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SYNTHESIS OF SOME 2-SUBSTITUTED-5-(BENZOTHIAZOL-2-YL)-1*H*-BENZIMIDAZOLE DERIVATIVES AND INVESTIGATION OF THEIR ANTIPROLIFERATIVE EFFECTS

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In addition to methods such as radiotherapy and surgery, another effective way that is preferred for the treatment of cancer is based on the use of chemotherapeutic agents. Although there are more than hundred drugs for cancer treatment, there is still a need to develop new anticancer drugs because of reasons such as drug resistance, side effects, low selectivity and severe toxicity. Benzimidazole and benzothiazole ring systems are the basis of chemical structures of many important drugs because of their different pharmacological properties. In this work, due to potential anticancer effects of benzothiazole and benzimidazole ring systems, eight new compounds that contain both these ring systems in the same chemical structure were synthesized. The structures of obtained compounds were characterized by the IR, NMR, and mass spectroscopy and by elemental analysis data. Cytotoxic effects of the synthesized compounds were tested on Caco-2 (ATCC HTB-37), A549 (ATCC CCL-185) and NIH3T3 (ATCC CRL-1658) cell lines. Two compounds (**3c** and **3e)** were determined as the most effective agents that showed activity comparable with that of the reference drug Cisplatin.

Keywords: cancer; cytotoxicity; benzimidazole; benzothiazole; Cisplatin, synthesis.

1. INTRODUCTION

Cancer is characterized by uncontrolled growth of abnormal cells anywhere in the body. According to statistics from the World Health Organization in 2018, the number of cancer cases in the world will increase up to 22 million by 2030 [1, 2]. Therefore, an important goal of today's research is the development of anticancer drugs that are highly efficient and low in toxicity [3, 4]. Benzimidazole is an important heterocyclic compound consisting of a fused benzene and imidazole ring, which is an integral part of vitamin B12. Because of the structural similarity of benzimidazoles and purine, they can easily interact with biomolecules of living systems. Therefore, they have considerable potential for the use in medicinal chemistry and are a critical pharmacophore in drug discovery [5, 6].

Bendamustine (Fig. 1) possessing benzimidazole moiety is a US FDA approved anti-cancer drug [7]. In addition, telmisartan is also known for its anticancer properties [8]. Furthermore, bisbenzimidazole derivatives that are commonly known as Hoechst 33342 and Hoechst 33258 (Fig. 1) compounds displayed potent anticancer activity [9, 10].

Benzothiazole nucleus has also attracted considerable attention in anticancer research [11 – 15]. In particular, nocodazole possessing anticancer activity contains benzothiazole ring. Fortress (NSC 710305) as an anticancer agent and its clinical trial analog NSC 703786 (5F 203) are compounds containing the benzothiazole ring. PMX 610 having anticancer activity and CJM 126 (Fig. 2) acting on human breast carcinoma cells also have a benzothiazole ring in the structure [16 – 18].

In the present work considering the above heterocyclic structures and studying their potential antiproliferative activity, eight new compounds containing benzimidazole and benzothiazole ring systems were synthesized and their antiproliferative properties were investigated.

2. RESULTS AND DISCUSSION

2.1. Chemistry

The synthetic routes of target benzimidazole-benzothiazole derivatives were shown in Scheme 1**.** Sodium bisulfite

