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Novel Synthesis and Anti-tumour Activity of 2-Hydrazino-1*H*-benzimidazoles

Sh. M. Abu-Bakr[1], *Kh. M. Abu-Zied[2],Mahmoud Youns, Amel Hashim[4], and Hoda I. El-Diwan[5]

[1,5]Chemistry of Natural and Microbial Products Department, National Research Centre.[2]Photochemistry Department, National Research Centre.[3,4]Faculty of Pharmacy, Biochemistry and Molecular Biology Department, Hellwan University, Cairo, Egypt.

ABSTRACT

As a continuation to our previous work concerning anti-tumour activity of benzimidazole we have synthesized series of new derivatives of 2-(1*H*-benzimidazol-2-yl)-*N*-(substituted)hydrazine- carbothioamide (**2**-**5**), ethyl [2-(1H-benzimidazol-2-yl)-1- (phenylcarbamoyl) hydrazinyl]acetate(**6**), **2**-[2-(1H-benzimidazol-2-yl)hydrazinylidene]-3-benzyl-1,3-thiazolidin-4-one(**7**), 1,2-dihydro-3*H*-[1,2,4]tria-zolo[4,3-*a*]benzimidazole-3-thione(**8**), 2,2'-(1,4-dioxido-1,4,2,3,5,6-dithiatetrazinane-2,6-diyl)bis- (1H-benzimidazole)dihydrochloric acid(**9**), 2,10-dihydro[1,2,4]triazino[4,3-a]benzimidazol-4(3H)-one(**10**), 1-[2-(1H-benzimidazol-2-yl)hydrazinyl]propan-2-one (**11**), 4-[2-(1H-benzimidazol-2-yl)hydrazinyl]-4-hydroxybut-3-en-2-one (**12**),N'-(1H-benzimidazol-2-yl)-2-chloroacetohydrazide **(13)**, and 5-[2-(1H-benzimidazol-2-yl)hydrazinyl]-1,6-dihydro-1,2,4-triazine-3(2H)-thione (**14**). The anti-tumor effect of compounds **2**-**6**,**8**-**13**, and**14** was studied against breast cancer (MCF7) and compound **3**[IC_{50}=3.241μM] was found to be more active than doxorubicin (IC_{50} = 17.12μM).

Keywords:2-Hydrazinobenzimidazole, triazolo-, triazene-, isothiocyanate, isocyanate, anti-tumour activity.

Corresponding author

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INTRODUCTION

Benzimidazole derivatives are endowedwith different types of biological activities especially antitumor activity [1-11]. Pyrrolo[1,2-*a*]benzimidazoles(PBIS) (I,II and III)represent a new class of antitumor agents exhibiting cytotoxicity activity against a variety of cancer cell line [12-17]. Also, other examples as the anticancer agents [Hoechst 33342],2'-(4-ethoxypyenyl)-6-(5-methyl-1-piprazin-1-yl-1*H*-benzimidazol-2-yl)-1*H*-benzimidazole (**IV**) [18,19] and the bisbenzimidazole dye[Hoechst 33258] (**V**) [20,21] are inhibitors of DNA topoisomerase **I**. 5, 6-Dichloro-2-pentafluoroethylbenzimidazole(**VI**) is an antitumor agent particularly against breast and prostatic cancer cell lines [22]. As a continuation to our previous work in synthesizing antitumor benzimidazole compounds, new 2-hydrazinylbenmidazole derivatives **2-6**, **8-14**were prepared and tested for their activity against MCF7 breast cancer cell line. Compound **3**(IC_{50} = 3.24μM) was discovered to be more potent than doxorubicin (Graph 1) and compound **4** was found to be active (Graph 2).

