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Short communication

Benzimidazole clubbed with triazolo-thiadiazoles and triazolo-thiadiazines: New anticancer agents

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ABSTRACT

Two series of Benzimidazole clubbed with triazolo-thiadiazoles (**5a**–**q**, **5r**, **5s** and **5x**–**a**[1]) and triazolo-thiadiazines (**5t**–**w**) were synthesized with an aim to produce promising anticancer agents. *In vitro* anticancer activities of synthesized compounds were investigated at the National Cancer Institute (NCI) against NCI 60 cell line panel; results showed good to remarkable broad-spectrum anticancer activity. Among them, the compound **5h** (NCS: 760452, 1-(1*H*-benzo [*d*] imidazol-2-yl)-3-(6-(2,4-dichlorophenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl) propan-1-one) exhibited significant growth inhibition with GI_{50} values ranging from 0.20 to 2.58 μM and found superior selectivity for the leukemia cell lines and further screened at 10-fold dilutions of five different concentrations (0.01, 0.1, 1, 10 and 100 μM). The **5h** may possibly be used as lead compound for developing new anticancer agents.

1. Introduction

Cancer, a disease of the cell cycle, is one of the major health problems in the world from decades. The effectiveness of many existing anticancer drugs is limited by their toxicity to normal rapidly growing cells and may develop resistance to that drug. Another drawback is that majority of the drugs currently in the market are not specific. Different classes of heterocyclic and fused heterocyclic compounds have been identified through molecular biology, empirical screening and rational drug development in search of anticancer agents during the recent times [1–3].

Among the heterocyclic compounds, benzimidazoles are of great importance due to their important biological actions as well as synthetic applications in medicinal chemistry. Benzimidazoles have been reported to have potential antitumor/antiproliferative/anticancer activity [4–12] along with antibacterial [13,14], antifungal [14–17], antiviral including anti-HIV [11,17] and antioxidant activities [16,18]. Similarly, triazolo-thiadiazoles and triazolo-thiadiazines are a class of fused heterocyclic compounds, which have attracted great interest in medicinal chemistry owing to their wide range of pharmacological activities including antitumor/antiproliferative/anticancer activities [19–28].

Benzimidazole attached with other heterocyclic moieties including fused rings resulted in compounds (hybrid molecules) with improved pharmacological profile [10–12]. Some biologically active antitumor agents having benzimidazole and other heterocyclic moiety, for example, Nocodazole (NSC-238189) (**1**) [29], FB642 (**2**) [30], A-620223 (**3**) [31], Hoechst-33258 (**4**) [32], ABT-888 (**5**) [33], Phortress (**6**) [34], SNS-032 (**7**) [35], Proxazole (**8**) [36], CYC116 (**9**) [37], thiadiazole derivative (**10**) [38], Levamisole (**11**) [39], imidazo [2,1-*b*] [1, 3,4]thiadiazole analogs (**12**) [40], triazolo[1,3,4]thiadiazole derivative (**13**) [41] and similarly synthetic target compounds (**5h**) are presented in Fig. 1. Here described in detail some rationally designed target compounds with marketed drug Treanda (bendamustine hydrochloride) as antitumor agents made up by Cephalon Inc. USA and comprises by mechlorethamine group and benzimidazole heterocyclic ring with a butyric acid substituent chemically as, 1*H*-benzimidazole-2-butanoicacid-5-[bis(2-chloroethyl)amino]-1-methyl monohydro chloride, this is a rationally designed purine analog and alkylator hybrid of chlorambucil chemotherapeutic agent. On March 20, 2008, it was approved by the FDA (U.S.) for the treatment of chronic lymphocytic leukemia (CLL) and about 6 months later, on October 31, 2008 this also approved for patients with indolent B-cell non-Hodgkin's lymphoma (NHL) [42,43]. The chemically drawn of newly prepared target compounds rationally designed with bendamustine along with chlorambucil represented in Fig. 2.

In view of these points, and in continuation of our work on benzimidazole derivatives bearing oxadiazole moiety [44] which

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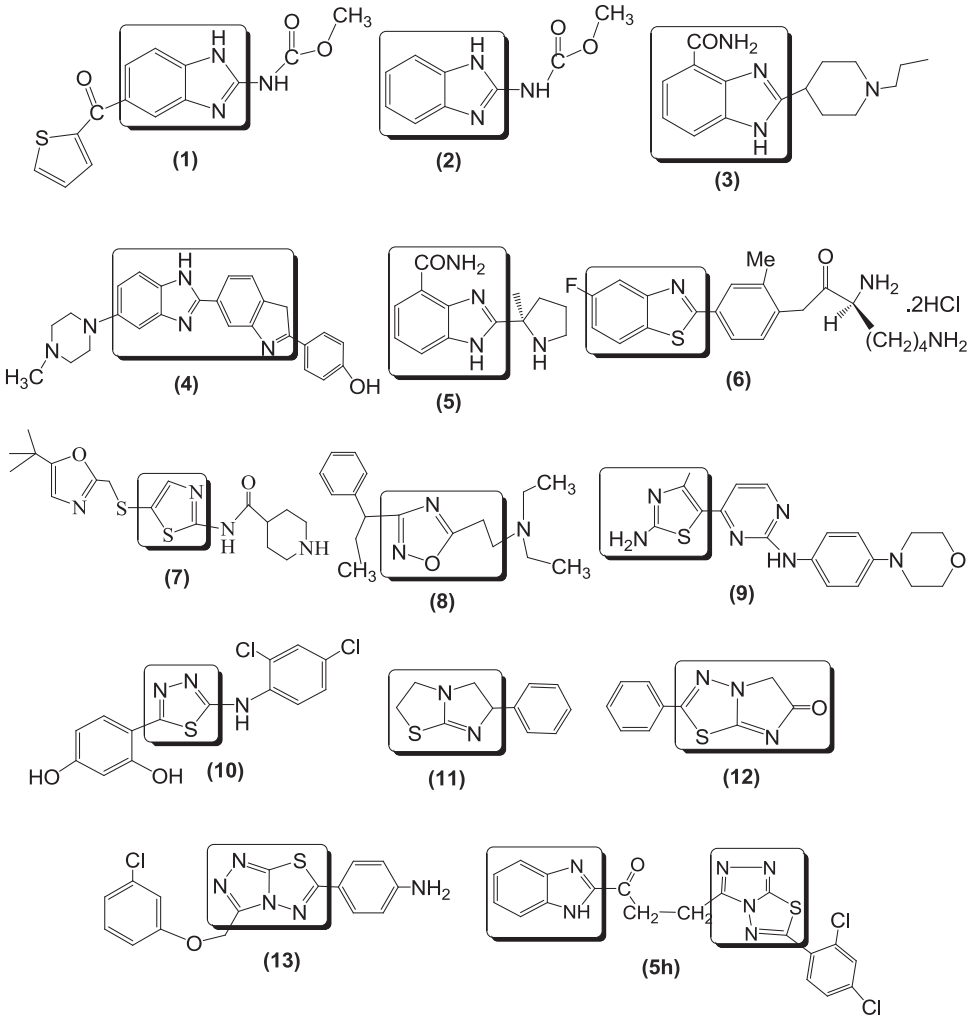

Fig. 1. Structure of some biologically active antitumor agents containing the benzimidazole and other heterocyclic scaffold and rationally designed targeted compounds (**5h**).

showed significant anticancer activities, it was thought worthwhile to study new benzimidazoles clubbed with fused heterocyclic ring systems; triazolo-thiadiazoles and triazolo-thiadiazines moieties, with the hope of getting promising anticancer agents. Therefore, a number of hybrid molecules were synthesized and screened for their *in vitro* anticancer activities at the Development Therapeutic Program (DTP), National Cancer Institute (NCI), Chemotherapeutic Research division, USA, against full NCI 60 cell line panel according their applied protocol. Twenty seven selected compounds among thirty two newly synthesized hybrid molecules were submitted to NCI and granted NCS codes (Table 1).

2. Chemistry

The title compounds were synthesized as outlined in Schemes 1 and 2 following reported methods with appropriate modifications [27,44–46]. The starting material, 4-(1*H*-benzo[*d*]imidazol-2-yl)-4-oxobutanoic acid (**1**) was synthesized by reacting *o*-phenylenediamine and α-ketoglutaric acid in presence of HCl. 4-(1*H*-benzo[*d*]imidazol-2-yl)-4-oxobutanehydrazide (**3**) was obtained by treating the compound **1** with hydrazine hydrate through ethyl-ester intermediate (**2**). The compound **3** was treated with CS_2/KOH using ethanol as solvent toget1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(5-mercapto-1,3,4-oxadiazol-2-yl)propan-1-one (**4**), and compound **5**, (3-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)-1-(1*H*-benzo[*d*]imidazol-2-yl) propan-1-one), was obtained by reacting hydrazine hydrate with compound **4** (Scheme 1). After that different acids were reacted with compound **5** to obtain the desired product as 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 (**5a**–**q**) and the compounds (**5r** and **5s**) obtained by reacting compound **5** with KOH/CS_2 and chloro-isothiocyanate, respectively. The Compound **5** treated with different types of α-chloro containing carbonyl compounds to produce 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 (**5t**–**w**) 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 (**5x**–**a**[1]) (Scheme 2). The products were purified by recrystallization with suitable solvent and found pure upon TLC examination. Their structures were established on the basis of modern analytical techniques (IR, 1H NMR, 13-NMR and mass spectral data). The elemental analysis results were within ±0.4% of the theoretical values.

3. Pharmacology

3.1. In vitro anticancer activity

All the selected 25 compounds were submitted to National Cancer Institute (NCI), USA for evaluation of their *in vitro* anticancer activity at single dose (1×10^{-5} M) against full NCI 60 cell lines

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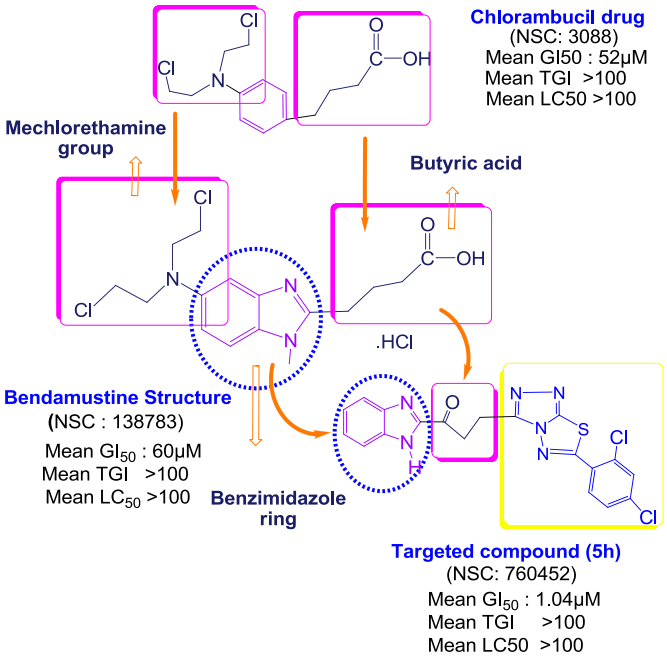


Fig. 2. Chemical structure of active chemotherapeutic anticancer agents (bendamustine, chlorambucil) and rationally designed template for targeted compound (**5h**).

panels representing on full nine human systems as leukemia, melanoma and cancers of lung, colon, brain, breast, ovary, kidney and prostate. The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose. The compounds added at a concentration (1×10^{-5} M) and the culture incubated for 48 h. End point determinations made with a protein binding dye, sulforhodamine B [47,48]. Results for each compound were reported as a mean graph of the percent growth of the treated cells. Results of each test agents are reported as percentage growth of the treated cells when compared with untreated control cells [49,50]. The output from the single dose screen is analyzed by the COMPARE program and compounds which exhibit significant growth inhibition at a single dose of 10 μM are further evaluated against the 60 cell panel at five concentration levels.