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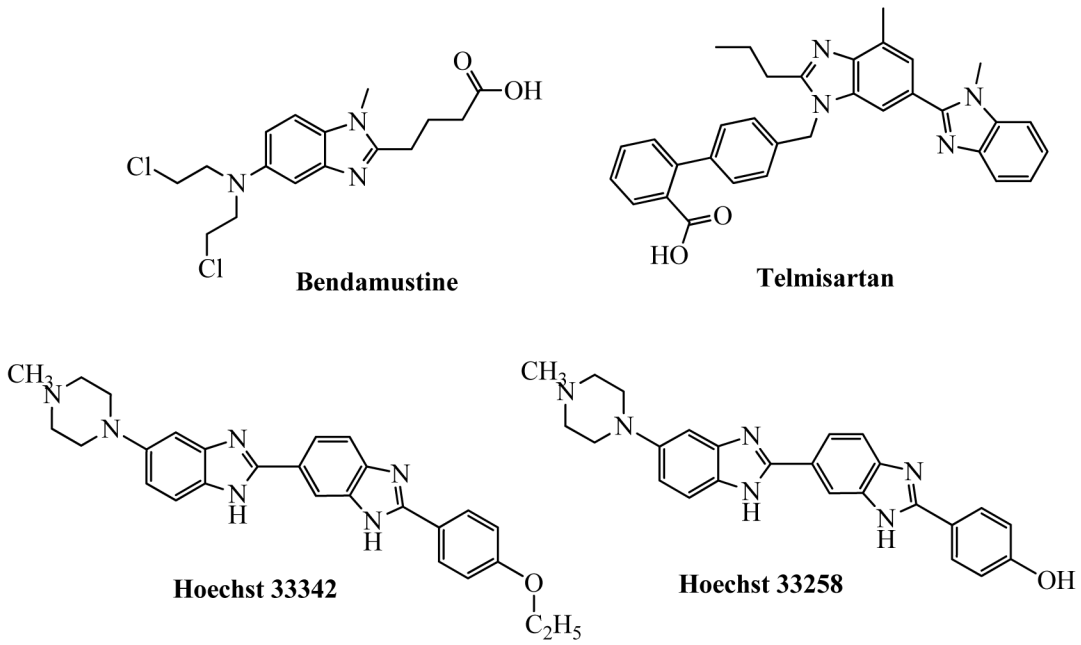


Fig. 1. Some benzimidazole derivatives with anticancer effect.

