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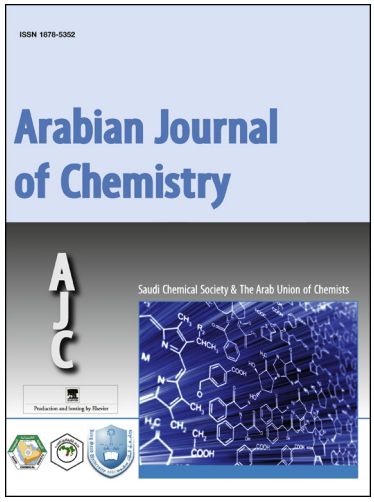

Original article

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Design and synthesis of benzimidazoles containing substituted oxadiazole, thiadiazole and triazolo-thiadiazines as a source of new anticancer agents

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Running title: **Bendamustine analogues as new anticancer agents**

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ABSTRACT

Based on the heterocyclic core of bendamustine, four series (**4a-g**, **5a-f**, **8a-b** and **9a-b**) of benzimidazole derivatives were designed and synthesized starting from 4-(1*H*-benzo[*d*]imidazol-2-yl)-4-oxobutanehydrazide. In the rational design of target molecules, the benzimidazole ring of bendamustine was retained and the bis-(chloroethyl) amine group (mechlorethanmine) was substituted with several biologically active scaffolds such as oxadiazole, thiadiazole, triazolo-thiadiazines etc., in the hope of obtaining novel cytotoxic agents with improved efficacy and safety. Cytoxic activities of the designed analogues were carried out at the National Cancer Institute (NCI), USA against full NCI 60 human cell lines. Among all the tested compounds, **4f (**761982/1) exhibited significant antiproliferative activity and was further screened at 10-fold dilutions of five different concentrations (0.01, 0.1, 1, 10 and 100µM) with GI_{50} values ranging from 0.09 to 16.2 µM and found superior for CNS cancer cell line SNB-75 (GI_{50} 0.09, TGI 1.39, LC_{50} >100 and $\log_{10}GI_{50}$ -7.0, $\log_{10}$TGI-5.86, $\log_{10}LC_{50}$>-4.00). Docking study was also performed to provide an insight about the binding mode into binding sites of topoisomerase enzyme. Hopefully in future, compound **4f** could be used as a lead compound for developing new anticancer agents.

Keywords: Bendamustine, Benzimidazole, Chlorambucil, Sulforhodamine B, Human cell lines.

1. Introduction

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Cancer is a disease in which uncharacteristic cells grow and proliferate in an uncontrolled manner. It can occur in all the living cells at any stage of life. Cancer is a major health problem across the globe and its prevalence is on the rise. Millions of people worldwide are suffering from this dreaded disease which not only affects the health of the patient, but also puts significant socioeconomic, mental and physical burden on the family members (Lee et al., 2002; Kidwai et al., 2002). Cancer is considered to be one of the leading causes of mortality due to diseases and the projected death toll due to cancer alone would touch approximately 10 million by the end of year 2020 (Schumacher et al., 2011). In spite of considerable development in the understanding of molecular mechanisms of pathogenesis of the disease, yet no specific treatment is available to cure the disease completely. The current approaches used to treat cancer involves surgery, radiotherapy and chemotherapy either alone or in combination, but due to metastasis i.e. invasion of nearby tissues by cancerous cells and spreading of disease to other parts of the body, can cure only 40% patients and thus associated with the high rate of mortality. The other approaches such as hormonal, antibodies treatments, biological response modifier, complementary and alternative medicine treatments are also practiced worldwide to curb this group of different and distinct diseases. Chemotherapy is considered to be the main weapon against the neoplastic diseases and the majority of the clinically used anticancer molecules are of synthetic origin. These chemo-preventive molecules act by various molecular mechanisms which may involve inhibition of initiation, promotion, progression and metastasis of cancerous cells but in the process can also kill normal cells leading to toxicity. There is an urgent need to design, synthesize potent and highly selective molecules to improve the current anticancer therapy with least or no toxicity to normal cells.

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Benzimidazole (a phenyl ring fused to an imidazole ring) is a well known bioactive heterocyclic ring system that is present in several natural and synthetic medicinal compounds. Benzimidazole is known to be a versatile scaffold that possess potential anticancer, antitumor and antiproliferative activities (Rafaat, 2010; Gumus et al., 2009; Sun et al., 2011; Abonia et al., 2011; Ramla et al., 2006; Demirayak et al., 2002; Romero-Castro et al., 2011) along with other useful biological actions. Similarly, oxadiazole, thiadiazole, triazolo-thiadiazines and triazolo-thiadiazoles are a course group of fused heterocyclic compounds, which have engrossed significant attention of medicinal chemists owing to their wide range of useful pharmacological actions particularly cytotoxic activities against DNA topoisomerase I (Formagio et al., 2008; Kumar et al., 2010; Sarhan et al., 2010).

Bendamustine hydrochloride, a potent DNA alkylating agent, is used clinically to treat chronic lymphocytic leukemia (CLL) and B-cell non-hodgkin's lymphoma (NHL) (Lissitchkov et al., 2006; Gandhi et al., 2009). The central core of Bendamustine is a benzimidazole heterocylic ring having a mechlorethamine side chain on the 5^{th} position, a butyric acid substituent on a 2^{nd} position and *N*- substituted methyl group on 1^{st} position. Thus, chemically bendamustine is; (1*H*-benzimidazole-2-butanoicacid-5-[bis(2-chloroethyl)amino]-1-methylmonohydro chloride) and was developed as a rational design of purine analogue and alkylation hybrid of chlorambucil (Fig. **1**). In view of these facts, we intended to synthesize some novel and potent anticancer agents based on the rationally designed template of bendamustine and chlorambucil i.e. molecules having benzimidazole residue as a pharmacophoric group (Romero-Castro et al., 2011) with butyric acid substituent and further clubbed with related heterocyclic rings like oxadiazole, thiadiazole, triazolo-thiadiazole and triazolo-thiadiazine (Fig. **1**). The designed template was further used to synthesize library of analogue molecules (**Scheme 1-3**) by incorporating

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heterocyclic ring systems of several antitumor agents reported in literature like proxazole (Duanmu et al., 1989), IMC-094332 (Britten et al., 2001) (having oxadiazole ring, **4a-g**), SNS-032 (Penning et al., 2008) (having thiazole ring, **5a-f**), levamisole (Tolner et al., 2001) and ATEK 10934 (Albert et al., 2007) (having diazole-thiazole & trizolo-thiadiazole ring; **8 a-b & 9a-b**) (Fig. **2**). Chemical structures of some other reported antitumor agents containing benzimidazole nucleus and other biologically active monocyclic or bicyclic heterocyclic ring system along with the similarly prepared molecules (**4f, 5e** and **9a**) are presented in chart 1. Molecules like , Nocodazole (NSC-238189) (**1**) (Duanmu et al., 1989), FB642 (**2**) (Britten et al., 2001), A-620223 (**3**) (Penning et al., 2001), Hoechst-33258 (**4**) (Tolner et al., 2001), ABT-888 (**5**) (Albert et al., 2007), Phortress (**6**) (Bradshaw & Westwell, 2004), SNS-032 (**7**) (Misra et al., 2004), Proxazole (**8**) (Dalip et al., 2011), CYC116 (**9**) (Griffiths et al., 2008), thiadiazole derivative (**10**) (Matysiak & Opolski, 2006), Levamisole (**11**) (Remrs et al., 1982), imidazo[2,1-b][1, 3,4] thiadiazole analogues (**12**) (Taher et al., 2012), triazolo [1,3,4] thiadiazole derivative (**13**) (Ibrahim et al., 2009), ATEK 10934 (**14**) (Ibrahim et al., 2009), IMC-094332 (**15**) (Maria et al., 2010) have shown potential to be used as antitumor agents and some of them are in the final stages of drug development.

Our previous and ongoing research work on the synthesis of benzimidazole derivatives bearing different heterocyclic rings in search of new anticancer agents have revealed that these compounds are selective towards Leukemia cancer subpanel with mean GI_{50} 6.59, GI_{50} 12.62 and GI_{50} 1.04 (Rashid et al., 2012; Husain et al., 2012; Husain et al., 2013; Rashid et al., 2012). From these results, we have observed that substituted benzimidazoles have the potential to provide the anticancer lead candidate or a drug molecule and thus are worthy of further studies. The study was undertaken with an aim to find new structures which could be used as a lead candidate in the

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field of cancer. We report herein the synthesis of some new molecules in which benzimidazole core of bendamustine drug is endowed with oxadiazole, triazolo-thiadiazine and triazolo-thiadiazole nuclei, in an attempt to significantly improve the anticancer activity of the marketed anticancer drug. The *in- vitro* anticancer activities of newly designed and synthesized compounds were carried out at the National Cancer Institute (NCI), Chemotherapeutic Research division, USA. The cytotoxic effects of the compounds were tested on nine human systems against full NCI 60 cell line panel. The tested compounds were also granted NCS code by NCI and are shown in Table 1.

2. Experimental

2.1. General information

The melting points of the all the synthesized compounds were measured on a liquid paraffin bath in open capillary tubes and are uncorrected. The progress of the chemical reaction as well as the purity of the target compounds was checked by using TLC plates, pre-coated with silica gel G in solvent systems of toluene: ethyl acetate: formic acid (5:4:1, v/v/v) and benzene: acetone (9:1, v/v). The spots on TLC plates were visualized after exposing to iodine vapors or under UV-light. Chemical synthesis was carried out in a scientific microwave synthesizer (model No. CATA-R, Catalyst systems, India). Flash chromatography technique was used to purify the target compounds using hexane and ethyl acetate mixture as an eluent mixture. ^{1}H- and ^{13}C-Nuclear magnetic resonance (NMR) spectra of the pure compounds in DMSO-d_6/$CDCl_3$ were recorded on Bruker spectrospin DPX-300 MHz instrument. Tetramethylsilane was used as an internal reference and the exchangeable protons of O*H* and N*H* were confirmed by the D_2O shaking test. Mass spectra were recorded on LCMS/MS (Perkin-Elmer and LABINDIA, Applied

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Biosystem) model no. API 3000 and is presented as *m/z*. IR spectra of the compounds were recorded on FT/IR (Jasco, Japan), model no.410. An elemental analysis was done on a Perkin-Elmer 240 analyzer and was found in the range of ± 0.5% for each element analyzed (C, H and N).

2.2. Synthesis

*2.2.1. 4-(1H-benzo[d]imidazol-2-yl)-4-oxobutanoicacid (**1**).* It was prepared as per the previously reported method (Husain et al., 2012). The identity of the compound was established after comparing its physical properties and spectral data which are found to be in good agreement with the reported literature values.

*2.2.2. Ethyl-4-(1H-benzo[d]imidazol-2-yl)-4-oxobutanoate (**2**).* It was prepared as per the previously reported method Husain et al., 2012). The identity of the compound was established after comparing its physical properties and mass spectral data with the literature values.

*2.1.3. 4-(1H-benzo[d]imidazol-2-yl)-4-oxobutanehydrazide (**3**).* It was prepared as per the previously reported method (Husain et al., 2012). The identity of the compound was established after comparing its physical properties and spectral data which are found to be in good agreement with the reported literature values.

*2.2.4. 1-(1H-benzo[d]imidazol-2-yl)-3-(5-(chloromethyl)-1,3,4-oxadiazol-2-yl) propan-1-one (**4**).* An equimolar mixture of 4-(1*H*-benzo[*d*]imidazol-2-yl)-4-oxobutanehydrazide (**3**; 0.001 mol) and a chloroacetic acid (0.001 mol) in $POCl_3$ (5 mL) was placed in a microwave reaction compatible glass vessel having a magnetic stirrer bar for mixing. The reaction mixture was irradiated at a power level of 6 (60%, 420 W) for 13 min in a scientific microwave synthesizer. The reaction mixture was cooled, poured slowly onto the crushed ice and finally neutralized with sodium bicarbonate solution to produce the solid precipitates. The mixture was filtered, washed

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with plenty of water and dried. Yield: 78%, mp 223-224°C, R_f = 0.61. IR (KBr, cm^{-1}): 3348 (N-H), 3020(-C-H, Ar-H), 2936(-C-H, CH_2), 1709(C=O), 1684(-C=N), 1573 (C=C), 1305(-N-N=C), 1166(C-O-C, asymmetric), 1027(C-O-C, symmetric), 712(C-Cl). ^{1}H-NMR ($CDCl_3$): 12.50(s, 1H, NH, D_2O exchangeable), 7.79(d, 1H, *J* = 7.8Hz, H-4, benzimidazole ring), 7.47(t, 1H, *J* = 7.2Hz, H-7, benzimidazole), 7.35(t, 2H, *J* = 5.4Hz, H-5,6, benzimidazole), 4.39(s, 2H, -CH_2Cl), 3.29(t, 2H, *J* = 6.9Hz, CH_2), 2.90(t, 2H, *J* = 6.6Hz, -CH_2C=O). ^{13}C-NMR ($CDCl_3$): 168.45(C=O), 158.23, 156.37(C, oxadiazole), 154.69(C=N), 136.14, 132.24, 132.15, 126.55, 126.12, 124.83(Ar-C), 51.02(CH_2Cl), 39.76(CH_2, CH_2CO), 28.51(CH_2). ESI-MS (*m/z*): 290 (M^+). Anal.calcd. for $C_{13}H_{11}ClN_4O_2$: C, 53.71; H, 3.81; N, 19.27. Found: C, 53.74; H, 3.90; N, 19.30. Eluent mixture ratio (9:1).

*2.2.5. General procedure for the synthesis of 1-(1H-benzo[d]imidazol-2-yl)-3-(5-(methyl substituted)-1,3,4-oxadiazol-2-yl)propan-1-one (**4a-g**).* An equimoloar amount compound **4** (0.003 mol) and a secondary amine (0.003 mol) were suspended in absolute ethanol (10 mL). The suspension was added to a magnetic stir bar equipped microwave reaction vessel and just before microwave irradiation, sodium acetate (0.001 mol) was added. The microwave was operated at a power level of 5 (50%, 350 W) for 8-14 min. The content of the reaction mixture was cooled, poured onto crushed ice and acidified with glacial acetic acid to obtain a solid mass. The solid product was filtered, repeatedly washed with water to flush the inorganic components and finally dried.

*2.2.5.1. 3-(5-((6-amino-9H-purin-9-yl)methyl)-1,3,4-oxadiazol-2-yl)-1-(1H-benzo[d]imidazol-2-yl)propan-1-one (**4a**).* Yield: 90%, mp 225-226°C, R_f = 0.67. IR (KBr, cm^{-1}): 3352(N-H), 3027(C-H, Ar-H), 2987(C-H, CH_2), 1716(C=O), 1682(C=N), 1568(C=C), 1312(N-N=C), 1160(C-O-C, asymmetric), 1022(C-O-C, symmetric), 834(C-N).^{1}H-NMR ($CDCl_3$): 12.63(s, 1H,

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NH,D_2O exchangeable, benzimidazole), 8.51(s, 2H, NH_2,D_2O exchangeable, adenine), 8.37(s, 1H, adenine), 7.97(s, 1H, adenine), 7.70(d, 1H, *J* = 7.5Hz, H-4, benzimidazole), 7.49(t, 1H, *J* = 7.8Hz, H-7, benzimidazole), 7.27(t, 2H, *J* = 7.5Hz, H-5,6, benzimidazole), 4.17(s, 2H, CH_2, adenine), 2.96(t, 2H, *J* = 7.2Hz, CH_2), 2.51(t, 2H, *J* = 7.2Hz, CH_2CO). ^{13}C-NMR ($CDCl_3$): 175.31(C=O), 161.74, 160.12(C, oxadiazole), 153.17(C=N), 141.32, 138.61, 137.52, 136.79, 134.92, 129.81, 129.03, 128.56, 127.63, 124.74, 122.87(Ar-C), 63.41(CH_2, adenine), 30.72(CH_2, CH_2CO), 28.45(CH_2). ESI-MS (*m/z*): 389(M^+). Anal.calcd. for $C_{18}H_{15}N_9O_2$: C, 55.52; H, 3.87; N, 32.38. Found: C, 55.86; H, 3.96; N, 32.47. Eluent mixture ratio (8:2).

