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SYNTHESIS AND STUDY OF ANTITUMOR ACTIVITY OF SOME NEW 2-(4-METHOXYBENZYL)-1H-BENZIMIDAZOLE DERIVATIVES BEARING TRIAZOLE, OXADIAZOLE AND ETHANOL MOIETY

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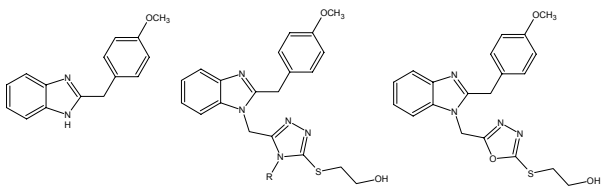
A series of novel 1,2-disubstituted benzimidazole derivatives containing hydrazinecarbothioamide (**7a-c**), 1,2,4-triazole (**8a-c**) and 1,3,4-oxadiazole (**5**) moiety were synthesized starting from 2-(4-methoxybenzyl)-1H-benzimidazole. Then, the compounds **8a-c**, **5** were converted to alkylated analogs (**9a-c**, **6**) in the presence of K_2CO_3 and 2-bromoethanol. The structures of all the synthesized compounds were elucidated by ^1H, ^{13}C NMR and mass spectroscopy. The newly synthesized compounds were tested on the five human cancer cells (breast (BT-20), melanoma (SK-Mel 128), prostate (DU-145), liver (SNU-398) andlung (A549)). The compound **7a** had some degree of cytotoxicity against BT-20.



INDRODUCTION

Cancer is the uncontrolled growth of cells and do not die. It's one of the leading health hazards which are affecting the world population. Large number of anticancer agents were used for treatment of different types of cancer. Due to the cytotoxic effects of these agents against normal cells, designing novel antitumor agents are of particular interest. Because of the structural similarities to adenine, guanine and the presence in the structure of vitamin B_{12}, benzimidazole nucleus is thought to easily show activity in living systems. Many studies has proven to be an valuable pharmacophore of the benzimidazole nucleus in medicinal chemistry.[1,2] Similarly, triazole, thiadiazole and oxadiazole rings are an important group of hetecylic compounds which have attracted considerable attention of medicinal chemist owing to their wide range of useful pharmacological activities, such as anticancer, anti proliferative, antibacterial, antifungal, anti-inflammatory, antimicrobial agents. [3-7]

Even though various chemical groups around the benzimidazole ring may be substituted at different seven positions, the literature survey showed that 1,2 and/or 5(or 6)-subtituted benzimidazole compounds have a wide range of biologically active such as antitumor; Bendamustine, antihistaminic; astemizole, antimicrobial; albendazole, antihypertensive; candesartan, antiinflamatuar; benoxaprofen analog, antiulcer; omeprazole.[1] In our previous studies, we synthesized some bioactive benzimidazole derivatives. Some of the compounds in these studies showed antitumor, antioxidant, antimicrobial and lipase inhibition activities.[2,7-9] As a continuous of our

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studies on synthesis and biological activity of benzimidazoles, we planned the synthesis of some new benzimidazole derivatives which have 4-methoxybenzyl functional group at 2-position and carbothioamide, oxadiazole, triazole functional groups at 1-position and also we have developed a new method for the synthesis of thioethanol derivatives (**6**, **9a-c**) not in the literature.

RESULTS AND DISCUSSION

In this study, we have synthesized some benzimidazole derivatives starting from iminoester-hydrochlorides and their antitumor activity have been investigated. The synthetic path for the target compounds is outlined in Scheme 1. The starting compound ethylimido-p-methoxyphenylacetate hydrochloride (**1**) was prepared according to the literature.[10] 2-(4-Methoxybenzyl)-1H-benzimidazole (**2**) was synthesized from the reaction of 1,2-phenylenediamine with compound **1**. Treatment of compound **2** with ethyl bromoacetate in dry acetone gave the ethyl [2-(4-methoxybenzyl)-1*H*-benzimidazol-1-yl]acetate **(3)**. Ester groups signals of the compound **3** were observed at 1.10 ppm ($-OCH_2C\underline{H}_3$), and 3.97 ppm ($-OC\underline{H}_2CH_3$) in the ^{1}H NMR spectrum. The carbon atoms of this group resonated at 14.33 and 61.54 ppm in the ^{13}C NMR spectrum. The compound **3** was converted by hydrazine hydrate to afford the desired hydrazine derivative **(4)**. In the ^{1}H NMR spectrum of compound **4**, new signals derived from hydrazide group appeared at 4.29 ppm ($-NHN\underline{H}_2$) and 9.48 ppm ($-N\underline{H}NH_2$) which proved by changing with D_2O.

