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Synthesis and antitumor activity of 1-substituted-2-methyl-5-nitrobenzimidazoles

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Abstract—Different substituents were introduced in position 1 of 2-methyl-5(6)-nitro-1*H*-benzimidazole (**2**) in order to obtain different side chains having different heterocyclic compounds, for example, thiadiazoles (**5**–**7**), tetrazoles (**8**, **9a**, **b**), triazoles (**11**–**13**), thiazoles (**14a**–**e**), triazines (**10**, **16**, **17**), and imidazoles (**18a**–**c**). The antitumor effect of compounds **1**, **2**, **2a**, **4**, **5**, **7**, **8**, **9a**, **10**, **13**, **14a**, **15**, **16**, and **18c** was studied against breast cancer (MCF7) and compounds **2** [IC_{50} = 4.52 μg] and **7** [IC_{50} = 8.29 μg] were found to be active.

1. Introduction

Benzimidazole derivatives are of wide interest because of their diverse biological activity and clinical applications.[1–6] Pyrrolo[1,2-*a*]benzimidazoles (PBIs) (**I**, **II**, and **III**) represent a new class of antitumor agents exhibiting cytotoxic activity against a variety of cancer cell lines. The mechanism of cytotoxicity involves reductive alkylation of DNA accompanied by cleavage of G and A bases.[7–14] 8-Amino-2-methyl-4,5-dihydroimidazo[1,5,4-*de*]quinoxalin-9-one (**IV**) exhibits antitumor activity against melanoma and breast cancer.[15] An anticancer agent, [Hoechst-33342], 2′-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bis-1*H*-benzimidazole (**V**), has been demonstrated being an inhibitor of topoisomerase I.[16,17] The bis-benzimidazole dye [Hoechst-33258] (**VI**)[18,19] not only shows in vitro antitumor activity, but also acts as inhibitor of DNA topoisomerase I.[20] The benzimidazole derivatives **VII**, **VIII**, and **IX** were prepared by our laboratory group and found to be cytotoxic against non-small lung cancer and breast cancer.[21,22]

The antitumor effect of compounds **1**, **2**, **2a**, **4**, **5**, **7**, **8**, **9a**, **10**, **13**, **14a**, **15**, **16** and **18c** was performed in the National Cancer Institute, Cancer Biology Department, Cairo University against breast cancer (MCF7) of human cell line. Compounds **2** [IC_{50} = 4.52 μg] and **7** [IC_{50} = 8.29 μg] were found to be active (Graphs 1–3).

2. Results and discussion

2.1. Chemistry

Nitration of 2-methyl-1*H*-benzimidazole (**1**), widely investigated,[23,24] gave 2-methyl-5(6)-nitro-1*H*-benzimidazole (**2**). Substitution at position 1 of the imino hydrogen eliminates the possibility for tautomerism and a definite assignment of structure has to take place. Benzylation of compound **2** was performed by its reflux with benzyl chloride in acetone and potassium carbonate to yield 1-benzyl-2-methyl-5-nitro-1*H*-benzimidazole (**2a**). To find out the position of attachment of the NO_2 group, NOE difference experiment has been performed for compound 1-benzyl-2-methyl-5-nitro-1*H*-benzimidazole (**2a**). The received spectrum showed the disappearance of H4 and H6 with the recognition of the H7 resonance at δ (ppm) 7.61 together with the phenyl protons, multiplet at δ (ppm) 7.13, thus proving that the NO_2 group is attached at position 5 of the benzimidazole ring (Fig. 1).

Keywords: Benzimidazole; Thiazoles; Triazoles; Imidazoles; Antitumor activity.
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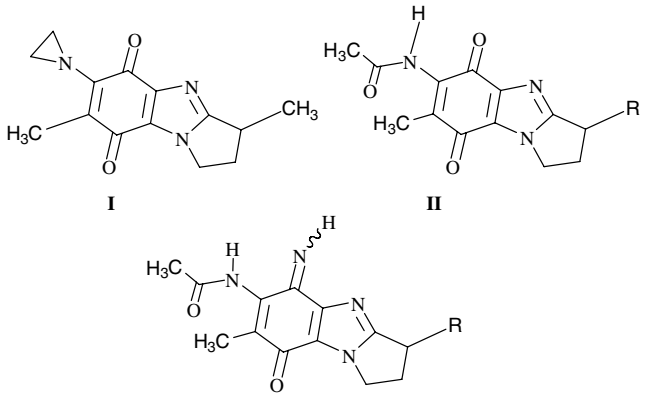
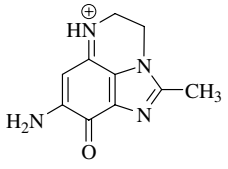


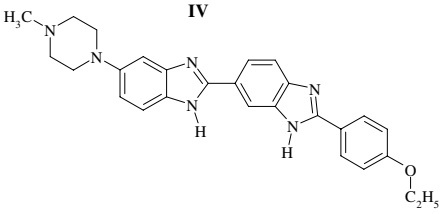
I II

III

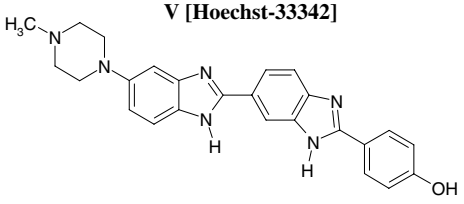
R=H, OH, OAc, carbamate, chloroacetate, propionate, benzoate, valerate, methoxyacetate



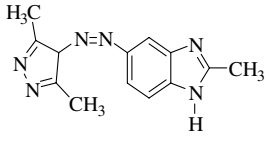
IV



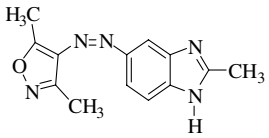
V [Hoechst-33342]



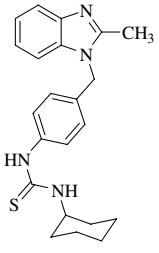
VI [Hoechst-33258]