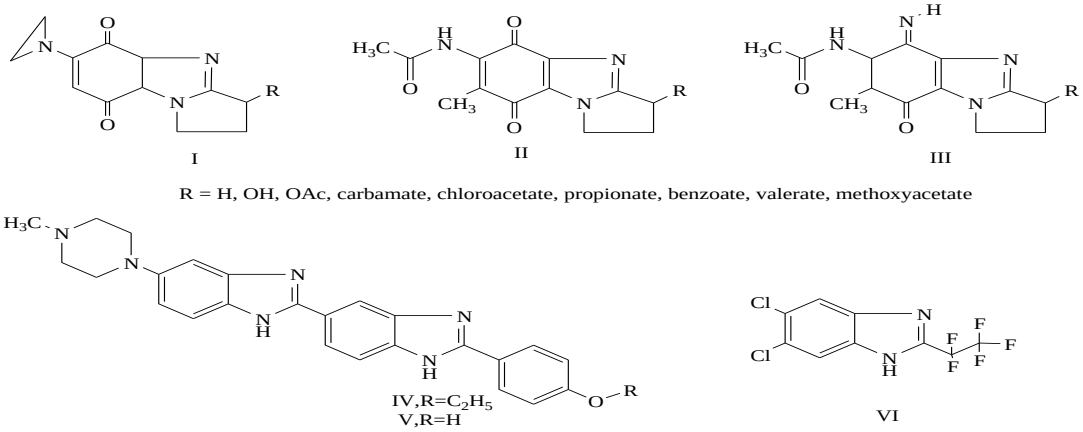
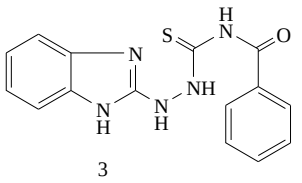
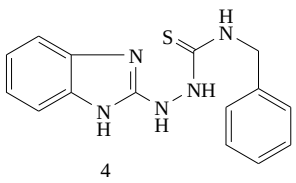
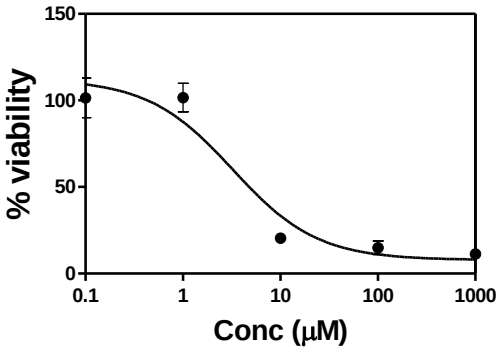
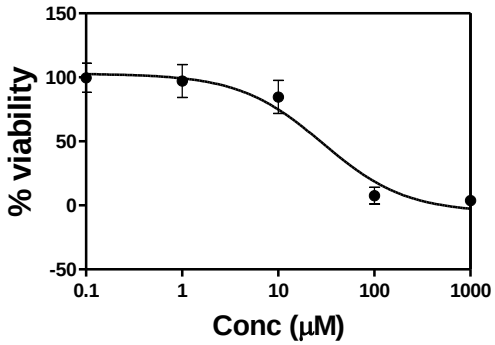


Fig. (1): Structural representation of Hoechst 33342(IV) and Hoechst 33258 (V) as potent specific topoisomerase I inhibitors with antitumor activity.









Graph 1 cytotoxic activity of compound 3Graph 2 cytotoxic activity of compound 4

Cytotoxicity Assessment

Methodology

Cell culture

MCF-7 human breast cancer cells was grown in RPMI-1640 medium, supplemented with 10% heat inactivated FBS, 50 units/mL of penicillin and 50 g/mL of streptomycin and maintained at 37°C in a humidified atmosphere containing 5% CO_2. The cells were maintained as "monolayer culture" by serial sub-culturing.

SRB cytotoxicity assay

Cytotoxicity was determined using SRB method as previously described [23-25]. Exponentially growing cells were collected using 0.25% Trypsin-EDTA and seeded in 96-well plates at 1000-2000 cells/well in RPMI-1640supplemented medium. After 24 h, cells were incubated for 72 h with various concentrations of the tested compounds. Following 72 h treatment, the cells will be fixed with 10% trichloroacetic acid for 1 h at 4 ºC. Wells were stained for 10 min at room temperature with 0.4% SRB dissolved in 1% acetic acid. The plates were air dried for 24 h and the dye was solubilized with Tris-HCl for 5 min on a shaker at 1600 rpm. The optical density (OD) of each well was measured spectrophotometric-ally at 564 nm with an ELISA micro-plate reader (ChroMate-4300, FL, USA). The IC_{50} valueswere calculated according to the equation for Boltzmann sigmoidal concentration–response curve using the nonlinear regression fitting models (Graph Pad, Prism Version 5).

RESULTS AND DISCUSSION

Chemistry

2-Hydrazinobenzimidazole (**1**) was prepared by refluxing 2-benzimidazolethiol with hydrazine hydrate [26] (95%) in acetic acid. When compound **1** reacted with (methyl, benzyl, benzoyl) isothiocyanate or phenylisocyanate [27] in ethanol, it afforded thiosemicarbazide derivatives **2-4** andsemicarbazide derivative **5** respectively. Compound **2**was present as tautomers. The IR spectra of compound **2** showed the absorption band at 1261(C=S) and in compound **3** the absorption bandat 1274 (C=S), 1677 (C=O). ^{1}H NMR spectra of compound **4** showed singlet signal at δ = 4.59 for (CH_2) and the ^{1}H NMR of compound **5** showed a multiplet signal at δ = 7.16-7.20 for the phenyl protons.

When compound**4**or**5**were reacted with ethylbromoacetate in ethanol and anhydrous sodium acetate, thethiazole derivatives **7** and/or hydrazinylacetate **6** were produced. Scheme (**1**)

The MS of compound **7**showed *m/z* 341(M+2+2, isotope of sulphur).The^{1}H NMR spectrum of compound **6** revealed the presence of a triplet signal at δ = 1.19 (CH_3), a singlet signal at 3.80 (CH_2) and a*quartet* signal atδ = 4.14 (CH_2, *J* = 6.90 Hz).

When compound **1** reacted with carbon disulphide in ethanolic sodium hydroxide solution [28] affordedthe triazolethione**8**.The IR spectra showed the absorption band at 3436, 3281 (2 NHs), 1202 (C=S).Refluxing compound **1** with thionyl- chloride or ethylbromoacetate [29] in presence of sodium ethoxide solution afforded the tetrazine **9**and triazine**10** respectively.