*2.2.5.2. 1-((5-(3-(1H-benzo[d]imidazol-2-yl)-3-oxopropyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-purin-6(9H) -one (**4b**).* Yield: 87%, mp 233-234°C, R_f = 0.65. IR (KBr, cm^{-1}): 3364 (N-H), 3086(C-H, Ar-H), 2952(C-H, CH_2), 1724(C=O), 1672(C=N), 1480(C=C), 1328(N-N=C), 1116 (C-O-C, asymmetric), 1020(C-O-C, symmetric). ^{1}H-NMR ($CDCl_3$): 12.31(s, 1H, NH, D_2O exchangeable, benzimidazole), 10.87(s, H, NH, D_2O exchangeable, guanine), 8.02(s, 1H, guanine), 7.85(s, 1H, guanine), 7.78(d, 1H, *J* = 7.8Hz, H-4, benzimidazole), 7.40(t, 1H, *J* = 7.8 Hz, H-7, benzimidazole), 7.27(t, 2H, *J* = 7.5Hz, H-5,6, benzimidazole), 3.92(s, 2H, CH_2, guanine), 3.23(t, 2H, *J* = 6.9Hz, CH_2), 2.85(t, 2H, *J* = 7.2Hz, CH_2CO). ^{13}C-NMR ($CDCl_3$): 168.73(C=O), 166. 23 (C=O, guanine), 158.45, 157.31(C, oxadiazole), 156.85(C=N), 137.93, 137.12, 136.87, 134.92, 127.53, 126.74, 124.56, 124.01, 122.87, 120.91(Ar-C), 57.37(CH_2, guanine), 38.61 (CH_2, CH_2CO), 25.43(CH_2). ESI-MS (*m/z*): 390(M^+). Anal.calcd. for $C_{18}H_{14}N_8O_3$: C, 55.38; H, 3.60; N, 28.71. Found: C, 55.41; H, 3.74; N, 28.92. Eluent mixture ratio (8:2).

*2.2.5.3. 3-(5-((1H-1,2,4-triazol-1-yl)methyl)-1,3,4-oxadiazol-2-yl)-1-(1H-benzo[d]imidazol-2-yl) propan -1-one (**4c**).* Yield: 92%, mp 221-222°C, R_f = 0.56. IR (KBr, cm^{-1}): 3321 (N-H), 3178

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(C-H, Ar-H), 2819(C-H, CH_2), 1724(C=O), 1662(C=N), 1523(C=C), 1384(N-N=C), 1191(C-O-C, asymmetric), 1033(C-O-C, symmetric), 848(C-N). ^{1}H-NMR ($CDCl_3$): 12.03(s, 1H, NH, D_2O exchangeable), 8.04(s, 1H, H-5, triazole), 7.88(s, 1H, H-3, triazole), 7.79(d, 1H, *J* = 8.1Hz, H-4, benzimidazole), 7.63(t, 1H, *J* = 7.2Hz, H-7, benzimidazole), 7.45(t, 2H, *J* = 7.2Hz, H-5,6, benzimidazole), 4.73(s, 2H, CH_2, triazole), 3.29(t, 2H, *J* = 6.9Hz, CH_2), 2.87(t, 2H, *J* = 6.9Hz, CH_2CO). ^{13}C-NMR ($CDCl_3$): 172.87(C=O), 165.11, 161.19(C, oxadiazole), 155.05(C=N), 132.83, 131.96, 129.86, 128.52, 124.73, 123.48, 117.67, 115.68(Ar-C), 60.24(C, triazole), 30.23 (CH_2, CH_2CO), 28.20(CH_2). ESI-MS (*m/z*): 323(M^+). Anal.calcd. for $C_{15}H_{13}N_7O_2$: C, 55.72; H, 4.05; N, 30.33. Found: C, 55.75; H, 4.13; N, 30.39. Eluent mixture ratio (7:3).

*2.2.5.4. 3-(5-((4H-1,2,4-triazol-4-ylamino)methyl)-1,3,4-oxadiazol-2-yl)-1-(1H-benzo[d]imidazol-2-yl) propan-1-one (**4d**).* Yield: 85%, mp 215-217°C, R_f = 0.53. IR (KBr, cm^{-1}): 3319 (N-H), 3104(C-H, Ar-H), 2994(CH_2), 1698(C=O), 1612(C=N), 1574(C=C), 1356(N-N=C), 1178 (C-O-C, asymmetric), 1036(C-O-C, symmetric), 834(C-N). ^{1}H-NMR ($CDCl_3$): 12.47(s, 1H, NH, D_2O exchangeable), 7.89(s, 2H, triazole), 7.61(d, 1H, *J* = 7.8Hz, H-4, benzimidazole), 7.33(t, 1H, *J* = 7.5Hz, H-7, benzimidazole), 7.26(t, 2H, *J* = 7.8 Hz, H-5,6, benzimidazole), 3.72(s, 2H, CH_2, CH_2NH), 3.21(t, 2H, *J* = 6.9Hz, CH_2), 2.92(t, 2H, *J* = 6.9Hz, CH_2CO), 2.63(s, IH, NH). ^{13}C-NMR ($CDCl_3$): 176.10(C=O), 163.41, 160.85(C, oxadiazole), 155.71(C=N), 137.32(C, aminotriazole), 129.63, 128.91, 124.73, 123.86, 121.18, 120.83(Ar-C), 66.51(CH_2, $NHCH_2$), 32.68(CH_2, CH_2CO), 28.38(CH_2). ESI-MS (*m/z*): 338(M^+). Anal.calcd. for $C_{15}H_{14}N_8O_2$: C, 53.25; H, 4.17; N, 33.12. Found: C, 53.43; H, 4.25; N, 33.71. Eluent mixture ratio (7:3).

*2.2.5.5. 3-(5-((1H-imidazol-1-yl)methyl)-1,3,4-oxadiazol-2-yl)-1-(1H-benzo[d]imidazol-2-yl) propan-1-one (**4e**).* Yield: 82%, mp 207-208°C, R_f = 0.57. IR (KBr, cm^{-1}): 3348(N-H), 3054 (C-

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H, Ar-H), 2942(C-H, CH_2), 1726 (C=O), 1685(C=N), 1532(C=C), 1328(N-N=C), 1176(C-O-C, asymme tric), 1042(C-O-C, symmetric). ^{1}H-NMR ($CDCl_3$): 11.87(s, 1H, NH, D_2O exchangeable), 8.07(s, 1H, H-2, imidazole), 7.74(d, 1H, *J* = 7.5Hz, H-4, benzimidazole), 7.30(t, 1H, *J* = 7.5Hz, H-7, benzimidazole), 7.27(t, 2H, *J* = 7.5Hz, H-5,6, benzimidazole), 6.65(d, 1H, *J* = 7.2Hz, H-5, imidazole), 6.48(d, 1H, *J* = 8.1Hz, H-4, imidazole), 4.03(s, 2H, CH_2, imidazole), 3.12(t, 2H, *J* = 7.2Hz, CH_2), 2.85(t, 2H, *J* = 6.9Hz, CH_2CO). ^{13}C-NMR ($CDCl_3$): 175.21(C=O), 161.92, 158.32 (C, oxadiazole), 154.63(C=N), 136.25, 132.53, 131.54(C, imidazole), 126.43, 125.82, 123.35, 122.61, 121.57, 119.64(Ar-C), 60.73(CH_2, imidazole), 35.31(CH_2, CH_2CO), 27.93(CH_2). ESI-MS (*m/z*): 322(M^+). Anal.calcd. for $C_{16}H_{14}N_6O_2$: C, 59.612; H, 4.37; N, 26.06. Found: C, 59.74; H, 4.25; N, 26.35. Eluent mixture ratio (8:2).

*2.2.5.6. 3-((5-(3-(1H-benzo[d]imidazol-2-yl)-3-oxopropyl)-1,3,4-oxadiazol-2-yl)methyl)-5-methyl pyrimiidine-2,4 (1H, 3H)-dione (**4f**).* Yield: 85%, mp236°C, R_f = 0.58. IR (KBr, cm^{-1}): 3344(N-H), 3105 (C-H, Ar-H), 29774(C-H, CH_2), 1724(C=O), 1662(C=N), 1566(C=C), 1384(N-N=C), 1180(C-O-C, asymmetric), 1064(C-O-C, symmetric). ^{1}H-NMR ($CDCl_3$): 14.10(s, 1H, NH, D_2O exchangeable, pyrimidine), 12.37(s, 1H, NH, D_2O exchangeable, benzimidazole), 8.03 (s, 1H, pyrimidine), 7.68(d, 1H, *J* = 7.7Hz, H-4, benzimidazole), 7.43(t, 1H, *J* = 7.2Hz, H-7, benzimidazole), 7.29(dd, 2H, *J* = 7.8Hz, *J* = 7.5Hz, H-5,6, benzimidazole), 4.09(s, 2H, CH_2, pyrimidine), 3.49(t, 2H, *J* = 6.9Hz, CH_2), 3.09(t, 2H, *J* = 7.0Hz, CH_2, CH_2CO), 2.57(s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$): 173.61(C=O), 173.14, 172.15(C=O, pyrimidine), 159.29, 159.13(C, oxadiazole), 156.41(C=N), 132.54, 130.94, 129.78, 129.84, 128.87, 128.48, 124.16, 115.70(Ar-C), 60.55(CH_2, pyrimidine), 30.40 (CH_2, CH_2CO), 28.05(CH_2), 14.31(CH_3). ESI-MS (*m/z*): 380 (M^+). Anal.calcd. for $C_{18}H_{16}N_6O_4$: C, 56.84; H, 4.24; N, 22.10. Found: C, 56.87; H, 4.33; N, 22.17. Eluent mixture ratio (9:1).

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*2.2.5.6. 1-((5-(3-(1H-benzo[d]imidazol-2-yl)-3-oxopropyl)-1,3,4-oxadiazol-2-yl)methyl)-4-amino pyrimidin-2(1H)-one (**4g**).* Yield: 82%, mp 234-235°C, R_f = 0.59. IR (KBr, cm^{-1}): 3372 (N-H), 3043(C-H, Ar-H), 2957(C-H, CH_2), 1703(C=O), 1674(C=N), 1587(C=C), 1372(N-N=C), 1184(C-O-C, asymmetric), 1065(C-O-C, symmetric). ^{1}H-NMR ($CDCl_3$): 12.17(s, 1H, NH, D_2O exchangeable), 8.34(d, 1H, *J* = 8.1Hz, pyrimidine), 8.01 (s, 2H, NH_2,D_2O exchangeable), 7.71 (d, 1H, *J* = 7.5Hz, H-4, benzimidazole), 7.33(t, 1H, *J* = 7.2Hz, H-7, benzimidazole), 7.24(t, 2H, *J* = 8.1Hz, H-5,6, benzimidazole), 6.72(d, 1H, *J* = 7.5Hz, pyrimidine), 4.13 (s, 2H, CH_2, pyrimidine), 3.28 (t, 2H, *J* = 7.2Hz, CH_2), 2.76(t, 2H, *J* = 6.9Hz, CH_2CO). ^{13}C-NMR ($CDCl_3$): 174.53(C=O), 165.01(CO, pyrimidine), 160.23, 159.10(C, oxadiazole), 155.41(C=N), 138.10, 135.29, 130.13, 129.69, 128.43, 123.14, 120.34, 118.72, 110.51(Ar-C), 55.72(CH_2, pyrimidine), 32.56(CH_2, CH_2CO), 27.13(CH_2). ESI-MS (*m/z*): 365 (M^+). Anal.calcd. for $C_{17}H_{15}N_7O_3$: C, 55.89; H, 4.14; N, 26.84. Found: C, 55.97; H, 3.64; N, 26.95. Eluent mixture ratio (8:2).

*2.2.6.. 1-(1H-benzo[d]imidazol-2-yl)-3-(5-mercapto-1,3,4-thiadiazol-2-yl)propan-1-one (**5**).* The compound **3** (0.015 mol**)** and potassium hydroxide (0.020 mol) were dissolved in ethylalcohol (40 mL) to form a solution. Afterwards, carbon disulphide (0.020 mol) was slowly introduced into the reaction mixture with constant stirring and left at room temperature for 10h. After that the ice cold conc. H_2SO_4 (5 mL) was gradually added in small increments over a period of 10 min and the resulting mixture was further stirred for 4 h at room temperature. It was then poured over crushed ice to obtain the solid precipitate which was recrystallized with carbinol.

Yield: 74%; Mp. 223-225°C; R_f = 0.43(T:E:F). IR (KBr, cm^{-1}): 3343(N-H), 3058(C-H, Ar-H), 2931(C-H, CH_2), 2589(S-H), 1694(C=O), 1651(C=N), 1607(C=C), 1380(N-N=C), 1067(C-S-C). ^{1}H-NMR (DMSO-d_6): δ 13.23(s, 1H, SH, D_2O exchangeable), 11.37(s, 1H, NH, D_2O exchangeable), 7.85(d, 1H, *J* = 7.5Hz, H-4, benzimidazole), 7.51(t, 1H, *J* = 6.9Hz, H-7,

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benzimidazole), 7.30(t, 2H, *J* = 7.5Hz, H-5,6, benzimidazole), 3.34(t, 2H, *J* = 6.9Hz, CH_2), 2.84(t, 2H, *J* = 6.9Hz, CH_2, CH_2CO). ^{13}C-NMR (DMSO-d_6): δ 175.11(C=O), 161.73, 155.61(2C, thiadiazole), 157.72(C=N), 133.87, 132.65, 130.61, 129.41, 124.72, 116.81(Ar-C), 32.45(CH_2, CH_2CO), 21.75(CH_2). ESI-MS (*m/z*): 290 (M^+). Anal. calcd. for $C_{12}H_{10}N_4OS_2$: C, 49.64; H, 3.48; N, 19.30. Found: C, 49.61; H, 3.51; N, 19.47.

*2.2.7. General procedure for the synthesis of 1-(1H-benzo[d]imidazol-2-yl)-3-(5-mercapto substituted-1,3,4-thiadiazol-2-yl) propan-1-one (**5a-f**).* A solution of compound **5** (0.001 mol) and an aryl or alkyl chloride compound (0.001 mol) in ethanolic alkali solvent (0.08g KOH in 15 mL ethanol) was placed in the scientific microwave synthesizer and irradiated at power level of 5 (50%, 350 W) for 10-14 min, The reaction mixture was decomposed by adding on to the crushed ice which on usual work up yielded the solid dried products (**5a-f**).

*2.2.7.1. 5-(3-(1H-benzo[d]imidazol-2-yl)-3-oxopropyl)-1,3,4-thiadiazol-2-yl-2-chloroethanethioate (**5a**).* Yield: 85%, mp 239°C, R_f = 0.45. IR (KBr, cm^{-1}): 3398(N-H), 3097(C-H, Ar-H), 2896(CH_2), 1724(C=O), 1612(C=N), 1504(C=C), 1384(N-N=C), 1060(C-S-C), 837(C-Cl), 759 (C-S). ^{1}H-NMR ($CDCl_3$): 11.75(s, 1H, NH, D_2O exchangeable), 7.81(d, 1H, *J* = 8.7Hz, H-4, benzimidazole), 7.49(t, 1H, *J* = 7.8Hz, H-7, benzimidazole), 7.35(t, 2H, *J* = 7.8Hz, H-5,6, benzimidazole), 3.72(s, 2H, CH_2Cl), 3.32(t, 2H, *J* = 7.2Hz, CH_2), 2.92(t, 2H, *J* = 6.9Hz, CH_2CO). ^{13}C-NMR (DMSO-d_6): 175.57(C=O), 168.76(C=O, $COCH_2Cl$), 162.24, 159.67(C, thiadiazole), 154.96(C=N), 132.21, 131.84, 130.19, 128.57, 124.65, 123.64 (Ar-C), 34.25(CH_2, CH_2Cl), 31.37(CH_2, CH_2CO), 29.32(CH_2). ESI-MS (*m/z*): 366(M^+). Anal.calcd. for $C_{14}H_{11}ClN_4O_2S_2$: C, 45.84; H, 3.03; N, 15.27. Found: C, 45.87; H, 3.08; N, 15.41. Eluent mixture ratio (8:2).