VII

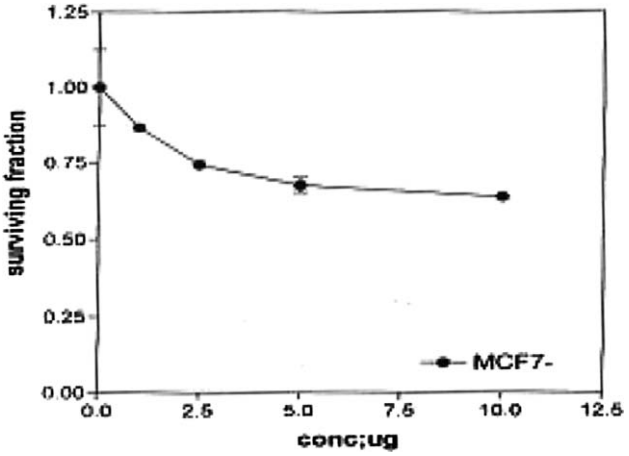


VIII

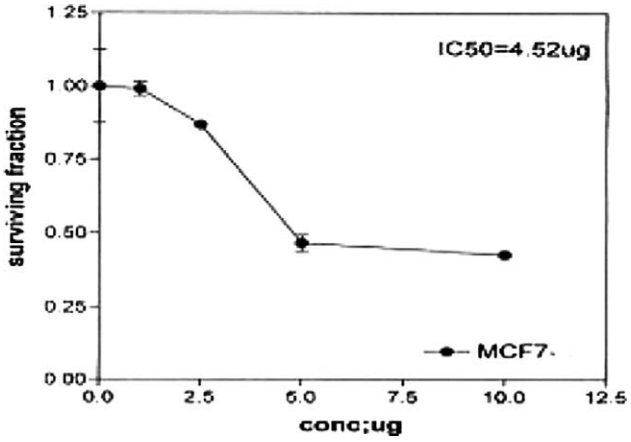


IX

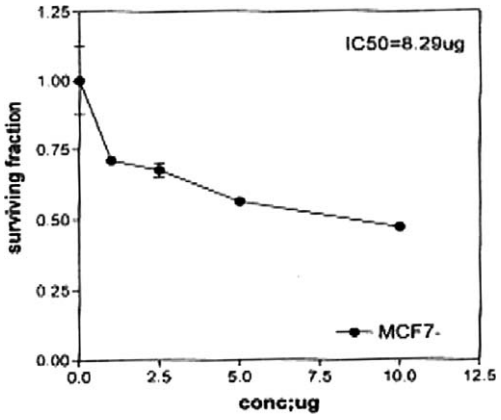
Compound **2** was cyanomethylated by chloroacetonitrile and sodium hydride in *N*,*N*-dimethylformamide to give 2-(2-methyl-5-nitro-1*H*-benzimidazol-1-yl)acetonitrile (**3**). Addition of hydrazine hydrate to the cyano group of compound **3** afforded the amidrazone derivative **4**, which was treated with carbon disulfide in methanol to give 1,3,4-thiadiazol-2-thione **5**. Substitution at



Graph 1. Cytotoxic activity of compound **1**.



Graph 2. Cytotoxic activity of compound **2**.



Graph 3. Cytotoxic activity of compound **7**.

the thiol group of compound **5** was performed by its reaction with ethyl bromoacetate or chloroacetone in *N*,*N*-dimethylformamide and sodium hydride to yield **6** and **7**, respectively (Scheme 1). The tetrazole derivative **8** was produced when compound **3** reacted with sodium azide in the presence of ammonium chloride in *N*,*N*-dimethylformamide. Introduction of methanesulfonyl and toluene-4-sulfonyl groups to the NH group of the tetrazole ring of compound **8** was achieved by its reaction

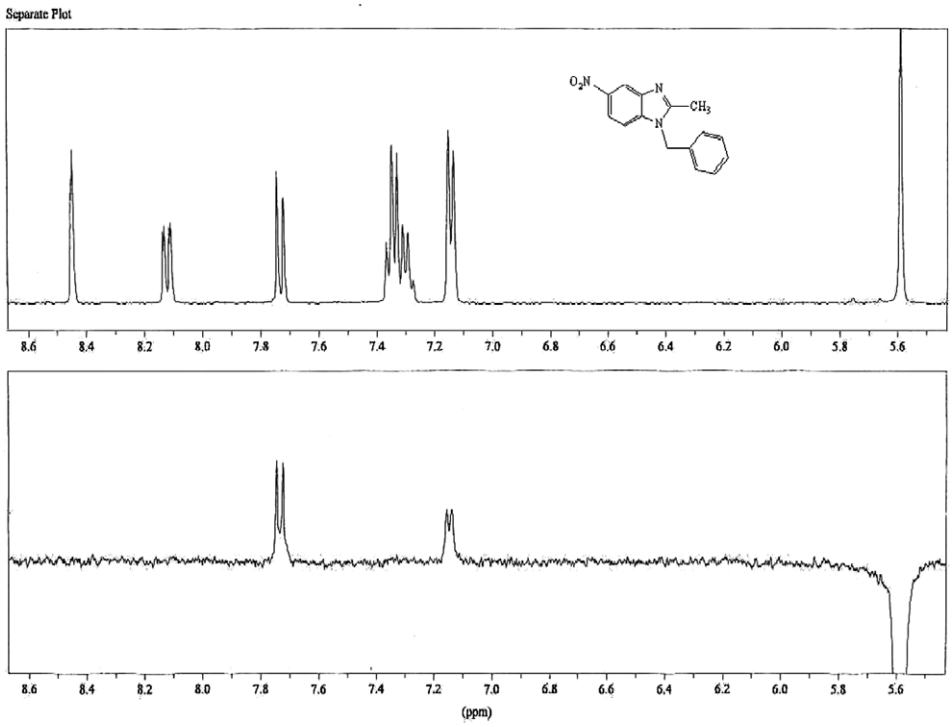
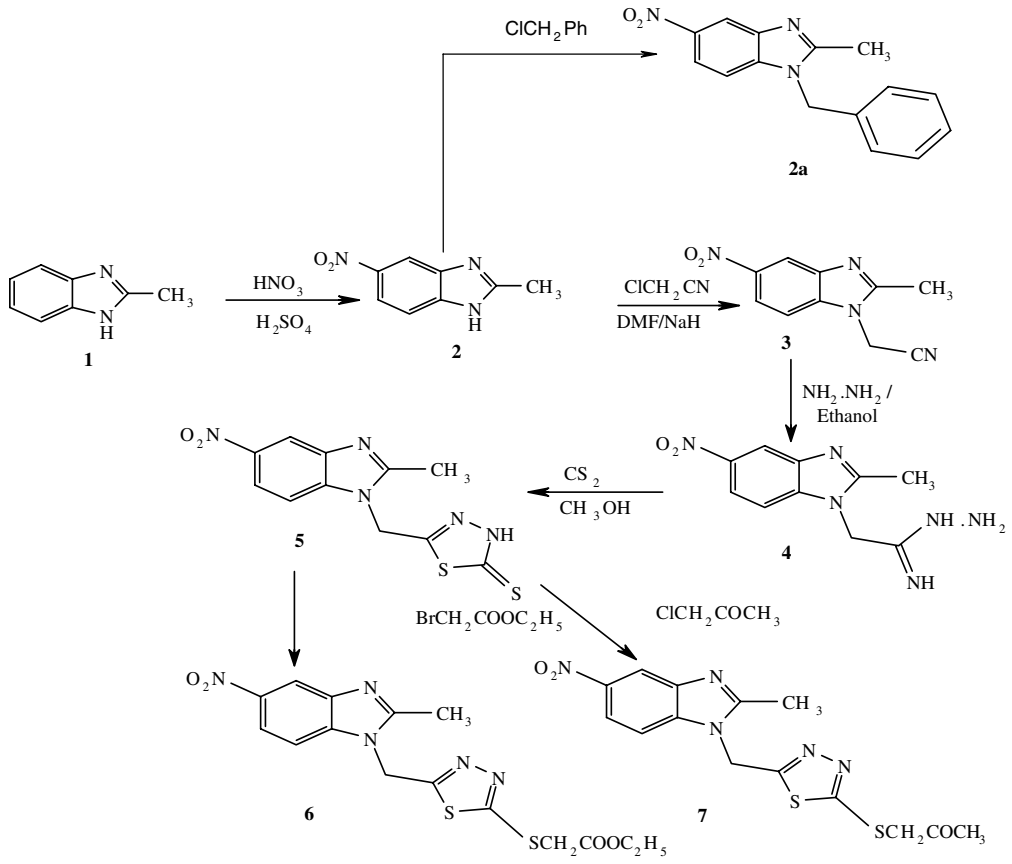