The MS of compound **9** displayed *m/z*461(M^+-2), IR spectrum showed the absorption band at 1244, 1199 (S=O). ^{1}H NMR spectrum of compound **10** revealed the presence of singlet signal at δ= 8.17 (=CH), 2 singlet signal at δ = 6.20, 10.40 (NHs, D_2O exchangeable), singlet signal δ=11.65 (OH, D_2O exchangeable).

When compound **1** reacted with chloroacetone in the presence of sodium hydride and N,N-dimethylformamide, it gave the hydrazinylpropane in keto and enol form **11**, **11'**. The^{1}H NMR spectrum of compound **11, 11'** revealed the presence of singlet signal atδ = 2.35 (CH_3), singlet signal at δ = 3.39 (CH_2), singlet signal at δ = 5.80 (=CH), three broad singlet signals at δ = 8.30, 9.65, 10.60 (3 NHs) and a broad singlet signal at δ = 12.70 (OH).

Also, compound **1** reacted with ethylacetoacetate in sodium ethoxide solution to give dione derivative **12.** The^{1}H NMR spectrum of compound **12** showed singlet signal atδ = 5.20 (CH_2).

When compound **1** reacted with chloroacetyl chloride in dry acetone and anhydrous potassium carbonate, chloroacetohyrazide **13** was obtained. The^{1}H NMR spectrum of compound **13** showed singlet signal atδ = 3.53 (CH_2). Compound **13** was cyclized by using thiosemicarbzide in ethanol, *N,N*-dimethyformamide and anhydrous potassium carbonate to give triazinethione **14**. The IR spectraof compound **14**showed the absorption band at 3327, 3298 (broad, s, NHs), 1253 (C=S). (Scheme 2)

The assignments of all newly synthesized compounds were confirmed by their different spectral data such as ^{1}HNMR, IR, mass spectra and microanalyses. See experimental.

Cytotoxicity

Compounds **2-6** and **8-14** were studied for their antitumor activity against MCF7 cell line. Compound 3 was found to be highly potent and compound **4** was active. Besides, compounds **11**, **9**, **10**, **12** and **2** have moderate activity in a decreasing order. Compounds **14**, **6**, **13** and **5** have no activity.

Structure-Activity Relationship

The high activity of compound **3,** the most active compound among the series, may be attributed to the presence of the side chain NHNHC(S)NHCOPh, which could be sterically favoured causing a good binding and fitting with the receptor.

The activity of compound **4** was less than compound **3**, which was probably due to lack of the C=O group in the side chain having instead of the lipophylic CH_2 group.

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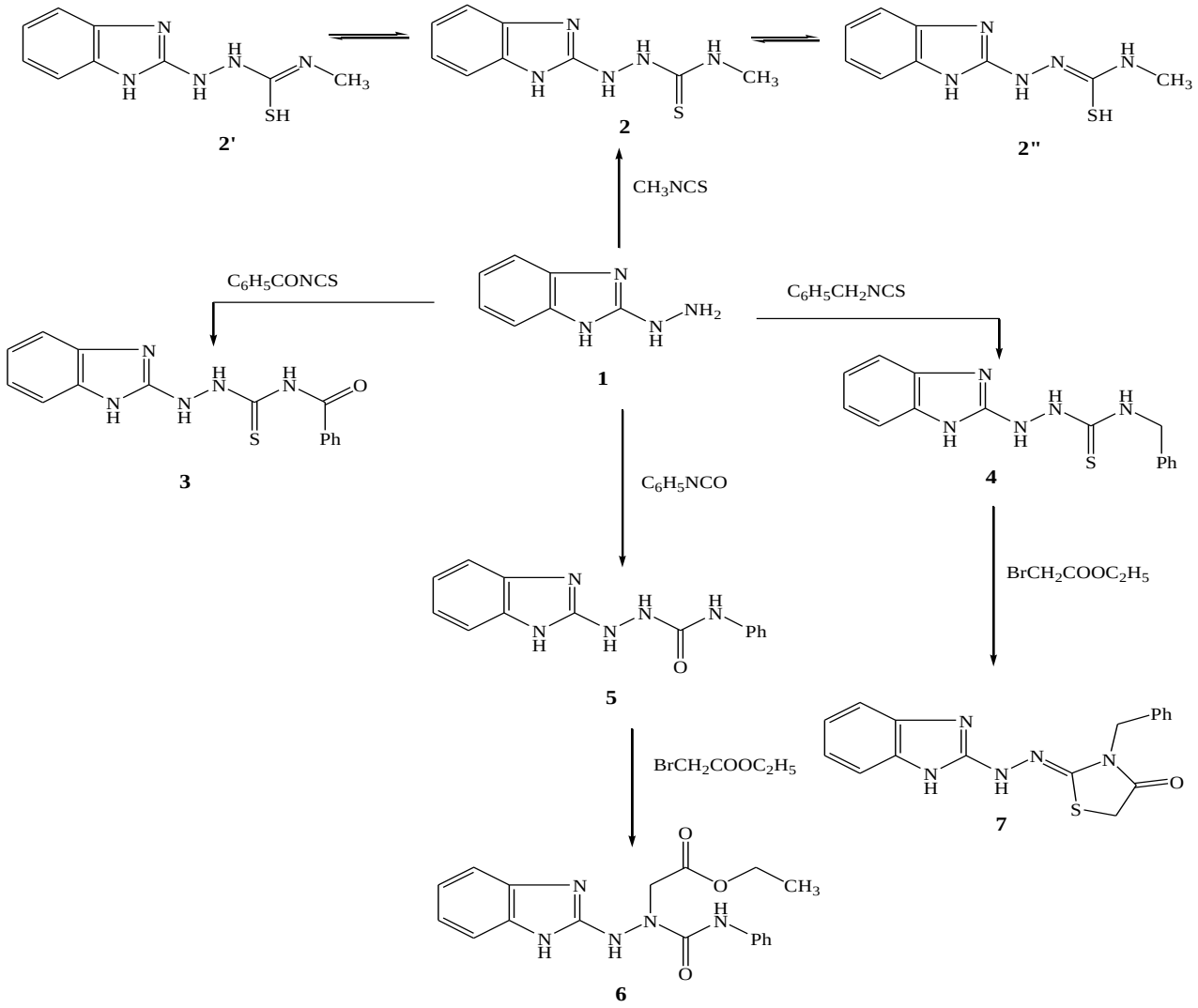
The presence of five or six member heterocyclic rings fused or connected to the benzimidazole moiety produced compounds of moderate anticancer activity e.g. compounds **8**, **9**, **10** and **14**.