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*2.2.7.2. 2-(5-(3-(1H-benzo[d]imidazol-2-yl)-3-oxopropyl)-1,3,4-thiadiazol-2-ylthio)acetic acid (**5b**).* Yield: 84%, mp 245-247°C, R_f = 0.43. IR (KBr, cm^{-1}): 3371(O-H), 3299(N-H), 3060 (C-H, Ar-H), 2968(C-H, CH_2), 1722(C=O), 1650(C=N), 1516(C=C), 1365(N-N=C), 1064(C-S-C), 718(C-S). ^{1}H-NMR ($CDCl_3$): 12.62(s, 1H, NH, D_2O exchangeable), 10.44(s, 1H, OH, D_2O exchangeable), 7.59(d, 1H, *J* = 7.5Hz, H-4, benzimidazole), 7.23(t, 1H, *J* = 7.8Hz, H-7, benzimidazole), 7.19(t, 2H, *J* = 7.5Hz, H-5,6, benzimidazole), 4.15(s, 2H, CH_2COOH), 2.95(t, 2H, *J* = 7.2Hz, CH_2), 2.58(t, 2H, *J* = 6.9Hz, CH_2CO). ^{13}C-NMR ($CDCl_3$): 173.45(C=O), 170.83 (C=O, COOH), 163.27, 162.35(C, thiadiazole), 154.21(C=N), 131.74, 129.51, 124.63, 123.45, 122.05, 121.67(Ar-C), 37.51(CH_2, CH_2COOH), 33.84(CH_2, CH_2CO), 28.30(CH_2). ESI-MS (*m/z*): 348(M^+). Anal.calcd. for $C_{14}H_{12}N_4O_3S_2$: C, 48.26; H, 3.47; N, 16.08. Found: C, 48.37; H, 4.05; N, 16.75. Eluent mixture ratio (9:1).

*2.2.7.3. 1-(1H-benzo[d]imidazol-2-yl)-3-(5-(2-bromoethylthio)-1,3,4-thiadiazol-2-yl)propan-1-one (**5c**).* Yield: 92%, mp 237-238°C, R_f = 0.52. IR (KBr, cm^{-1}): 3375(N-H), 3043 (C-H, Ar-H), 2952(C-H, CH_2), 1720(C=O), 1643(C=N), 1502(C=C), 1312(N-N=C), 1082(C-S-C), 720(C-S), 664(C-Br). ^{1}H-NMR ($CDCl_3$): 12.10(s, 1H, NH, D_2O exchangeable), 7.71(d, 1H, *J* = 7.8Hz, H-4, benzimidazole), 7.41(t, 1H, *J* = 7.5Hz, H-7, benzimidazole), 7.29(t, 2H, *J* = 7.8Hz, H-5,6, benzimidazole), 3.92(t, 2H, *J* = 6.9Hz, CH_2Br), 3.54(t, 2H, *J* = 6.9Hz, CH_2S), 3.23(t, 2H, *J* = 7.2Hz, CH_2), 2.87(t, 2H, *J* = 7.2Hz, CH_2CO). ^{13}C-NMR ($CDCl_3$): 174.61(C=O), 162.74, 160.53 (C, thiadiazole), 153.82(C=N), 132.54, 130.12, 128.32, 124.57, 123.42, 122.81(Ar-C), 45.23, 40.35 (CH_2CH_2Br), 31.92(CH_2, CH_2CO), 27.73(CH_2). ESI-MS (*m/z*): 397(M^+). Anal.calcd. for $C_{14}H_{13}BrN_4OS_2$: C, 42.32; H, 3.30; N, 14.10. Found: C, 41.86; H, 3.51; N, 14.23. Eluent mixture ratio (8:2).

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2.2.7.4. .5-(3-(1H-benzo[d]imidazol-2-yl)-3-oxopropyl)-1,3,4-thiadiazol-2-yl-benzothioate ***(5d)***. Yield: 80%, mp 235°C, R_f = 0.56. IR (KBr, cm^{-1}): 3367(N-H), 3058(C-H, Ar-H), 2977(C-H, CH_2), 1677(C=O), 1600(C=N), 1504(C=C), 1384(N-N=C), 1045(C-S-C), 752(C-S). ^{1}H-NMR ($CDCl_3$): 12.37 (s, 1H, NH, D_2O exchangeable), 8.14(d, 1H, *J* = 7.5Hz, H-4, benzimidazole), 7.79 (t, 1H, *J* = 8.1Hz, H-7, benzimidazole), 7.61(t, 2H, *J* = 7.2Hz, H-5,6, benzimidazole), 7.50-7.26 (m, 5H, phenyl), 3.29(t, 2H, *J* = 6.9Hz, CH_2), 2.90(t, 2H, *J* = 6.9Hz, CH_2CO). ^{13}C-NMR ($CDCl_3$): 175.85(C=O), 175.31(C=O, phenyl), 162.32, 160.72(C, thiadiazole), 154.67(C=N), 133.54, 132.27, 132.05, 131.97, 131.34, 130.72, 129.32, 128.73, 124.58, 123.21, 121.67, 120.67(Ar-C), 32.81(CH_2, CH_2CO), 27.94(CH_2). ESI-MS (*m/z*): 394(M^+). Anal.calcd. for $C_{19}H_{14}N_4O_2S_2$: C, 57.85; H, 3.59; N, 14.20. Found: C, 57.91; H, 3.52; N, 14.35. Eluent mixture ratio (8:2).

2.2.7.5. 1-(1H-benzo[d]imidazol-2-yl)-3-(5-(benzylthio)-1,3,4-thiadiazol-2-yl)propan-1-one ***(5e)***. Yield: 78%, mp 223-224°C, R_f = 0.66. IR (KBr, cm^{-1}): 3362(N-H), 3053(C-H, Ar-H), 2947 (C-H, CH_2), 1697(C=O), 1664(C=N), 1582(C=C), 1368(N-N=C), 1052(C-S-C), 724(C-S). ^{1}H-NMR ($CDCl_3$): 12.47(s, 1H, NH, D_2O exchangeable), 7.87(d, 1H, *J* = 7.8Hz, H-4, benzimidazole), 7.77(t, 1H, *J* = 8.1Hz, H-7, benzimidazole), 7.60(t, 2H, *J* = 7.8Hz, H-5,6, benzimidazole), 7.53-6.96(m, 5H, phenyl), 3.87(s, 2H, CH_2S), 3.42(t, 2H, *J* = 7.2Hz, CH_2), 2.88 (t, 2H, *J* = 7.2Hz, CH_2CO). ^{13}C-NMR ($CDCl_3$): 173.15(C=O), 163.87, 162.10(C, thiadiazole), 156.30 (C=N), 132.91, 132.14, 131.48, 130.17, 129.65, 128.71, 125.80, 124.01, 123.27, 123.12, 118.54, 117.93 (Ar-C), 41.35(CH_2, CH_2S), 31.53(CH_2, CH_2CO), 27.43(CH_2). ESI-MS (*m/z*): 380 (M^+). Anal. calcd. for $C_{19}H_{16}N_4OS_2$: C, 59.98; H, 4.24; N, 14.73. Found: C, 60.3; H, 4.51; N, 14.85. Eluent mixture ratio (6:4).

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*2.2.7.6. 2-(5-(3-(1H-benzo[d]imidazol-2-yl)-3-oxopropyl)-1,3,4-thiadiazol-2-ylthio)acetamide (**5f**).* Yield: 76%, mp 225-226°C, R_f = 0.54. IR (KBr, cm^{-1}): 3381(N-H), 3053(C-H, Ar-H), 2932 (C-H, CH_2), 1727(C=O), 1652(C=N), 1527(C=C), 1373(N-N=C), 1047(C-S-C), 720(C-S). ^{1}H-NMR($CDCl_3$): 12.51(s, 1H, NH, D_2O exchangeable), 8.31(s, 2H, NH_2, D_2O exchangeable), 7.90 (d, 1H, *J* = 7.5Hz, H-4, benzimidazole), 7.65(t, 1H, *J* = 7.2Hz, H-7, benzimidazole), 7.39(t, 2H, *J* = 7.8Hz, H-5,6, benzimidazole), 3.97(s, 2H, CH_2CONH_2), 3.19(t, 2H, *J* = 6.9Hz, CH_2), 2.85(t, 2H, *J* = 7.2Hz, CH_2CO). ^{13}C-NMR ($CDCl_3$): 173.21(C=O), 171.34(C=O, $CONH_2$), 161.51, 158.72(C-thiadiazole), 155.63(C=N), 130.42, 129.51, 128.53, 124.16, 123.93, 123.25 (Ar-C), 58.17(CH_2, CH_2CONH_2), 31.02(CH_2, CH_2CO), 25.31(CH_2). ESI-MS (*m/z*): 347(M^+). Anal. calcd. for $C_{14}H_{13}N_5O_2S_2$: C, 48.40; H, 3.77; N, 20.16. Found: C, 48.47; H, 3.75; N, 20.41. Eluent mixture ratio (7:3).

*2.2.8. 1-(1H-benzo[d]imidazol-2-yl)-3-(5-mercapto-1,3,4-oxadiazol-2-yl)propan-1-one (**6**).* It was prepared as per the previously reported method (Husain et al., 2013). The identity of the compound was established after comparing its physical properties and spectral data which are found to be in good agreement with the reported literature values.

2.2.9. 3-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)-1-(1H-benzo[d]imidazol-2-yl)propan-1-one (7). It was prepared as per the previously reported method (Husain et al., 2013). The identity of the compound was established after comparing its physical properties and spectral data which are found to be in good agreement with the reported literature values.

*2.2.10. General procedure for synthesis of 1-(1H-benzo[d]imidazol-2-yl)-3-(6-substituted-7H-[1,2,4] triazolo[3,4-b] [1,3,4]thiadiazin-3-yl)propan-1-one (**8a-b**).* An equimolar solution of compound **7** (0.003 mol) and α-chloro containing methyl compound (0.003 mol) was prepared

ACCEPTED MANUSCRIPT

in absolute ethanol (15 mL). The solution after refluxing for 3-4 h was cooled to room temperature and then neutralized with ammonia solution to yield a solid product.

*2.2.10.1. 1-(1H-benzo[d]imidazol-2-yl)-3-(6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl) propan-1-one (**8a**).* Yield: 65%, mp 240-241°C, R_f = 0.67.IR (KBr, cm^{-1}): 3354(N-H), 3027(C-H, Ar-H), 2968(C-H, CH_2), 1694(C=O), 1612(C=N), 1575(C=C), 1363(N=C-S), 1258 (N-N=C). ^{1}H-NMR ($CDCl_3$): 12.31(s, 1H, NH, D_2O exchangeable), 8.10-7.86(m, 5H, -C_6H_5), 7.78(d, 1H, *J* = 7.8Hz, H-4, benzimidazole), 7.48(t, 1H, *J* = 7.5Hz, H-7, benzimidazole), 7.30(t, 2H, *J* = 7.5Hz, H-5,6, benzimidazole), 3.73(s, 2H, CH_2, cyclic), 3.35(t, 2H, *J* = 7.2Hz, CH_2), 2.93 (t, 2H, *J* = 6.9Hz, CH_2, CH_2CO). ^{13}C-NMR ($CDCl_3$): 170.42(C=O), 161.54, 160.72(C, triazole), 156.20, 154.86(C=N), 135.38, 132.19, 131.94, 129.52, 129.12, 128.23, 127.43, 126.75, 125.73, 117.24, 116.71, 115.76(Ar-C), 51.75(CH_2, cyclic), 29.96(CH_2, CH_2CO), 27.97(CH_2). ESI-MS (*m/z*): 388 (M^+). Anal.calcd. for $C_{20}H_{16}N_6OS$: C, 61.84; H, 4.15; N, 21.63. Found: C, 61.95; H, 4.37; N, 21.69. Eluent mixture ratio (6:4).

*2.2.10.2. 1-(1H-benzo[d]imidazol-2-yl)-3-(6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-b] [1,3,4] thiadiazin-3-yl) propan-1-one (**8b**).* Yield: 60%, mp 231-232°C, R_f = 0.62.IR (KBr, cm^{-1}): 3361(N-H), 3037(C-H, Ar-H), 2965(C-H, CH_2), 1703(C=O), 1652(C=N), 1582(C=C), 1374 (N=C-S), 1237 (N-N=C). ^{1}H-NMR ($CDCl_3$): 12.03(s, 1H, NH, D_2O exchangeable), 7.99-7.69(m, 4H, phenyl), 7.45(d, 1H, *J* = 8.1Hz, H-4, benzimidazole), 7.32(t, 1H, *J* = 7.8Hz, H-7, benzimidazole), 7.24(t, 2H, *J* = 7.5Hz, H-5,6, benzimidazole), 4.11(s, 2H, CH_2,cyclic), 3.52(s, 3H, OCH_3), 3.24(t, 2H, *J* = 6.9 Hz, CH_2), 2.86(t, 2H, *J* = 6.9Hz, CH_2CO). ^{13}C-NMR ($CDCl_3$): 176.26(C=O), 159.04, 158.83(C, triazole), 156.20, 154.75(C=N), 133.29, 132.05, 129.69, 128.43, 123.14, 122.13, 121.63, 120.72, 117.38, 116.81, 115.97, 115.78(Ar-C), 50.52(OCH_3), 34.82(CH_2, cyclic), 28.80 (CH_2, CH_2CO), 26.90(CH_2). ESI-MS (*m/z*): 418(M^+). Anal.calcd. for

ACCEPTED MANUSCRIPT

$C_{21}H_{18}N_6O_2S$: C, 60.27; H, 4.34; N, 20.08. Found: C, 61.54; H, 4.73; N, 20.35. Eluent mixture ratio (7:3).

*2.2.11.General procedure for synthesis of 1-(1H-benzo[d]imidazol-2-yl)-3-(6-(substituted)-[1,2,4] triazolo[3,4-b] [1, 3, 4]thiadiazol-3-yl)propan-1-one (**9a-b**).* An equimolar solution of compound **7** (0.003 mol) and α-chloro containing carbonyl compounds (0.003 mol) was prepared in absolute ethanol (15 mL). The solution after refluxing for 3-4 h was cooled to room temperature and then neutralized with ammonia solution to yield a solid product...

*2.2.11.1. 1-(1H-benzo[d]imidazol-2-yl)-3-(6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)propan-1-one (**9a**).* Yield: 61%, mp 220-22°C, R_f = 0.62. IR (KBr, cm^{-1}): 3398(N-H), 3016 (C-H, Ar-H), 2904(C-H, CH_2), 1728(C=O), 1665(C=N), 1616(C=C), 1384(N=C-S), 1265(N-N=C). ^{1}H-NMR ($CDCl_3$): 11.01(s, 1H, NH, D_2O exchangeable), 7.83(d, 1H, *J* = 8.4Hz, H-4, benzimidazole), 7.73 (t, 1H, *J* = 8.1Hz, H-7, benzimidazole), 7.65(t, 2H, *J* = 7.2Hz, H-5,6, benzimidazole), 7.51-7.34(m, 5H, phenyl), 3.01(t, 2H, *J* = 8.1Hz, CH_2), 2.51(t, 2H, *J* = 8.1Hz, CH_2, CH_2CO). ^{13}C-NMR ($CDCl_3$): 170.37(C=O), 159.25, 156.25(C, triazole), 153.97(C=N), 148.68(C, thiadiazole), 132.51, 130.48, 129.51, 128.40, 128.01, 127.47, 126.95, 125.89, 123.58, 122.31, 117.38, 116.81 (Ar-C), 30.65(CH_2, CH_2CO), 28.03(CH_2). ESI-MS (*m/z*): 374(M^+). Anal. calcd. for $C_{19}H_{14}N_6OS$: C, 60.95; H, 3.77; N, 22.45. Found: C, 61.07; H, 3.80; N, 22.55. Eluent mixture ratio (6:4).

*2.2.11.2. 1-(1H-benzo[d]imidazol-2-yl)-3-(6-(4-hydroxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)propan-1-one (**9b**).* Yield: 68%, mp 231-232°C, R_f = 0.59. IR (KBr, cm^{-1}): 3371 (N-H), 3061 (C-H, Ar-H), 2951(C-H, CH_2), 1695(C=O), 1627(C=N), 1587(C=C), 1345 (N=C-S), 1250 (N-N=C). ^{1}H-NMR ($CDCl_3$): 11.97(s, 1H, NH, D_2O exchangeable), 7.75(d, 1H, *J* = 7.8 Hz, H-4, benzimidazole), 7.43(t, 1H, *J* = 7.8Hz, H-7, benzimidazole), 7.34(t, 2H, *J* =

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7.5Hz, H-5,6, benzimidazole), 7.27-7.03(m, 4H, phenyl), 6.15(s, 1H, OH), 3.27(t, 2H, *J* = 7.2Hz, CH_2), 2.95(t, 2H, *J* = 6.9Hz, CH_2CO). ^{13}C-NMR ($CDCl_3$): 169.27(C=O), 158.33, 157.51(C, triazole), 154.15 (C=N), 144.23(C, thiadiazole), 130.82, 128.63, 128.35, 127.47, 126.95, 125.89, 123.58, 122.28, 122.16, 117.38, 116.81, 115.97(Ar-C), 34.82(CH_2, CH_2CO), 28.25(CH_2). ESI-MS (*m/z*): 390 (M^+). Anal.calcd. for $C_{19}H_{14}N_6O_2S$: C, 58.48; H, 3.62; N, 21.523. Found: C, 58.48; H, 3.86; N, 21.74. Eluent mixture ratio (7:3).