4. Results and discussion

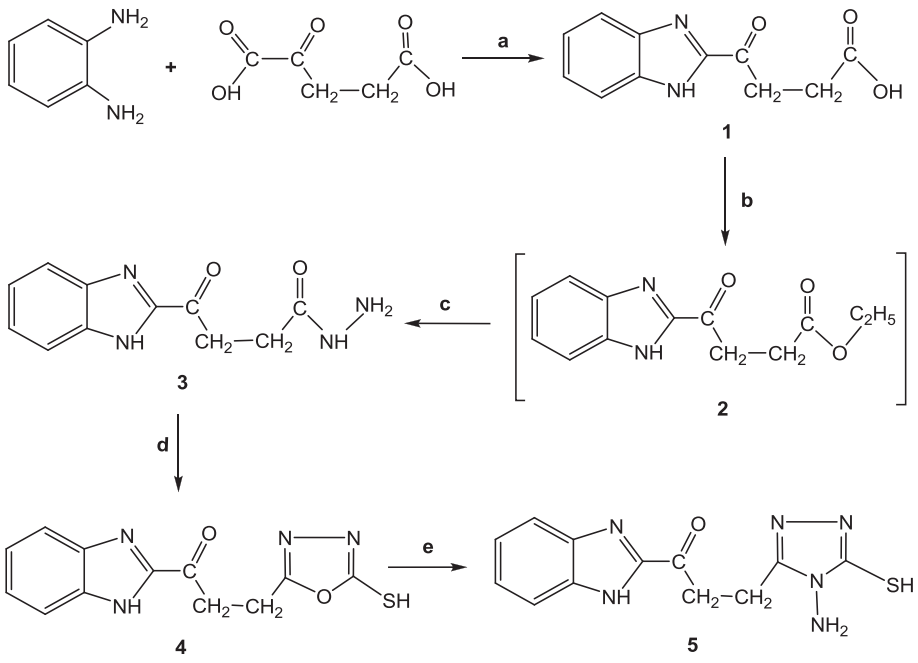
4.1. Chemistry

Thirty-two new compounds were prepared (Schemes 1 and 2) and their IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data and elemental analysis results found in full agreement with their assigned structures. In general, IR spectra of the compounds showed peaks at 3326 and 2557 cm^{-1} for NH and SH, respectively. In ^{1}H NMR spectra, there appeared a singlet at around δ 11.9 indicative of ring H–N and another singlet at δ 13.4 for S–H, both disappeared by addition of D_2O as confirmation for these groups. The appeared peak in IR spectra at around 1728 and 1600 cm^{-1} accounted for C=O and C=N. The chemical shift in ^{13}C NMR spectra at δ 173.56 and 156.24 could be accounted for C=O and C=N. The characteristic peaks at around 1384 and 1273 cm^{-1} for N=C–S and N–N=C as indicative the formation of thiadiazole and triazole ring. All the synthetic compounds showed two triplets at appropriate signals and chemical shifts in ^{1}H NMR spectra at around δ 2.8 (J = 6.9 Hz) and 3.3 (J = 7.2 Hz) and ^{13}C NMR spectra showed at around δ 30.9 and 22.4 which could be accounted for two methylene groups (–CH_2–CH_2–) forming a linker chain through which benzimidazole nuclei attached with triazole/thiadiazole nuclei made up the complete back bone of synthetic compounds. The presence of doublet, triplet at around δ 7.7, 7.5 and 7.2 (7.8 Hz, 7.5 Hz, 7.5 Hz) indicated benzimidazole hydrogens. The multiplets peaks shown at around δ 7.2–6.9 accounted for aromatic protons. The signals in ^{13}C NMR spectra which appeared at around δ 166.2 could be for thiadiazole carbon ring and other signals at δ 162.7, 158.3 indicative of triazole carbon ring. Other peaks were observed at appropriate δ values supporting the structure. The mass spectra (ESI MS) showed the

Table 1
Sensitivity, NSC: Code, growth percent, delta value, mean growth percent of NCI cancer cell lines treated with synthesized compounds (10 μM).

Compd.	NSC: Code	The most sensitive cell line	Growth % of the most sensitive cell line	Range of growth, %	Mean	Delta	Range	Activity[a]
4	759213/1	HOP-92(Non-Small Cell Lung Cancer)	69.36	69.36–128.74	104.14	34.78	59.38	Inactive
5a	760446/1	MCF7(Breast Cancer)	61.97	61.97–126.96	86.81	24.84	64.99	Active
5b	760447/1	UO-31(Renal Cancer)	63.03	63.03–143.70	93.15	30.12	80.67	Active
5c	760448/1	UO-31(Renal Cancer)	64.29	64.29–122.39	98.45	34.16	58.10	Active
5d	760449/1	NCI-H522(Non-Small Cell Lung Cancer)	30.17	30.17–107.44	70.41	40.24	77.27	Active
5e	760450/1	UO-31(Renal Cancer)	75.55	75.55–123.55	101.57	26.02	54.60	Inactive
5g	760451/1	UO-31(Renal Cancer)	76.24	76.24–112.60	103.05	26.81	58.58	Inactive
5h	760452/1	IGROV1(Ovarian Cancer)	0.38	−94.66–15.24	−47.05[b]	47.61	109.90	Active
5i	760455/1	NCI-H322M(Non-Small Cell Lung Cancer)	73.88	73.88–133.80	96.31	22.43	59.92	Inactive
5j	760453/1	UO-31(Renal Cancer)	54.88	54.88–125.77	89.16	34.28	70.89	Active
5k	760454/1	UO-31(Renal Cancer)	51.29	51.29–139.62	83.74	32.45	88.33	Active
5m	760456/1	UO-31(Renal Cancer)	79.38	79.38–130.64	103.36	23.98	51.26	Inactive
5n	760457/1	UO-31(Renal Cancer)	69.58	69.58–144.62	100.04	30.46	75.04	Inactive
5o	760458/1	UO-31(Renal Cancer)	67.68	67.68–134.34	101.48	33.80	66.66	Active
5p	760459/1	UO-31(Renal Cancer)	83.90	83.90–132.53	104.08	20.18	48.63	Inactive
5q	760460/1	UO-31(Renal Cancer)	73.78	73.78–129.94	103.98	30.20	56.16	Inactive
5r	760461/1	UO-31(Renal Cancer)	76.61	76.61–126.21	102.40	25.79	50.58	Inactive
5s	760462/1	UO-31(Renal Cancer)	64.18	64.18–114.65	92.73	28.55	54.64	Active
5t	760463/1	UO-31(Renal Cancer)	80.40	80.40–159.00	102.50	22.10	78.60	Inactive
5u	760464/1	UO-31(Renal Cancer)	79.85	79.85–126.44	104.38	24.53	46.59	Inactive
5v	760465/1	UO-31(Renal Cancer)	80.35	80.35–153.14	103.51	23.16	72.79	Inactive
5w	760466/1	UO-31(Renal Cancer)	78.94	78.94–143.32	103.75	24.81	64.38	Inactive
5x	760468/1	T-47D(Breast Cancer)	84.18	84.18–125.78	103.93	19.75	41.60	Inactive
5z	760469/1	UO-31(Renal Cancer)	80.06	80.06–123.52	104.16	24.10	50.92	Inactive
5a[1]	761987/1	HOP-62(Non-Small Cell Lung Cancer)	28.66	28.66–116.98	79.92	54.66	91.72	Active

[a] Compounds active of that particular cell lines, which showed growth inhibition ≤32% cell growth reduction following 48-h incubation with test compounds.
[b] Negative indicates the cell kill.



Reactions and conditions: (a) 4N HCl, methanol, water, reflux, r.t. (b) Abs. ethanol, conc. H_2SO_4 (c) $NH_2NH_2.H_2O$, ethanol (d) CS_2, KOH, ethanol (e) Hydrazine hydrate, ethanol.

Scheme 1. Protocol for synthesis of compound **5**.

presence of peak at definite *m/z* value in accordance to the molecular ion peak. In case of aryl groups having chloro-substituent (s) the molecular ion peak appeared as cluster of peaks.

4.2. In vitro anticancer activity

The synthesized compounds (25 in no.) were submitted to National Cancer Institute (NCI), USA for evaluation of their *in vitro* anticancer activity at single dose (1×10^{-5} M) against full NCI 60 cell lines panels representing on full nine human systems as leukemia, melanoma and cancers of lung, colon, brain, breast, ovary, kidney and prostate. The compounds added at a concentration (1×10^{-5} M) and the culture incubated for 48 h. End point determinations made with a protein binding dye, sulforhodamine B [47,48]. Results for each compound were reported as a mean graph of the percent growth of the treated cells. Results of each test agents are reported as percentage growth of the treated cells when compared with untreated control cells [49,50]. Compound **4** shown 69.36% growth inhibition against HOP-92 cell line (Non-Small Cell Lung Cancer), Compound **5a**, 61.97% against MCF7 cell lines (Breast Cancer), Compound **5b**, 63.03% against UO-31 cell lines (Renal Cancer), Compound **5c**, 64.29% against UO-31 cell lines (Renal Cancer), Compound **5d**, 30.17% against NCI-H522 cell lines (Non-Small Cell Lung Cancer), Compound **5e**, 75.55% against UO-31 cell lines (Renal Cancer), Compound **5g**, 76.24% against UO-31 cell lines (Renal Cancer), Compound **5h**, 0.38% against IGROV1 cell lines (Ovarian Cancer), Compound **5i**, 73.88% against NCI-H322M cell lines (Non-Small Cell Lung Cancer), Compound **5j**, 54.88% against UO-31 cell lines (Renal Cancer), Compound **5k**, 51.29% against UO-31 cell lines (Renal Cancer), Compound **5m**, 79.38% against UO-31 cell lines (Renal Cancer), Compound **5n**, 69.58% against UO-31 cell lines (Renal Cancer), Compound **5o**, 67.68% against UO-31 cell lines (Renal Cancer), Compound **5p**, 83.90% against UO-31 cell lines (Renal Cancer), Compound **5q**, 73.78% against UO-31 cell lines (Renal Cancer), Compound **5r**, 76.61% against UO-31 cell lines (Renal Cancer), Compound **5s**, 64.18% against UO-31 cell lines (Renal Cancer), Compound **5t**, 80.40% against UO-31 cell lines (Renal Cancer), Compound **5u**, 79.85% against UO-31 cell lines (Renal Cancer), Compound **5v**, 80.35% against UO-31 cell lines (Renal Cancer), Compound **5w**, 78.94% against UO-31 cell lines (Renal Cancer), Compound **5x**, 84.18% against T-47D cell lines (Breast Cancer) and Compound **5z**, 80.06% against UO-31 cell lines (Renal Cancer), Compound **5a**[1] exhibited 28.66% growth inhibition against HOP-62 cell lines (Non-Small Cell Lung Cancer) (Table 1). The compounds which reduced the growth of the cell lines to 32% or less (negative number indicate kills) is considered *in vitro* active [51,52]. Compound (**5h**, NCS: 760452) satisfied pre-determined threshold growth inhibition criteria and further selected for NCI full panel five dose assay at 10-fold dilutions of five different concentrations (0.01, 0.1, 1, 10 and 100 μM). The result of tested compound is given by three response parameters (GI_{50}, TGI and LC_{50}) for each cell line from log concentration vs % growth inhibition curves on nine cancer disease (Fig. 3). The GI_{50} value (growth inhibitory activity) corresponds to the concentration of the compound causing 50% decrease in net cell growth, the TGI value (cytostatic activity) is the concentration of the compound resulting in total growth inhibition and LC_{50} value (cytotoxic activity) is the concentration of the compound causing net 50% loss of initial cells at the end of the incubation period of 48 h. Furthermore, a mean graph midpoint (MG-MID) is calculated giving an averaged activity parameter overall cell lines.