The activity was abolished when the link between benzimidazole and the phenyl ring in the side chain at position 2, diminished from five to four atoms e.g. compound **5** was inactive while **3** and **4** were active.

The presence of the chlorine atom in the side had negative impact on the activity as compound **13** was inactive. In addition, when the side chain in compound **13** was cyclized accompanied by dehydrohalogenation, compound **14** was produced possessing a better activity.



Scheme (I)

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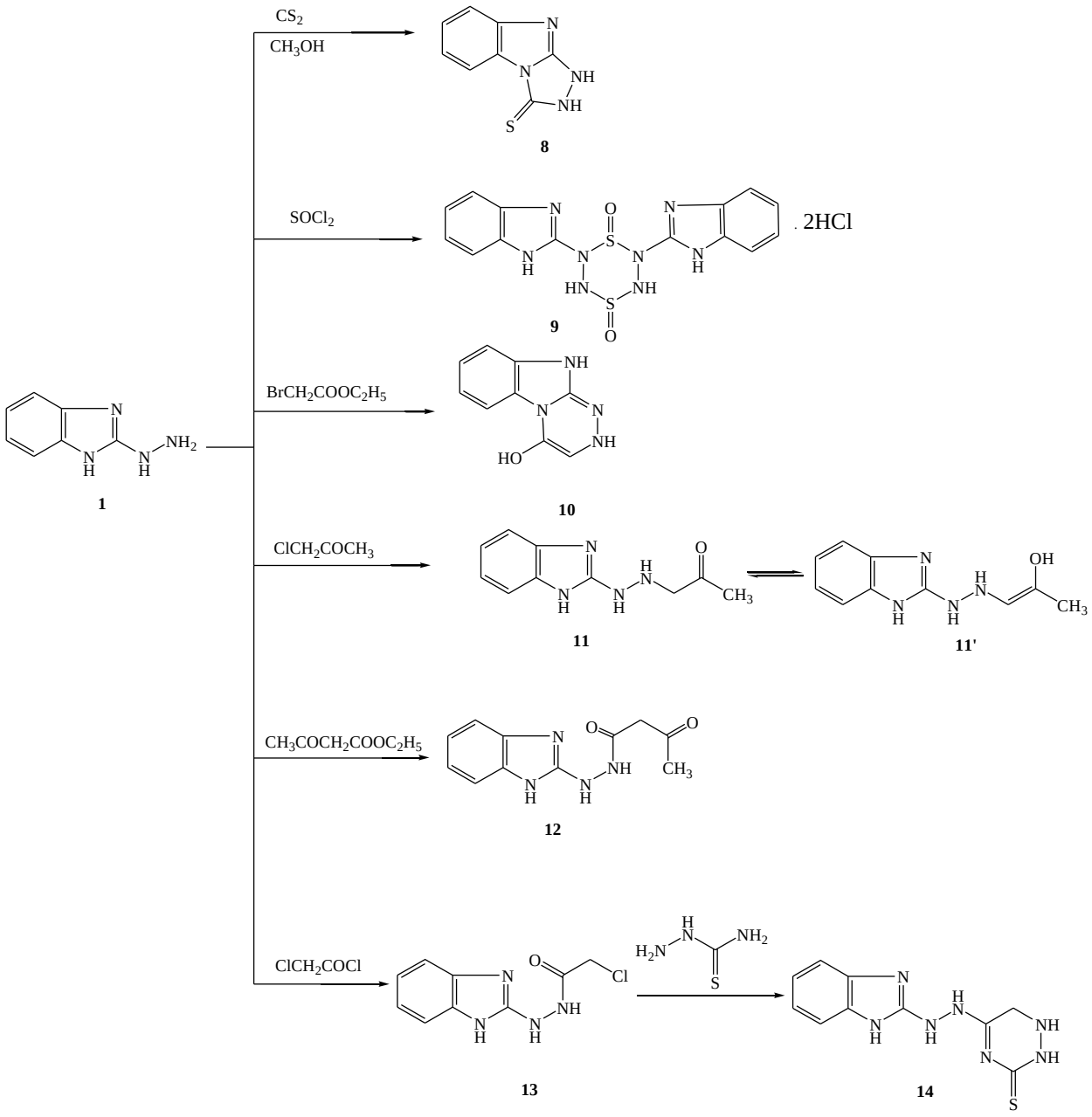