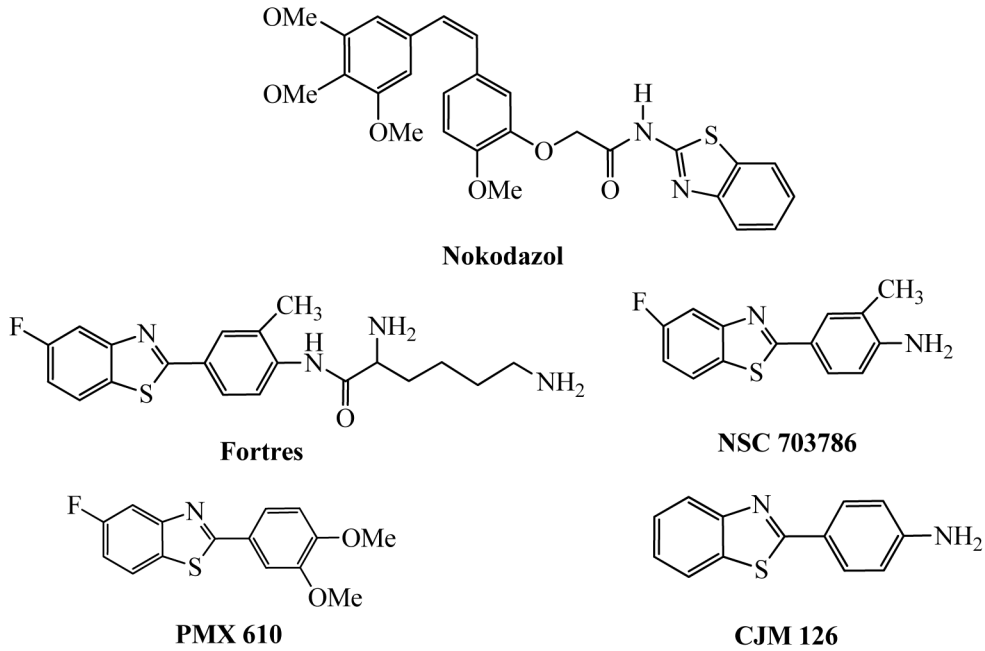
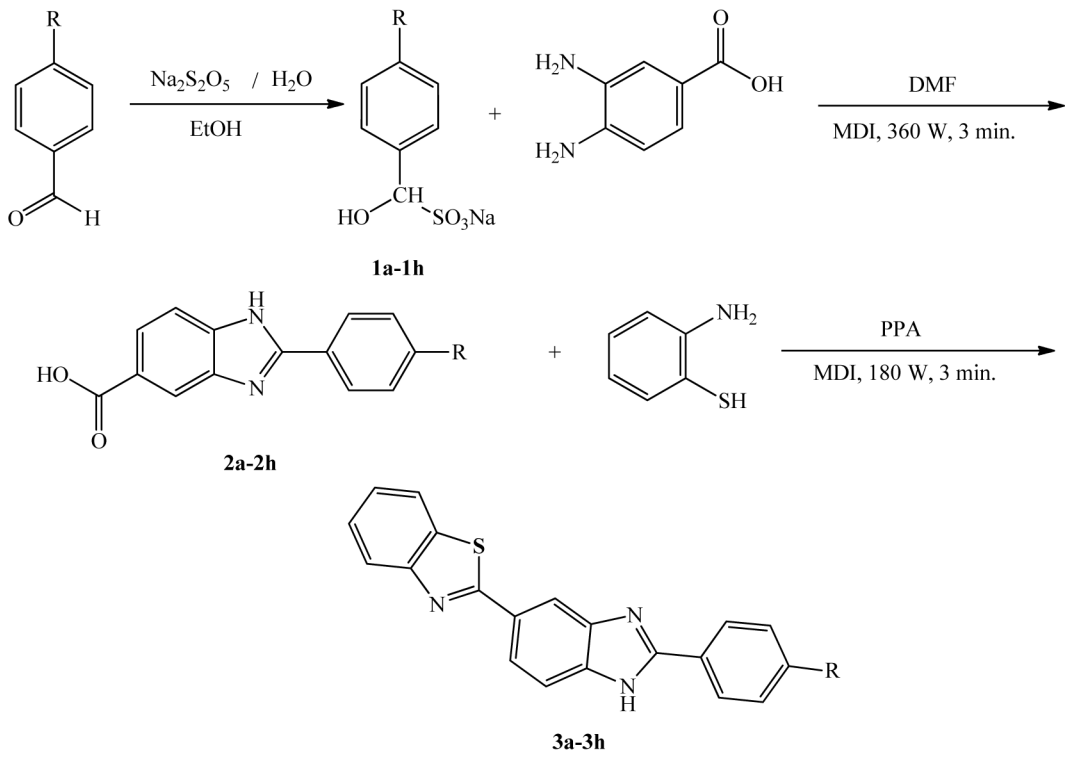
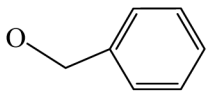


Fig. 2. Some benzothiazole derivatives possessing anticancer properties.

adducts of benzaldehyde derivatives were prepared in the first reaction step. In the second step, 2-substituted-1*H*-benzimidazole-5-carboxylic acid derivatives were obtained from benzaldehyde sodium bisulfite adducts and 3,4-diaminobenzoic acid. In the third step, 2-substituted-1*H*-benzimidazole-5-carboxylic acid derivatives were reacted with 2-aminothiophenol to give the final compounds. The IR spectra of the synthesized compounds show that the N-H bond of the benzimidazole ring system gives a weak band in the region of 3275 – 3312 cm^{-1}. The stretching vibrations at about 1408 – 1638 cm^{-1} were recorded for C=C and C=N double bonds. The stretching absorption belonging to 1,4-disubstituted benzene was determined at 804 – 845 cm^{-1}. The ^{1}H NMR spectrum showed a broad singlet at



Compound	R
3a	-H
3b	-Cl
3c	-F
3d	$-OCH_3$
3e	$-CH_3$
3f	$-OC_2H_5$
3g	-OH
3h	

Scheme 1. Synthesis route to target compounds **3a–3h.**

13.25 – 13.65 ppm due to NH proton of the benzimidazole ring and peaks in the region about 7 – 8 ppm due to aromatic protons in the structure. The mass spectra (ES-MS) of the compounds showed [M+1] mass peaks, in agreement with their molecular formula. All compounds gave satisfactory results of elemental analyses.