The title compound under investigation (**5h**, NCS: 760452) exhibited remarkable anticancer activity against all the tested cell lines representing nine different subpanels with GI_{50} values between 0.20 and 2.58 μM under sensitive range an outstanding activity (Table 2). With regard to the sensitivity against some individual cell lines the compound showed high activity against Leukemia CCRF-CEM and MOLT-4 cell lines with GI_{50} 0.26 and

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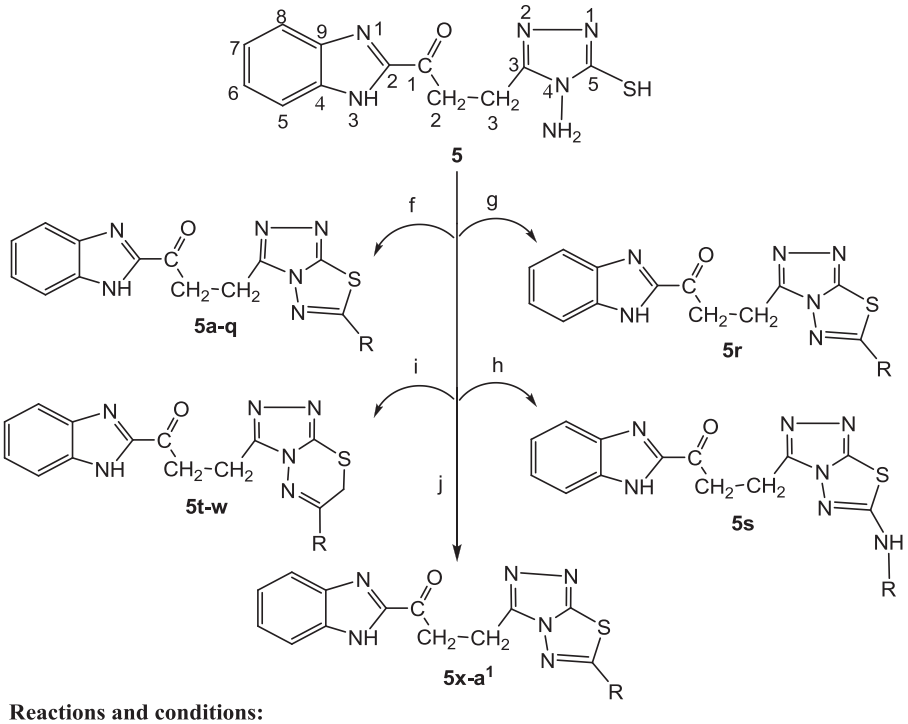
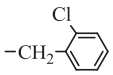
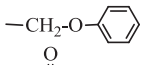
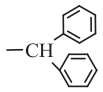
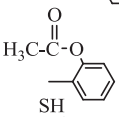
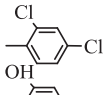
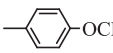
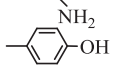


Reactions and conditions:

(f) R-COOH, $POCl_3$, reflux (g) KOH, CS_2, ethanol, reflux (h) Chloro-isothio cyanate, DMF, reflux (i) R-CH_2Cl, ethanol, reflux (j) R-COCl, ethanol, reflux.

Compd.	-R	Compd.	-R	Compd.	-R
5a		5b		5c	
5d		5e	$-CH_2CN$	5f	
5g	$-CH{=}CH_2$	5h		5i	
5j	$-CH{=}CHCH_3$	5k		5l	
5m	$-CH_2SH$	5n		5o	
5p	$-CH_2Cl$	5q	$-CH_2CH_2CH_2NH_2$	5r	$-SH$
5s		5t	$-NH_2$	5u	$-OH$
5v	$-OCH_2CH_3$	5w	$-Cl$	5x	$-CH_3$
5y	$-CH_2CH_2CH_2Cl$	5z	$-CH_2CH_2Cl$	5a[1]	$-OCH_2CH_3$

Scheme 2. Protocol for synthesis of title compounds.

0.28 μM respectively. Obtained data revealed an obvious sensitivity profile towards non-small cell Lung cancer subpanel (GI_{50} value ranging from 0.24 to 2.0 μM), least for NCI-H226 and maximum for A549/ATCC cell line. The compound proved to be sensitive towards all the tested Colon cancer cell lines with not more than 1.63 μM concentrations. All the tested Leukemia cancer cell lines were sensitive with not more than $GI_{50} > 1$ μM concentrations of the tested compound. The highest growth inhibitory activity was observed against the Colon SW-620 cancer cell line with GI_{50} value 0.20 μM. The all remaining subpanel cell line showed maximum sensitive towards tested compound with not more than 2.58 μM concentrations (Table 2). The criterion for selectivity of a compound depends upon the ratio obtained by dividing the full panel MID_a (the average sensitivity of all cell lines towards the test agent) by their individual subpanel MID_b (the average sensitivity of all cell lines of a particular subpanel towards the test agent). The ratios between 3 and 6 refer to moderate selectivity; ratios greater than 6 indicate high selectivity towards the corresponding cell line, while compounds not meeting either of these criteria rated non-selective [53]. As per this criterion, compound **5h** in the study was found to be mild selective towards Leukemia cancer subpanel. Over all the GI_{50} values of the screening process resulted in a sensitive range inferior of 2.58 μM denoting an outstanding activity and the values of LC_{50} are in most of the cell lines >100 μM (Table 2). The log molar concentration of the resulted screening as log GI_{50} ranged from −6.58 to −5.66, log TGI −6.26 to −5.18 except MOLT-4, RPMI-8226, SR and OVCAR-8 (>−4.00) and all Leukemia cell lines showed log $LC_{50} > -4.00$. A mean graph midpoint (MG-MID) calculated for

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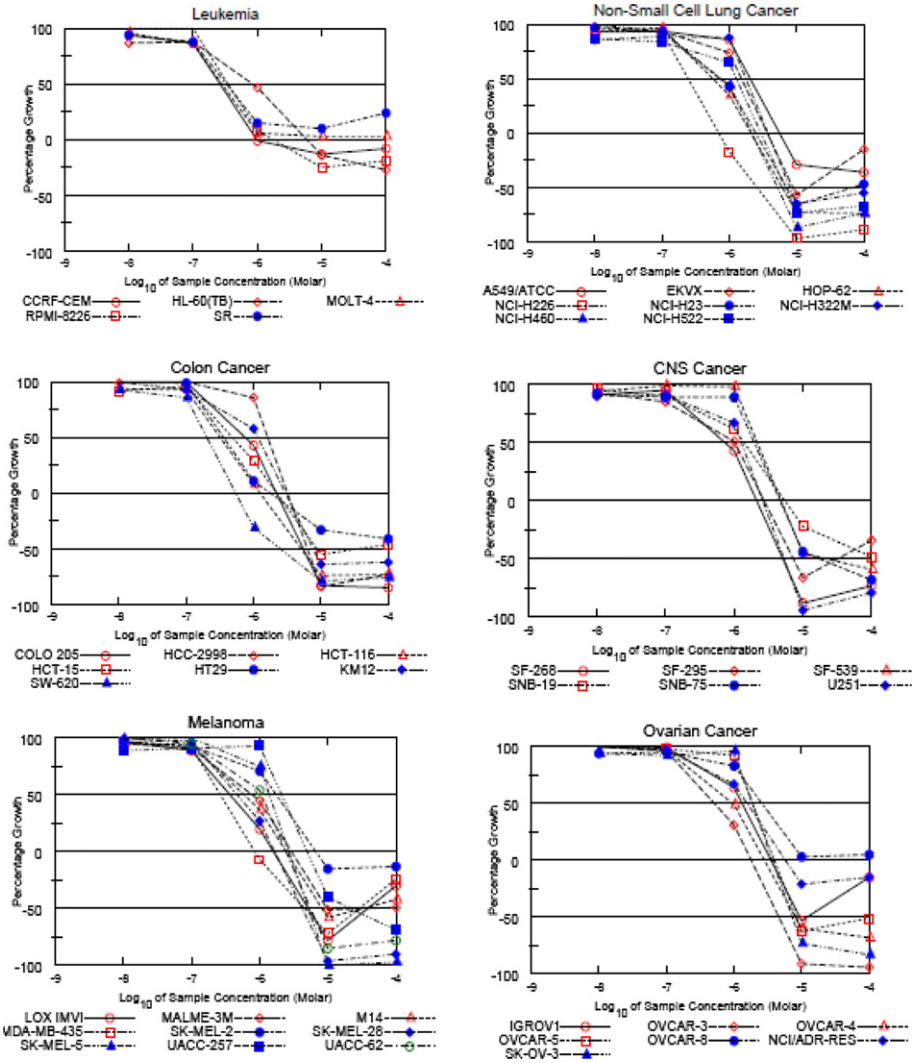

Fig. 3. Dose response curves (% growth verses sample concentration at NCI fixed protocol, μM) obtained from the NCI's *in vitro* disease-oriented human tumor cells line of compound (**5h**) on nine cancer disease. The different color and shape of NCI, subpanel cell lines indicative of growth percentage inhibition with concentration of sample. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

each of parameters, giving as log GI_{50} (−6.07), log TGI (−5.51) and log LC_{50} (−4.85) and insensitive cell lines are also included with the highest concentration (Table 3).

4.2.1. Structural activity relationship (SAR)

Following points could be drawn regarding the SAR of newly synthesized compounds in relation to their anticancer activity:

a. Benzimidazoles clubbed with triazolo-thiadiazole were found to have better anticancer activities than those of benzimidazoles clubbed with triazolo-thiadiazine.
b. The anticancer activity was influenced by the presence of electron with drawing group like chloro on *ortho*, *meta* or *para* position on aromatic ring. Compound **5s** having chloro group on *para* position of phenyl ring, 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(6-(4-chlorophenyl-amino)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)propan-1-one, showed increased in sensitivity of cell line (64.18%) and similarly the same group disubstituted on *meta* and *para* position, showed synergistic action in the sensitivity (−47.05%) as exhibited by the compound **5h**, 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(6-(2,4-dichlorophenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl) propan-1-one.
c. On the other hand the electron releasing groups like methyl attached to phenyl ring showed decrease in the sensitivity (84.18%) as shown by compound **5x**, 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(6-methyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl) propan-1-one.
d. Presence of diphenylmethane group (**5d**) or electron with drawing group (s) significantly improved anticancer activity against the human cancer cell lines of nine subpanels.
e. Compound **5h** may serve as novel template for development of potential and selective agents in the field of cancer chemotherapy.