Scheme (II)

EXPERIMENTAL

Solid compounds were re-crystallized to constant melting points and dried in vacuum in drying pistol containing sodium hydroxide and P_2O_5.

All melting points are uncorrected and were taken in open capillaries on a Gallen Kamp Apparatus.

Microanalyses were carried out at the Micro-analytical Unit National Research Centre and Faculty of Science, Cairo University.

IR spectra were carried out on FT/IR 300 E Jasco using KBr discs.

^{1}H-NMR spectra were measured in DMSO-d_6 or $CDCl_3$, using Joel Ex. 270 NMR spectrometer. Signals were measured with reference to TMS as an internal standard.

The Mass spectra were recorded on Finnigan SSQ 7000 spectrometer.

All reactions were followed up by TLC using $CHCl_3$/MeOH (9:1, v/v) and/or ethyl acetate/benzene (7:3) and detected under UV Lamp (λ_{max}254).

Table 1: IC_{50}(µM) values of compounds 2-6, 8-13 and 14 against breast cancer cell line

Compound no.	IC_{50}(µM)
2	39.67
3	3.24
4	28.93
5	350.0
6	70.10
8	48.93
9	32.19
10	35.20
11	31.39
12	36.75
13	243.90
14	50.24
Doxorubicin	17.12

General procedure: Compounds 2-5

Compound 1 (0.01 mol) was heated in 20 ml ethanol containing few drops of acetic acid. The proper isothiocyanate (methyl, benzyl, benzoyl or phenyl isocyanate) (0.01 mol) was added and the mixture was refluxed for 6 h.(followed by TLC). The collected product was recrystallized from appropriate solvent.

2-(1*H*-Benzimidazol-2-yl)-*N*-methylhydrazinecarbothioamide (2)

It crystallized from ethanol, yield 66% as green powder, mp.: 179-181°C; ^{1}H-NMR (DMSO-d_6)δ; 1.87(s, 1H, SH), 2.88(d,3H,CH_3), 7.1-7.31(m,2H, benzimidazole protons,C^5H, C^6H), 7.81-7.92(m,2H, benzimidazole protons,C^4H, C^7H), 8.30(s, 1H,NH, D_2O exchangeable),8.34(br,s, 1H,NH, D_2O exchangeable), and9.63(s, 1H,NH, D_2O exchangeable). IR (cm^{-1}); 3174 (broad NHs), 1660 (C=N), 1261 (C=S); MS (EI): m/z 221 (M^+, 70.5%), m/z 190 (58.5%). Anal. calc.for[$C_9H_{11}N_5S$]:C, 48.86; H, 4.97; N, 31.67; S, 14.47. Found: C, 48.46; H, 4.98; N, 31.64; S, 14.46.

N-{[2-(1*H*-Benzimidazol-2-yl)hydrazinyl]carbonothioyl}benzamide (3)

It crystallized from ethanol/diethyl ether(5:1), yield 60%, as dark brown powder, mp.230-233°C; ^{1}H-NMR (DMSO-d_6)δ;7.35(m,2H, aromatic protons),7.49 (m,3H, aromatic protons), 7.56(m,1H, benzimidazole proton,C^5H), 7.60(m,1H, benzimidazole proton,C^6H), 8.10

(d,1H,*J*=11.45Hz, benzimidazole proton,C^4H),8.2(d,1H,*J*=11.45Hz, benzimidazole proton, C^7H), 11.95(s, 1H,NH, D_2O exchangeable),12.32(s, 1H,NH, D_2O exchangeable), and 12.60 (s, 1H,NH, D_2O exchangeable). IR (cm^{-1}); 3220, 3200 (broad NHs), 1677 (C=O), 1626 (C=N), 1274 (C=S);MS (EI): m/z 313 (M^+, +2, 65%). Anal. calc.for [$C_{15}H_{13}N_5OS$]: C, 57.87; H, 4.18; N, 22.50; S, 10.28. Found:C, 57.84; H, 4.16; N, 22.52; S, 10.27.