2.2. Antiproliferative Activity Assay

The antiproliferative activity of synthesized compounds was evaluated against NIH3T3 (ATCC CRL-1658) mouse embryo fibroblast cell line, A549 (ATCC CCL-185) non-small cell lung cancer and Caco-2 (ATCC HTB-37) colon cancer cell lines using MTT in comparison to the well-known anticancer drug cisplatin as reference. The antiproliferative activitytesting results are listed in Table 1, which shows that the majority of the synthesized compounds exhibit low to moderate activity against the tested cancer cell lines. Compound **3e** showed the most potent activities against A549 and Caco-2 cells with IC_{50} values of 73.76 ± 2.54 μM and 73.15 ± 2.84 μM, respectively. This observation suggests that methyl group of the phenyl ring system may affect the antiproliferative activity to a certain

TABLE 1. Antiproliferative Activity (IC_{50} ± SD, μM)) of Compounds **3a–3h** and Cisplatin

Compound	A549	Caco-2	NIH3T3	SI (Caco-2)
3a	121.56 ± 4.08	470.18 ± 18.85	93.92 ± 4.14	0.20
3b	171.96 ± 6.52	347.00 ± 16.54	130.66 ± 6.28	0.38
3c	98.83 ± **3.71**	77.80 ± **3.19**	68.48 ± 2.99	0.88
3d	420.14 ± 17.42	435.16 ± 19.95	382.85 ± 16.65	0.88
3e	73.76 ± **2.54**	73.15 ± **2.84**	88.67 ± 4.11	**1.21**
3f	97.77 ± 3.98	166.42 ± 6.68	129.78 ± 6.21	0.78
3g	141.12 ± 6.18	172.05 ± 7.74	154.35 ± 6.99	0.90
3h	199.13 ± 8.76	400.15 ± 18.85	384.15 ± 17.78	0.96
Cisplatin	59.28 ± 1.72	**45.94** ± **2.17**	111.26 ± 4.86	**2.42**

extent. Furthermore, compound **3e** displayed anti-proliferative activity similar to cisplatin against NIH3T3. Compound **3c** displayed significant potency against A549 and Caco-2 cells with IC_{50} values of 98.83 ± 3.71 μM and 77.80 ± 3.19 μM, respectively. The effectiveness of synthesized compounds and cisplatin was compared in terms of their calculated selectivity index (SI) values. Compound **3e** exhibited selectivity similar to that of cisplatin. Compound **3e** was found to be the most valuable agent in the series studied in terms of the anticancer activity and toxicity.

3. EXPERIMENTAL

3.1. Chemistry

Chemicals and instruments. All reactants used in the syntheses were purchased from Merck Chemicals (Merck KGaA, Darmstadt, Germany) or Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA). Melting points of the synthesized compounds were determined by MP90 digital melting point apparatus (Mettler Toledo, Ohio, USA) and were uncorrected. ^{1}H NMR spectra were recorded by a Bruker 500 MHz digital FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO-d_6, respectively. The elemental compositions were recorded on a Leco CHNS-932 analyser (Leco, Michigan, USA). The IR spectra were obtained on a Shimadzu, IR Prestige-21 (Shimadzu, Tokyo, Japan). LC-MS-MS studies were performed on a Schimadzu, 8040 LC-MS-MS spectrophotometer (Shimadzu, Tokyo, Japan). The purity of compounds was checked by TLC on silica gel 60 F254 (Merck KGaA, Darmstadt, Germany).

