j	nd	nd
Cisplatin	0.07	0.08

nd could not determined in tested concentrations range

[a] Selected for DNA synthesis inhibition assay

Analysis of DNA synthesis inhibition

This immune staining assay is based on determination of the incorporation of bromo deoxyuridine (BrdU) into nuclear DNA in place of thymidine during the S-phase of the cell cycle using specific anti-BrdUantibodies (Malíková *et al.*, 2008). Hence, such method provides a colorimetric measurement for DNA synthesis inhibition ratio of the carcinogenic cells.

DNA syntheses of the HT-29 and C-6 cell lines were analysed for compounds **3g–3i** which indicated significant cytotoxic activity in MTT test. Cisplatin was used as a positive control. For 24 and 48 h time periods, HT-29 and C-6 cells were incubated with test compounds at three different concentrations (0.08, 0.16, and 0.32 μM) that were determined according to IC_{50} values. As seen in Figs. 1 and 2 tested compounds showed time- and dose-dependent inhibitory activity on DNA synthesis of both cell lines. The compounds **3g** and **3h**, which include *1H*-benzimidazol-2-thiol and benzoxazol-2-thiol substructures exhibited notable activity at a dose of 0.32 μM. For 48 h time period, these compounds indicated about 80 and 70 % DNA synthesis inhibitory activity on HT-29 and C-6 cell lines, respectively. Besides, for 24 h time period, they displayed higher than 50 % inhibitory activity on DNA synthesis of C-6 cells. On the other hand, benzothiazol-2-thiol substructure carrying compound **3i** showed the poorest inhibition on DNA synthesis of carcinogenic cells.

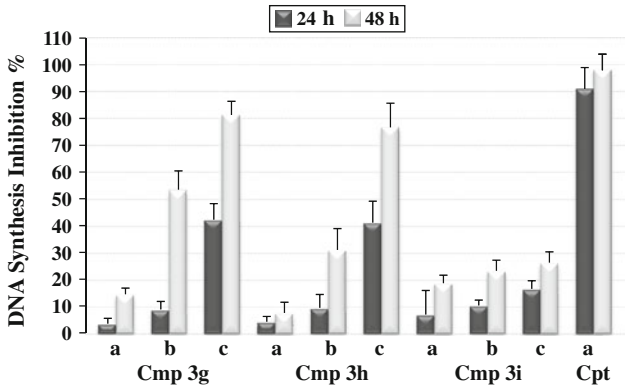


Fig. 1 Inhibitory effects of the compounds **3g–3i** on DNA synthesis of HT-29 cells: mean percent of absorbance of the untreated (assessed in the presence of DMSO used as a solvent and assumed as 0 %), and three different concentrations ($a = 0.8$ μM, $b = 1.6$ μM, $c = 3.2$ μM) of test compounds and single concentration ($a = 0.8$ μM) of cisplatin were given. Data points represent means for three independent experiments ± SD of nine independent wells. $p < 0.05$

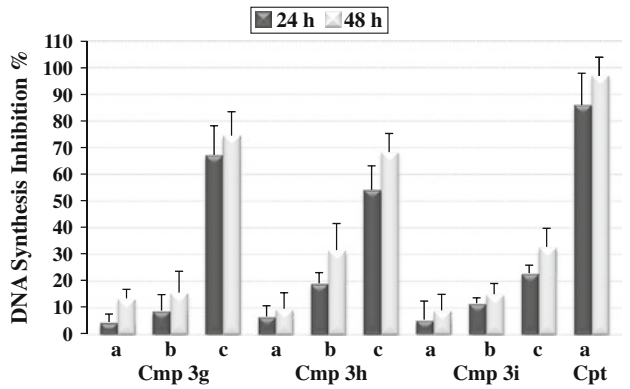


Fig. 2 Inhibitory effects of the compounds **3g–3i** on DNA synthesis of C-6 cells: mean percent of absorbance of the untreated (assessed in the presence of DMSO used as a solvent and assumed as 0 %), and three different concentrations ($a = 0.8$ μM, $b = 1.6$ μM, $c = 3.2$ μM) of test compounds and single concentration ($a = 0.8$ μM) of cisplatin were given. Data points represent means for three independent experiments ± SD of nine independent wells. $p < 0.05$

Polarization may explain the activity differences between compounds **3g**, **3h**, and **3i**. Presence of nitrogen and oxygen atoms provides more polarized character to a compound than sulfur atom. Thus, interaction with DNA of cancer cells, which designate the anticancer potency, may be easier for benzimidazole and benzoxazole than benzothiazole.