Figure 1. NOE spectrum of 1-benzyl-2-methyl-5-nitro-1*H*-benzimidazole (**2a**).



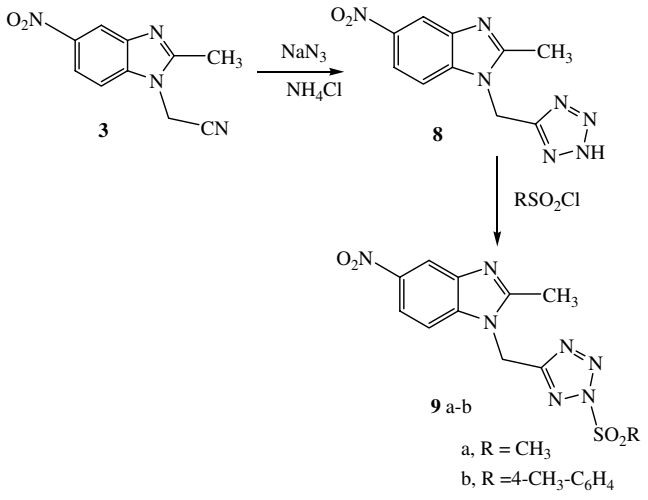
Scheme 1.

with methanesulfonyl chloride or *p*-toluenesulfonyl chloride in the presence of sodium hydride in *N*,*N*-dimethylformamide to afford **9a** and **9b**, respectively (Scheme 2). Cyclization of the amidrazone derivative **4** was performed by its reflux with ethyl bromoacetate, trimethyl orthoformate, thionyl chloride or ethyl chloroformate in ethanol and sodium ethoxide to obtain 1,2,4-triazin-5-one derivative **10**, 1,2,4-triazole derivative **11**, 1,2,3,5-thiatriazole derivative **12**, and 1,2,4-triazol-3-one derivative **13**, respectively (Scheme 3).

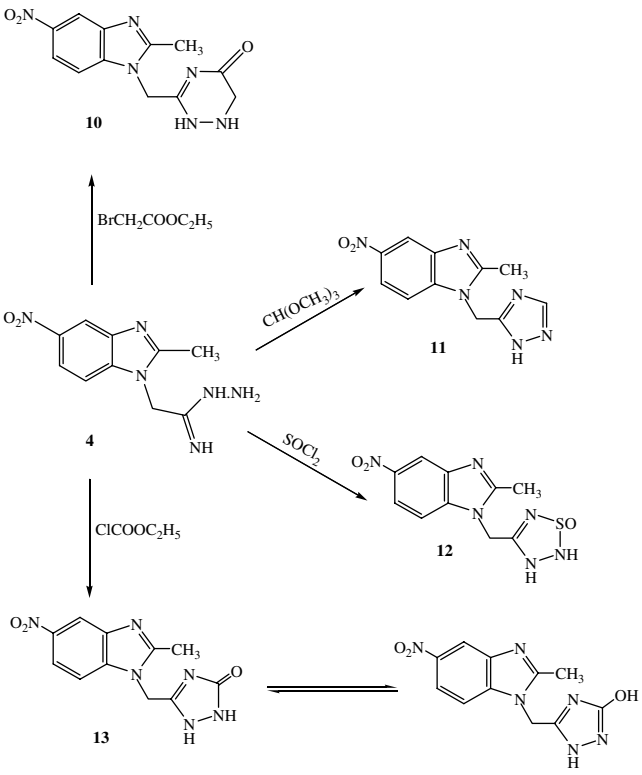
Substitution at position 1 of the benzimidazole ring by a substituted thiazol-2-thione ring could be performed by the reaction of compound **3** with sulfur and (methyl, ethyl, phenyl, benzoyl or *p*-methoxyphenyl isothiocyanates) yielded compounds **14a–e**, respectively (Scheme 4). 2-Chloro-1-(2-methyl-5-nitro-1*H*-benzimidazol-1-yl)ethanone (**15**) was synthesized in an excellent yield by substitution on the imino hydrogen at position 1 of compound **2** by its reaction with chloroacetyl chloride in acetone and anhydrous potassium carbonate. Different five- and six-membered heterocyclic rings substituting position 1 in compound **15** were synthesized by its reaction with thiosemicarbazide, semicarbazide, urea, thiourea or guanidine hydrochloride to produce 1,2,4-triazin-3-thione derivative **16**, 1,2,4-triazin-3-one derivative **17**, imidazol-2-one derivative **18a**, imidazolthione derivative **18b**, imidazol-2-imine derivative **18c**, respectively (Scheme 5). The structures of various compounds synthesized were assigned on the basis of their spectral data (^{1}H NMR, ^{13}C NMR, IR, and mass spectra) and microanalyses, which are given in the experimental part.

2.2. Cytotoxic activity

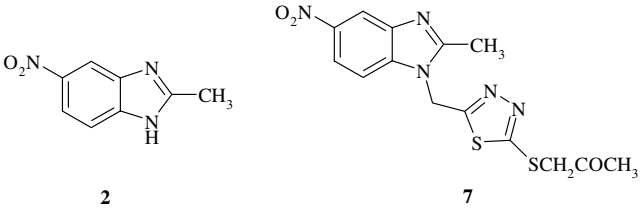
As a continuation to our previous work, El-Naem et al.,[21,22] we had for aim in this manuscript to study the effect of substitution at position 1 on the antitumor activity of benzimidazole derivatives, the antitumor effect of compounds **1**, **2**, **2a**, **4**, **5**, **7**, **8**, **9a**, **10**, **13**, **14a**, **15**, **16**, and **18c** was performed in the National Cancer Institute, Cancer Biology Department, Cairo University, against breast cancer (MCF7) of human cell line. Compounds **2** [IC_{50} = 4.52 μg] and **7** [IC_{50} = 8.29 μg] were found to be active.