Synthesis of sodium bisulfite addition products to aldehyde derivatives (1a–1h). The benzaldehyde derivative (0.02 mol, 2.1 g) was dissolved in ethanol (50 mL) and $Na_2S_2O_5$ (0.22 mol, 4.18 g) dissolved in water was added dropwise. When the drop was complete, the mixture was stirred at room temperature for 30 min in a magnetic stirrer. The precipitated product was filtered and dried.

Synthesis of 2-(4-substituted phenyl)-benzimidazole-5-carboxylic acid derivatives (2a–2h). Sodium bisulfite adduct (0.015 mol, 3.76 g) and 3,4-diamino benzoic acid (0.015 mol, 2.31 g) were dissolved in DMF (5 mL) with stirring. The reaction mixture was heated at 240°C and 10 bar for 3 min. After cooling, the mixture was poured into ice-cold water, the product was filtered, washed with water, dried, and recrystallized from EtOH.

Synthesis of 2-(2-(4-substituted phenyl) benzimidazol-5-yl) benzothiazole derivatives (3a-3h). 2-(4-Substituted phenyl)-benzimidazole-5-carboxylic acid (0.012 mol, 2.88 g) and 2-amino thiophenol polyphosphoric acid (0.012 mol, 1.52 g) were charged into a vial (30 mL) of microwave synthesis reactor (Anton-Paar, Monowave 300, Austria). The reaction mixture was heated under conditions of 250°C and 20 bar for 3 min. When the reaction was complete, the solution was cooled and basified with 10% NaOH solution. The precipitated product was separated by filtration and recrystallized from ethanol.

2-(2-Phenylbenzimidazole-5-yl) benzothiazole (3a). Yield: 65%; Mp, 165°C; FTIR (ATR, cm^{-1}): 3312 (N-H), 1626 – 1408 (C=C and C=N), 837 (1,4-disubstituted benzene); ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 7.43 – 8.30 (12 H, m, Ar-H), 13.25 (H, s, N-H); LC-MS (Es*) m/z*: M+1: 328.4; Anal. calcd. For $C_{20}H_{13}N_3S$ (%): C, 73.37; H, 4.00; N, 12.83; S, 9.79. Found (%): C, 73.70; H, 3.99; N, 12.80; S, 9.78.

2-(2-(4-Chlorophenyl)benzimidazole-5-yl) benzothiazole (3b). Yield: 72%; Mp, 238°C; FTIR (ATR, cm^{-1}): 3298 (N-H), 1626 – 1408 (C=C and C=N), 835 (1,4-disubstituted benzene); ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 7.68 – 8.24 (11 H, m, Ar-H), 13.45 (H, s, N-H); LC-MS (Es) *m/z*: M+1: 361.8; Anal. calcd. For $C_{20}H_{12}ClN_3S$ (%): C, 66.39; H, 3.34; N, 9.80; S, 11.61. Found (%): C, 66.23; H, 3.35; N, 9.78; S, 11.58.

2-(2-(4-Fluorophenyl)benzimidazole-5-yl) benzothiazole (3c). Yield: 70 %; Mp, 225°C; FTIR (ATR, cm^{-1}): 3306 (N-H), 1628 – 1431 (C=C and C=N), 839 (1,4-disubstituted

benzene); ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 7.38 – 8.31 (11 H, m, Ar-H), 13.45 (H, s, N-H); LC-MS (Es) *m/z*: M+1: 345.3; Anal. calcd. For $C_{20}H_{12}FN_3S$ (%): C, 69.55; H, 3.50; N, 12.17; S, 9.28. Found (%): C, 69.65; H, 3.49; N, 12.18; S, 9.30.

2-(2-(4-Methoxyphenyl)benzimidazole-5-yl) benzothiazole (3d). Yield: 37%; Mp, 288°C; FTIR (ATR, cm^{-1}): 3296 (N-H), 1632 – 1408 (C=C and C=N), 837 (1,4-disubstituted benzene); ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 3.59 (3H, s, OCH_3), 6.95 – 8.34 (11 H, m, Ar-H), 13.25 (H, s, N-H); LC-MS (Es) *m/z*: M+1: 357.3; Anal. calcd. For $C_{21}H_{15}N_3OS$ (%): C, 70.57; H, 4.23; N, 11.76; S, 8.97. Found (%): C, 70.81; H, 4.22; N, 11.79; S, 8.98.