2.3. In vitro anticancer methodology

The medium RPMI 1640 having a 5% fetal bovine serum and 2mM L-glutamine was used to grow the human tumor cell lines. The Microtiter plates were inoculated with the cancer cells followed by incubation at ideal conditions for growth, such as 37°C temperature, 5% CO_2, 95% air and 100% relative humidity for a period of 24 h before treatment with the tested compounds. After the incubation for 24 h, cell population of each cell line at the time of sample addition (Tz) was measured by fixing two plates of each cell line with TCA in situ. The sample was dissolved in DMSO at 400-fold of the desired final maximum test concentration and stored in frozen conditions until its further use in experiments. An aliquot of frozen concentrate of test sample was shaken to liquefy the content at the time of sample addition to cell lines and diluted to two fold with the complete medium containing 50 µg/mL gentamicin of the desired final maximum concentration. In addition to this, another four, 10-fold or ½ log serial dilutions were prepared to have five different and a control. The different dilutions of sample in aliquots of 100 µL were added to the appropriate Microtiter wells having 100 µL of medium to obtain the desired final sample concentrations. After the addition of sample, the plates were again incubated for an additional 48 h at the standard temperature, air and humidity conditions. An accurately measured 50 µL of cold 50% (w/v) TCA (final concentration, 10% TCA) was gently added to fix the cells

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in situ and incubated at 4°C for a duration of 60 min. The supernatant was rejected and the plates were thoroughly washed five times with tap water and finally dried in air. A 4 % (w/v) solution of Sulforhodamine B (SRB) (100 µL) prepared in 1% acetic acid was added to each Microtiter well and the plates were incubated at room temperature for another 10 min. A 10 mM trizma base was subsequently added to solublize the bound stain and the absorption of resulting mixture was recorded at 515 nm using an automated micro plate reader (Grever et al., 1992; Monks et al., 1991). A total of seven absorbance (optical density) readings were recorded at [time zero, (Tz), control growth (C) and test growth in the presence of sample at five concentration levels (Ti)]. The Percentage Growth (PG) i.e. the effect of the compound on growth of a cell line was calculated as by using the following formula:

If (Mean OD test - Mean ODtzero) > 0. then
PG = 100 x (Mean ODtest - Mean ODtzero)/(Mean ODctrl - Mean ODtzero)

If (Mean ODtest -Mean ODtzero) < 0. then
PG = 100 x (Mean ODtest - Mean ODtzero)/Mean ODtzero

Where: Mean OD_{tzero} represents an average of optical density (OD) measurements of SRB-derived color just before exposure of cells to the screened sample. Mean OD_{test} is the average of optical OD measurements of SRB-derived color after 48 h exposure of cells to the screened sample. Mean OD_{ctrl} stands for an average of OD measurements of SRB-derived color after 48 h with no exposure of cells to the test compound. Each concentration was expressed as the $\log_{10}$ (molar or ug/mL and the response parameters values of GI_{50}, TGI and LC_{50} were interpolated which indicate the concentrations at which the PG is +50, 0 and -50 respectively.

The IC_{50} value has been renamed by NCI in to special concentration parameters (GI_{50}, TGI and LC_{50}). Percentage growth inhibition was calculated as follows:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz

[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz

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Three dose response parameters viz. GI_{50}, TGI and LC_{50} were calculated for each cell line sub panel. GI_{50} or Growth inhibition of 50 % was deduced from [(Ti-Tz)/(C-Tz)] x 100 = 50 (It is the drug concentration which results in a 50% decrease in the net protein increase). Total growth inhibition (TGI) was calculated from the Ti = Tz (i.e. a concentration at which the total growth inhibition is 100%) while LC_{50} was calculated from [(Ti-Tz)/Tz] x 100 = -50 (concentration of the drug which results in a 50% reduction in the measured protein at the end of the drug exposure as compared to that in the beginning) indicating a net loss of the cells (Holbeck et al., 2010; Boyd & Paul, 1995). The dose response curve is plotted to get a fair idea about the growth percentage inhibition of cell lines at a particular concentration of the tested sample. The points at which the curve crosses the horizontal grid lines correspond to parameters; GI_{50} (crosses at +50 line), TGI (crosses at 0 line) and LC_{50} (crosses at -50 line), respectively.

2.4. Molecular docking studies

The molecular docking studies were performed with the help of a Maestro 9.0 docking software (Schrodinger Inc. USA) on the 3D structure of DNA topoisomerase complex enzyme A windows 7 based 64 bit operating systems using an HCl computer [Intel (R) Core (TM) i5-2400 CPU @ 3.10 GHz, 8GB memory] was used to carry out the docking studies. The 3D structure of DNA topoisomerase enzyme for the study was downloaded from the Protein Data Bank (PDB ID: 1SC7). It has 96% similarity with the human cell enzyme and all active site residues in the vicinity of cofactor have exact counterparts. The downloaded structure was further refined for ideal docking results (Staker et al., 2005). The PDB enzyme structure was thoroughly analyzed for missing atoms, bonds and/or contacts. All the residues and water molecules except ligand from the enzyme structure were removed manually. A builder molecule was used to construct the ligand molecules and then to obtain a stable structure, the energy of the molecule was also

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minimized. With the help of a grid box, the active sites were generated on the molecule. The conformation corresponding to the lowest energy was selected and subjected to an energy minimization.

3. Results and discussion

3.1. Chemistry

The target compounds were prepared as per the synthetic route outlined in scheme **1-3**. The starting material 4-(1*H*-benzo[*d*]imidazol-2-yl)-4-oxobutanoic acid (**1**) was synthesized by oxidative cyclization of 1,2 diaminobenzene with α-ketoglutaric acid in an acidified solution (4NHCl). The compound (**1**) was converted to an ethyl ester (**2**) by simple Fischer esterification reaction followed by treatment with hydrazine hydrate to obtain e 4-(1*H*-benzo[*d*]imidazol-2-yl)-4-oxobutane hydrazide (**3**). The hydrazide (**3**) upon reaction with chloroacetic acid in the presence of cyclizing agent, ($POCl_3$) under microwave irradiation yielded a compound (**4**). The chloro group at the 5^{th} position of the oxadiazole ring of compound 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(5-(chloromethyl)-1,3,4-oxadiazol-2-yl) propan-1-one (**4)** was further substituted with eight heterocyclic secondary amines. The reaction of compound (**4**) with substituted secondary amines was carried out in in presence of NaOAc by employing microwave radiations to accomplish the synthesis of 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(5-(methylsubstituted)-1,3,4-oxadiazol-2-yl)propan-1-ones (**4a-g**) (Scheme **1**). The compounds of the series (**5a-f**); (1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(5-mercaptosubstituted-1,3,4-thiadiazol-2-yl) propan-1-one) were synthesized by reacting compound 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(5-mercapto-1,3,4-thiadiazol-2-yl)propan-1-one (**5**) with six different chloro compounds (Scheme **2**). Compound (**3**) was also cyclized with CS_2/KOH in ethanol to produce, 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(5-mercapto-1,3,4-oxadiazol-2-yl) propan-1-one (**6**) which on further treatment with hydrazine

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hydrate gave a compound **7**, (3-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)-1-(1*H*-benzo[*d*]imidazol-2-yl)propan-1-one). Benzimidazole clubbed triazolo compounds (**7)** were then condensed with three substituted methyl chloride and three carbonyl chloride compounds to get benzimidazole bearing substituted triazolo-thiadiazine and triazolo-thiadiazole derivatives i.e. 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(6-substituted-7*H*-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazin-3-yl)propan-1-one (**8a-b**) and 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(6-(substituted)-[1,2,4]triazolo [3, 4-b][1,3,4]thiadiazol-3-yl)propan-1-one (**9a-b**), respectively (Scheme **3**).

The structures of newly prepared compounds were elucidated using modern, sophisticated analytical techniques viz. FT-IR, ^{1}H &^{13}C-NMR and mass spectral data. The results of C, H, N analysis were consistent with the proposed structures and found within ±0.4% as compared with the theoretical values. In general, for all the synthesized compounds, the absorption bands for carbonyl (C=O) and secondary amino (N-H) were observed in the range 1666-1728 and 3317-3429 cm^{-1}, respectively. The absorption peaks around 2599 and 1660 cm^{-1} were accounted for S-H and C=N. In ^{1}H-NMR spectra, the presence of a singlet around δ 12.3 is indicative of ring N-*H* and another singlet further downfield, at δ 13.4 is assigned to S-*H*, both these signals were disappeared upon D_2O shaking, which confirm the presence of these groups in the synthesized molecules. The appearance of peaks at δ 173.1 and 154.9 in ^{13}C-NMR spectra could be related to C=O and C=N. The distinct bands observed in IR spectra nearly at 1384, 1060 and 1028 cm^{-1} were accounted for N-N=C, C-S-C and C-O-C which indicates the incorporation of oxadiazole and thiadiazole ring in the benzimidazole analogs. ^{1}H-NMR spectra of all the compounds exhibited two triplets at appropriate chemical around δ 2.8 (J = 6.9Hz) and 3.2 (J = 7.2Hz) and also in ^{13}C-NMR spectra two signals were obtained around δ 30.2 and 27.9, which could be assigned tor two methylene groups (-CH_2-CH_2-) spacer, linker or bridge groups between the

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benzimidazole ring and the other heterocyclic rings such as oxadiazole, thiadiazole, triazolo-thiadiazole and triazolo-thiadiazine etc. The signals for benzimidazole hydrogens in proton NMR appeared as doublet, triplet at around δ 7.6, 7.4 and 7.2 (7.8Hz, 7.5Hz, 7.5Hz). The characteristic signals in ^{13}C-NMR spectra which appeared at around δ 161.1, 159.1 are related tor oxadiazole carbon ring and other signals at δ 162.3, 160.7 are indicative of the thiadiazole carbon ring. The 3D, optimization and viewer of few designed molecules such as **4f**, **5e, 9a** and clinically used anticancer drug, bendamustine has been shown with space fill model (Fig. **3**).

3.2. In vitro anticancer screening

A total of fifteen compounds were submitted to NCI for *in vitro* anticancer screening against 60 human cell lines obtained from nine clinically isolated cancer types and were granted NCS- codes (Table 1). The prepared compounds were tested at a single dose and added at a concentration ($1x10^{-5}$ M) followed by incubation of culture for the duration of 48 h. A protein binding dye ‘‘sulforhodamine B’’ was used for the end point determinations (Shoemaker, 2006; Grever et al., 1992). The result of each tested compound on the growth of cells is expressed in terms of percent growth of treated cells in comparison to the untreated control cells.

Those compounds which diminished or inhibited the cell line growth to 32% or less were regarded (a negative value is suggestive of cell kills) as *in-vitro* active (Table **1**) (Corona et al., 2009). Among all the tested compounds, **4b**, **4f**, **4g**, **5e**, **8b** and **9a** were observed to be active against CCRF-CEM (leukemia), MDA-MB-435 (melanoma), MLT-4 (leukemia), CCRF-CEM (leukemia) and K-562 (leukemia) cell lines respectively. Except compound **4f**, all other agents exhibited low antiproliferative activity. The compound **4f** (NSC: 761982/1) was the most active anticancer agent which met the pre – determined criteria of growth inhibition and thus was further chosen for the NCI full panel of five dose assay method at 10-fold dilutions of five

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different concentrations (0.01, 0.1, 1, 10 and 100 µM) (Table **2**). Compound **4f** showed remarkable antiproliferative activity at all the five dosage levels and therefore, further referred to Biological Evaluation Committee of NCI for advanced study (Monks et al., 1991).

The compound **4f** (NSC: 761982/1) displayed remarkable significant cytotoxic potential against all the investigated cell lines which represent diverse sub-panels with GI_{50} values obtained between 0.09 to 16.2 µM falling within the sensitive range and exhibiting an outstanding antiproliferative activity (Table **3**). The compound was also found to be sensitive against some individual cell lines and demonstrated the highest activity against CNS cancer cell lines, such as SNB-75 (GI_{50} 0.09, TGI 1.39, LC_{50}>100 and $\log_{10}GI_{50}$ -7.0, $\log_{10}TGI$ -5.86, $\log_{10}LC_{50}$ >-4.00). The analyzed date also indicated an evident sensitivity profile against colon cancer subpanel (GI_{50} value vary from 0.23-15.20 µM), least for HT29 and highest for HCT-15 cell lines. The tested compound **4f** was also noted to be quite sensitive against other cell lines like leukemia, melanoma, CNS, prostate, breast cancer, etc., and in each case the concentration required by the compound to exhibit the activity was observed to be under 2 µM. All the screened melanoma cancer cell lines were sensitive against the tested compound and GI_{50} value was observed to be less than 1.56 µM. The maximum inhibition of growth of cell lines was noted against the SNB-75 CNS cancer cell line (GI_{50} value 0.09 µM) and the least growth inhibitory activity against ovarian cancer, NCI/ADR-RES ovarian cancer cell line (GI_{50} value 16.2 µM).). The rest of all other subpanel cell lines exhibited maximum sensitivity against the tested compound with not more than 16.2 µM concentrations (Table **3**). LC_{50} values for the majority of the cell lines was greater than 100 µM with exception to COLO 205 and DU-145, where LC_{50} was observed to be very low (20.2 µM & 24.3 µM, respectively) (Table **3**). The log molar concentration ($\log GI_{50}$) values of compound **4f** against various cell lines ranged from -7.00

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to -4.79. The minimum concentration (-7.00) was observed against CNS cancer subpanel of SNB-75 cell line, while for NCI/ADR-RES cell line of ovarian cancer subpanel, log GI_{50} was the highest (-4.79). The majority of the cell lines of sub panel showed logTGI and $logLC_{50}$ values to be more than > -4.00 and except COLO 205 (colon cancer) and DU-145 (prostate cancer) cell lines for which $logLC_{50}$ were noted to be -4.69 and -4.61), respectively. Furthermore, a mean graph midpoint (MG-MID) value of **4f** was also calculated for $logGI_{50}$, logTGI and $logLC_{50}$ parameters. MG-MID value is the averaged activity parameter of GI_{50}, TGI, or LC_{50} values of all cell lines in the subpanel or the full panel towards the tested compound. The values were found on the lower side, $logGI_{50}$ (-6.04), logTGI (-4.38) and $logLC_{50}$ (-4.02) which indicates efficacy of the screened compound (Table **4**). The selective index, which is a ratio of average sensitivity of all cell lines to the average sensitivity of all cell lines of a particular subpanel towards the tested compound, was also calculated to measure the compound selectivity towards cell lines (Rostom 2006). The selective index values between 3-6 indicate moderate selectivity; ratios >6 is considered to have high selectivity towards the corresponding cell line, while compounds not meeting either of these criteria are referred to as nonselective i.e. mild selectivity towards the corresponding subpanel. Compound **4f**, in the study exhibited moderate selectivity towards prostate cancer cell lines with a selective index 3.66 and observed to be mild selective against breast cancer, melanoma, leukemia and CNS cancer with a selective index of 2.78, 2.71, 2.55 and 2.03, respectively (Table **3**). A dose response curve of synthetic compound **4f** (after exposure to various cancer cell lines in NCI60 panel was plotted between log_{10} of respective molar concentration of the sample versus percentage growth (PGs). The horizontal grid lines for comparison purpose are drawn across the plot at percentage growth values of +50, 0 and -50. The curved lines were coded with different colors as per origin of tissues such as; blue color for lung

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cancer; red color for leukemia cell line; the gray color for central nervous system cancer; green color for colon cancer; purple color for ovarian cancer; pink color for breast cancer cell line; coral color for melanoma; golden color for renal cancer; turquoise color for prostate cancer cell lines (Fig. **4**). The plots of percentage growth in cancer cell lines vs sample concentration at five different dose levels (1 log dilutions from 10^{-4} mol/L to 10^{-8} mol/L) after treatment with compound **4f** is shown in Fig. **5**.