2-(1*H*-Benzimidazol-2-yl)-*N*-benzylhydrazinecarbothioamide(4)

It crystallized from ethanol, yield 71%, as brown powder, mp.123-125°C; ^{1}H-NMR (DMSO-d_6)δ;4.06(s, 1H,NH, D_2O exchangeable),4.59 (s,2H,CH_2), 6.90-7.40(m, 9H,(5 aromatic protons + 4 benzimidazol protons,C^4H,C^5H,C^6H,C^7H)),8.75(s, 1H,NH, D_2O exchangeable), 9.57(s, 1H,NH, D_2O exchangeable), and 12.50(br. s, 1H,NH, D_2O exchangeable).IR (cm^{-1}); 3322,3169(NHs), 1625 (C=N), 1232 (C=S); MS (EI): m/z 297 (M^+, 83.1%). Anal. calc.for[$C_{15}H_{15}N_5S$]: C, 60.60; H, 5.05; N, 23.56; S, 10.77. Found: 60.63; H, 5.03; N, 23.54; S, 10.76.

2-(1*H*-Benzimidazol-2-yl)-*N*-phenylhydrazinecarboxamide (5)

It crystallized from ethanol, yield 50%, as yellow powder, mp.:152-154°C; ^{1}H-NMR (DMSO-d_6)δ;7.14 (d,1H,*J*=11.36Hz, benzimidazole proton, C^7H),7.16-7.20(m,5H, aromatic protons), 7.37-7.39(m,2H, benzimidazole protons,C^5H,C^6H),7.45(d,1H,*J*=11.36Hz, benzimidazole proton, C^4H),8.78(s, 1H,NH, D_2O exchangeable),10.1(s, 1H,NH, D_2O exchangeable), and 11.10 (s, 1H,NH, D_2O exchangeable).IR (cm^{-1}); 3351, 3210, (broad NHs), 1685(C=O), 1600 (C=N).MS (EI): m/z 267 (M^+, 80,3%). Anal. calc.for[$C_{14}H_{13}N_5O$]: C, 62.92; H, 4.86; N, 26.21. Found; 62.90; H, 4.88; N, 26.19.

General procedure for synthesis of compounds 6 and 7

To a solution of compounds **4** or **5** (0.01 mol) in absolute ethanol (10 ml), ethyl bromoacetate (0.01 mol), and anhydrous sodium acetate (0.02 mol) were added. The reaction mixture was refluxed on water bath for 6h, then cooled, diluted with water and allowed to stand overnight. The precipitate so formed was collected upon filtration and crystallized from suitable solvent.

Ethyl [2-(1*H*-benzimidazol-2-yl)-1-(phenylcarbamoyl)hydrazinyl]acetate (6)

It crystallized from ethanol, yield 50%, as brown powder, mp.:202-205°C; ^{1}H-NMR (DMSO-d_6)δ;1.19(t, 3H, *J*= 6.90 Hz, $CH_2\underline{CH_3}$), 3.80 (s, 2H,CH_2), 4.14 (Q, 2H, *J*= 6.90 Hz, CH_2), 6.98(m,1H, aromatic proton),7.35-750(m,2H, benzimidazole protons,C^5H,C^6H), 7.71-7.73(m,4H, aromatic protons), 7.79 (m, 2H,*J*=110.85Hz, benzimidazole protons, C^4H, C^7H),8.37(s, 1H,NH, D_2O exchangeable), 8.93(s, 1H,NH, D_2O exchangeable),and9.3(s, 1H,NH, D_2O exchangeable).IR (cm^{-1}); 3204, 3180 (broad NHs), 1700,1682 (2C=O), 1640 (C=N);MS (EI): m/z 353 (M^+, 61.5%), *m/z* 260.3 (100%). Anal. calc.for[$C_{18}H_{19}N_5O_3$]: C, 61.18; H, 5.38; N, 19.83. Found:C, 61.15; H, 5.35; N, 19.82.

2-[2-(1*H*-Benzimidazol-2-yl)hydrazinylidene]-3-benzyl-1,3-thiazolidin-4-one (7)

It crystallized from acetone, yield 45%, as yellow powder, mp.:> 300 °C; ^{1}H-NMR (DMSO-d_6)δ;3.98(s, 2H,CH_2), 4.06 (s, 2H, CH_2), 7.19-7.24(m, 3H, aromatic protons), 7.25-7.27(m, 4H, benzimidazole protons,C^5H,C^6H+2H aromatic protons), 7.31 (d,1H,*J*=10.85Hz, benzimidazole proton, C^4H), 7.35 (d,1H,*J*=10.85Hz, benzimidazole proton, C^7H),7.91(s, 1H,NH, D_2O exchangeable), 10.45(s, 1H,NH, D_2O exchangeable).IR (cm^{-1}); 3200 (broad NHs), 1671 (C=O), 1631, 1620 (C=N);MS (EI): m/z 341 (M^++2+2, 82.21%). Anal. calc.for[$C_{17}H_{15}N_5OS$]: C, 60.53; H,4.45; N,20.77; S, 9.49. Found: C,60.51; H, 4.46; N, 20.76; S, 9.48.