Antimicrobial activity

Antimicrobial effects of the compounds are shown in Table 2. Synthesized compounds (**3a–3j**) exhibited poor antibacterial activity against both gram (+) and gram (−) bacteria. On the other hand, the compounds **3a–3f** showed significant antifungal activity against *Candida albicans*. MIC values (100 μg/mL) of these compounds were the same with that of the reference Ketoconazole. The other compounds **3g–3j** exhibited moderate antifungal activity (MIC = 200 μg/mL). Results of this study showed parallelism with our recent study (Ozkay *et al.*, 2011). According to observed results, it is cleared that azole ring systems (imidazole, triazole, tetrazole, and thiadiazole) carrying compounds **3a–3f** possess more antifungal potency than benzazole (benzimidazole, benzoxazole, and benzothiazole) rings bearing compounds **3g–3j**. The reason for such finding may be explained with the optimum lipophilicity, which is a key property that influences the ability of a drug to reach the target by transmembrane diffusion and to have a major effect on the biological activity (Testa *et al.*, 2000; Patil *et al.*, 2010). Lower lipophilic character of compounds **3a–3f** probably makes intracellular transport easier through the cell membrane of fungi, whereas increasing lipophilicity of the compounds **3g–3j** due to benzazole rings may hinder transmembrane diffusion.

Table 2 MIC values (μg/mL) of the compounds **3a–3j** against various microorganisms

Comp	A	B	C	D	E
3a	200	400	400	200	100
3b	200	400	400	100	100
3c	200	400	400	100	100
3d	200	400	400	100	100
3e	200	400	400	100	100
3f	200	400	400	200	100
3g	200	400	400	200	200
3h	200	400	400	200	200
3i	200	400	400	200	200
3j	200	400	400	200	200
Ref[1]	50	100	100	50	–
Ref[2]	–	–	–	–	100

A: *S. aureus,* B: *E. faecalis,* C: *E. Coli,* D: *P. aeruginosa,* E: *C. albicans,* Ref[1]: Chloramphenicol, Ref[2]: Ketoconazole

Materials and methods

Chemistry

All reagents were used as purchased from commercial suppliers (Merck, Acros or Sigma-Aldrich) without further purification. Melting points (m.p.) were determined by using an Electrothermal 9100 digital melting point apparatus and were uncorrected. During the synthesis, the

compounds were routinely checked for purity by TLC on silica gel 60. IR spectra were recorded on a Shimadzu, 8400 FTIR spectrometer as KBr pellets. ^{1}H NMR spectra were recorded on a Bruker UltraShield 500 MHz spectrometer in DMSO-d_6. MS data were obtained on an Agilent 1100 Series LC/MSD Trap VL&SL spectrometer. Elemental analyses (C, H, and N) were determined on a Perkin Elmer analyser.

Synthesis of the compounds (**3a**–**3j**)

3-Amino-5-methyl-isoxazole (4.9 g, 50 mmol) was dissolved in 50 mL of tetrahydrofuran and triethylamine (8.5 mL, 60 mmol) was added. This mixture was allowed to stir on an ice bath. Chloroacetyl chloride (0.06 mol, 4.8 mL) in 10 mL of tetrahydrofuran was added drop by drop. After completion of dropping, reaction mixture was stirred for 1 h at room temperature. Tetrahydrofuran was evaporated and the residue was recrystallized from ethanol to give 2-chloro-*N*-(5-methyl-isoxazol-3-yl)acetamide (**2**). A mixture of the starting compound (**1**) (0.349 g, 2 mmol), appropriate thiol-azole derivative (2 mmol), and K_2CO_3 (0.276 g, 2 mmol) in acetone (30 mL) was refluxed for 2 h. After cooling, the solution was evaporated until dryness. The residue was washed with cold water and recrystallized from ethanol to give target compounds (**3a**–**3j**).

*2-(1-Methyl-1H-imidazol-2-yl)sulfanyl-N-(5-methyl-isoxazol-3-yl)acetamide (**3a**)*

Yield 74 %. m.p. 143–144 °C. IR (KBr) ν_{max}(cm^{-1}): 3341 (N–H), 1672 (C=O), 1611–1413 (C=C and C=N). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 2.35 (3H, s, isoxazole C_3–CH_3), 2.93 (3H, s, imidazole N–CH_3), 4.44 (2H, m, CO–CH_2), 6.62 (H, s, isoxazole C_2–H), 7.94 (H, d, $J = 8.16$, imidazole C_3–H), 8.24 (H, d, $J = 8.05$, imidazole C_2–H), 10.70 (H, s, NH–CO). Es-Ms (*m*/*z*): M+1: 253.1. Anal. calcd. for $C_{10}H_{12}N_4O_2S$: C, 47.61; H, 4.79; N, 22.21. Found: C, 47.58; H, 4.78; N, 22.14.