Scheme 2.



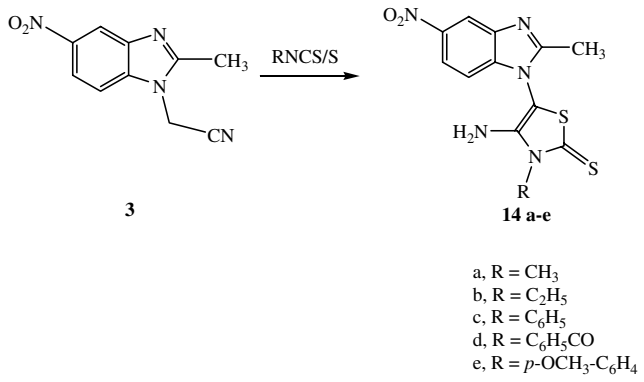
Scheme 3.



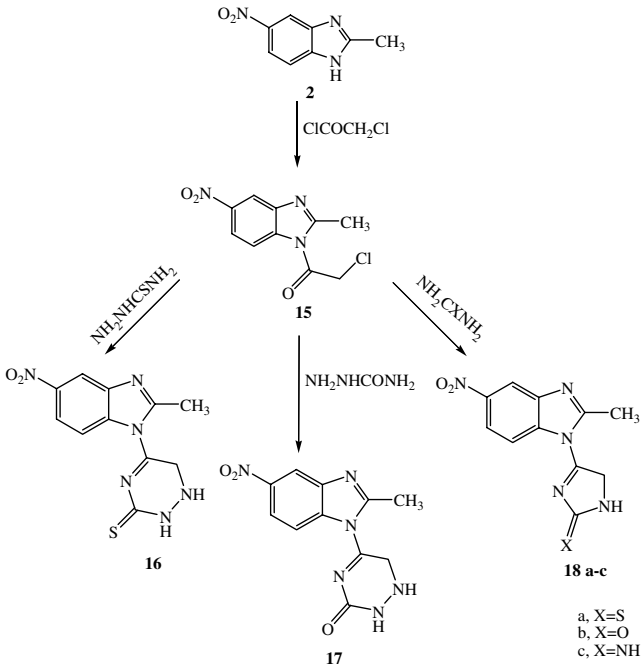
3. Conclusion

By examining the activity of the compounds, we can deduce three main conclusions:

(1) Substitution at position 5 by NO_2 group increases the activity as shown from the higher activity of compound **2** than compound **1**.
(2) The presence of the free NH group of the benzimidazole ring increases the activity as compound **2** is the most active one among the tested compounds.
(3) Among the 1-substituted-5-nitrobenzimidazoles, substitution by a thiadiazole ring at position 1 of the benzimidazole through a methylene group and having 2-propanoyl group at position 2 increases the activity as compound **7** was the most active one, especially more active than the unsubstituted thaidiazole derivative **5**.



Scheme 4.



Scheme 5.

4. Experimental

Melting points were taken on a capillary melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were measured in DMSO-d_6 on JEOL-300 spectrometer with Me_4Si as an internal standard. Mass spectra were obtained with a Schimadzu GCS-QP 1000EX spectrometer at 70 ev. The IR spectra were recorded with a Philips Infracord Spectro-photometer Model PU9712 in KBr discs. Elemental analyses were performed at the Microanalytical Laboratory of the National Research Centre.

2-Methyl-1*H*-benzimidazole (**1**) was obtained by refluxing *o*-phenylene diamine with acetic acid in 1:1 molar ratio in 4N hydrochloric acid.[24] 2-Methyl-5-nitro-1*H*-benzimidazole (**2**) was prepared according to Bapat et al.'s method.[24]

4.1. 1-Benzyl-2-methyl-5-nitro-1*H*-benzimidazole (2a)

To a solution of compound **2** (10 g, 56.5 mmol) and anhydrous potassium carbonate (7.8 g, 56.5 mmol) in acetone (30 ml), benzyl chloride (6.5 ml, 56.5 mmol) was added dropwise. The mixture was stirred at room temperature for about 8 h. The mixture was then poured onto water and extracted with ethyl acetate, dried over sodium sulfate anhydrous, and concentrated under vacuum to give pure compound **3** as a yellow solid. R_f 0.75 (petroleum ether/ethyl acetate/methanol, 1:1:1/2). Yield: 2.59 (s, 3H, CH_3) δ 13.5 g (89%); mp 152–154 °C;^{1}H NMR (300 MHz, DMSO-d_6), 5.58 (s, 2H, CH_2), 7.13 (dd, J_1 = 9 Hz, J_2 = 1.8 Hz, 2H, H′2 + H′6), 7.45 (m, 3H, H′3 + H′4 + H′5), 7.61 (d, J = 9 Hz, 1H, H7), 8.22 (dd, J_{6-7} = 9 Hz, J_{6-4} = 1.8 Hz, 1H, H6), 15.01 (CH_3), 47.8 (CH_2), 111.9–129.9 δ 8.44 (s, 1H, H4); ^{13}C NMR (DMSO-d_6) (Ar.C), 143.3 (N–C–N), 157.5 (C=N); IR (cm^{-1}): 1618 (C–N), 1518–1343 (NO_2); Anal. Calcd for $C_{15}H_{13}N_3O_2$: C, 67.40; H, 4.90; N, 14.72. Found: C, 67.35; H, 4.85; N, 15.87.

4.2. 2-(2-Methyl-5-nitro-1H-benzimidazol-1-yl)acetonitrile (3)

To a solution of compound **2** (10 g, 56.5 mmol) and sodium hydride (1.5 g, 56.6 mmol) in (30 ml) *N*,*N*-dimethylformamide, chloroacetonitrile (3.55 ml, 56.6 mmol) was added dropwise and the reaction mixture was stirred for 8 h at room temperature, then poured onto ice water. The obtained precipitate was filtered off, dried, and recrystallized from ethanol as a gray powder. R_f = 0.86 (petroleum ether/ethyl acetate, 1:1). Yield: 10.5 g (85%); mp 185 °C;^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H, CH_3), 5.60 (s, 2H, CH_2), 7.61 (d, J = 9 Hz, 1H, H7), 8.02 (d, J = 9 Hz, 1H, H6), 8.34 (s, 1H, H4); IR (cm^{-1}): 2245 (C≡N), 1662 (C=N), 1499, 1380 (NO_2); Anal. Calcd for $C_8H_8N_4O_2$ (216): C, 55.55; H, 3.73; N, 25.91. Found: C, 55.60; H, 3.81; N, 25.86.