1,2-Dihydro-3*H*-[1,2,4]triazolo[4,3-*a*]benzimidazole-3-thione(8)

Carbon disulphide (0.015 mol) was added drop wise with constantstirringto a solution of compound **1**(0.01 mol) in ethanolicpotassium hydroxide solution (0.01 mol in 50 ml). The reaction mixture was heated on water bath at 70^{o}C for about8 h until the evolution of hydrogen disulphide caused. The reaction mixture was concentrated to one fourth of its volume and poured into ice and acidified with dilute hydrochloric acid. The precipitate thus obtained wasfiltered, washed with water and purification by recrystallization from ethanol as a green powder. Yield; 80%;mp. ; 272-275°C;^{1}H-NMR (DMSO-d_6)δ; 6.70-6.85(m, 2H, benzimidazol protons,C^5H,C^6), 7.13(d,1H,*J*=10.85Hz, benzimidazole proton, C^4H),7.19(d,1H,*J*=10.85Hz, benzimidazole proton, C^7H),7.35(s, 1H,NH, D_2O exchangeable), and8.17(s, 1H,NH, D_2O exchangeable).IR (cm^{-1}); 3436, 3281, (broad NHs), 1648 (C=N), 1202 (C=S); MS (EI): m/z 192 (M^++2, 64.7%). Anal. calc. for[$C_8H_6N_4S$] C,50.52; H,3.15; N, 29.47; S, 16.84. Found:C,50.50; H, 3.17; N, 29.46; S, 16.83.

General procedure for compounds 9 and 10

To a solution of compound **1**(0.01 mol) in ethanol (10 ml), was treated with sodium ethoxide (0.01 mol sodium metal in 5ml absolute ethanol), and thionyl chloride (0.01 mol) or ethyl bromoacetate (0.01 mol). The mixture was heated on water bath for 8 h., then cooled to room temperature, diluted with water and the product was extracted with ethyl acetate. The organic layer was dried using anhydrous sodium sulphate and excess of ethyl acetate was removed under reduced pressure. The collected solid material was re-crystallized from appropriate solvent to afford the titled compounds in good yield.

2,2'-(1,4-Dioxido-1,4,2,3,5,6-dithiatetrazinane-2,6-diyl)bis(1H-benzimidazole)dihydrochloric acid (9)

It crystallized from ethanol, yield 65%, as yellow powder, mp: > 300°C; ^{1}H-NMR (DMSO-d_6)δ;7.15-7.18 (m, 4H,benzimidazole proton),7.54-7.57 (m, 4H, benzimidazole protons), 8.17 (br.s, 1H, NH,D_2O exchangeable),9.85 (s, 1H,NH, D_2O exchangeable),10.55 (s, 1H, NH, D_2O exchangeable), 10.75(br.s, 1H,NH, D_2O exchangeable). IR (cm^{-1}); 3369 (broad NHs), 1621, 1685 (C=N), 1244, 1199 (S=O). MS (EI): m/z 461 (M^+-2, 824%), m/z 333 (42.8%). Anal. calc. for[$C_{14}H_{14}Cl_2N_8O_2S_2$]: C,36.44; H,3.03; N, 24.29; S, 13.87, Cl, 15.40. Found: C, 36.43; H, 3.02; N, 24.28; S, 13.87, Cl, 15.38.

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2,10-Dihydro[1,2,4]triazino[4,3-a]benzimidazol-4(3H)-one (10)

It crystallized from benzene, yield 55%, as brown powder, mp.:226-228°C; ^{1}H-NMR (DMSO-d_6)δ;6.20 (br.s, 1H,NH, D_2O exchangeable), 7.15(m,2H,C^5H,C^6H, benzimidazole protons),7.55-7.57(m, 2H, C^4H,C^7H benzimidazole protons,),8.17 (s, 1H, =CH), 10.40(s, 1H,NH, D_2O exchangeable),11.65(s, 1H,OH, D_2O exchangeable).IR (cm^{-1}); 3377 (br OH), 3391 (broad NHs), 1683(C=O), 1627 (C=N).MS (EI): m/z 188 (M^+, 47%), m/z 144 (94.7%). Anal. calc. for[$C_9H_8N_4O$]: C; 57.44, H; 4.25, N; 29.78. Found:C; 57.41, H; 4.23, N; 29.77.

1-[2-(1*H*-Benzimidazol-2-yl)hydrazinyl]propan-2-one (11)

To an well stirred solution of compound **1**(0.01 mol), sodium hydride (0.01 mol) in N,N-dimethylformamide (20 ml), chloroacetone(0.01 mol) was slowly added drop wise. The mixture was stirred at room temperature for 9hr. and then poured over ice water. The resulting precipitate was collected by filtration and recrystallized from methanol to afford the title compound as a brown powder, yield; 48%; mp.;251-253°C.^{1}H-NMR (DMSO-d_6)δ;2.35 (s, 3H, CH_3), 3.39 (s,2H,CH_2),5.8 (s, 1H,CH=), 7.5 (m, 2H,C^5H,C^6H, benzimidazole protons),7.67(d,1H,*J*=10.85Hz, C^4H benzimidazole proton),7.96(d,1H, *J*=10.85Hz, C^7H benzimidazole proton),8.3 (br.s, 1H,NH, D_2O exchangeable), 9.65 (br.s, 1H,NH, D_2O exchangeable), 10.6 (br.s, 1H,NH, D_2O exchangeable), 11.77 (br.s, 1H, OH, D_2O exchangeable).IR (cm^{-1}); 3747 (br. OH), 3250, 3221 (broad NHs), 1680(C=O), 1624 (C=N).MS (EI): m/z 206.1 (M^++2, 63%), m/z 188 (38%). Anal. calc. for[$C_{10}H_{12}N_4O$]: C; 58.82, H; 5.88, N; 27.45. Found:C; 58.80, H; 5.85, N; 27.43.