*2-(4H-1,2,4-triazol-3-yl)sulfanyl-N-(5-methyl-isoxazol-3-yl)acetamide (**3b**)*

Yield 79 %. m.p. 196–197 °C. IR (KBr) ν_{max}(cm^{-1}): 3346 (N–H), 1671 (C=O), 1604–1412 (C=C and C=N). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 2.35 (3H, s, isoxazole C_3–CH_3), 4.46 (2H, s, CO–CH_2), 6.64 (H, s, isoxazole C_2–H), 8.62 (H, s, triazole C_2–H), 10.65 (H, s, NH–CO), 12.91 (br, H, triazole NH). Es-Ms (*m*/*z*): M+1: 240.2. Anal. calcd. for $C_8H_9N_5O_2S$: C, 40.16; H, 3.79; N, 29.27. Found: C, 40.08; H, 3.80; N, 29.31.

*2-(4-Methyl-4H-1,2,4-triazol-3-yl)sulfanyl-N-(5-methyl-isoxazol-3-yl)acetamide (**3c**)*

Yield 82 %. m.p. 184–185 °C. IR (KBr) ν_{max}(cm^{-1}): 3337 (N–H), 1668 (C=O), 1605–1408 (C=C and C=N). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ(ppm): 2.36 (3H, s, isoxazole C_3–CH_3), 3.65 (3H, s, triazole N–CH_3), 4.46 (2H, s, CO–CH_2), 6.63 (H, s, isoxazole C_2–H), 8.61 (H, s, triazole C_2–H), 10.67 (H, s, NH–CO). Es-Ms (*m*/*z*): M+1: 254.1. Anal. calcd. for $C_9H_{11}N_5O_2S$: C, 42.68; H, 4.38; N, 27.65. Found: C, 42.78; H, 4.38; N, 27.61.

*2-(1-Methyl-1H-1,2,3,4-tetrazol-5-yl)sulfanyl-N-(5-methyl-isoxazol-3-yl)acetamide (**3d**)*

Yield 76 %. m.p. 198–199 °C. IR (KBr) ν_{max}(cm^{-1}): 3364 (N–H), 1670 (C=O), 1605–1410 (C=C and C=N). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 2.37 (3H, s, isoxazole C_3–CH_3), 4.05 (3H, s, tetrazole N–CH_3), 4.49 (2H, s, CO–CH_2), 6.62 (H, s, isoxazole C_2–H), 10.68 (H, s, NH–CO). Es-Ms (*m*/*z*): M+1: 255.1 Anal. calcd. for $C_8H_{10}N_6O_2S$: C, 37.79; H, 3.96; N, 33.05. Found: C, 37.71; H, 3.98; N, 33.01.

*2-(1-Phenyl-1H-1,2,3,4-tetrazol-5-yl)sulfanyl-N-(5-methyl-isoxazol-3-yl)acetamide (**3e**)*

Yield 83 %. m.p. 179–180 °C. IR (KBr) ν_{max}(cm^{-1}): 3368 (N–H), 1672 (C=O), 1607–1409 (C=C and C=N). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 2.36 (3H, s, isoxazole C_3–CH_3), 4.45 (2H, s, CO–CH_2), 6.61 (H, s, isoxazole C_2–H), 7.60–7.66 (m, 3H, tetrazole N–Ph $C_{3,4,5}$–H), 8.02 (d, 2H, $J = 8.48$ Hz, tetrazole N–Ph $C_{2,6}$–H), 10.70 (H, s, NH–CO). Es-Ms (*m*/*z*): M+1: 317.2. Anal. calcd. for $C_{13}H_{12}N_6O_2S$: C, 49.36; H, 3.82; N, 26.57. Found: C, 49.48; H, 3.81; N, 26.51.

*2-(5-Methyl-1,3,4-thiadiazol-2-yl)sulfanyl-N-(5-methyl-isoxazol-3-yl)acetamide (**3f**)*

Yield 72 %. m.p. 225 °C. IR (KBr) ν_{max}(cm^{-1}): 3364 (N–H), 1673 (C=O), 1606–1411 (C=C and C=N). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 2.35 (3H, s, isoxazole C_3–CH_3), 2.71 (3H, s, thiadiazole C_2–CH_3), 4.48 (2H, s, CO–CH_2), 6.63 (H, s, isoxazole C_2–H), 10.68 (H, s, NH–CO). Es-Ms (*m*/*z*): M+1: 271.1. Anal. calcd. for $C_9H_{10}N_4O_2S_2$: C, 39.99; H, 3.73; N, 20.73. Found: C, 39.88; H, 3.74; N, 20.71.