4.3. 1-Hydrazinyl-2-(methyl-5-nitro-1*H*-benzimidazol-1-yl)ethenamine (4)

To a solution of compound **3** (10 g, 46.3 mmol) in ethanol (8.3 ml, 138 mmol), hydrazine hydrate (99%) was added. The mixture was refluxed for 5 h and the solvent was removed under reduced pressure. The precipitate was crystallized from ethanol as a yellow solid. R_f 0.25 (petroleum ether/ethyl acetate/methanol, 1:1:1/2). Yield: 11 g (78%); mp 195–197 °C; ^{1}H NMR (DMSO-d_6) δ 2.49 (s, 3H, CH_3), 3.18 (br s, 2H, NH_2), 4.60 (s, 2H, CH_2), 7.63 (d, J = 9 Hz, 1H, H7), 7.99 (d, J = 9 Hz, 1H, H6), 8.34 (s, 1H, H4); IR (cm^{-1}): 3387, 3295 (NH_2), 3226 (NH), 1619, 1559 (C=N groups), 1518, 1335 (NO_2) ; Anal. Calcd for $C_{10}H_{12}N_6O_2$ (248): C, 48.38; H, 4.87; N, 33.85. Found: C, 48.27; H, 4.80; N, 33.79.

4.4. 5-[(2-Methyl-5-nitro-1*H*-benzimidazol-1-yl)methyl]1,3,4-thiadiazol-2(3*H*)-thione (5)

To a solution of compound **7** (2 g, 8 mmol) in methanol (30 ml), (5 ml, 16 mmol) of carbon disulfide was added.

The mixture was refluxed for 5 h, the solvent was removed under reduced pressure. The precipitate was crystallized from methanol as a green powder. $R_f = 0.65$ (petroleum ether/ethyl acetate/methanol, 1:1:1/2). Yield: 2 g (80%); mp 235 °C; ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H, CH_3), 5.80 (s, 2H, CH_2), 7.80 (d, $J = 9$ Hz, 1H, H7), 8.20 (dd, $J_{6-7} = 9$ Hz, $J_{6-4} = 1.8$ Hz, 1H, H6), 8.42 (s, 1H, H4); ^{13}C NMR (DMSO-d_6) δ 15.1 (CH_3), 43.2 (CH_2), 111–118.9 (Ar.C), 145.5 (C=N), 193.1 (C=S); IR (cm^{-1}): 3424 (NH), 1619, 1598 (C=N groups), 1522, 1332 (NO_2), 1280 (C=S) ; Anal. Calcd for $C_{11}H_9N_5O_2S_2$ (307): C, 42.95; H, 2.91; N, 22.83; S, 20.91. Found: C, 42.89; H, 2.99; N, 22.79; S, 20.87.

4.5. General procedure for the preparation of the compounds 6 and 7

To a stirred solution of compound **4** (2 g, 6.5 mmol) and sodium hydride (0.4 g, 6.5 mmol) in *N*,*N*-dimethylformamide (20 ml), ethyl bromoacetate or chloroacetone (6.5 mmol) was slowly added dropwise. The mixture was stirred at room temperature for 8 h, the mixture was then poured onto ice water, and the resulting precipitate was collected by filtration and recrystallized from appropriate solvent.

4.6. Ethyl-2-[5-((2-methyl-5-nitro-1*H*-benzimidazol-1-yl)methyl)-1,3,4-thiadiazol-2-yl-thio]acetate (6)

Crystallized from chloroform as a green powder. $R_f = 0.83$ (petroleum ether/ethyl acetate/methanol, 2:1:1/2). Yield: 2.8 g (84%); mp 252–253 °C; ^{1}H NMR (DMSO-d_6) δ 1.33 (t, $J = 6.9$ Hz, 3H, CH_3), 2.44 (s, 3H, CH_3), 3.84 (s, 2H, CH_2), 4.56 (q, $J = 6.9$ Hz, 2H, CH_2), 5.76 (s, 2H, CH_2), 7.60 (d, $J = 9$ Hz, 1H, H7), 8.06 (d, $J = 9$ Hz, 1H, H6), 8.34 (s, 1H, H4) ; Anal. Calcd for $C_{15}H_{15}N_5O_4S_2$ (393): C, 45.79; H, 3.84; N, 17.80; O, 16.27; S, 16.30. Found: C, 45.61; H, 3.91; N, 17.98; S, 16.38.

4.7. 1-[5-((2-Methyl-5-nitro-1*H*-benzimidazol-1-yl)methyl)-1,3,4-thiadiazol-2-ylthio]propan-2-one (7)

Crystallized from chloroform/methanol (2:5) as a yellow solid. R_f 0.71 (petroleum ether/ethyl acetate/methanol, 2:1:1/2). Yield: 2.7 g (87%); mp 202–204 °C; ^{1}H NMR (DMSO-d_6) δ 2.08 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 4.61 (s, 2H, CH_2), 5.71 (s, 2H, CH_2), 7.63 (d, $J = 9$ Hz, 1H, H7), 8.06 (d, $J = 9$ Hz, 1H, H6), 8.34 (s, 1H, H4); Anal. Calcd for $C_{14}H_{13}N_5O_3S_2$ (363): C, 46.27; H, 3.61; N, 19.27; S, 17.65. Found: C, 46.19; H, 3.58; N, 19.15; S, 17.55.

4.8. 1-[(2*H*-Tetrazol-5-yl)methyl]-2-methyl-5-nitro-1*H*-benzimidazole (8)

A mixture of compound **3** (4 g, 18.5 mmol), sodium azide (1.2 g, 18.5 mmol), and ammonium chloride (0.98 g, 18.5 mmol) in *N*,*N*-dimethylformamide (10 ml) was refluxed for 7 h at 125 °C. The solvent was removed under reduced pressure, the residue was dissolved in (100 ml) water and carefully acidified with concn. hydrochloric acid to pH 2. The solution was cooled to 5 °C in ice bath to precipitate **8**. Compound **8** recrystallized from aqueous methanol as white crystals. $R_f = 0.15$ (petroleum ether/ethyl acetate/methanol, 1:1:1/2). Yield: 4.4 g (75%); mp 251–252 °C; ^{1}H NMR (DMSO-d_6) δ 2.65 (s, 3H, CH_3), 4.50 (br s,1H, NH), 5.95 (s, 2H, CH_2), 7.77 (d, $J = 9$ Hz, 1H, H7), 8.27 (dd, $J_{6-7} = 9$ Hz, $J_{6-4} = 1.8$ Hz, 1H, H6), 8.43 (s, 1H, H4); ^{13}C NMR (DMSO-d_6) δ 15.01 (CH_3), 38.5 (CH_2) 111.9–112.3 (Ar.C), 141–144.01 (C–N), 155.3, 158.7 (C=N groups). IR (cm^{-1}): 3429 (NH), 1621, 1598 (C=N groups), 1519–1336 (NO_2); MS: *m*/*z* 259, 60.9% (M^+); *m*/*z* 190, 100% (M^+-tetrazole ring); *m*/*z* 144, 91.8% [M^+-(tetrazole ring + NO_2)]; Anal. Calcd for $C_{10}H_9N_7O_2$(259): C, 46.33; H, 3.50; N, 37.82. Found: C, 46.41; H, 3.59; N, 37.78.