4-[2-(1*H*-Benzimidazol-2-yl)hydrazinyl]-4-hydroxybut-3-en-2-one (12)

To a solution of compound **1** (0.01 mol) and ethyl acetoacetate (0.01 mol) in sodium ethoxide solution (prepared by dissolving sodium metal (0.01 mol) in absolute ethanol (30ml) was heated under reflux for 5 hr. The reaction mixture was allowed to cool to room temperature, poured onto cold water (100 ml) and neutralized by acetic acid. The solid so formed was filtered off, dried and crystallized from ethanol as dark brown powder, yield; 57%, mp; 280-283°C. ^{1}H-NMR (DMSO-d_6)δ;2.16 (s,3H,CH_3), 3.99 (br.s, 1H, NH), 5.20(s,2H, CH_2), 7.13 (m, 2H,benzimidazole protons), 7.48 (m,2H,benzimidazole protons),8.40(br.s, 1H,NH, D_2O exchangeable), 9.30 (br.s, 1H, NH, D_2O exchangeable), 10.50(br.s, 1H,NH, D_2O exchangeable). IR (cm^{-1}); 3730.01 (broad OH), 3307 (broad NHs), 1654 (C=O), 1624 (C=N);MS (EI): m/z 233 (M^++1, 78.3%), m/z 214 (26.31%). Anal. calc. for[$C_{11}H_{12}N_4O_2$]: C; 56.89, H; 5.17, N; 24.13. Found: C; 56.88, H; 5.15, N; 24.10.

N'-(1*H*-Benzimidazol-2-yl)-2-chloroacetohydrazide (13)

To a solution of compound **1**(0.01 mol) and anhydrous potassium carbonate (0.01 mol) in dry acetone (20ml), chloroacetylchloride (0.01 mol) was added drop wise. The mixture was stirred at room temperature for about 6hr. Filter excess potassium carbonate The filtrate was then poured onto water and the organic layer was extracted with ethyl acetate, driedover anhydrous sodium sulphate and concentrated under vacuum whereby a yellow precipitate was obtained in 51% yield. Its mp.; 128-130°C. ^{1}H-NMR (DMSO-d_6)δ;3.53(s,2H,CH_2),7.27-7.41(m,2H,C^5H,C^6H, benzimidazole protons),7.42(d,1H,*J*=10.85Hz,

C^4H,benzimidazole proton),7.44(d,1H,*J*=10.85Hz, C^7H,benzimidazole proton),8.30(br.s, 1H, NH, D_2O exchangeable), 9.70 (br.s, 1H,NH, D_2O exchangeable), 10.40 (br.s, 1H,NH, D_2O exchangeable). IR (cm^{-1}); 3240, 3247 (broad NHs),1667 (C=O), 1624 (C=N); MS (EI): m/z 224.5 (M^+, 68%), m/z 162 (59.08%). Anal. calc. for[$C_9H_9ClN_4O$]: C,48.10; H, 4.00; N, 24.94; Cl, 15.81. Found; C; 48.07, H; 4.02, N; 24.93; Cl, 15.80.

5-[2-(1*H*-Benzimidazol-2-yl)hydrazinyl]-1,6-dihydro-1,2,4-triazine-3(2H)-thione (14)

To a solution of compound **13** (0.01 mol) and anhydrous potassium carbonate (0.01 mol) in dry *N,N*-dimethylformamide (15ml), thiosemicarbazide (0.01 mol) was added and the reaction mixture was heated under reflux for 8-9hr. The reaction mixture was left to cool to room temperature and poured onto ice water. The formed precipitate was collected by filtration, dried, and crystallized from ethanol to obtain the titled compound as a brownprecipitate in47% yield. Its mp.; 186-189°C. ^{1}H-NMR (DMSO-d_6)δ;2.82(s,2H,CH_2),7.0(s, 1H, NH, D_2O exchangeable), 7.08(m, 2H,C^5H,C^6H, benzimidazole protons),7.23(s, 1H, NH, D_2O exchangeable), 7.72(s, 1H,NH, D_2O exchangeable), 7.91(m,2H,C^4H,C^7H,benzimidazole protons), 10.15 (br.s, 1H. NH, D_2O exchangeable). IR (cm^{-1}); 3327, 3298 (broad NHs),1650, 1624 (C=N), 1253 (C=S); MS (EI): m/z 261 (M^+, 66%), m/z 170 (47.4%). Anal. calc. for. [$C_{10}H_{11}N_7S$](261): C; 45.97, H; 4.21, N; 37.54,S;12.26. Found: C; 45.95, H; 4.20, N; 37.52,S;12.24.

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