*2-(1H-benzimidazol-2-yl)sulfanyl-N-(5-methyl-isoxazol-3-yl)acetamide (**3g**)*

Yield 83 %. m.p. 187–188 °C. IR (KBr) ν_{max}(cm^{-1}): 3388 (N–H), 1675 (C=O), 1611–1407 (C=C and C=N). ^{1}H NMR

(500 MHz) (DMSO-d_6) δ(ppm): 2.35 (3H, s, isoxazole C_3–CH_3), 4.46 (2H, s, CO–CH_2), 6.64 (H, s, isoxazole C_2–H), 7.29–7.33 (m, 2H, benzimidazole $C_{3,4}$–H), 7.76–7.80 (m, 2H, benzimidazole $C_{2,5}$–H), 10.72 (H, s, NH–CO), 12.81 (br, H, benzimidazole N–H). Es-Ms (*m/z*): M+1: 289.2. Anal. calcd. for $C_{13}H_{12}N_4O_2S$: C, 54.15; H, 4.19; N, 19.43. Found: C, 53.98; H, 4.18; N, 19.39.

*2-(Benzoxazol-2-yl)sulfanyl-N-(5-methyl-isoxazol-3-yl)acetamide (**3h**)*

Yield 86 %. m.p. 193 °C. IR (KBr) $\nu_{max}(cm^{-1})$: 3382 (N–H), 1678 (C=O), 1604–1411 (C=C and C=N). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 2.37 (3H, s, isoxazole C_3–CH_3), 4.46 (2H, s, CO–CH_2), 6.63 (H, s, isoxazole C_2–H), 7.35–7.39 (2H, m, benzoxazole $C_{3,4}$–H), 7.62–7.65 (2H, m, benzoxazole $C_{2,5}$–H), 10.72 (H, s, NH–CO). Es-Ms (*m/z*): M+1: 290.12. Anal. calcd. for $C_{13}H_{11}N_3O_3S$: C, 53.97; H, 3.83; N, 14.52. Found: C, 53.78; H, 3.85; N, 14.56.

*2-(Benzothiazol-2-yl)sulfanyl-N-(5-methyl-isoxazol-3-yl)acetamide (**3i**)*

Yield 82 %. m.p. 180–181 °C. IR (KBr) $\nu_{max}(cm^{-1})$: 3362 (N–H), 1674 (C=O), 1602–1409 (C=C and C=N). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 2.36 (3H, s, isoxazole C_3–CH_3), 4.47 (2H, s, CO–CH_2), 6.64 (H, s, isoxazole C_2–H), 7.38–7.42 (2H, m, benzothiazole $C_{3,4}$–H), 7.84 (H, d, $J = 8.06$, benzothiazole C_5–H), 8.09 (H, d, $J = 8.02$, benzothiazole C_2–H), 10.72 (H, s, NH–CO). Es-Ms (*m/z*): M+1: 306.1. Anal. calcd. for $C_{13}H_{11}N_3O_2S_2$: C, 51.13; H, 3.63; N, 13.76. Found: C, 51.28; H, 3.64; N, 13.81.

*2-Phenylsulfanyl-N-(5-methyl-isoxazol-3-yl)acetamide (**3j**)*

Yield 77 %. m.p. 110–111 °C. IR (KBr) $\nu_{max}(cm^{-1})$: 3314 (N–H), 1666 (C=O), 1610–1408 (C=C and C=N). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 2.36 (3H, s, isoxazole C_3–CH_3), 4.47 (2H, s, CO–CH_2), 6.63 (H, s, isoxazole C_2–H), 7.28–7.41 (5H, m, C_6H_5–H), 10.70 (H, s, NH–CO). Es-Ms (*m/z*): M+1: 249.2. Anal. calcd. for $C_{12}H_{12}N_2O_2S$: C, 58.05; H, 4.87; N, 11.28. Found: C, 57.89; H, 4.88; N, 11.31.

Anticancer activity screening

Cell cultures

C-6 cells were maintained in 90 % Dulbecco's Modified Eagle's Medium (DMEM) (Sigma, Deisenhofen, Germany), and 10 % (v/v) of fetal bovine serum (FBS) (Gibco, UK). HT-29 cells were cultured in 90 % McCoy's 5A (Sigma, Deisenhofen, Germany) and 10 % Fetal Bovine Serum (FBS) (Gibco, UK). All media were supplemented with penicillin/streptomycin at 100 units/mL and cells were incubated at 37 °C in a 5 % CO_2/95 % air humidified atmosphere.