The dose response curve of compound **4f** (NSC: 761982) plotted for seven subpanels of colon cancer (Fig. **6**), illustrates the endpoint calculations for $GI_{50,}$ TGI and LC_{50} at five dose concentrations. A value equal to '0' growth percent suggests no net growth or multiplication during the whole assay and the number of cells at remain equal to quantity at time zero. The calculated endpoints for the cell line COLO 205 of colon subpanel (red open circle) was found as GI_{50} = 0.35, TGI = 1.36 and LC_{50} = 20.2. All other cell lines of the colon cancer subpanel such as HCC-2998 (red open diamond), HCT-116 (red open triangle), HCT-15 (red open square), HT29 (solid blue circle), KM12 (solid blue diamond), SW-620 (solid blue triangle) were found to be less sensitive than COLO 205 against the screened compound (Fig. **6**). The *in vtiro* anticancer activity of compound **4f**, the most potent and active compound among the entire library of synthesized compounds based on rational design was also compared with the biological data obtained from the NCI web site for the clinically used anticancer drugs (Bendamustine and Chlorambucil) in terms of potency (μmol/L) by three response parameters and the results are presented in Table **5**. The tabulated results clearly indicate that the compound **4f** has lower mean values of log molar concentration for response parameters viz. GI_{50} and TGI_{50} and slightly higher mean value of LC_{50} as compared to clinically used anticancer agents, bendamustine and chloramabucil. Also, the mean graph midpoint GI_{50} value (arithmetical mean value of treated

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cancer cell lines) of the most potent compound **4f** was observed to be only 2.09 μM, which is much lower than the reference anticancer agents (60 and 52 μM, respectively) suggesting that the benzimidazole endowed 1,3,4 oxadiazole compound holds promise as a potential anticancer agent. It has been reported in literature that benzimidazole derivatives act by inhibiting DNA topoisomerase complex (Selcen et al., 2009; Singh and Tandon 2011) and their binding mode to DNA varies from intercalation to groove binding based on the number of benzimidazole rings (Kubota et al., 1999). Therefore, there is a high probability that antiproliferative effects of compound **4f,** a benzimidazole derivative which is attached to pyrimidine and 1,3,4 oxadiazole rings could be due to DNA intercalation. However, the studies focusing on the mechanism of action of these derivatives are currently under progress in our lab.

3.3. Structural activity relationship (SAR)

On the basis of the obtained results, it can be concluded that, benzimidazole analogues endowed with oxadiazole possess excellent antiproliferative activities as compared to the other benzimidazole clubbed thiadiazole, triazolo-thiadiazines and triazolo-thiadiazoles derivatives. It was observed that the presence of electron withdrawing groups like oxygen at *ortho* (2^{nd} position), *meta* (3^{rd}position) or *para* (4^{th}position) position on aromatic ring of target compounds influence the antiproliferative activity. Compound **4g** having free oxygen group at *ortho* (2-one) position of phenyl ring, chemically as, 1-((5-(3-(1*H*-benzo [*d*] imidazol-2-yl)-3-oxopropyl)-1,3,4-oxadiazol-2-yl)methyl)-4-amino pyrimidin-2(1*H*)-one, demonstrated high sensitivity (78.75%) against NCI cancer cell lines panel and likewise when the same group is disubstituted on *ortho* and *para* (2,4-dione) positions of phenyl ring, an augment in the sensitivity (20.03%) was observed for the compound, 3-(5-(3-(1*H*-benzo [*d*] imidazol-2-yl)-3-oxopropyl)-1,3,4-

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oxadiazol-2-yl)methyl)-5-methylpyrimidine-2,4(1*H*, 3*H*)-dione (**4f**). Contrary to this, the electron donating groups like methoxy (-OCH_3) attached at *para* (4^{th}position) position of the phenyl ring, a decrease in the sensitivity (93.97%) was observed for compound **8b**, namely, 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(6-(4-methoxy phenyl)-7*H*-[1,2,4] triazolo [3,4-b] [1, 3] thiazin-3-yl) propan-1-one. Similarly, the presence of methyl group also decreased the sensitivity (94.39%) as observed for the compound **5e,** namely, 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(5-(benzylthio)-1,3,4-thiadiazol-2-yl) propan-1-one. However, the sensitivity of unsubstituted aromatic ring such as in compound **9a** noted to be increased to (92.86%) in comparison to the compounds bearing electron donating groups such as methoxy and methyl as in compounds **8b** and **5e**.

3.3. Molecular docking studies

A number of research studies conducted elsewhere have reported that benzimidazole derivatives act by inhibiting DNA topoisomerase complex (Selcen et al., 2009; Singh and Tandon 2011). Therefore, the molecular interactions of the compound **4f** with the target protein, topoisomerase enzyme complex were studied with the help of Maestro 9.0 molecular docking software. The most fitting binding modes of compound **4f** in the active site of topoisomerase enzyme (1SC7) are presented in Fig. **7** and **8**.

Docking of the most active synthesized compound **4f** into the enzyme active site yielded a number of molecular interactions showing hydrogen bond, π interaction and hydrophobic interactions between the drug and enzyme and are considered to be accountable for the noted affinity of the compound. Though, compound **4f** lacks Zwitter ion but it is able to form hydrogen bonds with the enzyme through its secondary amino group of benzimidazole/pyrimidine rings and carbonyl group with the Arg 364 residue which is the same residue where the natural

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inhibitor binds (Staker et al., 2005). In the hydrogen bond interaction between the nitrogen (-N-) of the imidazole ring of the compound **4f** and the carboxyl group (C=O) of the side chain residue of Arg 364 (1.73 Å), the former acts as a hydrogen bond donor while the later behaves as a hydrogen bond acceptor. Further, in the second Hydrogen bond interaction, the carbonyl group (C=O) of the compound **4f** acts as the hydrogen bond acceptor and an amino group (N-H) of the side chain residue of Arg 364 (2.39 Å) is a hydrogen bond donor (Fig. **7**). The amide group of compound **4f** seems to have an important role in strong hydrogen bonding because the lone pair electrons on nitrogen atom of the amide delocalized into the carbonyl group of compound. Pi- π interactions were also observed between the compound and the binding site of enzyme, which are considered to play a significant role in the inhibitory activity. As it can be seen in the Lig plot (Fig. **8**) of compound which shows the interactions with binding site, it appears that oxadiazole and phenyl ring of benzimidazole are properly oriented towards the more lipophilic area of 1SC7 binding site and form CH-π interaction with Arg 364 (4.83 Å) and DNA pointed DG 12 (4.29 Å) resides . In addition to this, several hydrophobic interactions were also observed between the phenyl ring, oxadiazole and pyrimidine ring of compound **4f** with the amino acid residues of the enzyme topoisomerase including DA 14, DA 13, Tgp 11, Lys 532, Thr 718, Ile 535 and Asp 533 and shown in Fig. **7** and **8**. The compound **4f** was found to have a glide score value of -5.39, indicating a high affinity and better interaction between the compound and the enzyme.

4. Conclusion

A total of 22 novel heterocyclic compounds based on the benzimidazole nucleus were prepared, characterized and among them 15 molecules were chosen for studying their anticipated antiproliferative activity against various human cell lines by *in vitro* methods at NCI, USA.

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Notably, compound **4f** (3-(5-(3-(1*H*-benzo[*d*]imidazol-2-yl)-3-oxopropyl)-1,3,4-oxadiazol-2-yl)methyl)-5-methylpyrimidine-2,4 (1*H*, 3*H*)-dione) was identified as lead candidate exhibiting excellent antiproliferative activity with MG-MID value GI_{50}(2.09), $\log_{10}GI_{50}$ (-6.04), $\log_{10}TGI$(-4.38) and $\log_{10}LC_{50}$(-4.02). The cytotoxic effects of **4f** molecule were at par with the marketed anticancer drugs, chlorambucil and bendamustine. Furthermore, molecular docking studies performed with the help of Maestro 9.0 software program (Schrodinger Inc. USA) provided an insight into the binding patterns of the compound **4f** into the binding sites of the DNA-topoisomerase complex. In view of these outcomes, the further investigations on compound **4f** may be carried in search of potential new anticancer agents.

ABBREVIATIONS

mp, Melting point; TLC, Thin layer chromatography; IR, infra-red spectroscopy; ^{1}H NMR, Hydrogen Nuclear magnetic resonance; ^{13}C NMR, Carbon Nuclear magnetic resonance; ESI-MS, Electron spray ionization method of Mass spectroscopy; PGs, Percentage Growth; MG-MID, Mean graph midpoint; CLL, Chronic lymphocytic leukemia; NHL, Non-Hodgkin's lymphoma; NCI, National cancer institute; DTP, Developmental Therapeutics Program; TGI, Total growth inhibition; LC_{50}, 50% Lethal concentration; GI_{50}, 50% Growth inhibition; FDA, Food and drug administration; MWI, Microwave irradiation; SRB, Sulforhodamine B.

Conflict Of Interest: Authors declare no conflict of interest.

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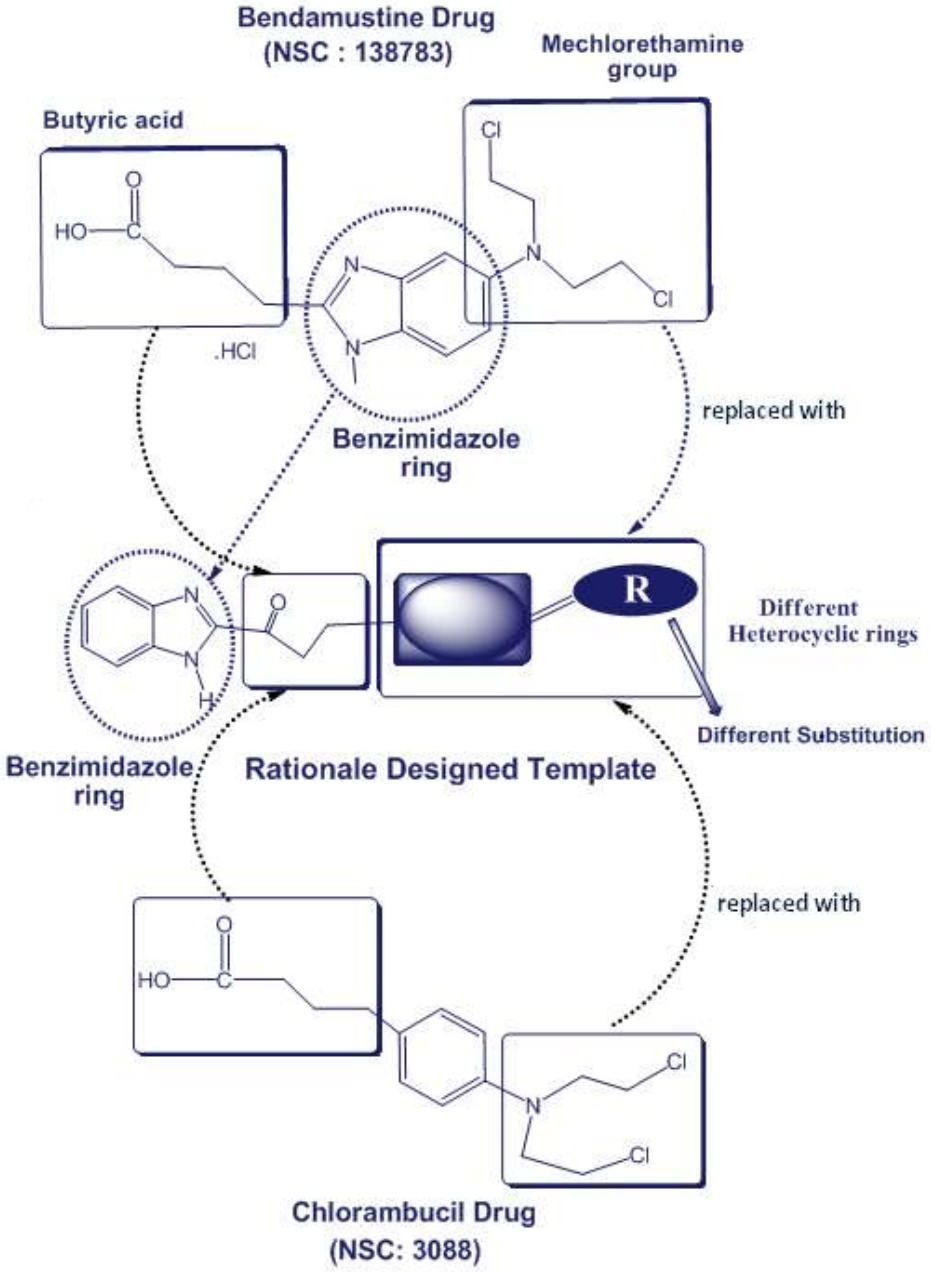

Fig. (1). Rationally designed template for targeted molecules obtained from the marketed anticancer drugs like bendamustine and chlormabucil.

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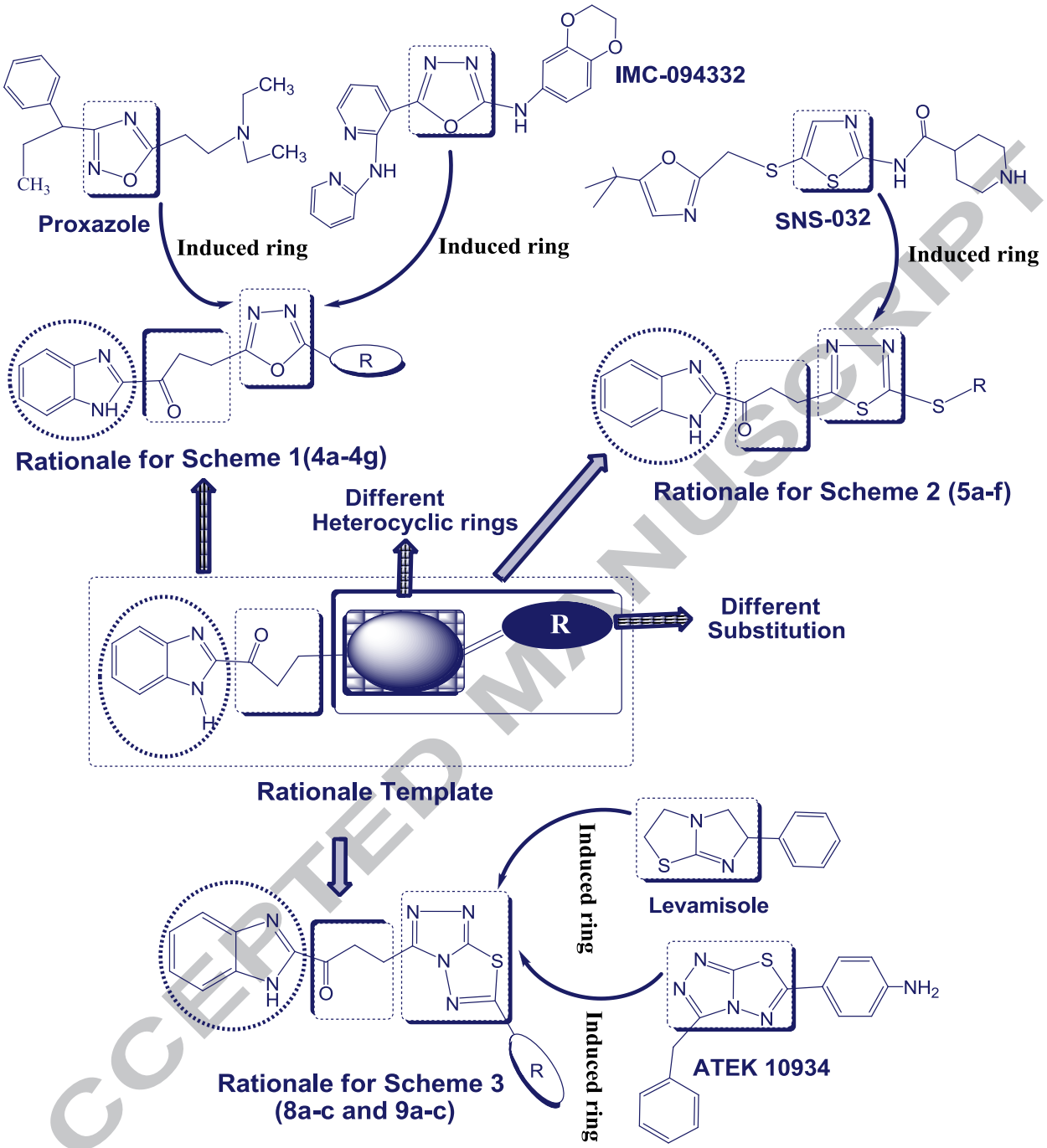

Fig. (2). Rationale template for the different synthetic schemes.