With the conversion of compound **4** into 5-{[2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]methyl}-1,3,4-oxadiazole-2-thiol (5), the $-N\underline{H}N\underline{H}_2$ signals disappeared and the presence of –SH proton signal at 14.12 ppm in ^{1}H NMR spectrum confirmed the synthesis of compound **5**. Treatment of compound **4** with methylisothiocyanate, ethylisothiocyanate and phenylisothiocyanate resulted in their carbothioamide derivatives **7a-c**, recpectively. Due to carbothioamide moiety at the related chemical shift values, additional signals were observed in the ^{1}H and ^{13}C NMR spectrums of compounds **7a-c**. The compounds **7a-c** were converted to corresponding 1,2,4-triazole derivatives in the presence of 2N NaOH. The $-NHNH_2$ proton signals of compounds (**7a-c**) disappered; instead, -SH protons of compounds **8a-c** appeared at 13.44, 13.50, 13.79 ppm as singlet in the ^{1}H NMR spectrums. Reaction of 2-bromoethanol with 1,2,4-triazole and 1,3,4-oxadiazole derivatives in the presence of K_2CO_3 yielded the thioethanol analogs **6**, **9a-c**. The -OH protons of the compounds **6**, **9a-c** observed at 5.07 and 4.99 ppm as triplet, at 5.03 and 4.97 ppm as singlet in the ^{1}H NMR spectrums. And also the $–CH_2CH_2-$ proton and carbon singnals appeared at the related chemical shift values in the ^{1}H and ^{13}C NMR spectrums. In addition, the molecular ion peaks of the synthesized compounds were observed in the mass spectrums.