5. Conclusion

Two series of benzimidazole clubbed with triazolo-thiadiazole and triazolo-thiadiazine rings, comprising of 32 new compounds were successfully synthesized and among them 25 compounds were evaluated for their *in vitro* anticancer activity at the NCI, USA. Compounds showed good to remarkable and broad-spectrum anticancer activity. One compound (**5h**), namely 1-(1*H*-benzo [*d*]imidazol-2-yl)-3-(6-(2,4-dichlorophenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)propan-1-one emerged as lead compound with broad

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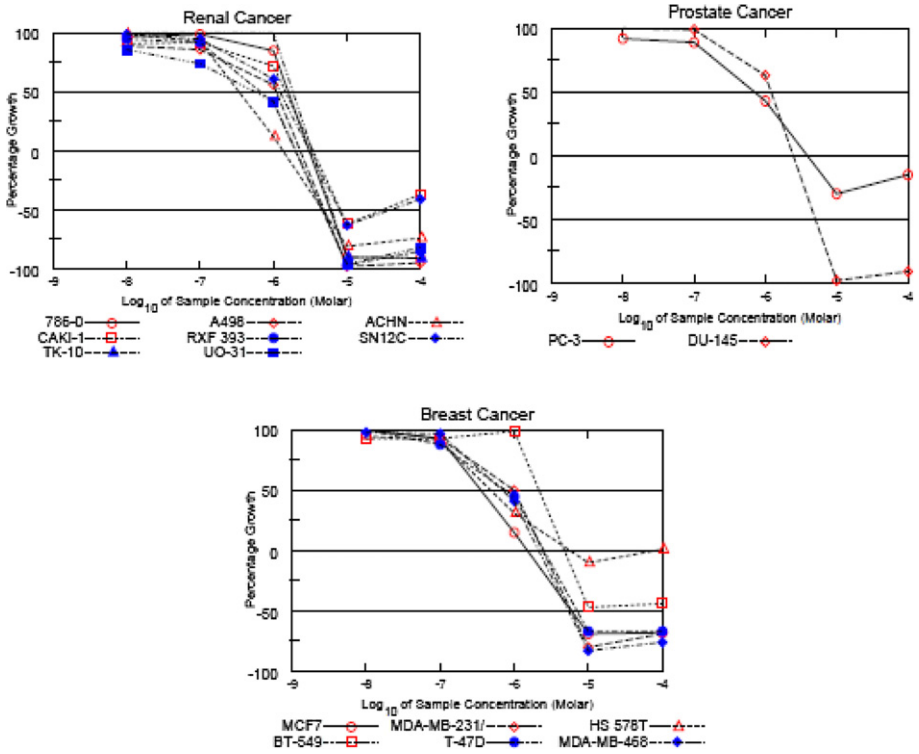

Fig. 3. (*continued*).

spectrum of anticancer activities against tumor cell lines (MG-MID −6.07, −5.51 and −4.85 value of $\log_{10}$ GI_{50}, $\log_{10}$ TGI and $\log_{10}$ LC_{50} respectively). Based on these observations, it could be concluded that the compound (**5h**) may be used as template for development of novel anticancer agents.

6. Experimental protocols

6.1. General materials and instrumentations

All chemicals (reagents and solvents) were procured from E. Merck Ltd. and S.D. Fine Chemicals (India). Melting points were taken on a liquid paraffin bath in open capillary tubes and are uncorrected. Progress of the reactions was monitored by using TLC plates (silica gel G), Toluene:Ethyl acetate:Formic acid (5:4:1, v/v/v) and benzene: acetone (9:1, v/v) used as solvent systems. The spots were located by exposure to iodine vapors or under UV-light. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded on Bruker spectrospin DPX-300 MHz in DMSO-d_6/$CDCl_3$; chemical shift (δ) values reported in parts per million (*ppm*) and coupling constants (*J*) in *Hz* using tetramethylsilane as internal reference. The splitting pattern abbreviations are as follows: s, singlet; bs, broad singlet; d, doublet; dd, double doublet; t, triplet; q, quadruplet; m, multiplet. The exchangeable protons (O*H* and N*H*) confirmed by the addition of D_2O. Mass spectra recorded on LCMS/MS (Perkin–Elmer and LABINDIA, Applied Biosystems) model no. API 3000, presented as *m/z*. IR spectra recorded on FT/IR (Jasco, Japan), model no.410. Elemental analyses performed on a Perkin–Elmer 240 analyzer and found in the range of ±0.4% for each element analyzed (C, H and N).

6.2. Synthesis

6.2.1. Synthesis of 4-(1H-benzo[d]imidazol-2-yl)-4-oxobutanoic acid (*1*)

A solution of *o*-phenylenediamine (0.01 mol), α-ketoglutaric acid (equimolar; 0.01 mol) in methanol/water mixture (1:1) and HCl (4 N; 5 mL) was refluxed for 6 h and then left to cool to room temperature. 10% NaOH solution was added slowly to neutralize the reaction mixture, when a solid mass precipitated out, which was filtered, washed with water and recrystallized with ethanol. Yield: 87%, m.p. 261–262 °C, R_f 0.71 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3394 (O–H), 3326 (N–H), 3114 (C–H, Ar–H), 2972 (C–H, CH_2), 1728 (C=O), 1600 (C=N), 1562 (C=C); ^{1}H NMR (DMSO-d_6): 12.97 (s, 1H, OH, D_2O exchangeable), 12.34 (s, 1H, NH, D_2O exchangeable), 7.66 (d, 1H, J = 7.5 Hz, H-4, benzimidazole), 7.48 (t, 1H, J = 7.2 Hz, H-7, benzimidazole), 7.27 (t, 2H, J = 8.7 Hz, H-5,6, benzimidazole), 3.05 (t, 2H, J = 6.9 Hz, CH_2), 2.76 (t, 2H, J = 6.9 Hz, CH_2); ^{13}C NMR (DMSO-d_6): 175.57 (C=O), 168.21 (C=O, COOH), 159.67 (C=N), 138.27, 132.21, 130.19, 128.57, 124.65, 123.64 (Ar–C), 34.23 (CH_2, CH_2CO), 31.36 (CH_2, CH_2COOH); ESI MS (*m/z*): 218 (M^+); Anal. calcd. for $C_{11}H_{10}N_2O_3$: C, 60.55; H, 4.62; N, 12.84. Found: C, 60.63; H, 4.65; N, 12.98.

6.2.2. Synthesis of ethyl-4-(1H-benzo[d]imidazol-2-yl)-4-oxobutanoate (*2*)

A solution of compound (**1**) (0.01 mol) in absolute ethanol (25 mL) was refluxed for 10 h in presence of conc. H_2SO_4, (0.1 mL). After completion of the reaction, it was cooled to room temperature, diluted with cold water and then neutralized with $NaHCO_3$ to pH 7. A precipitate formed which was filtered and crystallized from ethanol. Yield: 81%, m.p. 253–254 °C, R_f = 0.60, [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3330 (N–H), 3047 (C–H, Ar–H), 2958 (C–H, CH_2), 1718 (C=O), 1640 (C=N), 1570 (C=C); ^{1}H NMR (DMSO-d_6): 12.51 (bs, 1H, NH, D_2O exchangeable), 7.73 (d, 1H, J = 7.8 Hz, H-4, benzimidazole), 7.54 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.12 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 3.82 (q, 2H, J = 5.4 Hz, CH_2), 3.03 (t, 2H, J = 7.2 Hz, CH_2), 2.71 (t, 2H, J = 7.2 Hz, CH_2), 1.15 (t, 3H, J = 1.2 Hz, CH_3); ^{13}C NMR (DMSO-d_6):177.61 (C=O), 165.37 (C=O, ester), 156.47 (C=N), 138.32, 137.93, 129.13, 128.17, 124.55, 123.74 (Ar–C), 30.81 (CH_2, CH_2CO), 28.36 (CH_2, CH_2COO), 69.07 (CH_2, ester),

Table 2
Calculated values of GI_{50}, TGI, LC_{50} of the cell lines, NCI: full cell lines panel, MG-MID and selectivity ratio of the compound (**5h**, NCS: 760452).

Panel	Cell line	$GI_{50}(10^{-6}$ M)				TGI (10^{-6} M)	LC_{50} (10^{-6} M)[c]
		Concentration per cell line	Subpanel concentration	Subpanel MID[b]	Selectivity ratio		
Leukemia	CCRF-CEM	0.265				0.974	>100
	HL-60(TB)	0.862	2.094	0.418	2.485	5.93	>100
	MOLT-4	0.282				>100	>100
	RPMI-8226	0.352				1.76	>100
	SR	0.333				>100	>100
Non-Small Cell Lung Cancer	A549/ATCC	2.07				5.63	>100
	EKVX	1.53				3.69	Nt
	HOP-62	0.576	8.945	1.118	0.929	2.12	6.16
	NCI-H226	0.244				0.696	2.59
	NCI-H23	0.719				2.48	Nt
	NCI-H322M	1.76				3.74	7.94
	NCI-H460	0.766				2.17	5.21
	NCI-H522	1.28				2.96	6.82
Colon Cancer	COLO 205	0.767				2.21	5.50
	HCC-2998	1.63				3.20	6.29
	HCT-116	0.320	4.932	0.704	1.476	1.24	5.06
	HCT-15	0.489				2.21	Nt
	HT29	0.362				1.81	>100
	KM12	1.16				2.99	7.70
	SW-620	0.204				0.545	2.48
CNS Cancer	SF-268	0.740				2.14	5.15
	SF-295	1.03				2.73	Nt
	SF-539	2.17	8.55	1.425	0.729	4.86	22.8
	SNB-19	1.37				5.44	>100
	SNB-75	1.96				4.65	17.1
	U251	1.28				2.61	5.34
Melanoma	LOX IMVI	0.363				1.59	Nt
	MALME-3M	0.762				2.94	Nt
	M14	0.596				2.45	Nt
	MDA-MB-435	0.265				0.842	Nt
	SK-MEL-2	1.76	8.77	0.974	1.067	6.67	>100
	SK-MEL-28	0.474				1.67	4.24
	SK-MEL-5	1.39				2.69	5.19
	UACC-257	2.10				5.01	22.5
	UACC-62	1.06				2.44	5.61
Ovarian Cancer	IGROV1	1.31				3.51	Nt
	OVCAR-3	0.514				1.80	4.60
	OVCAR-4	0.971	10.675	1.525	0.681	2.82	8.04
	OVCAR-5	1.88				3.98	8.39
	OVCAR-8	2.58				>100	>100
	NCI/ADR-RES	1.56				5.80	>100
	SK-OV-3	1.86				3.68	7.28
Renal Cancer	786-0	1.59				3.06	5.92
	A498	1.10				2.32	4.89
	ACHN	0.339	8.969	1.121	0.927	1.35	4.67
	CAKI-1	1.45				3.44	Nt
	RXF 393	0.674				1.99	4.55
	SN12C	1.23				3.10	Nt
	TK-10	2.01				3.55	6.30
	UO-31	0.576				2.02	4.63
Prostate Cancer	PC-3	0.695	1.895	0.947	1.097	3.90	>100
	DU-145	1.20				2.46	5.02
Breast Cancer	MCF7	0.355				1.50	5.97
	MDA-MB-231/ATCC	1.00				2.42	5.87
	HS 578T	0.478	5.445	0.907	1.145	–	>100
	BT-549	2.16				4.77	>100
	T-47D	0.753				2.51	7.06
	MDA-MB-468	0.699				2.15	5.41
MID[a]		1.04					

[a] Mean graph midpoint (arithmetical mean value of treated cancer cell lines) representing the average sensitivity of all cell lines (full panel) towards the test agent in μM.
[b] Average sensitivity of all cell lines of a particular subpanel towards the test agent in μM.
[c] Nt indicates not tested.