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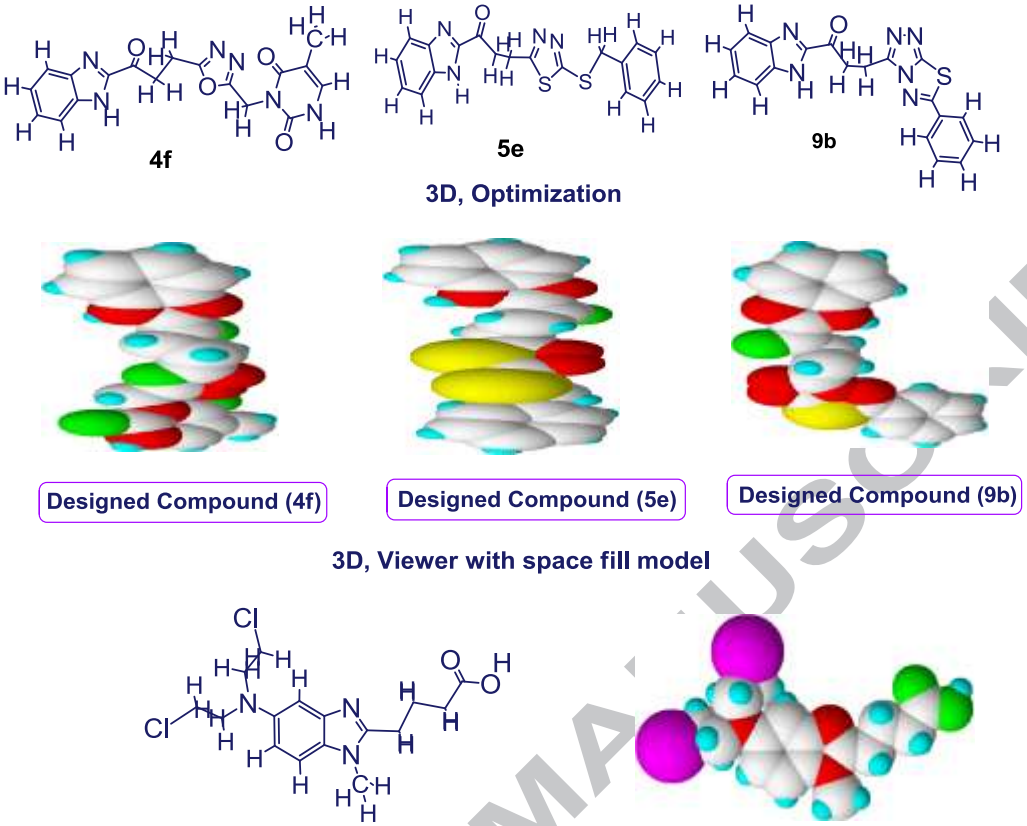

Fig. (3). The 3D, optimization and 3D, viewer with space fill model of designed molecules **4f, 5e, 9b** and marketed anticancer drug (Bendamustine). The different groups of structure showed with different colors as such carbon with white color, hydrogen with cyan color, nitrogen with red color, oxygen with lime color, sulfur with yellow color and chlorine with magenta color.

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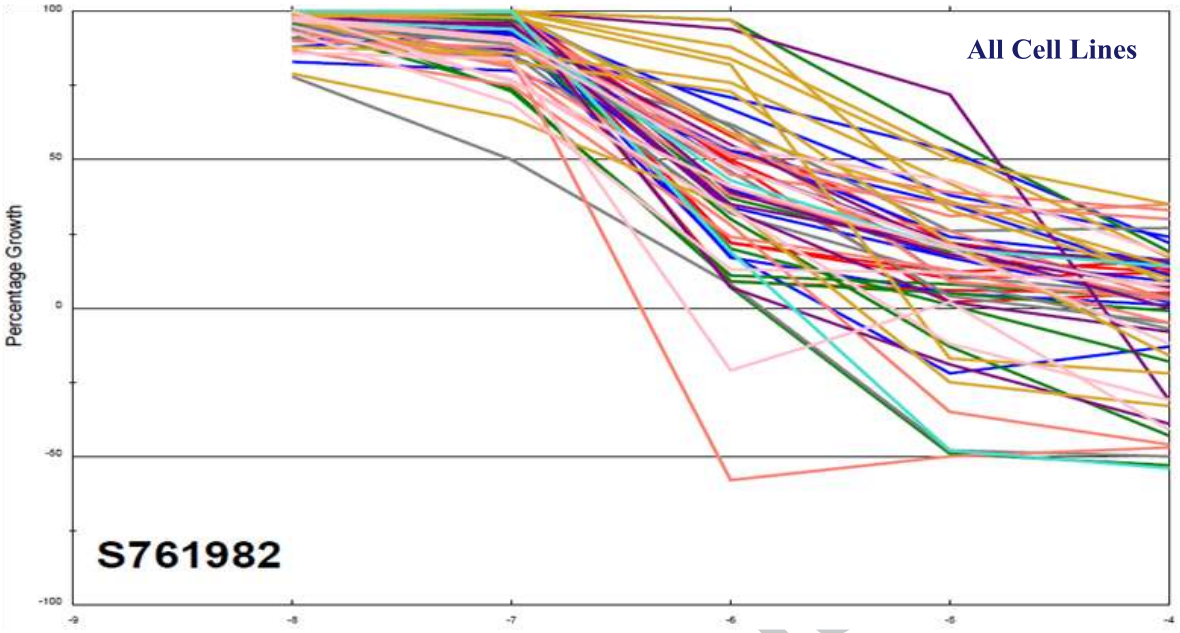

Fig. (4). Dose response curves for all cell lines in the NCI 60 panel after exposed with compound **4f** (NSC: 761982) of different subpanel with originated colors and shapes.

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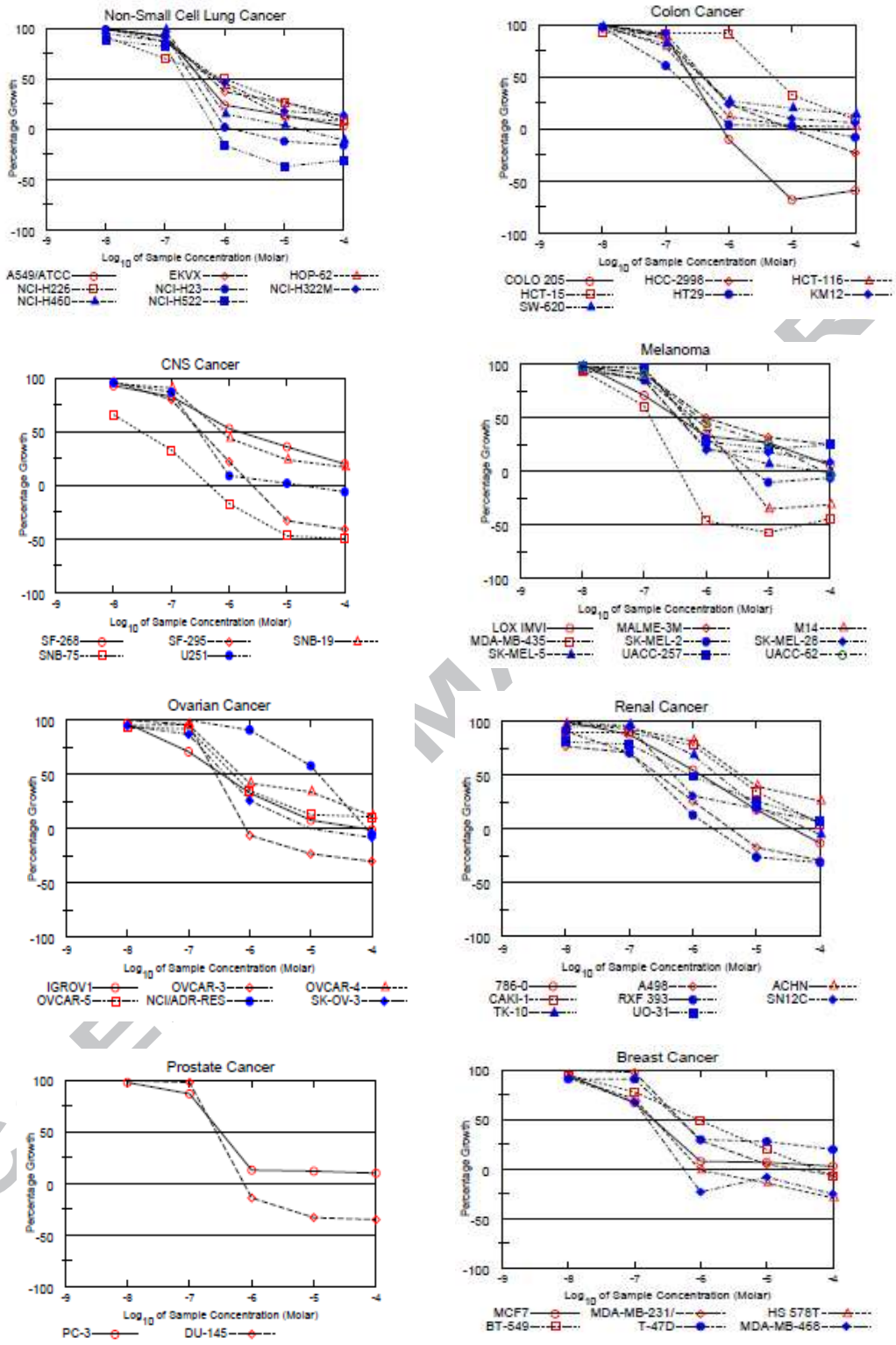

Fig. (5). Dose response curves at NCI fixed protocol, (μM) for all cell lines with different subpanel in the NCI 60 panel after treatment with compound **4f** (NCS: 761982/1). The curves of tested compound obtained from the NCI's *in-vitro* disease-oriented human tumor cells line on nine cancer disease at five concentrations (1 log dilutions from 10^{-4} mol/L to 10^{-8} mol/L). The tissue originated color and shapes of NCI, subpanel cell lines indicative of growth percentage inhibition with concentration of tested sample.

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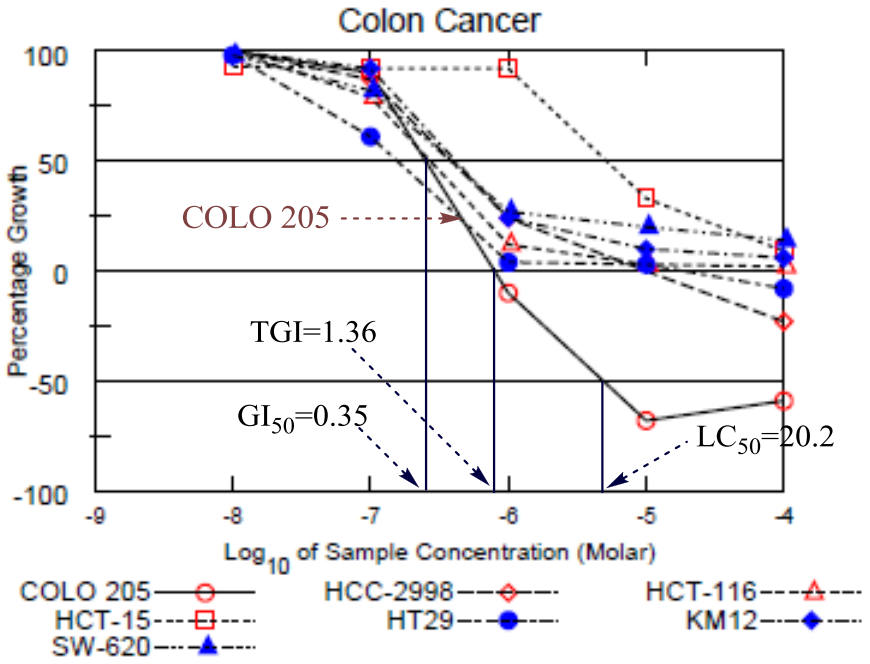

Fig. (6). Dose response curve of Colon cancer subpanel of compound **4f** (NSC: 761982) showing end point calculations for COLO 205 cell line at five dose concentration (1log dilution from 10^{-4} mol/L to 10^{-8} mol/L).

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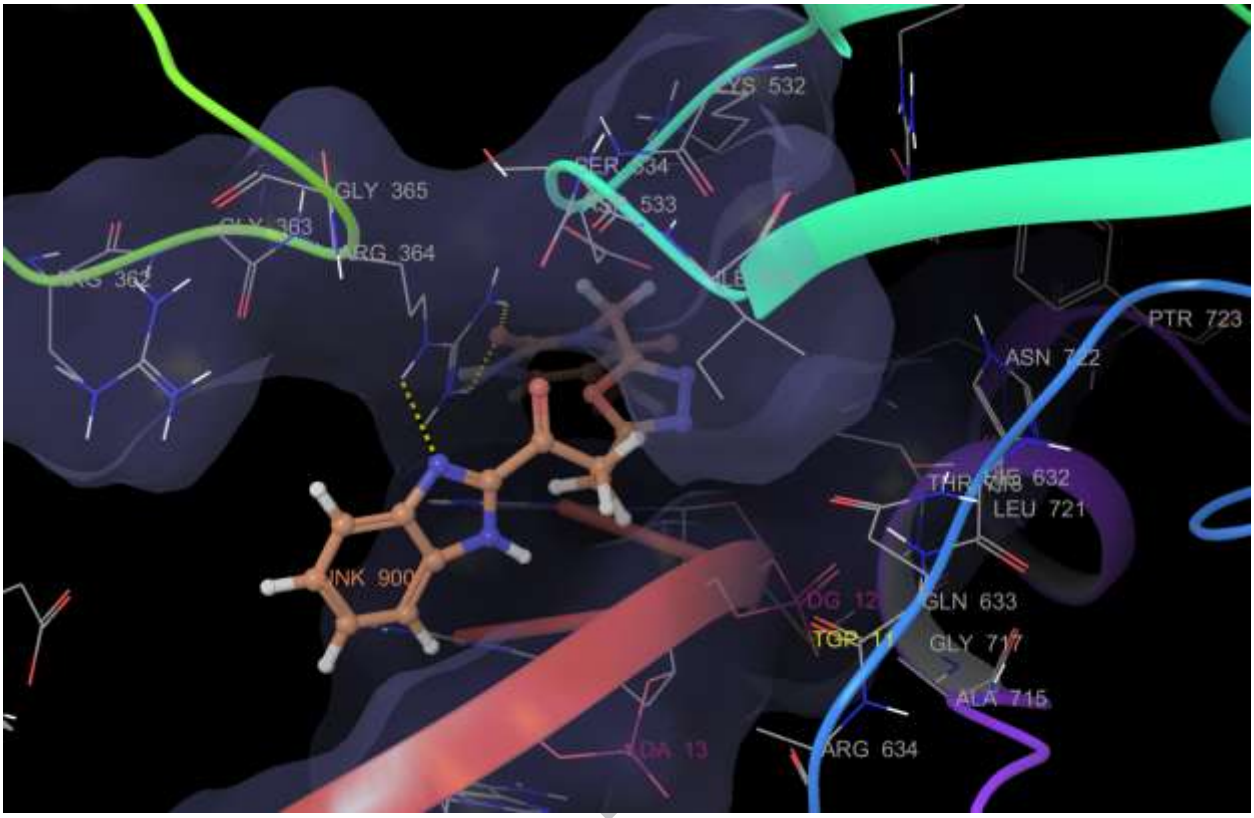

Fig. (7). Binding mode of compound **4f** into the binding sites of topoisomerase enzyme (PDB code: 1SC7) showing hydrogen bond (yellow dotted lines) with Arg 364 and pi interaction with Arg 364 and DG 12.