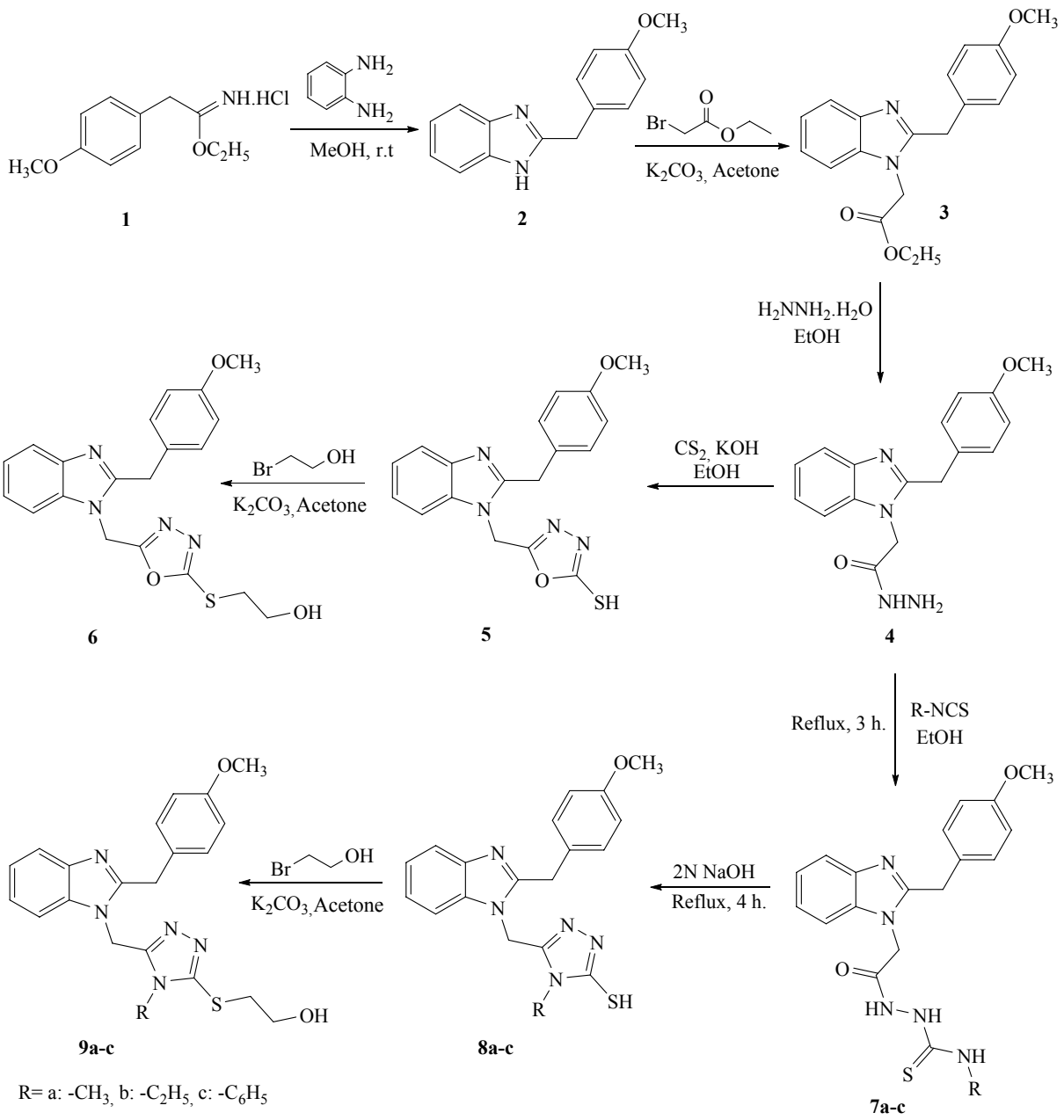
Antitumor activity results

The cytotoxicity test results of 14 compounds are shown in Table 1 and indicate that all compounds appear to have no strong growth inhibition effect on the human cancer cell lines tested. Compared to CC_{50} values of the anticancer drug cisplatin, only one the compound **7a** (2-{[2-(4-methoxybenzyl)-1*H*-benzimidazol-1-yl]acetyl}-*N*-ethylhydrazinecarbothioamide) had some degree of cytotoxicity against BT-20 cell.

Table 1

CC_{50}values of the synthesized compounds

Compound no	CC_{50}values (μM)				
	BT-20	DU-145	SNU-398	SK-MEL128	A549
2	171,0	154,2	126,2	163,7	154,2
3	123,9	157,8	94,4	127,4	158,1
4	170,6	169,4	161,8	192,3	161,1
5	195,9	175,8	171,0	193,2	166,7
6	164,4	153,1	123,0	121,1	157,4
7a	88,1	159,2	156,7	191,9	163,7
7b	167,5	164,4	133,7	173,4	165,6
7c	193,6	191,4	112,2	166,7	162,6
8a	192,3	147,6	159,2	154,2	154,9
8b	132,7	166,0	128,5	165,6	164,8
8c	196,8	191,9	104,5	154,2	154,9
9a	153,8	160,7	159,6	172,2	140,3
9b	134,6	138,0	130,9	132,4	152,4
9c	175,0	190,1	122,5	160,7	166,0
Cisplatin	4,5	6,0	12,9	16,9	44,9



Scheme 1 – The synthetic routes for the synthesized compounds.

EXPERIMENTAL

All the chemicals were obtained from commercial suppliers are used without further purification. Melting points were uncorrected and determined in open capillaries on a Büchi oil-heated melting point apparatus. Reactions are monitored by thin-layer chromatography (TLC) using precoated aluminum sheets (silica gel 60 F 2.54 0.2 mm thickness). The mobile phase was ethyl acetate and hexane (2:1 or 3:1) and detection was made using UV light. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian-Mercury 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer using DMSO-d_6 as solvent and TMS as internal standart. All chemical shifts are reported in ppm. The Mass spectra were obtained for the synthesized compounds on Thermo Scientific Quantum Access max LC-MS spectrometer. Elemental analyses were performed on a Carla Erba 1106 CHN analyser (Heraeus, Hanau, Germany); the experimental values were in agreement (± 0.4 %) with calculated ones.

Procedure for the preparation of 2-(4-methoxybenzyl)-1H-benzimidazole (2)

A mixture of 1,2-phenylenediamine (0.010 mol) and compound **1** (0.012 mol) in dry methanol (25 mL) was stirred at room temperature for 5 h. After the completion of the reaction (monitored by TLC, ethylacetate: hexane, 3: 1). The resulted reaction mixture was poured onto water. The precipitate formed was filtered off and recrystallized from ethanol–water (1: 3) to give pure product in 90 % yield (2.14 g), m.p. 162-163°C (Lit.[11] 56 % yield, mp: 165-165.5°C); ^{1}H NMR (*400 MHz, DMSO-d_6*) δ (ppm): 3.69 (s, 3H, -OCH_3), 4.07 (s, 2H, -NCH_2), 6.86 (d, 2H, ArH, J= 8.8 Hz), 7.08-7.10 (m, 4H, ArH), 7.43 (brs, 2H, ArH), 12.18 (s, 1H, NH). ^{13}C NMR (*100 MHz, DMSO-d_6*) δ (ppm): 34.54 (-CH_2-), 55.49

(-OCH_3), 114.33, 129.96, 130.22, 154.38, 158.41. LC-MS, m/z: 239.30 [M^++1]. Anal. Calcd. for $C_{15}H_{14}N_2O$: C, 75.61; H, 5.92; N, 11.76. Found: C, 75.65; H, 5.90; N, 11.73.