20.31 (CH_3); ESI MS (*m*/*z*): 246 (M^+); Anal. calcd. for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38. Found: C, 62.77; H, 5.81; N, 11.35.

*6.2.3. Synthesis of 4-(1H-benzo[d]imidazol-2-yl)-4-oxobutane hydrazide (**3**)*

A solution of compound **2** (0.01 mol) and hydrazine hydrate (0.015 mol) in ethanol was refluxed for 8 h. The reaction mixture was cooled, a solid precipitate separated out, which was filtered, dried and recrystallized from methanol. Yield: 85%, m.p. 241–242 °C, $R_f = 0.53$ [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3494 (N–H, NH_2), 3359 (N–H, NH), 3151 (C–H, Ar–H), 2947 (C–H, CH_2), 1691 (C=O), 1643 (C=N), 1600 (C=C); ^1H NMR (DMSO-d_6): 11.51 (s, 1H, NH, benzimidazole, D_2O exchangeable), 10.72 (s, 1H, NH, hydrazide, D_2O exchangeable), 9.05 (bs, 2H, NH_2,

Table 3
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 (**5h**, NCS: 760452).

Cancer disease	Used cell lines	log_{10} GI_{50}	log_{10} TGI	log_{10} LC_{50}[a]
Leukemia	CCRF-CEM	−6.58	−6.01	>−4.00
	HL-60(TB)	−6.06	5.23	>−4.00
	MOLT-4	−6.55	>−4.00	>−4.00
	RPMI-8226	−6.45	−5.75	>−4.00
	SR	−6.48	>−4.00	>−4.00
Non-Small Cell Lung Cancer	A549/ATCC	−5.68	−5.25	>−4.00
	EKVX	−5.81	−5.43	Nt
	HOP-62	−6.24	−5.67	−5.21
	NCI-H226	−6.61	−6.16	−5.59
	NCI-H23	−6.14	−5.61	Nt
	NCI-H322M	−5.75	−5.43	−5.10
	NCI-H460	−6.12	−5.66	−5.28
	NCI-H522	−5.89	−5.53	−5.17
Colon Cancer	COLO 205	−6.12	−5.66	−5.26
	HCC-2998	−5.79	−5.50	−5.20
	HCT-116	−6.50	−5.91	−5.30
	HCT-15	−6.31	−5.65	Nt
	HT29	−6.44	−5.74	>−4.00
	KM12	−5.94	−5.52	−5.11
	SW-620	−6.69	−6.26	−5.60
CNS Cancer	SF-268	−6.13	−5.67	−5.29
	SF-295	−5.99	−5.56	Nt
	SF-539	−5.66	−5.31	−4.64
	SNB-19	−5.86	−5.26	>−4.00
	SNB-75	−5.71	−5.33	−4.77
	U251	−5.89	−5.58	−5.27
Melanoma	LOX IMVI	−6.44	−5.80	Nt
	MALME-3M	−6.12	−5.53	Nt
	M14	−6.22	−5.61	Nt
	MDA-MB-435	−6.58	−6.07	Nt
	SK-MEL-2	−5.75	−5.18	>−4.00
	SK-MEL-28	−6.32	−5.78	−5.37
	SK-MEL-5	−5.86	−5.57	−5.28
	UACC-257	−5.68	−5.30	−4.65
	UACC-62	−5.97	−5.61	−5.25
Ovarian Cancer	IGROV1	−5.88	−5.46	Nt
	OVCAR-3	−6.29	−5.74	−5.34
	OVCAR-4	−6.01	−5.55	−5.09
	OVCAR-5	−5.72	−5.40	−5.08
	OVCAR-8	−5.59	> −4.00	>−4.00
	NCI/ADR-RES	−5.81	−5.24	>−4.00
	SK-OV-3	−5.73	−5.43	−5.14
Renal Cancer	786-0	−5.80	−5.51	−5.23
	A498	−5.96	−5.63	−5.31
	ACHN	−6.47	−5.87	−5.33
	CAKI-1	−5.84	−5.46	Nt
	RXF 393	−6.17	−5.70	−5.34
	SN12C	−5.91	−5.51	Nt
	TK-10	−5.70	−5.45	−5.20
	UO-31	−6.24	−5.69	−5.33
Prostate Cancer	PC-3	−6.16	−5.41	>−4.00
	DU-145	−5.92	−5.61	−5.30
Breast Cancer	MCF7	−6.45	−5.82	−5.22
	MDA-MB-231/ATCC	−6.00	−5.62	−5.23
	HS 578T	−6.32	–	>−4.00
	BT-549	−5.67	−5.32	>−4.00
	T-47D	−6.12	−5.60	−5.15
	MDA-MB-468	−6.16	−5.67	−5.27
MID		−6.07	−5.51	−4.85
Delta		0.62	0.75	0.75
Range		1.1	2.26	1.6

[a] Nt indicates compound not evaluated on that particular cell lines; showed values >100 μM.

D_2O exchangeable), 7.68 (d, 1H, J = 7.8 Hz, H-4, benzimidazole), 7.50 (t, 1H, J = 7.2 Hz, H-7, benzimidazole), 7.27 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 3.02 (t, 2H, J = 7.5 Hz, CH_2), 2.52 (t, 2H, J = 7.8 Hz, CH_2CO); ^{13}C NMR (DMSO-d_6):179.15 (C═O), 162.72 (C═O, hydrazide), 156.32 (C═N), 133.99, 132.28, 129.59, 128.45, 122.92, 116.85 (Ar−C), 30.17 (CH_2, CH_2CO), 22.27 (CH_2, hydrazide); ESI MS (m/z): 232 (M^+); Anal. calcd. for $C_{11}H_{12}N_4O_2$: C, 56.89; H, 5.21; N, 24.12. Found: C, 56.73; H, 5.27; N, 24.15.

*6.2.4. Synthesis of 1-(1H-benzo[d]imidazol-2-yl)-3-(5-mercapto-1,3,4-oxadiazol-2-yl)propan-1-one (**4**)*

A mixture of compound **3** (0.01 mol) and CS_2/KOH (0.01 mol) in ethanol/water (1:1) refluxed for 6 h. After completion of reaction, the reaction mixture was reduced to half of its original volume and cold water (20 mL) added to it. The contents were then acidified with conc. HCl. A solid precipitate obtained which was filtered, washed with water and recrystallized from methanol. Yield: 75%; m.p. 220–221 °C; R_f = 0.47 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3341 (NH), 3066 (CH), 2900 (CH_2), 2599 (SH), 1685 (C═O), 1654 (C═N), 1627 (C═C), 1280 (N−N═C), 1161 (C−O−C, asymmetric), 1060 (C−O−C, symmetric); ^{1}H NMR ($CDCl_3$): 13.40 (s, 1H, SH, D_2O exchangeable), 11.31 (s, 1H, NH, D_2O exchangeable), 7.81 (d, 1H, J = 8.1 Hz, H-4, benzimidazole), 7.49 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.32 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 3.31 (t, 2H, J = 6.9 Hz, CH_2), 2.92 (t, 2H, J = 6.9 Hz, CH_2CO); ^{13}C NMR (DMSO-d_6): 179.14 (C═O), 162.77, 156.31 (C-oxadiazole), 159.84 (C═N), 133.93, 132.26, 129.62, 128.45, 122.96, 116.86 (Ar−C), 30.14 (CH_2, CH_2CO), 22.25 (CH_2); ESI MS (m/z): 274 (M^+); Anal. calcd. for $C_{12}H_{10}N_4O_2S$: C, 52.54; H, 3.67; N, 20.43. Found: C, 52.61; H, 4.05; N, 20.51.

*6.2.5. Synthesis of 3-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)-1-(1H-benzo[d]imidazol-2-yl) propan-1-one (**5**)*

To a solution of compounds **4** (0.01 mol) in *n*-butanol (40 mL), hydrazine hydrate (0.03 mol) was added. The reaction mixture was refluxed for 5 h and after completion of the reaction, KOH (0.015 mol) was added to reaction media. A solid precipitate separated out, which was acidified with conc. HCl, filtered, washed with H_2O and recrystallized from dichloromethane. Yield: 70%; m.p. 231–232 °C; R_f = 0.45 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3429 (NH), 3105 (CH), 2904 (CH_2), 2557 (SH), 1728 (C═O), 1670 (C═N), 1566 (C═C), 1303 (N−N═C); ^{1}H NMR (DMSO-d_6): 13.43 (s, 1H, SH, D_2O exchangeable), 12.40 (s, 1H, NH, benzimidazole, D_2O exchangeable), 7.73 (d, 1H, J = 7.8 Hz, H-4-benzimidazole), 7.51 (t, 1H, J = 7.5 Hz, H-7-benzimidazole), 7.32 (t, 2H, J = 7.8 Hz, H-5,6-benzimidazole), 3.53 (s, 2H, NH_2), 3.20 (t, 2H, J = 6.3 Hz, CH_2), 2.76 (t, 2H, J = 7.2 Hz, CH_2CO); ^{13}C NMR (DMSO-d_6): 166.16 (C═O), 160.32, 152.14 (C-triazole), 155.06 (C═N), 132.29, 131.94, 130.03, 128.59, 123.54, 115.80 (Ar−C), 29.24 (CH_2, CH_2CO), 21.41 (CH_2); ESI MS (m/z): 288 (M^+); Anal. calcd. for $C_{12}H_{12}N_6OS$: C, 49.99; H, 4.19; N, 29.15. Found: C, 49.85; H, 4.25; N, 29.37.

*6.2.6. 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 (**5a**–**q**)*

Compounds (**5a**–**q**) were prepared by reacting compound **5** (0.003 mol) and different types of aliphatic/aromatic acids (equimolar; 0.003 mol) in phosphorus oxychloride (5 mL) under reflux for 5–6 h. After completion of reaction, the reaction mixture was cooled to room temperature and then gradually poured on to crushed ice with continuous stirring. The obtained solid precipitate was treated with dilute sodium hydroxide solution and washed thoroughly with cold water to remove the inorganic component. The compound so obtained was filtered, dried and recrystallized from ethanol.

*6.2.6.1. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-(2-aminophenyl)-[1,2,4] triazolo[3,4-b][1,3,4]thiadia-zol-3-yl)propan-1-one (**5a**).* Yield: 66%; m.p. 215–216 °C; R_f = 0.53 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3357 (NH), 3063 (CH), 2955 (CH_2), 1711 (C═O), 1657 (C═N), 1563 (C═C), 1385 (N═C−S), 1320 (N−N═C);

^{1}H NMR (DMSO-d_6): 12.37 (s, 1H, NH, D_2O exchangeable), 7.71 (d, 1H, J = 7.8 Hz, H-4, benzimidazole), 7.48 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.30 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 7.27–6.95 (m, 4H, phenyl), 5.92 (s, 2H, NH_2), 3.23 (t, 2H, J = 6.9 Hz, CH_2), 2.87 (t, 2H, J = 6.9 Hz, CH_2CO); ^{13}C NMR (DMSO-d_6): 170.41 (C=O), 169.23 (C-thiadiazole), 161.23, 158.01 (C-triazole), 154.93 (C=N), 138.21, 133.05, 132.75, 131.45, 129.84, 129.31, 128.53, 127.47, 124.37, 123.92, 117.32, 115.03 (Ar–C), 33.73 (CH_2, CH_2CO), 24.56 (CH_2); ESI MS (m/z): 389 (M^+); Anal. calcd. for $C_{19}H_{15}N_7OS$: C, 58.60; H, 3.88; N, 25.18. Found: C, 58.71; H, 3.97; N, 25.21.