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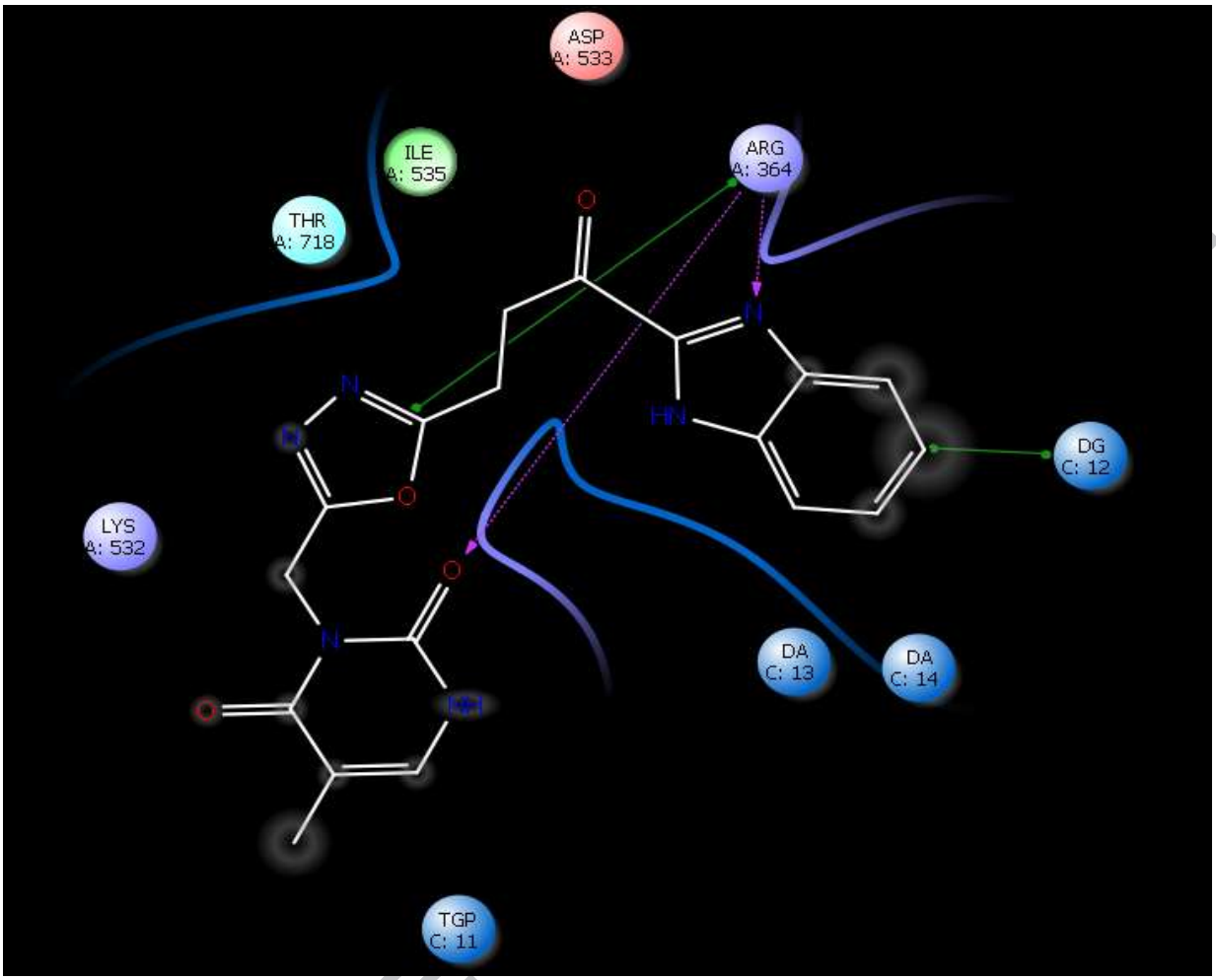

Fig. (8). Lig plot of compound **4f** showing interaction into the binding sites of topoisomerase enzyme (PDB code: 1SC7), hydrogen bond (pink dotted line) with Arg 364 (1.73Å and 2.39Å) and pi interaction (green solid line) with Arg 364 (4.83Å) and DG 12 (4.29Å).

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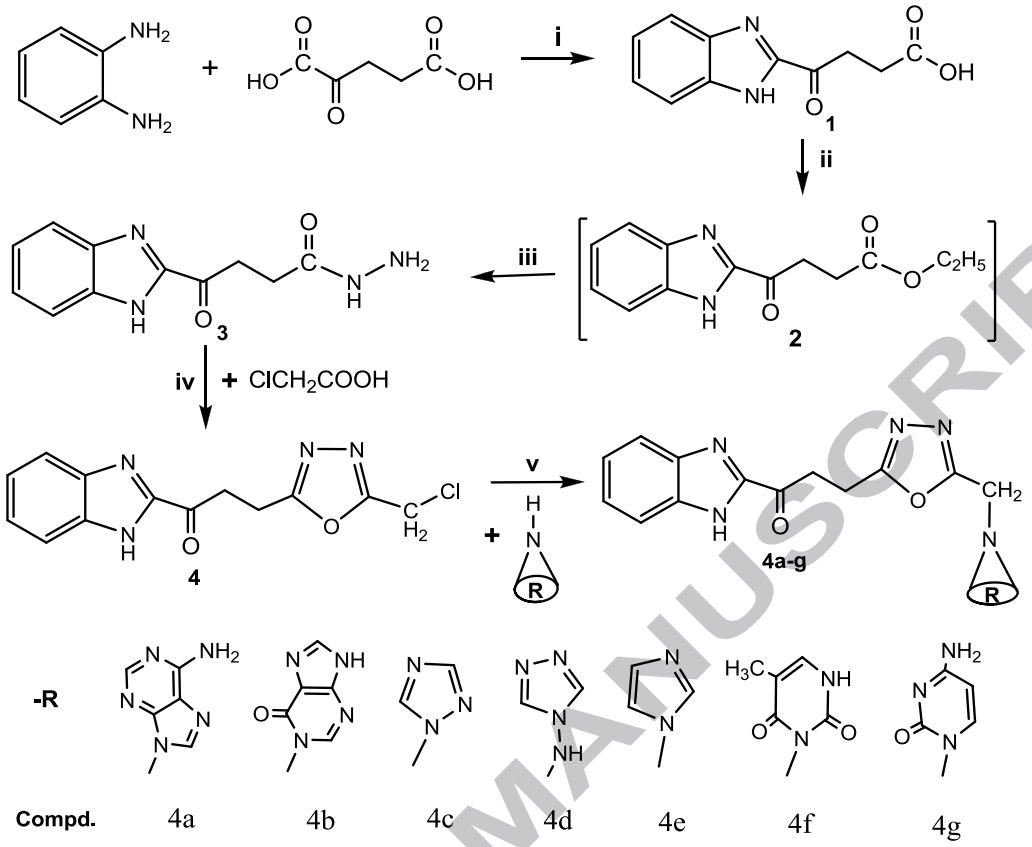

Reactions and conditions:
(i) 4NHCl, methanol, water, reflux, r.t. (ii) Abs. ethanol, conc. H_2SO_4 (iii) $NH_2NH_2.H_2O$, ethanol (iv) $POCl_3$, reflux (v) Abs. ethanol, sodium acetate.

Scheme 1. The route for synthetic protocol of title compounds (**4a-g**)

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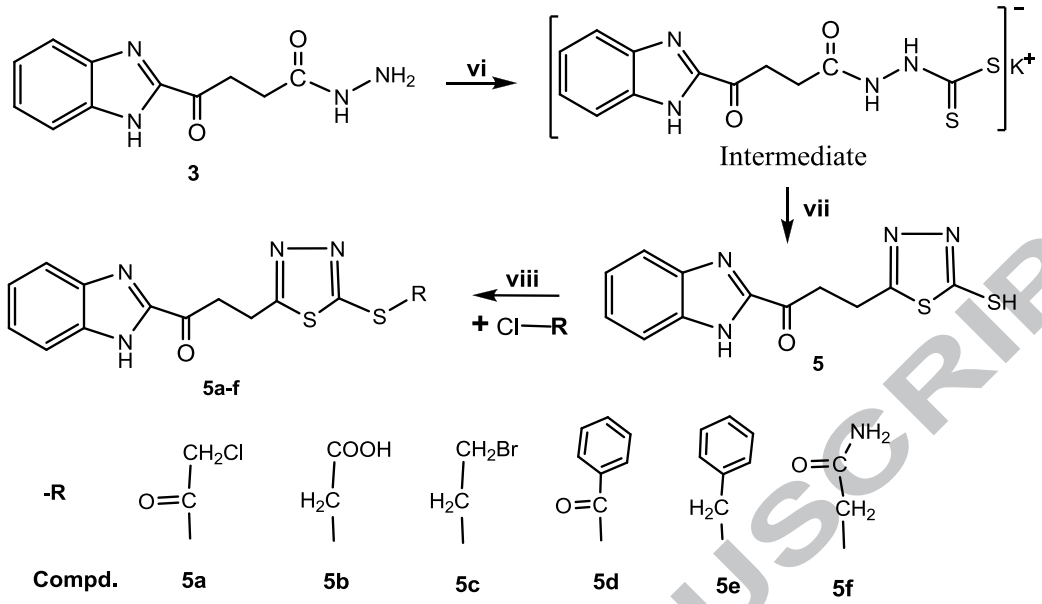

Reactions and conditions:

(vi) CS_2, KOH, reflux, r.t. (vii) Conc. H_2SO_4 (viii) KOH, Ab. ethanol.

Scheme 2. The route for synthetic protocol of title compounds (**5a-f**)

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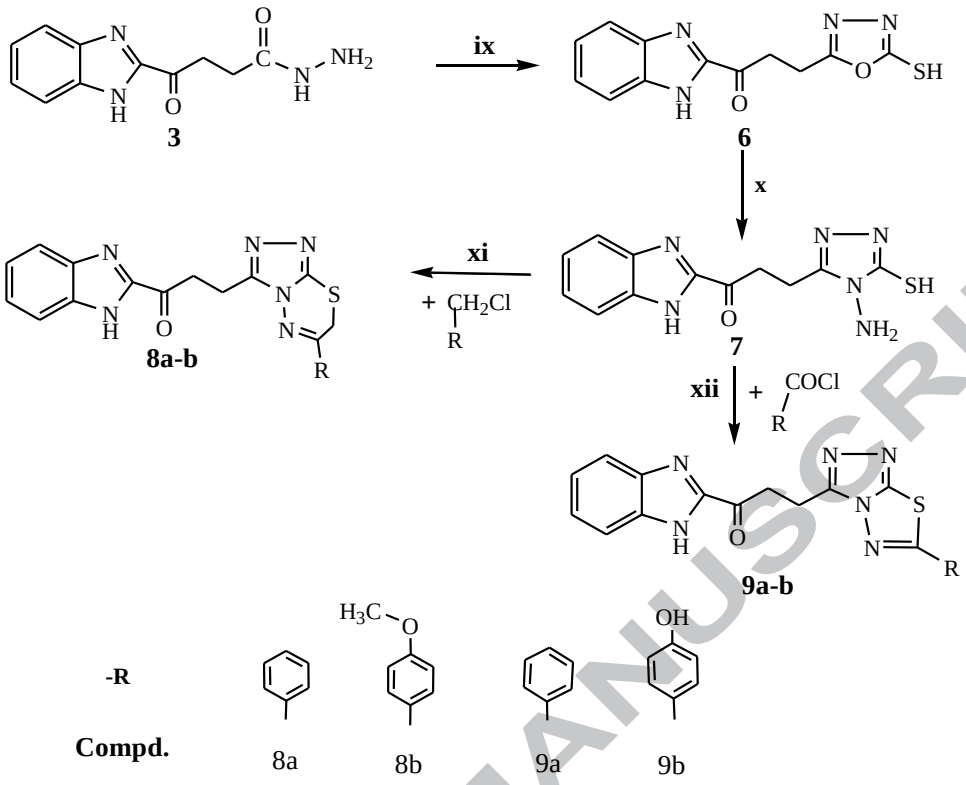

Reactions and conditions:
(ix) CS_2, KOH, ethanol (x) Hydrazine hydrate, Abs.ethanol (xi) Abs. ethanol, reflux (xii) Abs. ethanol, reflux.

Scheme 3.The route for synthetic protocol of title compounds (**8a-b** and **9a-b**)

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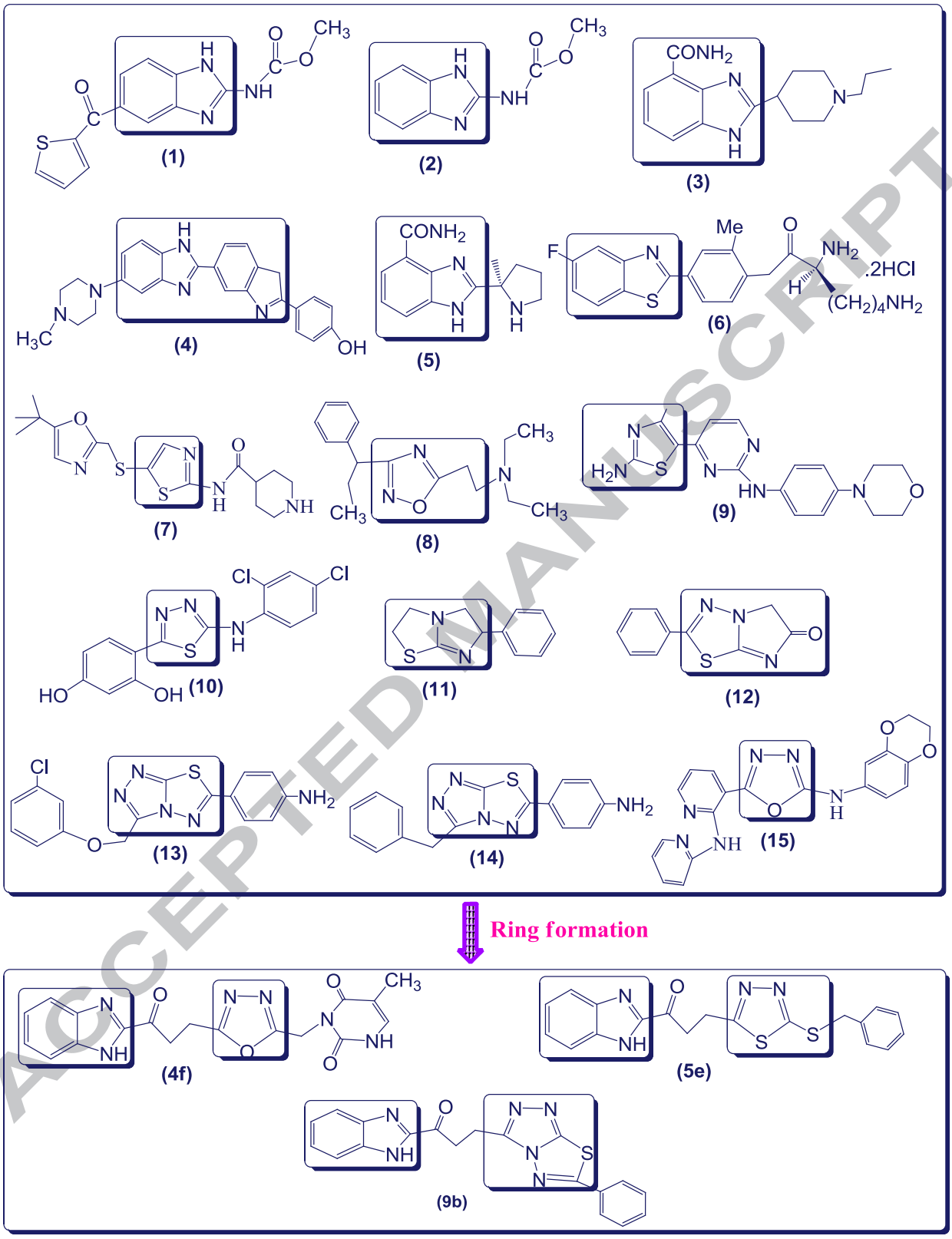

Chart 1. Reported structure (**1-15**) of some biologically active anticancer agents containing benzimidazole, oxadiazole, thiadiazole and triazolothiadiazole scaffold, and rationale designing target compounds (**4f, 5e & 9b**) having above hybrid nuclei.

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Table 1. NSC: Code, sensitivity, growth percent, mean growth percent of NCI cancer cell lines treated with synthesized compounds (10μM) and activity.