MTT assay

A tetrazolium salt, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), was used as a colorimetric substrate for measuring cytotoxicity. HT-29 and C-6 cells were cultured in 96 well plates and 0.01–5 μM of test compounds or Cisplatin were added. The plates were incubated for 24 h at 37 °C in 5 % CO_2 humidified incubator together with untreated control sample. After incubation period, 20 μL MTT dye was added and the plates were measured with a ELx808-IU Bio-Tek apparatus at 540 nm. Control cell viability was regarded as 100 %. Stock solutions of the test compounds were dissolved in dimethyl sulfoxide (DMSO) and further concentrations were prepared in cell culture media. All experiments were repeated three times. For each of the compound doses, four independent wells were used. Percent viability was defined as the relative absorbance of treated versus untreated control cells.

DNA synthesis inhibition assay

DNA synthesis inhibitory effects of the synthesized compounds were performed in the 96-well flat-bottomed microtiter plates by using a BrdU colorimetric kit. HT-29 and C-6 cells were collected from cell cultures by 0.25 % trypsin/EDTA solution and counted in a hemocytometer. Suspensions of cell lines were seeded into 96-well flat-bottomed microtiter plates at a density of 1×10^3 cells/mL. The tumor cell lines were cultured in the presence of various doses of the test compounds or Cisplatin. Microtiter plates were incubated at 37 °C in a 5 % CO_2/95 % air humidified atmosphere for 24 h. At the end of incubation period, the cells were labeled with 10 μL BrdU solution for 2 h and then fixed. Anti-BrdU-POD (100 μL) was added and incubated for 90 min. Finally, microtiter plates were washed with phosphate buffer saline (PBS) three times and the cells were incubated with substrate solution until the color was sufficient for photometric detection. Absorbance of the samples was measured with an ELx808-IU Bio-Tek apparatus at 492 nm. As a control solvent, DMSO was added to the cells during the time course. The values of blank wells were subtracted from each well of treated and control cells. The absorbance values of background control wells did not exceed 0.1. All experiments were repeated three times. For each dose of the compounds, triplicate wells were used.

Statistics

The SPSS for Windows 11.5 computer program was used for statistical analyses. Statistical comparison of the results obtained from controls, groups, and time periods parameters were carried out by the one-way analyses of variance (ANOVA) test and post hoc analyses of group differences were performed by using Tukey test. Results were expressed as mean ± SD.

Microbiology

Final products were tested for their in vitro growth inhibitory activity against human pathogenic as gram (+) bacteria *Staphylococcus aureus* NRRL B-767, and *Enterococcus faecalis* ATCC 29212, as gram (−) bacteria, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 254992, as yeast *Candida albicans* (obtained from Faculty of Medicine Osmangazi University, Eskisehir, Turkey). Chloramphenicol and Ketoconazole were used as control drugs.

Antimicrobial activity assay

Antimicrobial activity test was performed according to CLSI reference M7-A7 broth microdilution method (Wayne, 2006) as described in our previous study (Ozkay *et al.,* 2011). Twice MIC readings were carried out for each chemical agent. The compounds were dissolved in DMSO for antibacterial and antimicrobial assays. Further dilutions of the compounds and standard drugs in test medium were prepared at the required quantities of 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.5625 μg/mL concentrations with Mueller–Hinton broth and Sabouroud dextrose broth. In order to ensure that the solvent per se had no effect on bacteria or yeast growth, a control test was also performed containing inoculated broth supplemented with only DMSO at the same dilutions used in our experiments and found inactive in culture medium.

Conclusion

Rapid development of resistance to a number of anticancer and antimicrobial agents, remain pressing problems worldwide. Thus, chemotherapeutic drug development area has been always hot and there is a vital need to discover new chemotherapeutic agents to avert the emergence of resistance and ideally shorten the duration of therapy. Prompted from this respect, a compact system, which bears isoxazole and varying azoles or benzazoles on a chemical skeleton, was designed in order to perform anticancer and antimicrobial evaluations. According to results of anticancer screening, it can be declared that the compounds **3g** and **3h** have the highest anticancer potency as a result of their significant cytotoxicity and their notable inhibitory activity on DNA synthesis of carcinogenic cells. Another important finding of the present study is antifungal activity of the compounds **3a**–**3f** against *Candida albicans*. Such compounds were found to be as active as Ketoconazole with a MIC value of 100 μg/mL. Furthermore, no correlation between the results of cytotoxicity and antifungal effect assays revealed that the antifungal activity of the compounds is not due to their general toxicity but can be ascribed to the selectivity of their antifungal properties.

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