*6.2.6.2. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-(2-chlorobenzyl)-[1,2,4] triazolo[3,4-b][1,3,4] thiadiazol-3-yl)propan-1-one (**5b**).* Yield: 65%; m.p. 212–213 °C; R_f = 0.56 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3373 (NH), 3042 (CH), 2930 (CH_2), 1720 (C=O), 1625 (C=N), 1561 (C=C), 1383 (N=C–S), 1280 (N–N=C), 713 (CCl); ^{1}H NMR (DMSO-d_6): 11.97 (s, 1H, NH, D_2O exchangeable), 7.87 (d, 1H, J = 8.1 Hz, H-4, benzimidazole), 7.64 (t, 1H, J = 8.1 Hz, H-7, benzimidazole), 7.36 (t, 2H, J = 7.8 Hz, H-5,6, benzimidazole), 7.30–7.01 (m, 4H, phenyl), 4.10 (s, 2H, CH_2), 3.68 (t, 2H, J = 6.9 Hz, CH_2), 2.88 (t, 2H, J = 6.9 Hz, CH_2CO); ^{13}C NMR (DMSO-d_6): 174.61 (C=O), 166.23 (C-thiadiazole), 160.42, 158.74 (C-triazole), 156.52 (C=N), 136.13, 135.98, 134.57, 132.45, 130.35, 129.17, 128.86, 127.45, 124.90, 124.21, 123.16, 115.38 (Ar–C), 34.81 (CH_2, CH_2CO), 30.51 (CH_2), 22.14 (CH_2); ESI MS (m/z): 422 (M^+); Anal. calcd. for $C_{20}H_{15}ClN_6OS$: C, 56.80; H, 3.58; N, 19.87. Found: C, 57.15; H, 3.65; N, 19.95.

*6.2.6.3. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-(phenoxymethyl)-[1,2,4] triazolo[3,4-b][1,3,4] thiadiazol-3-yl) propan-1-one (**5c**).* Yield: 67%; m.p. 200–201 °C; R_f = 0.52 [benzene:acetone (9:1)]; IR (KBr, cm^{-1}): 3341 (NH), 3010 (CH), 2963 (CH_2), 1710 (C=O), 1647 (C=N), 1573 (C=C), 1373 (N=C–S), 1314 (N–N=C), 1247 (C–O–C, asymmetric), 1040 (C–O–C, symmetric), 1193 (OCH_2); ^{1}H NMR (DMSO-d_6): 12.27 (s, 1H, NH, D_2O exchangeable), 7.81 (d, 1H, J = 7.8 Hz, H-4, benzimidazole), 7.54 (t, 1H, J = 7.8 Hz, H-7, benzimidazole), 7.41 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 7.31–6.95 (m, 5H, phenyl), 5.44 (s, 2H, CH_2), 3.31 (t, 2H, J = 7.2 Hz, CH_2), 2.94 (t, 2H, J = 6.9 Hz, CH_2CO); ^{13}C NMR (DMSO-d_6): 179.15 (C=O), 167.23 (C-thiadiazole), 163.42, 159.32 (C-triazole), 156.17 (C=N), 141.57, 134.12, 133.61, 132.41, 129.70, 128.23, 127.74, 124.75, 123.82, 122.34, 117.52, 112.93 (Ar–C), 51.35 (OCH_2), 30.92 (CH_2, CH_2CO), 23.41 (CH_2); ESI MS (m/z): 404 (M^+); Anal. calcd. for $C_{20}H_{16}N_6O_2S$: C, 59.39; H, 3.99; N, 20.78. Found: C, 59.45; H, 3.83; N, 20.89.

*6.2.6.4. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-benzhydryl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)propan-1-one (**5d**).* Yield: 65%; m.p. 197–198 °C; R_f = 0.59 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3326 (NH), 3050 (CH), 2890 (CH_2), 2873 (CH), 1724 (C=O), 1633 (C=N), 1537 (C=C), 1380 (N=C–S), 1306 (N–N=C); ^{1}H NMR (DMSO-d_6): 12.01 (s, 1H, NH, D_2O exchangeable), 8.05 (d, 1H, J = 7.5 Hz, H-4, benzimidazole), 7.75 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.57 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 7.33–6.80 (m, 10H, phenyl), 5.61 (s, H, CH), 3.24 (t, 2H, J = 7.2 Hz, CH_2), 2.71 (t, 2H, J = 7.2 Hz, CH_2CO); ^{13}C NMR (DMSO-d_6): 172.46 (C=O), 169.72 (C-thiadiazole), 162.72, 159.86 (C-triazole), 156.39 (C=N), 140.23, 134.72, 134.01, 133.91, 132.31, 131.82, 130.51, 129.45, 128.41, 125.74, 124.86, 124.21, 123.37, 122.30, 120.15, 118.41, 111.32 (Ar–C), 47.24 (CH), 36.10 (CH_2, CH_2CO), 22.71 (CH_2); ESI MS (m/z): 464 (M^+); Anal. calcd. for $C_{26}H_{20}N_6OS$: C, 67.22; H, 4.34; N, 18.09. Found: C, 67.25; H, 4.45; N, 18.17.

*6.2.6.5. 2-(3-(3-(1H-Benzo[d]imidazol-2-yl)-3-oxopropyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)acetonitrile (**5e**).* Yield: 63%; m.p. 217–218 °C; R_f = 0.53 [benzene:acetone (9:1)]; IR (KBr, cm^{-1}): 3344 (NH), 3076 (CH), 2966 (CH_2), 2335 (CN), 1718 (C=O), 1664 (C=N), 1569 (C=C), 1384 (N=C–S), 1263 (N–N=C); ^{1}H NMR ($CDCl_3$):11.76 (s, 1H, NH, D_2O exchangeable), 7.81 (d, 1H, J = 8.7 Hz, H-4, benzimidazole), 7.52 (t, 1H, J = 7.8 Hz, H-7, benzimidazole), 7.33 (t, 2H, J = 7.8 Hz, H-5,6, benzimidazole), 3.72 (s, 2H, CH_2CN), 3.34 (t, 2H, J = 7.2 Hz, CH_2), 2.95 (t, 2H, J = 6.9 Hz, CH_2CO); ^{13}C NMR (DMSO-d_6): 174.39 (C=O), 172.89 (C-thiadiazole), 160.73, 160.42 (C-triazole), 155.01 (C=N), 132.11, 131.83, 129.97, 128.50, 115.72 (Ar–C), 60.24 (CN), 30.01 (CH_2, CH_2CO), 28.07 (CH_2), 14.60 (CH_2, CH_2CN); ESI MS (m/z): 337 (M^+); Anal. calcd. for $C_{15}H_{11}N_7OS$: C, 53.40; H, 3.29; N, 29.06. Found: C, 53.65; H, 3.41; N, 29.11.

*6.2.6.6. 2-(3-(3-(1H-Benzo[d]imidazol-2-yl)-3-oxopropyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)phenyl acetate (**5f**).* Yield: 66%; m.p. 195–196 °C; R_f = 0.61 [benzene:acetone (9:1)]; IR (KBr, cm^{-1}): 3327 (NH), 3053 (CH), 2941 (CH_2), 1692 (C=O), 1628 (C=N), 1526 (C=C), 1384 (N=C–S), 1260 (N–N=C); ^{1}H NMR ($CDCl_3$): 12.05 (s, 1H, NH, D_2O exchangeable), 8.13–7.78 (m, 4H, phenyl), 7.73 (d, 1H, J = 7.5 Hz, H-4, benzimidazole), 7.51 (t, 1H, J = 7.8 Hz, H-7, benzimidazole), 7.35 (t, 2H, J = 8.1 Hz, H-5,6, benzimidazole), 3.27 (t, 2H, J = 6.9 Hz, CH_2), 2.74 (t, 2H, J = 6.9 Hz, CH_2CO), 2.53 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): 175.21 (C=O), 172.45 (CO, $COCH_3$), 169.81 (C-thiadiazole), 162.52, 158.21 (C-triazole), 156.37 (C=N), 132.10, 129.83, 128.46, 126.87, 125.23, 123.41, 122.35, 121.89, 120.53, 118.76, 118.21, 116.71 (Ar–C), 32.13 (CH_2, CH_2CO), 24.17 (CH_2), 18.61 (CH_3); ESI MS (m/z): 432 (M^+); Anal. calcd. for $C_{21}H_{16}N_6O_3S$: C, 58.32; H, 3.73; N, 19.43. Found: C, 57.43; H, 3.83; N, 19.47.

*6.2.6.7. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-vinyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)- propan-1-one (**5g**).* Yield: 70%; m.p. 186–187 °C; R_f = 0.51 [benzene:acetone (9:1)]; IR (KBr, cm^{-1}): 3328 (NH), 3012 (CH), 2974 (HC=CH_2), 2904 (CH_2), 1728 (C=O), 1666 (C=N), 1569 (C=C), 1342 (N=C–S), 1230 (N–N=C); ^{1}H NMR ($CDCl_3$):12.41 (s, 1H, NH, D_2O exchangeable), 7.80 (d, 1H, J = 7.8 Hz, H-4, benzimidazole), 7.51 (t, 1H, J = 7.2 Hz, H-7, benzimidazole), 7.33 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 4.06 (t, 1H, J = 7.2 Hz, CH), 3.92 (d, 2H, J = 6.9 Hz, CH_2=CH), 3.29 (t, 2H, J = 6.9 Hz, CH_2), 2.90 (t, 2H, J = 6.9 Hz, CH_2CO); ^{13}C NMR ($CDCl_3$): 171.51 (C=O), 167.31 (C-thiadiazole), 160.41, 157.13 (C-triazole), 153.47 (C=N), 133.27, 128.91 (CH=CH_2) 128.78, 128.13, 124.31, 123.82, 120.53, 118.46 (Ar–C), 34.73 (CH_2, CH_2CO), 22.53 (CH_2); ESI MS (m/z): 324 (M^+); Anal. calcd. for $C_{15}H_{12}N_6OS$: C, 55.54; H, 3.73; N, 25.91. Found: C, 55.68; H, 3.85; N, 25.97.

*6.2.6.8. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-(2,4-dichlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl)propan-1-one (**5h**).* Yield: 69%; m.p. 225–226 °C; R_f = 0.41 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3336 (NH), 3093 (CH), 2900 (CH_2), 1685 (C=O), 1654 (C=N), 1627 (C=C), 1373 (N=C–S), 1234 (N–N=C); ^{1}H NMR ($CDCl_3$): 12.17 (s, 1H, NH, D_2O exchangeable), 8.20 (s, 1H, H-3-phenyl), 8.11 (d, 1H, J = 7.8 Hz, H-5-phenyl), 7.70 (d, 1H, J = 7.5 Hz, H-4, benzimidazole), 7.51 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.36 (t, 2H, J = 7.8 Hz, H-5,6, benzimidazole), 3.29 (t, 2H, J = 6.9 Hz, CH_2), 2.90 (t, 2H, J = 6.9 Hz, CH_2CO); ^{13}C NMR ($CDCl_3$): 173.63 (C=O), 173.01 (C-thiadiazole), 157.29, 156.42 (C-triazole), 154.21 (C=N), 138.21, 132.97, 129.25, 128.15, 126.91, 126.08, 125.95, 125.21, 124.01, 117.40, 116.63, 109.97 (Ar–C), 35.10 (CH_2, CH_2CO), 25.03 (CH_2); ESI MS (m/z): 443 (M^+); Anal. calcd. for $C_{19}H_{12}Cl_2N_6OS$: C, 51.48; H, 2.73; N, 18.96. Found: C, 51.49; H, 3.21; N, 18.87.