4.9. General procedure for the preparation of the compounds 9a–b

To a stirred solution of compound **8** (1.5 g, 5.8 mmol) and sodium hydride (0.14 g, 5.8 mmol) in *N*,*N*-dimethylformamide (20 ml), methanesulfonyl chloride or *p*-toluenesulfonyl chloride (5.8 mmol) was slowly added dropwise. The mixture was stirred at room temperature for 8 h then refluxed on a water bath for 3 h. After cooling, the mixture was then poured onto ice water and the resulting precipitate was collected by filtration and recrystallized from appropriate solvent to obtain **9a**–**b**, respectively.

4.10. 1-(2-Methanesulfonyl-2*H*-tetrazol-5-ylmethyl)-2-methyl-5-nitro-1*H*-benzimidazole (9a)

Crystallized from methanol/water (5:1) as a yellow powder. $R_f = 0.55$ (petroleum ether/ethyl acetate/methanol, 1:1:1/2). Yield: 1.5 g (76%); mp 181–183 °C; ^{1}H NMR (DMSO-d_6) δ 2.44 (s, 3H, CH_3), 3.35 (s, 3 H, CH_3), 5.76 (s, 2H, CH_2), 7.60 (d, $J = 9.1$ Hz, 1H, H7), 8.05 (d, $J = 9.1$ Hz, 1H, H6), 8.35 (s, 1H, H4); Anal. Calcd for $C_{11}H_{11}N_7O_4S$ (337): C, 39.17; H, 3.29; N, 29.07; S, 9.51. Found: C, 39.21; H, 3.31; N, 29.10; S, 9.48.

4.11. 2-Methyl-5-nitro-1-[1-(toluene-4-sulfonyl)-2*H*-tetrazol-5-ylmethyl]-1H- benzimid-azole (9b)

Crystallized from chloroform as gray crystals. $R_f = 0.45$ (petroleum ether/ethyl acetate/methanol, 1:1:1/2). Yield: 1.7 g (70%); mp 219–221 °C; ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H, CH_3), 3.35 (s, 3H, CH_3), 5.49 (s, 2H, CH_2), 7.13 (d, $J = 7.6$ Hz, 2H, H′2 + H′6), 7.29 (d, $J = 7.6$ Hz, 2H, H′3 + H′5), 7.92 (d, $J = 9$ Hz, 1H, H7), 8.16 (d, $J = 9$ Hz, 1H, H6), 8.54 (s, 1H, H4); Anal. Calcd for $C_{17}H_{15}N_7O_4S$ (413): C, 49.34; H, 3.66; N, 23.73; S, 7.76. Found: C, 49.29; H, 3.70; N, 23.67; S, 7.61.

4.12. General procedure for the preparation of the compounds 10, 11, 12, and 13

A solution of compound **4** (2 g, 8.06 mmol) in ethanol was treated with sodium ethoxide (2 g, 80.6 mmol sodium metal in 10 ml ethanol) and (8.06 mmol) of ethyl bromoacetate, trimethyl orthoformate, thionyl chloride

or ethyl chloroformate. The mixture was heated on a water bath for 6 h. The mixture was diluted with water and extracted with ethyl acetate, the organic layers were dried over anhydrous sodium sulfate and the ethyl acetate was removed under reduced pressure and recrystallized from appropriate solvent.

4.13. 1,2-Dihydro-3-[(2-methyl-5-nitro-1*H*-benzimidazol-1-yl)methyl]-1,2,4-triazin-5(6*H*)-one (10)

Crystallized from acetone as a yellow solid. R_f 0.35 (petroleum ether/ethyl acetate/methanol, 1:1:1/2). Yield: 1.7 g (73%); mp 171–173 °C; ^{1}H NMR (DMSO-d_6) δ 2.49 (s, 3H, CH_3), 3.36 (s, 2H, CH_2), 4.49 (s, 2H, CH_2), 7.62 (d, J = 9 Hz, 1H, H7), 8.04 (d, J = 9 Hz, 1H, H6), 8.35 (s, 1H, H4); IR (cm^{-1}): 3332, 3219 (NH groups), 1662 (C=O), 1627–1594 (C=N groups), 1517, 1390 (NO_2); Anal. Calcd for $C_{12}H_{12}N_6O_3$ (288): C, 50.00; H, 4.20; N, 29.15. Found: C, 49.97; H, 4.11; N, 29.26.

4.14. 2-Methyl-5-nitro-1-(2H-1,2,4-triazol-3-ylmethyl)-1H-benzimidazole (11)

Crystallized from dichloromethane as a brown solid. R_f 0.38 (petroleum ether/ethyl acetate/methanol, 1:1:1/2). Yield: 1.8 g (86%); mp 123–126 °C; ^{1}H NMR (DMSO-d_6) δ 2.49 (s, 3H, CH_3), 4.52 (s, 2H, CH_2), 7.14 (s, 1H, CH=N), 7.62 (d, J = 9 Hz, 1H, H7), 8.04 (d, J = 9 Hz, 1H, H6), 8.35 (s, 1H, H4); IR (cm^{-1}): 3422 (NH), 1617, 1597 (C=N groups), 1523, 1339 (NO_2); Anal. Calcd for $C_{12}H_{13}N_6O_2$ (273): C, 52.74; H, 4.79; N, 30.75. Found: C, 52.89; H, 4.60; N, 30.61.

4.15. 2-Methyl-5-nitro-1-(1-oxo-2,3-dihydro-1,2,3,5-thiatriazol-4-ylmethyl)-1*H*- benz-imidazole (12)

Crystallized from dichloromethane/methanol (2:1) as a yellow solid. R_f 0.31 (petroleum ether/ethyl acetate/methanol, 1:1:1/2). Yield: 1.8 g (76%); mp 209–211 °C; IR (cm^{-1}): 3382, 3295 (NH groups), 1619, 1597 (C=N groups), 1518, 1401 (NO_2), 1335 (SO); Anal. Calcd for $C_{10}H_{10}N_6O_3S$ (294): C, 40.81; H, 3.42; N, 28.56; S, 10.90. Found: C, 40.74; H, 3.32; N, 28.23; S, 10.95.