2-(2-(4-Methylphenyl)benzimidazole-5-yl) benzothiazole (3e). Yield: 63%; Mp, 245°C; FTIR (ATR, cm^{-1}): 3273 (N-H), 1638 – 1412 (C=C and C=N), 806 (1,4-disubstituted benzene); ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 2.36 (3H, s, CH_3), 7.37 – 8.11 (11 H, m, Ar-H), 13.35 (H, s, N-H); LC-MS (Es) *m/z*: M+1: 341.4; Anal. calcd. For $C_{21}H_{15}N_3S$ (%): C, 73.87; H, 4.43; N, 12.31; S, 9.39. Found (%): C, 73.75; H, 4.44; N, 12.30; S, 9.38.

2-(2-(4-Ethoxyphenyl) benzimidazole-5-yl) benzothiazole (3f). Yield: 55%; Mp 280°C; FTIR (ATR, cm^{-1}): 3275 (N-H), 1632 – 1410 (C=C and C=N), 845 (1,4-disubstituted benzene); ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 1.32 (3H, t, CH_3), 4.01 (2H, q, OCH_2), 7.37 – 8.11 (11 H, m, Ar-H), 13.35 (H, s, N-H); LC-MS (Es) *m/z*: M+1: 371.3. Anal. calcd. For $C_{22}H_{17}N_3OS$ (%): C, 71.14; H, 4.61; N, 11.31; S, 8.63. Found (%): C, 71.25; H, 4.62; N, 11.29; S, 8.65.

2-(2-(4-Hydroxyphenyl) benzimidazole-5-yl) benzothiazole (3g). Yield: 45%; Mp, 347°C; FTIR (ATR, cm^{-1}): 3445 (O-H), 3275 (N-H), 1632 – 1410 (C=C and C=N), 804 (1,4-disubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 7.36 – 8.26 (11 H, m, Ar-H), 9.19 (1H, s, OH), 13.35 (H, s, N-H); LC-MS (Es) *m/z*: M+1: 343.5; Anal. calcd. For $C_{22}H_{13}N_3OS$ (%): C, 69.95; H, 3.82; N, 12.24; S, 9.34. Found (%): C, 70.07; H, 3.81; N, 12.22; S, 9.35.

2-(2-(4-Benzyloxyphenyl) benzimidazol-5-yl) benzothiazole (3h). Yield: 40%; Mp, 180°C; FTIR (ATR, cm^{-1}): 3290 (N-H), 1632 – 1410 (C=C and C=N), 832 (1,4-disubstituted benzene); ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 5.51 (2H, s, CH_2), 7.73 – 8.15 (16 H, m, Ar-H), 13.65 (H, s, N-H); LC-MS (Es) *m/z*: M+1: 343.5; Anal. calcd. For $C_{27}H_{19}N_3OS$ (%): C, 74.80; H, 4.42; N, 9.69; S, 7.40. Found (%): C, 74.72; H, 4.41; N, 9.67; S, 7.38.

3.2. Antiproliferative Activity Assay

The antiproliferative properties of compounds **3a-3h** were screened according to the MTT assay. The MTT assay was performed as previously described [19]. Cisplatin was used as a reference drug. A459 (ATCC CCL-185), Caco-2 (ATCC HTB-37) and NIH3T3 (ATCC CRL-1658) cell lines were used in the MTT assay.

In concluding, we have synthesized 8 original compounds carrying benzimidazole and benzothiazole ring systems in the common chemical structure. Structures of the obtained compounds were determined by ^{1}H NMR, IR, and mass spectroscopy methods and by elemental analysis data. In particular, the cytotoxic activity of compounds **3c** and **3e** carrying fluoro and methyl substituents in the variable group is promising.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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