Procedure for the preparation of ethyl[2-(4-methoxybenzyl)-1*H*-benzimidazol-1-yl]acetate (3)

To a solution of compound **2** (0.010 mol) in acetone, K_2CO_3 (0.025 mol) was added and the mixture was stirred at room temperature for 1 h. Then, ethylbromoacetate (0.011 mol) was added and stirred at room temperature for 8 h. The reaction was monitored by TLC (ethylacetate: hexane, 3: 1). The resulted reaction mixture was poured onto water. The precipitate formed was filtered off and recrystallized from ethanol–water (1: 3) to give pure product in 85 % yield (2.75 g), m.p. 139-140°C (CAS Registry Number: 954518-81-1); ^{1}H NMR (*400 MHz, DMSO-d_6*) δ (ppm): 1.10 (t, 3H, -CH_3, J= 6.8 Hz), 3.69 (s, 3H, -OCH_3), 3.97 (q, 2H, -CH_2, J= 7.2 Hz), 4.16 (s, 2H, -CH_2-), 5.11 (s, 2H, -NCH_2-), 6.82-6.85 (m, 2H, ArH), 7.15- 7.18 (m, 4H, ArH), 7.40- 7.42 (m,1H, ArH), 7.55- 7.57 (m, 1H, ArH). ^{13}C NMR (*100 MHz, DMSO-d_6*) δ (ppm): 14.33 (-CH_3), 32.46 (-CH_2-), 44.92 (-NCH_2-), 55.46 (-OCH_3), 61.54 (-OCH_2-), 110.48, 114.25, 118.97, 121.98, 122.35, 128.66, 130.29, 136.15, 142.56, 154.49, 158.41, 168.17. LC-MS, m/z: 325.35 [M^++1]. Anal. Calcd. for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64. Found: C, 70.32; H, 6.23; N, 8.69.

Procedure for the preparation of 2-[2-(4-methoxybenzyl)-1*H*-benzimidazol-1-yl]acetohydrazide (4)

To a solution of compound **3** (0.010 mol) in ethanol (10 mL), hydrazine monohydrate (0.035 mol) was added and was stirred at room temperature for 2 h. The reaction was monitored by TLC (ethanol:ethylacetate, 2: 1). The resulted reaction mixture was filtered, dried and then recrystallized from ethanol–water (1: 2) to give pure product in 60 % yield (1.86 g), m.p. 167-168°C; ^{1}H NMR (*400 MHz, DMSO-d_6*) δ (ppm): 3.70 (s, 3H, -OCH_3), 4.16 (s, 2H, -CH_2-), 4.29 (s, 2H, -NH_2), 4.75 (s, 2H, -CH_2-), 6.83-6.86 (m, 2H, ArH), 7.13-7.20 (m, 4H, ArH), 7.37-7.40 (m, 1H, ArH), 7.52-7.54 (m, 1H, ArH), 9.48 (s, 1H, NH). ^{13}C NMR (*100 MHz, DMSO-d_6*) *δ* (ppm): 32.52 (-CH_2-), 44.85 (-NCH_2-), 55.47 (-OCH_3), 110.38, 114.28, 118.92, 121.83, 122.13, 129.05, 130.35, 136.05, 142.66, 154.87, 158.38, 166.37. LC-MS, m/z: 311.37 [M^++1]. Anal. Calcd. for $C_{17}H_{18}N_4O_2$: C, 65.79; H, 5.85; N, 18.05. Found: C, 65.83; H, 5.87; N, 18.01.

Procedure for the preparation of 5-{[2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]methyl}-1,3,4-oxadiazole-2-thiol (5)

To a solution of compound **4** (0.010 mol) in ethanol (20 mL), CS_2 (0.60 mL, 0.010 mol) and a solution of KOH (0.56 g, 0.010 mol) in 50 mL water were added, then the reaction mixture was refluxed for 3 h. The reaction mixture was cooled at room temperature and was acidified with cold dilute HCl (1:1). Then, the solid formed was filtered off, washed with plenty of water and dried. The product was recrystallized from ethanol–water (1: 1). Yield %70 (2.46 g), m.p. 204-206 °C; ^{1}H NMR (*400 MHz, DMSO-d_6*) *δ* (ppm): 3.69 (s, 3H, -OCH_3), 4.27 (s, 2H, -CH_2-), 5.66 (s, 2H, -NCH_2), 6.79 (d, 2H, ArH, J=8.4 Hz), 7.14 (d, 2H, ArH, J= 8.4 Hz), 7.18-7.25 (m, 4H, ArH). ^{13}C NMR (*100 MHz, DMSO-d_6*) δ (ppm): 33.09 (-CH_2-), 39.50 (-NCH_2-), 56.28 (-OCH_3), 111.56, 115.08, 119.76, 123.52, 123.86, 128.91, 131.05, 136.39, 155.23, 159.32, 160.12, 179.03. LC-MS, m/z: 353.37 [M^++1]. Anal. Calcd. for $C_{18}H_{16}N_4O_2S$: C, 61.35; H, 4.58; N, 15.90. Found: C, 61.30; H, 4.62; N, 15.95.