*6.2.6.9. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-(2-mercaptophenyl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl)propan-1-one (**5i**).* Yield: 60%; m.p. 189–190 °C; R_f = 0.63 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3356 (NH), 3080 (CH), 2925 (CH_2), 2571

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(SH), 1703 (C=O), 1643 (C=N), 1534 (C=C), 1380 (N=C–S), 1290 (N–N=C); ^{1}H NMR ($CDCl_3$): 12.51 (s, 1H, NH, D_2O exchangeable), 7.83 (d, 1H, J = 7.8 Hz, H-4, benzimidazole), 7.73 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.61–7.39 (m, 4H, phenyl), 7.27 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 4.63 (s, H, SH), 3.04 (t, 2H, J = 6.9 Hz, CH_2), 2.61 (t, 2H, J = 7.2 Hz, CH_2CO); ^{13}C NMR ($CDCl_3$): 172.60 (C=O), 169.71 (C-thiadiazole), 158.37, 157.52 (C-triazole), 155.43 (C=N), 136.73, 133.53, 130.23, 129.85, 126.57, 125.81, 124.35, 123.71, 122.13, 118.42, 111.60, 110.87 (Ar–C), 36.23 (CH_2, CH_2CO), 23.45 (CH_2); ESI MS (m/z): 406 (M^+); Anal. calcd. for $C_{19}H_{14}N_6OS_2$: C, 56.14; H, 3.47; N, 20.67. Found: C, 56.17; H, 3.53; N, 20.85.

*6.2.6.10. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-(prop-1-enyl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl)propan-1-one (**5j**).* Yield: 64%; m.p. 210–211 °C; R_f = 0.62 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3321 (NH), 3109 (=CH), 3004 (CH), 2908 (CH_2), 2877 (CH_3), 1658 (C=O), 1631 (C=N), 1566 (C=C), 1384 (N=C–S), 1145 (N–N=C); ^{1}H NMR ($CDCl_3$): 12.63 (s, 1H, NH, D_2O exchangeable), 8.11 (d, 1H, J = 7.5 Hz, H-4, benzimidazole), 7.61 (t, 1H, J = 7.2 Hz, H-7, benzimidazole), 7.48 (t, 2H, J = 7.2 Hz, H-5,6, benzimidazole), 4.54 (d, 1H, J = 8.7 Hz, CH), 4.17 (m, 1H, $CHCH_3$), 3.31 (t, 2H, J = 6.6 Hz, CH_2), 2.90 (t, 2H, J = 6.9 Hz, CH_2CO), 1.26 (d, 3H, J = 6.9 Hz, CH_3); ^{13}C NMR ($CDCl_3$):174.15 (C=O), 170.53 (C-thiadiazole), 160.71, 158.43 (C-triazole), 154.71 (C=N), 135.46, 132.67 (CH=CH), 130.21, 129.75, 125.17, 124.83, 120.37, 119.71 (Ar–C), 32.75 (CH_2, CH_2CO), 25.31 (CH_2), 14.67 (CH_3); ESI MS (m/z): 338 (M^+); Anal. calcd. for $C_{16}H_{14}N_6OS$: C, 56.79; H, 4.17; N, 24.84. Found: C, 56.81; H, 4.31; N, 24.73.

*6.2.6.11. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-(2-hydroxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl)propan-1-one (**5k**).* Yield: 66%; m.p. 198 °C; R_f = 0.62 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3391 (OH), 3317 (NH), 3025 (CH), 2913 (CH_2), 1690 (C=O), 1636 (C=N), 1585 (C=C), 1380 (N=C–S), 1236 (N–N=C); ^{1}H NMR (DMSO-d_6): 12.53 (s, 1H, NH, D_2O exchangeable), 7.84 (d, 1H, J = 7.8 Hz, H-4, benzimidazole), 7.63 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.54 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 7.45–7.23 (m, 4H, phenyl), 6.52 (s, 1H, OH), 3.21 (t, 2H, J = 6.9 Hz, CH_2), 2.87 (t, 2H, J = 6.9 Hz, CH_2CO); ^{13}C NMR (DMSO-d_6): 170.87 (C=O), 170.10 (C-thiadiazole), 163.51, 157.85 (C-triazole), 155.23 (C=N), 138.53, 133.56, 130.63, 129.41, 127.45, 124.81, 124.15, 120.35, 118.71, 116.31, 115.67, 113.75 (Ar–C), 37.27 (CH_2, CH_2CO), 24.10 (CH_2); ESI MS (m/z): 390 (M^+); Anal. calcd. for $C_{19}H_{14}N_6O_2S$: C, 58.45; H, 3.61; N, 21.53. Found: C, 59.12; H, 3.67; N, 21.47.

*6.2.6.12. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-(5-amino-2-hydroxyphenyl)-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazol-3-yl)propan-1-one (**5l**).* Yield: 70%; m.p. 215 °C; R_f = 0.49 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3495 (OH), 3371 (NH), 3071 (CH), 2890 (CH_2), 1711 (C=O), 1651 (C=N), 1582 (C=C), 1378 (N=C–S), 1280 (N–N=C); ^{1}H NMR ($CDCl_3$): 12.01 (s, 1H, NH, D_2O exchangeable), 8.04 (d, 1H, J = 7.5 Hz, H-4, benzimidazole), 7.81 (t, 1H, J = 7.8 Hz, H-7, benzimidazole), 7.54 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 7.42 (s, 1H, H-6-phenyl), 7.34 (d, 1H, J = 7.8 Hz, H-3-phenyl), 7.22 (d, 1H, J = 7.5 Hz, H-4, phenyl), 6.83 (s, 1H, OH), 6.29 (s, 2H, NH_2), 3.17 (t, 2H, J = 6.9 Hz, CH_2), 2.94 (t, 2H, J = 6.9 Hz, CH_2CO); ^{13}C NMR ($CDCl_3$): 172.56 (C=O), 169.43 (C-thiadiazole), 162.71, 156.93 (C-triazole), 154.75 (C=N), 139.50, 137.35, 131.26, 129.31, 128.57, 123.62, 123.51, 122.48, 120.73, 118.35, 116.63, 115.17 (Ar–C), 36.21 (CH_2, CH_2CO), 23.63 (CH_2); ESI MS (m/z): 405 (M^+); Anal. calcd. for $C_{19}H_{15}N_7O_2S$: C, 56.29; H, 3.73; N, 24.18. Found: C, 57.15; H, 3.77; N, 24.27.

*6.2.6.13. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-(mercaptomethyl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl)propan-1-one (**5m**).* Yield: 63%; m.p. 204–205 °C; R_f = 0.55 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3321 (NH), 3097 (CH), 2896 (CH_2), 2545 (SH), 1724 (C=O), 1612 (C=N), 1504 (C=C), 1384 (N=C–S), 1253 (N–N=C); ^{1}H NMR ($CDCl_3$): 12.05 (s, 1H, NH, D_2O exchangeable), 7.78 (d, 1H, J = 7.8 Hz, H-4, benzimidazole), 7.51 (t, 1H, J = 7.8 Hz, H-7, benzimidazole), 7.33 (t, 2H, J = 8.4 Hz, H-5,6, benzimidazole), 3.72 (s, 1H, CH_2SH), 3.32 (t, 2H, J = 6.9 Hz, CH_2), 2.92 (t, 2H, J = 7.2 Hz, CH_2CO), 1.73 (s, 1H, SH); ^{13}C NMR ($CDCl_3$): 172.60 (C=O), 170.14 (C-thiadiazole), 158.19, 157.07 (C-triazole), 155.42 (C=N), 131.64, 130.78, 129.84, 128.85, 124.18, 116.70 (Ar–C), 51.80 (CH_2SH), 30.30 (CH_2, CH_2CO), 28.06 (CH_2); ESI MS (m/z): 344 (M^+); Anal. calcd. for $C_{14}H_{12}N_6OS_2$: C, 48.82; H, 3.51; N, 24.40. Found: C, 48.93; H, 3.54; N, 24.87.

*6.2.6.14. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-(4-methoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)propan-1-one (**5n**).* Yield: 67%; m.p. 199 °C; R_f = 0.57 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3370 (NH), 3065 (CH), 2931 (CH_2), 1705 (C=O), 1654 (C=N), 1573 (C=C), 1382 (N=C–S), 1281 (N–N=C); ^{1}H NMR (DMSO-d_6): 12.13 (s, 1H, NH, D_2O exchangeable), 8.11 (d, 1H, J = 7.8 Hz, H-4, benzimidazole), 7.61 (t, 1H, J = 7.2 Hz, H-7, benzimidazole), 7.50 (t, 2H, J = 7.2 Hz, H-5,6, benzimidazole), 7.40–7.26 (m, 4H, phenyl), 3.71 (s, 3H, CH_3), 3.29 (t, 2H, J = 6.9 Hz, CH_2), 2.90 (t, 2H, J = 6.9 Hz, CH_2CO); ^{13}C NMR (DMSO-d_6): 175.32 (C=O), 164.23 (C-thiadiazole), 162.42, 157.53 (C-triazole), 155.13 (C=N), 133.62, 130.75, 130.45, 128.57, 127.83, 124.13, 122.34, 120.65, 119.37, 115.27, 113.41, 112.19 (Ar–C), 61.31 (OCH_3), 34.17 (CH_2, CH_2CO), 23.83 (CH_2). ESI MS (m/z): 404 (M^+); Anal. calcd. for $C_{20}H_{16}N_6O_2S$: C, 59.39; H, 3.99; N, 20.78. Found: C, 59.43; H, 3.87; N, 20.91.

*6.2.6.15. 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 (**5o**).* Yield: 72%; m.p. 207–208 °C; R_f = 0.56 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3483 (OH), 3321 (NH), 3027 (CH), 2890 (CH_2), 1728 (C=O), 1671 (C=N), 1620 (C=C), 1370 (N=C–S), 1283 (N–N=C); ^{1}H NMR (DMSO-d_6): 12.32 (s, 1H, NH, D_2O exchangeable), 7.81 (d, 1H, J = 7.8 Hz, H-4, benzimidazole), 7.63 (t, 1H, J = 7.8 Hz, H-7, benzimidazole), 7.54 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 7.31–6.92 (m, 4H, phenyl), 6.23 (s, 1H, OH), 3.32 (t, 2H, J = 7.2 Hz, CH_2), 2.94 (t, 2H, J = 7.2 Hz, CH_2CO); ^{13}C NMR (DMSO-d_6): 172.15 (C=O), 168.43 (C-thiadiazole), 163.52, 157.75 (C-triazole), 153.87 (C=N), 141.65, 135.73, 130.48, 128.25, 127.62, 123.17, 122.54, 120.19, 119.38, 113.27, 112.43, 111.15 (Ar–C), 38.05 (CH_2, CH_2CO), 26.73 (CH_2); ESI MS (m/z): 390 (M^+); Anal. calcd. for $C_{19}H_{14}N_6O_2S$: C, 58.45; H, 3.61; N, 21.53. Found: C, 58.48; H, 3.75; N, 21.63.