4.16. 1,2-Dihydro-5-[(2-methyl-5-nitro-1H-benzimidazol-1-yl)methyl]-1,2,4-triazol-3-one (13)

Crystallized from acetone as a brown solid. R_f 0.33 (petroleum ether/ethyl acetate/methanol, 1:1:1/2). Yield: 1.6 g (72%); mp 144–146 °C; IR (cm^{-1}): 3306–3104 (NH groups + enolic OH), 1718 (C=O), 1621, 1597 (C=N groups), 1523, 1339 (NO_2); Anal. Calcd for $C_{11}H_{10}N_6O_3$ (274): C, 48.18; H, 3.68; N, 30.65. Found: C, 48.23; H, 3.58; N, 30.56.

4.17. General procedure for the preparation of the compounds 14a–e

To a well-stirred solution of compound **3** (2 g, 9.26 mmol), finely divided sulfur (0.3 g, 9.26 mmol), and triethylamine (0.9 ml, 9.26 mmol) in absolute ethanol (30 ml), the proper isothiocyanate (methyl, ethyl, phenyl, benzoyl or *p*-methoxyphenyl) (9.26 mmol) was gradually added. The reaction mixture was heated under reflux for 15–30 min. during which a yellowish green crystalline product separated. After cooling, the product was filtered, washed with ether, dried, and recrystallized from appropriate solvent.

4.18. 4-Amino-3-methyl-5-(2-methyl-5-nitro-1*H*-benzimidazol-1-yl)thiazol-2(3*H*)-thione (14a)

Crystallized from ethanol as a green solid. R_f 0.16 (petroleum ether/ethyl acetate, 1:1). Yield: 2.2 g (73%); mp 215 °C; ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H, CH_3), 3.35 (s, 3H, CH_3), 4.61 (s, 2H, NH_2), 7.69 (d, J = 9 Hz, 1H, H7), 8.06 (d, J = 9 Hz, 1H, H6), 8.30 (s, 1H, H4); IR (cm^{-1}): 3366, 3269 (NH_2), 1629, 1573 (C=N groups), 1502, 1434 (NO_2), 1353 (C=S); Anal. Calcd for $C_{12}H_{11}N_5O_2S_2$ (321): C, 44.85; H, 3.45; N, 21.79; S, 19.95. Found: C, 44.90; H, 3.49; N, 21.82; S, 19.91.

4.19. 4-Amino-3-ethyl-5-(2-methyl-5-nitro-1*H*-benzimidazol-1-yl)thiazol-2(3*H*)-thione (14b)

Crystallized from aqueous methanol as a brown solid. R_f 0.25 (petroleum ether/ethyl acetate, 1:1). Yield: 2.3 g (74%); mp 231 °C; ^{1}H NMR (DMSO-d_6) δ 1.30 (t, J = 6.9 Hz, 3H, CH_3), 2.45 (s, 3H, CH_3), 4.49 (q, J = 6.9 Hz, 2H, CH_2), 5.71 (s, 2H, NH_2), 7.62 (d, J = 9.1 Hz, 1H, H7), 8.06 (d, J = 9.1 Hz, 1H, H6), 8.38 (s, 1H, H4); Anal. Calcd for $C_{13}H_{13}N_5O_2S_2$ (335): C, 46.55; H, 3.91; N, 20.88; S, 19.12. Found: C, 45.65; H, 3.86; N, 20.80; S, 19.06.

4.20. 4-Amino-5-(2-methyl-5-nitro-1*H*-benzimidazol-1-yl)-3-phenylthiazol-2(3*H*)-thione (14c)

Crystallized from ethanol/ether (5:1) as a yellow powder. R_f = 0.17 (petroleum ether/ethyl acetate, 1:1). Yield: 2.8 g (80%); mp 100 °C; ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H, CH_3), 4.51 (br s, 2H, NH_2), 7.13–7.36 (m, 5H, aromatic protons), 7.90 (d, J = 9 Hz, 1H, H7), 8.15 (d, J = 9 Hz, 1H, H6), 8.54 (s, 1H, H4); Anal. Calcd for $C_{17}H_{13}N_5O_2S_2$ (383): C, 53.25; H, 3.42; N, 18.26; S, 16.72. Found: C, 53.31; H, 3.57; N, 18.33; S, 16.82.

4.21. (4-Amino-5-(2-methyl-5-nitro-1*H*-benzimidazol-1-yl)-2-thioxothiazol-3(2*H*)-yl)phenylmethanone (14d)

Crystallized from acetone as a gray powder. R_f = 0.13 (petroleum ether/ethyl acetate, 1:1). Yield: 2.8 g (73%); mp 148–150 °C; ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H, CH_3), 4.52 (br s, 2H, NH_2), 7.16–7.54 (m, 5H, aromatic protons), 7.92 (d, J = 9 Hz, 1H, H7), 8.15 (d, J = 9 Hz, 1H, H6), 8.44 (s, 1H, H4); IR (cm^{-1}): 3403, 3232 (NH_2), 1703 (C=O), 1621, 1611 (C=N groups), 1518, 1423 (NO_2), 1338 (C=S); Anal. Calcd for $C_{18}H_{13}N_5O_2S_2$ (411): C, 52.54; H, 3.18; N, 17.02; S, 15.59. Found: C, 52.49; H, 3.20; N, 17.11; S, 15.48.

4.22. 4-Amino-3-(4-methoxyphenyl)-5-(2-methyl-5-nitro-1*H*-benzimidazol-1-yl)thiazol-2(3*H*)-thione (14e)

Crystallized from ethanol as a yellow solid. R_f 0.15 (petroleum ether/ethyl acetate, 1:1). Yield: 3 g (79%); mp 95 °C;

^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H, CH_3), 3.35 (s, 3H, OCH_3), 4.50 (br s, 2H, NH_2), 7.13–7.36 (m, 4H, aromatic protons), 7.90 (d, J = 9.1 Hz, 1H, H7), 8.18 (d, J = 9.1 Hz, 1H, H6), 8.54 (s, 1H, H4); Anal. Calcd for $C_{18}H_{15}N_5O_3S_2$ (413): C, 52.29; H, 3.66; N, 16.94; S, 15.51. Found: C, 52.39; H, 3.59; N, 16.88; S, 15.47.