Procedure for the preparation of 2-[(5-{[2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]methyl}-1,3,4-oxadiazol-2-yl)thio]ethanol (6)

To a solution of compound **5** (0.010 mol) in acetone, K_2CO_3 (0.020 mol) was added and the mixture was stirred at room temperature for 30 min. Then, 2-bromoethanol (0.012 mol) was added and stirred at room temperature for 10 h. The reaction was monitored by TLC (ethylacetate). The K_2CO_3 was filtered off from the reaction mixture. After evaporating the solvent under reduced pressure, a solid appeared. The product was recrystallized with acetone. Yield % 60 (2.37 g), m.p. 154°C; ^{1}H NMR (*400 MHz, DMSO-d_6*) δ (ppm): 3.21 (t, 2H, -SCH_2-, J=5.6 Hz), 3.62 (q, 2H, -OCH_2-, J= 5.6 Hz), 3.69 (s, 3H, -OCH_3), 4.25 (s, 2H, -CH_2-), 5.07 (t, 1H, -OH, J=5.2 Hz), 5.76 (s, 2H, -NCH_2-), 6.79 (d, 2H, J= 8 Hz), 7.12-7.19 (m, 4H, ArH), 7.50 (d, 1H, ArH, J= 6.4 Hz), 7.58 (d, 1H, ArH, J= 6.4 Hz). ^{13}C NMR (*100 MHz, DMSO-d_6*) *δ* (ppm): 32.34 (-SCH_2-), 35.34 (-CH_2-), 38.36 (-NCH_2-), 55.44 (-OCH_3), 59.64 (-CH_2OH), 110.55, 114.23, 119.19, 122.38, 122.72, 128.47, 130.20, 135.61, 142.63, 154.33, 158.42, 163.29, 164.92. LC-MS, m/z: 397.33 [M^++1]. Anal. Calcd. for $C_{20}H_{20}N_4O_3S$: C, 60.59; H, 5.08; N, 14.13. Found: C, 60.55; H, 5.11; N, 14.09.

General method for the synthesis of the carbothioamide derivatives (7a-c)

To a solution of compound **4** (0.010 mol) in ethanol (25 mL), methylisothiocyanate (for **7a**), ethylisothiocyanate (for **7b**) or phenylisothiocyanate (for **7c**) was added. Then the reaction mixture was refluxed for 3 h. The reaction was monitored by TLC (ethylacetate; ethanol, 2: 1). The resulted reaction mixture was cooled at room temperature and was filtered, dried and then recrystallized from ethanol–water (1 : 2).

2-{[2-(4-Methoxybenzyl)-1H-benzimidazol-1-yl]acetyl}-N-ethylhydrazinecarbothioamide **(7a)**
Yield 84 % (3.22 g), m. p. 201-202°C; ^{1}H NMR (*400 MHz, DMSO-d_6*) δ(ppm): 2.88 (s, 3H, -NCH_3), 3.69 (s, 3H, -CH_3), 4.13 (s, 2H, -CH_2-), 4.80 (s, 2H, -NCH_2-), 6.82-6.87 (m, 2H, ArH), 7.12-7.19 (m, 4H, ArH), 7.40 (d, 1H, ArH, J=7.6 Hz), 7.53 (d, 1H, ArH, J= 8.0 Hz), 8.03 (s, 1H, -NH), 9.30 (s, 1H, -NH), 10.23 (s, 1H, -NH). ^{13}C NMR (*100 MHz, DMSO-d_6*) *δ* (ppm): 31.37 (-NCH_3), 32.49 (-CH_2-), 44.78 (-NCH_2-), 55.50 (-OCH_3), 110.48, 114.34, 118.95, 121.92, 122.20, 128.96, 130.32, 136.06, 142.62, 154.83, 158.42, 166.99, 175.25. LC-MS, m/z: 384.38 [M^++1]. Anal. Calcd. for $C_{19}H_{21}N_5O_2S$: C, 59.51; H, 5.52; N, 18.26. Found: C, 59.49; H, 5.55; N, 18.29.