Compd.	NSC: Code	The most sensitive cell line	Growth % of the most sensitive cell line [b]	Range of growth, %	Mean	Activity [a]
3	758071/1	UO-31 (Renal Cancer)	86.64	86.64-134.77	102.34	inactive
4	755135/1	MALME-3M (Melanoma)	82.96	82.96- 121.44	102.35	inactive
4a	755140/1	UO-31 (Renal Cancer)	84.84	84.84 - 129.33	106.04	inactive
4b	755141/1	CCRF-CEM (Leukemia)	28.46	28.46- 125.48	96.09	active
4c	755142/1	SR (Leukemia)	82.01	82.01-126.78	105.73	inactive
4d	Nt[c]	-	-	-	-	-
4e	755143/1	MALME-3M (Melanoma)	77.82	77.82-123.77	104.78	inactive
4f	761982/1	MDA-MB-435 (Melanoma)	-45.79	-45.79- 90.70	20.03	active
4g	761983/1	MOLT-4 (Leukemia)	8.54	8.54 -104.24	78.75	active
5a	Nt[c]	-	-	-	-	-
5b	Nt[c]	-	-	-	-	-
5c	Nt[c]	-	-	-	-	-
5d	755144/1	UO-31 (Renal Cancer)	85.04	85.04 - 121.47	102.43	inactive
5e	755145/1	CCRF-CEM (Leukemia)	68.09	68.09- 116.72	94.39	active
5f	755146/1	UO-31 (Renal Cancer)	85.11	85.11-124.27	107.30	inactive
6	759213/1	HL-60(TB) (Leukemia)	72.55	72.55- 128.74	104.14	inactive
7	Nt[c]	-	-	-	-	-
8a	760467/1	UO-31 (Renal Cancer)	72.63	72.63- 126.41	97.18	inactive
8b	761989/1	K-562 (Leukemia)	3.14	3.14-129.13	93.97	active
9a	761988/1	K-562 (Leukemia)	5.84	5.84-116.27	92.86	active
9b	Nt[c]	-	-	-	-	-

[a] The compound which showed growth inhibition ≤ 32%, active for that particular cell line.[b] Percent cell growth reduction following 48h incubation with test compounds (used sulphorhodamine B procedure).[c]Not selected by NCI for anticancer screening.

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Table 2. NCI: DTP, The percentages of growth and inhibition of testing compound (**4f,** 761982/1) over the full panel of tumor cell lines at a single dose (10μM).

Panel	Cell Line Name	Developmental Therapeutics Program One Dose Mean Graph Value (10μM)	
		Growth Percent	Growth Inhibition Percent[a]
Leukemia	HL-60(TB)	-0.43	100.43
	K-562	10.38	89.62
	MOLT-4	9.42	90.58
	RPMI-8226	17.25	82.75
	SR	4.57	95.43
Non-Small Cell Lung Cancer	A549/ATCC	21.13	78.87
	EKVX	54.89	45.11
	HOP-62	17.72	82.28
	HOP-92	53.21	46.79
	NCI-H226	31.72	68.28
	NCI-H23	36.88	63.12
	NCI-H460	9.27	90.73
	NCI-H522	12.71	87.29
Colon Cancer	COLO 205	-32.71	132.71
	HCC-2998	-27.87	127.87
	HCT-116	6.25	93.75
	HCT-15	65.29	34.71
	HT29	8.43	91.57
	KM12	4.98	95.02
	SW-620	28.93	71.07
CNS Cancer	SF-268	36.63	63.37
	SF-295	21.47	78.53
	SF-539	-15.22	115.22
	SNB-19	33.18	66.82
	SNB-75	-32.99	132.99
	U251	16.65	83.35
Melanoma	LOX IMVI	35.59	64.41
	MALME-3M	47.85	52.15
	M14	-16.65	116.65
	MDA-MB-435	-45.79	145.79
	SK-MEL-2	25.22	74.78
	SK-MEL-28	40.86	59.14
	SK-MEL-5	13.22	86.78
	UACC-257	81.40	18.60
	UACC-62	41.05	58.95
Ovarian Cancer	IGROV1	47.82	52.18
	OVCAR-3	-14.80	114.80
	OVCAR-5	13.80	86.20
	OVCAR-8	21.03	78.97
	NCI/ADR-RES	90.70	9.30
	SK-OV-3	-7.95	107.95
Renal Cancer	786-0	28.74	71.26
	A498	11.54	88.46
	ACHN	61.12	38.88
	CAKI-1	63.47	36.53
	RXF 393	-7.83	107.83
	SN12C	30.25	69.75
	TK-10	64.11	35.89
	UO-31	44.15	55.85
Prostate Cancer	PC-3	19.30	80.70
	DU-145	-35.32	135.32
Breast Cancer	MCF7	9.44	90.56
	MDA-MB-231/ATCC	26.05	73.95
	HS 578T	10.15	89.85
	BT-549	18.86	81.14
	T-47D	34.34	65.66
	MDA-MB-468	-1.54	101.54
	Mean	20.03	

[a]Percentage inhibition calculated by simple abstraction of % activity from 100 and % inhibition above 100 (100-200%) means that compound show lethality at cancer cells.

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Table 3. Calculated values of GI_{50}, TGI, LC_{50} (in μM) of the NCI cell lines panel, MG-MID value and selectivity index of the compound (**4f,** 761982/1).

Panel	Cell line	GI_{50} (μM)				TGI (μM)	LC_{50} (μM)
		Concentration per cell line	Subpanel Concentration	Subpanel MID[b]	Selectivity Index		
Leukemia	**CCRF-CEM**	0.459				>100	>100
	HL-60(TB)	0.966				>100	>100
	K-562	1.03	4.932	0.82	2.55	>100	>100
	MOLT-4	1.69				>100	>100
	RPMI-8226	0.340				>100	>100
	SR	0.447				>100	>100
Non-SmallCell Lung Cancer	**A549/ATCC**	0.542				>100	>100
	EKVX	3.90				>100	>100
	HOP-62	1.37				>100	>100
	OP-92	1.44	22.248	2.47	0.85	>100	>100
	NCI-H226	12.7				>100	>100
	NCI-H23	0.627				>100	>100
	NCI-H322M	0.904				>100	>100
	NCI-H460	0.394				>100	>100
	NCI-H522	0.371				2.86	>100
Colon Cancer	**COLO 205**	0.354				1.36	20.2
	HCC-2998	0.459				4.88	>100
	HCT-116	0.233	17.503	2.50	0.84	80.6	>100
	HCT-15	15.2				>100	>100
	HT29	0.237				>100	>100
	KM12	0.415				10.9	>100
	SW-620	0.605				>100	>100
CNS Cancer	**SF-295**	1.61				36.2	>100
	SF-539	0.799				29.9	>100
	SNB-19	2.21	5.1791	1.03	2.03	>100	>100
	SNB-75	0.099				1.39	>100
	U251	0.461				>100	>100
Melanoma	**LOX IMVI**	0.543				>100	>100
	MALME-3M	1.26				>100	>100
	M14	0.386				2.80	>100
	MDA-MB-435	0.174	6.97	0.77	2.71	0.393	nt[c]
	SK-MEL-2	1.53				>100	>100
	SK-MEL-28	0.829				>100	>100
	SK-MEL-5	0.304				54.9	>100
	UACC-257	1.29				>100	>100
	UACC-62	0.654				>100	>100
Ovarian Cancer	**IGROV1**	0.914				>100	>100
	OVCAR-3	0.353				1.82	>100
	OVCAR-4	0.558	20.637	2.95	0.71	>100	>100
	OVCAR-5	1.42				>100	>100
	OVCAR-8	0.661				>100	>100
	NCI/ADR-RES	16.2				49.7	>100
	SK-OV-3	0.531				15.5	>100
Renal Cancer	**786-0**	2.84				38.7	>100
	A498	0.296				3.78	>100
	ACHN	10.0				>100	>100
	CAKI-1	11.5	40.686	5.08	0.41	>100	>100
	RXF 393	2.11				6.77	>100
	SN12C	2.51				>100	>100
	TK-10	5.42				>100	>100
	UO-31	6.01				>100	>100
Prostate Cancer	**PC-3**	0.725				>100	>100
	DU-145	0.424	1.149	0.57	3.66	1.94	24.3
Breast Cancer	**MCF7**	0.216				>100	>100
	MDA-MB-231/ATCC	0.902				>100	>100
		0.413	4.502	0.75	2.78	5.41	>100
	HS 578T	0.539				42.1	>100
	BT-549	2.22				>100	>100
	T-47D	0.212				nt[c]	>100
	MDA-MB-468						
Total Cell lines and total concentration	**59**	123.81					
MID[a]		**2.09**					

[a]The average sensitivity of all cell lines towards the test agent in μM.[b]The average sensitivity of all cell lines of a particular subpanel towards the test agent in μM.[c]Not tested cell line.

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Table 4. Values of the log molar concentration of response parameter ($log_{10}GI_{50}$, $log_{10}TGI$ and $log_{10}LC_{50}$) of the title compound (**4f,** 761982/1).

Cancer disease	Used Cell lines	Potency[a] in µmol/L		
		Log_{10} GI_{50}	Log_{10} TGI	Log_{10} LC_{50}
Leukemia	CCRF-CEM	-6.34	> -4.00	> -4.00
	HL-60(TB)	-6.01	> -4.00	> -4.00
	K-562	-5.99	> -4.00	> -4.00
	MOLT-4	-5.77	> -4.00	> -4.00
	RPMI-8226	-6.47	> -4.00	> -4.00
	SR	-6.35	> -4.00	> -4.00
Non-Small Cell Lung Cancer	A549/ATCC	-6.27	> -4.00	> -4.00
	EKVX	-5.41	> -4.00	> -4.00
	HOP-62	-5.86	> -4.00	> -4.00
	OP-92	-5.84	> -4.00	> -4.00
	NCI-H226	-4.89	> -4.00	> -4.00
	NCI-H23	-6.20	> -4.00	> -4.00
	NCI-H322M	-6.04	> -4.00	> -4.00
	NCI-H460	-6.40	> -4.00	> -4.00
	NCI-H522	-6.43	-5.54	> -4.00
Colon Cancer	COLO 205	-6.45	-5.87	-4.69
	HCC-2998	-6.34	-5.31	> -4.00
	HCT-116	-6.63	-4.09	> -4.00
	HCT-15	-4.82	> -4.00	> -4.00
	HT29	-6.63	> -4.00	> -4.00
	KM12	-6.38	-4.96	> -4.00
	SW-620	-6.22	> -4.00	> -4.00
CNS Cancer	SF-295	-5.79	-4.44	> -4.00
	SF-539	-6.10	-4.53	> -4.00
	SNB-19	-5.66	> -4.00	> -4.00
	SNB-75	-7.00	-5.86	> -4.00
	U251	-6.34	> -4.00	> -4.00
Melanoma	LOX IMVI	-6.26	> -4.00	> -4.00
	MALME-3M	-5.90	> -4.00	> -4.00
	M14	-6.41	-5.55	> -4.00
	MDA-MB-435	-6.76	-6.41	nt[b]
	SK-MEL-2	-5.81	> -4.00	> -4.00
	SK-MEL-28	-6.08	> -4.00	> -4.00
	SK-MEL-5	-6.52	-4.26	> -4.00
	UACC-257	-5.89	> -4.00	> -4.00
	UACC-62	-6.18	> -4.00	> -4.00
Ovarian Cancer	IGROV1	-6.04	> -4.00	> -4.00
	OVCAR-3	-6.45	-5.74	> -4.00
	OVCAR-4	-6.25	> -4.00	> -4.00
	OVCAR-5	-5.85	> -4.00	> -4.00
	OVCAR-8	-6.18	> -4.00	> -4.00
	NCI/ADR-RES	-4.79	-4.30	> -4.00
	SK-OV-3	-6.28	-4.81	> -4.00
Renal Cancer	786-0	-5.55	-4.41	> -4.00
	A498	-6.53	-5.42	> -4.00
	ACHN	-5.00	> -4.00	> -4.00
	CAKI-1	-4.94	> -4.00	> -4.00
	RXF 393	-5.68	-5.17	> -4.00
	SN12C	-5.60	> -4.00	> -4.00
	TK-10	-5.27	> -4.00	> -4.00
	UO-31	-5.22	> -4.00	> -4.00
Prostate Cancer	PC-3	-6.14	> -4.00	> -4.00
	DU-145	-6.37	-5.71	-4.61
Breast Cancer	MCF7	-6.67	> -4.00	> -4.00
	MDA-MB-231/ATCC	-6.04	> -4.00	> -4.00
	HS 578T	-6.38	-5.27	> -4.00
	BT-549	-6.27	-4.38	> -4.00
	T-47D	-5.65	> -4.00	> -4.00
	MDA-MB-468	-6.67	nt[b]	> -4.00
MID		-6.04	-4.38	-4.02
Delta		0.96	2.03	0.67

[a]Values determined using the optimal concentration range (s) for each end point.[b]Not tested cell lines.

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Table 5. Comparison of *in vitro* anticancer activity between **4f** and marketed anticancer drugs (bendamustine and chlorambucil)

Compd.	NSC[a] No.	Log (High Conc.)	Potency in log_{10}(M) unit in µmol/L				No. of Expts	N.C.L.[e]	MID[f] GI_{50}
			Unit	GI_{50} EDP[d] (mean value)	TGI EDP[d] (mean value)	LC_{50} EDP[d] (mean value)			
4f	761982	-4.79	log_{10}(M)	-6.04	-4.38	-4.02	2	59	2.09
BENDA[b]	138783	-4.0	log_{10}(M)	-4.153	-4.018	-4.004	3	60	60
CHLB[c]	3088	-5.0	log_{10}(M)	-4.758	-4.282	-4.062	2	59	52

[a]National service center number.[b]Bendamustine hydrochloride (Treanda).[c]Chlorambucil (Leukeran).[d]Endpoint.[e]Number of cell lines.[f]Mean graph midpoint (arithmetical mean value of treated cancer cell lines). Data was obtained from the Developmental Therapeutics Program, National Cancer Institute (NCI) main web site for comparison purpose.

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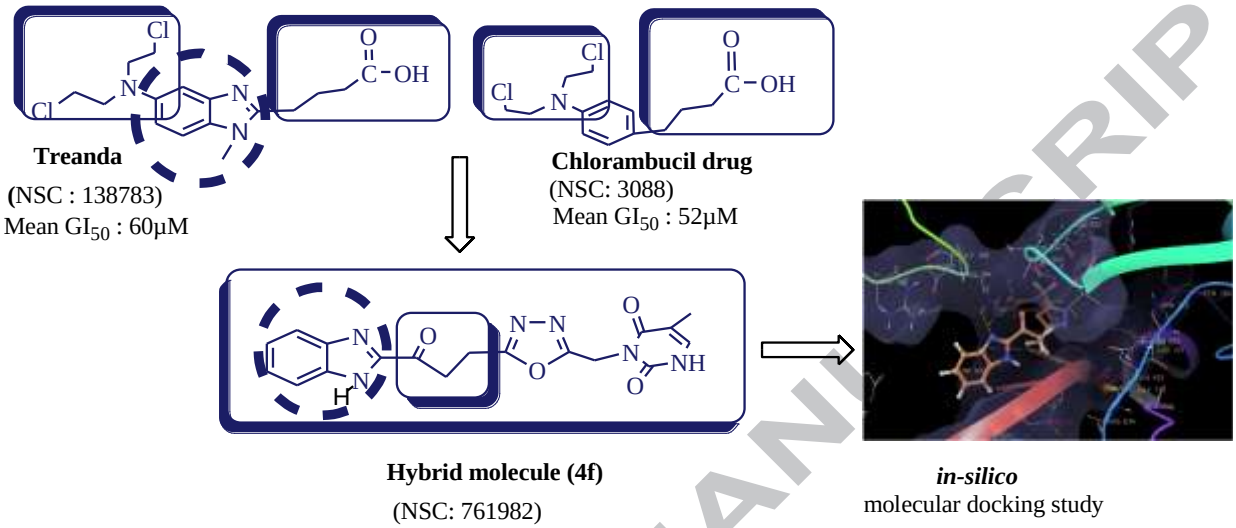
Graphical Abstract:

Design and synthesis of benzimidazoles containing substituted oxadiazole, thiadiazole and triazolo-thiadiazines as a source of new anticancer agents



New benzimidazole derivatives clubbed with biologically active heterocyclic moities (**4a-g, 5a-f, 8a-b** & **9a-b**) were designed and synthesized under microwave irradiation. Initial screening of compounds showed good to remarkable anticancer activity by NCI panel. Compound **4f** exhibited significant growth inhibition and was further referred for five dose level study. The present study may result in the development of new anticancer agents in the near future.

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