4.23. 2-Chloro-1-(2-methyl-5-nitro-1*H*-benzimidazol-1-yl)ethanone (15)

To a solution of compound **2** (10 g, 56.5 mmol) and anhydrous potassium carbonate (7.8 g, 56.5 mmol) in dry acetone (30 ml), chloroacetyl chloride (4.5 ml, 56.5 mmol) was added dropwise. The mixture was stirred at room temperature for about 8 h. The mixture was then poured onto water and extracted with ethyl acetate, dried over anhydrous sodium sulfate, and concentrated under vacuum to give pure compound **15** as a yellow powder. R_f = 0.56 (petroleum ether/ethyl acetate, 1:1). Yield: 12.6 g (87%); mp 158 °C; ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H, CH_3), 4.21 (s, 2H, CH_2), 7.62 (d, J = 9 Hz, 1H, H7), 8.05 (d, J = 9 Hz, 1H, H6), 8.34 (s, 1H, H4); IR (cm^{-1}): 1664 (C=O), 1628 (C=N), 1521, 1334 (NO_2); Anal. Calcd for $C_{10}H_8ClN_3O_3$ (255.5): C, 47.35; H, 3.18; Cl, 13.98; N, 16.57. Found: C, 47.31; H, 3.21; Cl, 13.91; N, 16.60.

4.24. General procedure for the preparation of the compounds 16, 17, and 18a–c

To a solution of compound **15** (2 g, 7.8 mmol) and potassium carbonate (1.08 g, 7.8 mmol) in *N*,*N*-dimethylformamide (20 ml), appropriate amine (thiosemicarbazide, semicarbazide, urea, thiourea or guanidine hydrochloride) (7.8 mmol) was added and the reaction mixture was heated under reflux for 8 h. The reaction mixture was left to cool, poured onto ice water. The precipitate was collected by filtration and recrystallized from appropriate solvent.

4.25. 1,2-Dihydro-5-(2-methyl-5-nitro-1*H*-benzimidazol-1-yl)-1,2,4-triazin-3(6*H*)-thione (16)

Crystallized from acetone as a brown powder. R_f = 0.65 (petroleum ether/ethyl acetate, 1:1). Yield: 1.7 g (75%); mp 160 °C; ^{1}H HMR (DMSO-d_6) δ 2.49 (s, 3H, CH_3), 3.69 (s, 2H, CH_2), 7.62 (d, J = 9 Hz, 1H, H7), 8.06 (d, J = 9 Hz, 1H, H6), 8.34 (s, 1H, H4); IR (cm^{-1}): 3419, 3234 (NH groups), 1663, 1611 (C=N), 1537, 1358 (NO_2), 1300 (C=S); Anal. Calcd for $C_{11}H_{16}N_6O_2S$ (290): C, 45.51; H, 3.47; N, 28.95; S, 11.05. Found: C, 45.31; H, 3.35; N, 28.77; S, 11.09.

4.26. 1,2-Dihydro-5-(2-methyl-5-nitro-1*H*-benzimidazol-1-yl)-1,2,4-triazin-3(6*H*)-thione (17)

Crystallized from ethanol as a brown powder. R_f = 0.35 (petroleum ether/ethyl acetate, 1:1). Yield: 1.9 g (88%); mp 250–252 °C; ^{1}H HMR (DMSO-d_6) δ 2.49 (s, 3H, CH_3), 4.49 (s, 2H, CH_2), 7.62 (d, J = 6.9 Hz, 1H, H7), 8.07 (d, J = 6.9 Hz, 1H, H6), 8.34 (s, 1H, H4); IR (cm^{-1}): 3414, 3252 (NH groups), 1715 (C=O), 1659, 1607 (C=N groups), 1500, 1338 (NO_2); Anal. Calcd for $C_{11}H_{10}N_6O_3$ (274): C, 48.18; H, 3.68; N, 30.65. Found: C, 48.22; H, 3.72; N, 30.69.

4.27. 4-(2-Methyl-5-nitro-1*H*-benzimidazol-1-yl)-1*H*-imidazol-2(5*H*)-thione (18a)

Crystallized from methanol as yellow crystals. R_f = 0.31 (petroleum ether/ethyl acetate, 1:1). Yield: 1.8 g (88%); mp 143–145 °C; IR (cm^{-1}): 3327 (NH), 1706 (CO), 1687, 1632 (C=N groups), 1517–1335 (NO_2); Anal. Calcd for $C_{11}H_9N_5O_3$ (259): C, 50.97; H, 3.50; N, 27.02. Found: C, 50.88; H, 3.61; N, 27.11.

4.28. 4-(2-Methyl-5-nitro-1*H*-benzimidazol-1-yl)-1*H*-imidazol-2(5*H*)-thione (18b)

Crystallized from ethanol/diethyl ether (4:1) as brown crystals. R_f = 0.72 (petroleum ether/ethyl acetate, 1:1). Yield: 1.6 g (74%); mp 210 °C; IR (cm^{-1}): 3327 (NH), 1662, 1626 (C=N groups), 1516, 1336 (NO_2), 1278 (C=S); Anal. Calcd for $C_{20}H_{20}N_4OS$ (275): C, 47.99; H, 3.30; N, 25.44; S, 11.65. Found: C, 47.84; H, 3.39; N, 25.38; S, 11.70.

4.29. 4-(2-Methyl-5-nitro-1*H*-benzimidazol-1-yl)-1*H*-imidazol-2(5*H*)-imine (18c)

Crystallized from acetone as a yellow powder. R_f = 0.28 (petroleum ether/ethyl acetate, 1:1). Yield: 1.6 g (79%); mp 171–173 °C; ^{1}H HMR (DMSO-d_6) δ 2.49 (s, 3H, CH_3), 3.28 (s, 2H, CH_2), 7.61 (d, J = 9 Hz, 1H, H7), 8.05 (d, J = 9 Hz, 1H, H6), 8.34 (s, 1H, H4); Anal. Calcd for $C_{11}H_{16}N_6O_2$ (258): C, 51.16; H, 3.90; N, 32.54. Found: C, 51.23; H, 3.86; N, 32.4.

5. Cytotoxic activity

5.1. Materials and methods

Potential cytotoxicity of compounds **1**, **2**, **2a**, **4**, **5**, **7**, **8**, **9a**, **10**, **13**, **14a**, **15**, **16** and **18c** was tested using the method of Skehan et al.[25] Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the compounds to allow attachment of cell to wall of the plate. Different concentrations of the compound under test (0, 1, 2.5, 5, and 10 μg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed, and stained with Sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris–EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concn. is plotted to get the survival curve of each tumor cell line after the specified compound (Graphs 1–14 we select only two graphs of high activity table of compound **2** and table of compound **7** compared to Graph **1** of compound **1**).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.06.033.

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