N-Ethyl-2-{[2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]acetyl}hydrazine carbothioamide **(7b)**
Yield 87% (3.45 g), m.p. 211-212°C; ^{1}H NMR (*400 MHz, DMSO-d_6*) δ(ppm): 1.06 (t, 3H, -CH_3, J=6.8 Hz), 3.46 (brs, 2H, -CH_2-), 3.69 (s, 3H, -OCH_3), 4.14 (s, 2H, -CH_2-), 4.88 (s, 2H, -NCH_2-), 6.81-6.87 (m, 2H, ArH), 7.14-7.19 (m, 4H, ArH), 7.41 (d, 1H, ArH, J= 7.6 Hz), 7.53 (d, 1H, ArH, J= 6.8 Hz), 8.04 (s, 1H, -NH), 9.24 (s, 1H, -NH), 10.22 (s, 1H, -NH). ^{13}C NMR (*100 MHz, DMSO-d_6*) δ(ppm): 14.89 (-NCH_3), 32.48 (-CH_2-), 38.94 (-NCH_2-), 44.78 (-NCH_2-), 55.49 (-OCH_3), 110.43, 114.31, 118.89, 121.90, 122.15, 128.96, 130.34, 136.06, 142.65, 154.84, 158.41, 166.87, 170.47. LC-MS, m/z: 398.38 [M^++1]. Anal. Calcd. for $C_{20}H_{23}N_5O_2S$: C, 60.43; H, 5.83; N, 17.62. Found: C, 60.39; H, 5.85; N, 17.57.

2-{[2-(4-Methoxybenzyl)-1H-benzimidazol-1-yl]acetyl}-N-phenylhydrazine carbothioamide **(7c)**
Yield 76 % (3.38 g), m.p. 211-212°C; ^{1}H NMR (*400 MHz, DMSO-d_6*) δ(ppm): 3.70 (s, 3H, -OCH_3), 4.19 (s, 2H, -CH_2-),

4.97 (s, 2H, -NCH_2), 6.79-6.89 (m, 2H, ArH), 7.16-7.57 (m, 6H, ArH), 9.72 (s, 1H, -NH), 9.84 (s, 1H, -NH), 10.55 (s, 1H, -NH). ^{13}C NMR (*100 MHz, DMSO-d_6*) δ(ppm): 32.53 (-CH_2-), 44.88 (-NCH_2-), 55.50 (-OCH_3), 110.54, 114.35, 118.96, 121.97, 122.24, 128.71, 130.36, 136.10, 139.40, 142.62, 154.89, 155.04, 158.44, 167.03, 170.47. LC-MS, m/z:446.47 [M^++1]. Anal. Calcd. for $C_{24}H_{23}N_5O_2S$: C, 64.70; H, 5.20; N, 15.72. Found: C, 64.66; H, 5.24; N, 15.69.

General method for the synthesis of the 1,2,4-triazole derivatives (8a-c)

A solution of the corresponding carbothioamide derivatives (**7a-c**) (0.010 mol) in ethanol (15 mL) was refluxed in the presence of 2 N 15 mL NaOH for 4 h. Then, the resulted reaction mixture was cooled at room temperature and acidified to pH 4-5 with 37 % HCl. The separated product was filtered, washed with plenty of water and recrystallized from ethanol–water (1: 2).

5-{[2-(4-Methoxybenzyl)-1H-benzimidazol-1-yl]methyl}-4-methyl-4H-1,2,4-triazole-3-thiol **(8a)**

Yield 76 % (2.77 g), m.p. 244-245°C; ^{1}H NMR (*400 MHz, DMSO-d_6*) δ(ppm): 3.42 (s, 3H, -NCH_3), 3.69 (s, 3H, -OCH_3), 4.25 (s, 2H, -CH_2-), 5.65 (s, 2H, -NCH_2-), 6.78 (d, 2H, ArH, J= 8.8 Hz), 7.15-7.22 (m, 4H, ArH), 7.52-7.59 (m, 2H, ArH), 13.44 (s, 1H, -SH). ^{13}C NMR (*100 MHz, DMSO-d_6*) δ(ppm): 30.34 (-NCH_3), 32.06 (-CH_2-), 39.31-40.59 (DMSO-d_6+-NCH_2-), 55.48 (-OCH_3), 111.09, 114.19, 118.53, 122.76, 122.99, 128.09, 130.26, 135.55, 135.56, 148.41, 154.61, 158.49, 167.84. LC-MS, m/z: 366.32 [M^++1]. Anal. Calcd. for $C_{19}H_{19}N_5OS$: C, 62.44; H, 5.24; N, 19.16. Found: C, 62.41; H, 5.27; N, 19.20.

4-Ethyl-5-{[2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]methyl}-4H-1,2,4-triazole-3-thiol **(8b)**

Yield 75 % (2.85 g), m.p. 240-241°C; ^{1}H NMR (*400 MHz, DMSO-d_6*) δ(ppm): 1.036 (brs, 3H, -CH_3), 3.69 (s, 3H, -OCH_3), 3.92 (q, 2H, -CH_2-, J= 6.0 Hz), 4.21 (s, 2H, -CH_2-), 5.63 (s, 2H, -NCH_2-), 6.77 (d, 2H, ArH, J= 7.2 Hz), 7.11-7.17 (m, 4H, ArH), 7.46 (s, 1H, ArH), 7.58 (s, 1H, ArH), 13.50 (s, 1H, -SH). ^{13}C NMR (*100 MHz, DMSO-d_6*) *δ* (ppm): 13.53 (-CH_3), 32.46 (-CH_2-), 38.94 (-CH_2-), 39.34-40.59 (DMSO-d_6+-NCH_2-), 55.46 (-OCH_3), 110.63, 114.15, 119.13, 122.16, 122.53, 128.49, 130.17, 135.97, 142.61, 148.00, 154. 55, 158.40, 167.32. LC-MS, m/z: 380.39 [M^++1]. Anal. Calcd. for $C_{20}H_{21}N_5OS$: C, 63.30; H, 5.58; N, 18.46. Found: C, 63.35; H, 5.61; N, 18.49.

5-{[2-(4-Methoxybenzyl)-1H-benzimidazol-1-yl]methyl}-4-phenyl-4H-1,2,4-triazole-3-thiol **(8c)**

Yield 80 % (3.42 g), m.p. 227-228°C; ^{1}H NMR (*400 MHz, DMSO-d_6*) δ(ppm): 3.69 (s, 3H, -OCH_3), 3.89 (s, 2H, -CH_2^-), 5.26 (s, 2H, -NCH_2-), 6.78 (d, 1H, ArH, J= 8.4 Hz), 7.00 (d, 1H, ArH, J= 8.4 Hz), 7.12-7.35 (m, 4H, ArH), 7.51-7.53 (m, 2H, ArH), 13.79 (s, 1H, -SH); ^{13}C NMR (*100 MHz, DMSO-d_6*) δ(ppm): 32.24 (CH_2), 39.38 (NCH_2), 55.50 (OCH_3), 110.67, 114.24, 118.97, 122.06, 122.34, 128.39, 128.42, 129.96, 130.08, 130.20, 133.33, 135.79, 142.46, 147.89, 154.13, 158.38, 168.91. LC-MS, m/z: 428.41 [M^++1]. Anal. Calcd. for $C_{24}H_{21}N_5OS$: C, 67.42; H, 4.95; N;16.38. Found: C, 67.38; H, 4.94; N, 16.41.

General method for the synthesis of the thioethanol derivatives (9a-c)

A solution of corresponding compounds **8a-c** (0.010 mol) in acetone was stirred in the presence of K_2CO_3 at room temperature for 30 min. Then, 2-bromoethanol (0.012 mol) was added and stirred for 10 h. The reaction was monitored by TLC (ethylacetate). The K_2CO_3 was filtered off from the reaction mixture. After evaporating the solvent under reduced pressure, a solid appeared. The product was recrystallized with acetone.

2-[(5-{[2-(4-Methoxybenzyl)-1H-benzimidazol-1-yl]methyl}-4-methyl-4H-1,2,4-triazol-3-yl)thio]ethanol **(9a)** Yield 40 % (1.64 g), m.p. 180-181 °C; ^{1}H NMR (*400 MHz, DMSO-d_6*) δ(ppm): 3.12 (t, 2H, -SCH_2-, J= 6.4 Hz), 3.38 (s, 3H, -NCH_3), 3.58 (q, 2H, -OCH_2-, J= 6 Hz), 3.69 (s, 3H, -OCH_3), 4.21 (s, 2H, -CH_2-), 4.99 (t, 1H, -OH, J=5.2 Hz), 5.61 (s, 2H, -NCH_2-), 6.80 (d, 2H, ArH, J= 8.8 Hz), 7.12-7.17 (m, 4H, ArH), 7.45-7.47 (m, 1H, ArH), 7.45-7.47 (m, 1H, ArH). ^{13}C NMR (*100 MHz, DMSO-d_6*) δ(ppm): 30.60 (NCH_3), 32.52 (SCH_2), 35.90 (CH_2), 38.84 (NCH_2), 55.45 (OCH_3), 60.29 (OCH_2), 110.71, 114.18, 119.08, 122.03, 122.37, 128.77, 130.18 (2C), 135.92, 142.71, 151.07, 151.85, 154.68, 158.34. LC-MS, m/z: 410.46 [M^++1]. Anal. Calcd. for $C_{21}H_{23}N_5OS$: C, 61.59; H, 5.66; N;17.10. Found: C, 61.63; H, 5.69; N, 17.07.

2-[(4-Ethyl-5-{[2-(4-methoxybenzyl)-1H-benzimidazol-1-yl]methyl}-4H-1,2,4-triazol-3-yl)thio]ethanol **(9b)** Yield 35 % (1.48 g), m.p. 114-115 °C; ^{1}H NMR (*400 MHz, DMSO-d_6*) δ(ppm): 0.86 (t, 3H, -CH_3, J= 7.2 Hz), 3.18 (t, 2H, -SCH_2-, J= 6.4 Hz), 3.62 (brs, 2H, -OCH_2-), 3.69 (s, 3H, -OCH_3), 3.81 (q, 2H, -OCH_2-, J= 7.2 Hz), 4.21 (s, 2H, -CH_2-), 5.03 (brs, 1H, -OH), 5.66 (s, 2H, -NCH_2-), 6.80 (d, 1H, ArH, J= 8.4 Hz), 7.12 (d, 1H, ArH, J= 8.4 Hz), 7.16-7.19 (m, 4H, ArH), 7.46-7.48 (m, 1H, ArH), 7.56-7.59 (m, 1H, ArH). ^{13}C NMR (*100 MHz, DMSO-d_6*) δ(ppm): 15.86 (CH_3), 33.42 (SCH_2), 36.75 (CH_2), 39.72 (NCH_2), 40.12 (NCH_2), 56.38 (OCH_3), 61.22 (OCH_2), 111.61, 115.14, 120.01, 123.10, 123.46, 129.57, 131.18, 136.74 , 143.45, 151.75, 152.17, 155.57, 159.33. LC-MS, m/z: 424.7 [M^++1]. Anal. Calcd. for $C_{22}H_{25}N_5OS$: C, 62.39; H, 5.95; N;16.54. Found: C, 62.43; H, 5.91; N, 16.51.

2-[(5-{[2-(4-Methoxybenzyl)-1H-benzimidazol-1-yl]methyl}-4-phenyl-4H-1,2,4-triazol-3-yl)thio]ethanol **(9c)** Yield 38 % (1.79 g), m.p. 169-170 °C; ^{1}H NMR (*400 MHz, DMSO-d_6*) δ(ppm): 3.16 (brs, 2H, -SCH_2-), 3.59 (q, 2H, -OCH_2-, J= 5.2 Hz), 3.68 (s, 3H, -OCH_3), 3.79 (s, 2H, -CH_2-), 4.97 (s, 1H, -OH), 5.41 (s, 2H, -NCH_2-), 6.79 (d, 1H, ArH, J= 7.6 Hz), 6.99 (d, 1H, ArH, J= 7.2 Hz), 7.05-7.21 (m, 4H, ArH), 7.47 (d, 2H, ArH, J= 7.2 Hz). ^{13}C NMR (*100 MHz, DMSO-d_6*) δ(ppm): 32.19 (SCH_2), 35.10 (CH_2), 39.33 (NCH_2), 55.46 (OCH_3), 60.14 (OCH_2), 110.58, 114.24, 118.91, 121.93, 122.21, 127.45, 128.52, 130.15, 130.35, 130.68, 132.59, 135.55, 142.51, 151.55, 152.33, 154.01, 158.37. LC-MS, m/z: 472.55 [M^++1]. Anal. Calcd. for $C_{26}H_{25}N_5OS$: C, 66.22; H, 5.34; N,14.85. Found: C, 66.26; H, 5.31; N, 14.89.

Antitumor activity testing

Tumor cell growth inhibition in the presence of the compounds was performed essentially as described[12] using human cancer cell lines of breast (BT-20), melanoma (SK-Mel 128), prostate (DU-145), liver (SNU-398) and lung (A549). Briefly, $1X10^4$ viable cells from each cell line in RPMI-1640 growth medium supplemented with 10 % FBS were seeded in a 96-well plate and incubated for 24 h at 37 °C, % 5 CO_2 incubator. Next day, the growth medium was decanted and cells were incubated with serial dilutions of the compounds at 200, 100, 20, 4 ve 0.8 μM concentrations in triplicates (0.1 ml/well). For this the compounds were first dissolved in dimethyl sulphoxide (DMSO) and then diluted in the growth medium; the Cisplatin (Koçak Farma, Tekirdağ, Turkey) was used as the cell cytotoxicity control drug. After 48 h of incubation at 37 °C, the treated and untreated cells (negative controls) were incubated with 5 mg/ml MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) for

3 h at 37 °C for the living cells to metabolize and convert soluble MTT salt into formazan crystals purple in color. The medium was then replaced with 100 µl DMSO per well. After 30 minutes, the absorbance of developing color was determined by measuring the optical density (OD) at 570–630 nm using a multiwell spectrophotometer (Tecan Sunrise). The percent cell cytotoxicity for each concentration of the compound was calculated by the formula of [1- (Test OD/Control OD) ×100]. CC_{50} values which is the dose of drug producing a 50 % reduction in absorbance compared to the control were determined from the non-linear dose-response curves using the GraphPad software program.

CONCLUSIONS

In conclusion, we have synthesized a new series of 2-(4-methoxybenzyl)-1H-benzimidazole derivatives containing triazole, oxadiazole and ethanol moiety in good yields which were characterized by ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analysis. Also, the synthesized compounds were tested on the five human cancer cells. The antitumor resultings showed that the compound 7a had some degree of cytotoxicity against BT-20 cell.

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