*6.2.6.16. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-(chloromethyl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl)propan-1-one (**5p**).* Yield: 68%; m.p. 209 °C; R_f = 0.44 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3301 (NH), 3012 (CH), 2904 (CH_2), 1728 (C=O), 1670 (C=N), 1608 (C=C), 1384 (N=C–S), 1180 (N–N=C), 759 (CCl); ^{1}H NMR ($CDCl_3$): 11.94 (s, 1H, NH, D_2O exchangeable), 7.81 (d, 1H, J = 8.4 Hz, H-4, benzimidazole), 7.49 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.32 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 3.72 (s, 2H, CH_2Cl), 3.34 (t, 2H, J = 7.2 Hz, CH_2), 2.92 (t, 2H, J = 6.6 Hz, CH_2CO); ^{13}C NMR ($CDCl_3$): 173.61 (C=O), 173.14 (C-thiadiazole), 159.19, 159.07 (C-triazole), 156.42 (C=N), 132.64, 130.84, 129.88, 128.87, 124.16, 115.70 (Ar–C), 60.52 (CH_2, CH_2Cl), 30.30 (CH_2, CH_2CO), 28.02 (CH_2); ESI MS (m/z): 346 (M^+); Anal. calcd. for $C_{14}H_{11}ClN_6OS$: C, 48.49; H, 3.20; N, 24.23. Found: C, 48.63; H, 3.35; N, 24.26.

*6.2.6.17. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-(3-aminopropyl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl)propan-1-one (**5q**).* Yield: 65%;

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m.p. 221–222 °C; R_f = 0.55 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3398 (NH), 2974 (CH), 2850 (CH_2), 1728 (C=O), 1653 (C=N), 1508 (C=C), 1338 (N=C–S), 1145 (N–N=C); ^{1}H NMR (DMSO-d_6):12.77 (s, 1H, NH, D_2O exchangeable), 7.79 (d, 1H, J = 7.8 Hz, H-4, benzimidazole), 7.28 (t, 1H, J = 7.8 Hz, H-7, benzimidazole), 7.07 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 6.46 (s, 2H, NH_2), 3.32 (t, 2H, J = 7.2 Hz, CH_2), 3.01 (t, 2H, J = 6.9 Hz, $CH_2\gamma$, $CH_2\gamma CH_2CH_2NH_2$), 2.87 (t, 2H, J = 7.2 Hz, CH_2CO), 2.74 (t, 2H, J = 6.9 Hz, $CH_{2\alpha}NH_2$), 2.30 (m, 2H, $CH_{2\beta}$, $CH_{2\beta}CH_2NH_2$); ^{13}C NMR (DMSO-d_6): 176.25 (C=O), 170.53 (C-thiadiazole), 164.46, 158.31 (C-triazole), 155.17 (C=N), 130.45, 129.52, 128.63, 122.73, 121.68, 117.21 (Ar–C), 50.13, 42.37, 27.56 ($CH_2CH_2CH_2NH_2$), 33.21 (CH_2, CH_2CO), 24.15 (CH_2); ESI MS (m/z): 355 (M^+); Anal. calcd. for $C_{16}H_{17}N_7OS$: C, 54.07; H, 4.82; N, 27.59. Found: C, 54.13; H, 4.87; N, 27.68.

*6.2.7. Synthesis of 1-(1H-benzo[d]imidazol-2-yl)-3-(6-mercapto-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl)propan-1-one (**5r**)*

A mixture of compound **5** (0.003 mol) and KOH/CS_2 (0.003 mol) in ethanol (15 mL) was refluxed for 4 h. After completion of the reaction, excess solvent was removed under reduced pressure and then cold water (20 mL) added into reaction media with stirring. A solid separated out which was filtered off, washed with water, dried and recrystallized from methanol. Yield: 63%; m.p. 232 °C; R_f = 0.61 [benzene:acetone (9:1)]; IR (KBr, cm^{-1}): 3398 (NH), 3097 (CH), 2835 (CH_2), 2545 (SH), 1724 (C=O), 1612 (C=N), 1504 (C=C), 1334 (N=C–S), 1176 (N–N=C); ^{1}H NMR ($CDCl_3$): 12.12 (s, 1H, NH + SH, D_2O exchangeable), 7.80 (d, 1H, J = 8.1 Hz, H-4, benzimidazole), 7.51 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.35 (t, 2H, J = 7.8 Hz, H-5,6, benzimidazole), 3.34 (t, 2H, J = 7.2 Hz, CH_2), 2.95 (t, 2H, J = 7.2 Hz, CH_2CO); ^{13}C NMR ($CDCl_3$): 174.21 (C=O), 171.16 (C-thiadiazole), 163.61, 157.32 (C-triazole), 155.03 (C=N), 131.23, 129.58, 128.47, 120.37, 119.71, 115.13 (Ar–C), 31.64 (CH_2, CH_2CO), 22.57 (CH_2); ESI MS (m/z): 330 (M^+); Anal. calcd. for $C_{13}H_{10}N_6OS_2$: C, 47.26; H, 3.05; N, 25.44. Found: C, 48.31; H, 3.17; N, 25.51.

*6.2.8. Synthesis of 1-(1H-benzo[d]imidazol-2-yl)-3-(6-(4-chlorophenylthio)-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazol-3-yl)propan-1-one (**5s**)*

Compound **5** (0.003 mol) and *p*-chlorophenyl isothiocyanate (0.003 mol) were completely dissolved in dimethylformamide and then refluxed for 10 h. After completion of the reaction, the reaction mixture was cooled to room temperature and then gradually poured on to crushed ice with stirring. A solid product separated out which was filtered, washed thoroughly with water, dried and crystallized from ethanol. Yield: 60%; m.p. 223–224 °C; R_f = 0.46 [benzene:acetone (9:1)]; IR (KBr, cm^{-1}): 3310 (NH), 3073 (CH), 2907 (CH_2), 1711 (C=O), 1671 (C=N), 1543 (C=C), 1384 (N=C–S), 1171 (N–N=C), 751 (CCl); ^{1}H NMR ($CDCl_3$): 11.95 (s, 1H, NH, benzimidazole, D_2O exchangeable), 7.73 (d, 1H, J = 7.8 Hz, H-4, benzimidazole), 7.57 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.48 (t, 2H, J = 8.1 Hz, H-5,6, benzimidazole), 7.39–7.12 (m, 4H, phenyl), 5.18 (s, 1H, NH, D_2O exchangeable), 3.27 (t, 2H, J = 7.2 Hz, CH_2), 2.81 (t, 2H, J = 6.9 Hz, CH_2CO); ^{13}C NMR ($CDCl_3$): 173.15 (C=O), 168.37 (C-thiadiazole), 165.25, 158.36 (C-triazole), 153.87 (C=N), 133.21, 132.51, 130.41, 129.92, 128.27, 124.71, 123.67, 120.56, 118.71, 115.32, 112.43, 111.64 (Ar–C), 36.47 (CH_2, CH_2CO), 23.51 (CH_2); ESI MS (m/z): 423 (M^+); Anal. calcd. for $C_{19}H_{14}ClN_7OS$: C, 53.84; H, 3.33; N, 23.13. Found: C, 53.89; H, 4.65; N, 23.27.

*6.2.9. General method 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 (**5t**–**5w**)*

A solution of compound **5** (0.003 mol) and different types of α-chloro containing carbonyl methyl compound (equimolar; 0.003 mol) in absolute ethanol (15 mL) was reflux for 3–4 h. After completion of the reaction, the reaction solution was cooled to room temperature and then neutralized with ammonia solution. A solid product obtained which was filtered, washed with water, dried and recrystallized from dichloromethane.

*6.2.9.1. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-amino-7H-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazin-3-yl)propan-1-one (**5t**).* Yield: 62%; m.p. 238 °C; R_f = 0.57 [benzene:acetone (9:1)]; IR (KBr, cm^{-1}): 3372 (NH), 3061 (CH), 2913 (CH_2), 1690 (C=O), 1651 (C=N), 1583 (C=C), 1383 (N=C–S), 1287 (N–N=C); ^{1}H NMR ($CDCl_3$): 12.25 (s, 1H, NH, D_2O exchangeable), 7.36 (d, 1H, J = 7.5 Hz, H-4, benzimidazole), 7.18 (d, 1H, J = 7.2 Hz, H-7, benzimidazole), 7.02 (t, 2H, J = 8.7 Hz, H-5,6, benzimidazole), 6.91 (s, 2H, NH_2), 3.78 (s, 2H, CH_2, cyclic), 2.91 (t, 2H, J = 7.2 Hz, CH_2), 2.27 (t, 2H, J = 6.9 Hz, CH_2CO); ^{13}C NMR ($CDCl_3$): 171.37 (C=O), 160.13, 157.74 (C-triazole), 156.27, 155.32 (C=N), 131.53, 130.15, 127.21, 126.77, 119.61, 118.37 (Ar–C), 50.32 (CH_2, cyclic), 32.73 (CH_2, CH_2CO), 25.47 (CH_2); ESI MS (m/z): 327 (M^+); Anal. calcd. for $C_{14}H_{13}N_7OS$: C, 51.36; H, 4.00; N, 29.95. Found: C, 51.37; H, 4.10; N, 29.99.

*6.2.9.2. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-hydroxy-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)propan-1-one (**5u**).* Yield: 66%; m.p. 215 °C; R_f = 0.53 [benzene:acetone (9:1)]; IR (KBr, cm^{-1}): 3475 (OH), 3367 (NH), 3058 (CH), 2904 (CH_2), 1677 (C=O), 1600 (C=N), 1504 (C=C), 1384 (N=C–S), 1180 (N–N=C); ^{1}H NMR ($CDCl_3$): 12.10 (s, 1H, NH, D_2O exchangeable), 7.71 (d, 1H, J = 8.1 Hz, H-4, benzimidazole), 7.38 (t, 1H, J = 8.1 Hz, H-7, benzimidazole), 7.27 (dd, 2H, J = 6.3 Hz, J = 7.2 Hz, H-5,6, benzimidazole), 3.69 (s, 2H, CH_2, cyclic), 3.20 (t, 2H, J = 7.2 Hz, CH_2), 2.84 (t, 2H, J = 6.9 Hz, CH_2CO), 2.59 (s, 1H, OH); ^{13}C NMR ($CDCl_3$): 173.53 (C=O), 161.25, 158.75 (C-triazole), 155.31, 154.85 (C=N), 130.41, 129.57, 124.32, 123.83, 120.13, 119.85 (Ar–C), 47.83 (CH_2, cyclic), 33.82 (CH_2, CH_2CO), 24.81 (CH_2); ESI MS (m/z): 328 (M^+); Anal. calcd. for $C_{14}H_{12}N_6O_2S$: C, 51.21; H, 3.68; N, 25.59. Found: C, 51.45; H, 3.71; N, 26.82.

*6.2.9.3. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-ethoxy-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)propan-1-one (**5v**).* Yield: 61%; m.p. 226–227 °C; R_f = 0.55 [benzene:acetone (9:1)]; IR (KBr, cm^{-1}): 3321 (NH), 3070 (CH), 2974 (CH_2), 1728 (C=O), 1643 (C=N), 1573 (C=C), 1330 (N=C–S), 1218 (N–N=C), 1149 (C–O–C, asymmetric), 1064 (C–O–C, symmetric); ^{1}H NMR ($CDCl_3$): 11.94 (s, 1H, NH, D_2O exchangeable), 7.78 (d, 1H, J = 8.4 Hz, H-4, benzimidazole), 7.46 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.32 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 4.14 (q, 2H, J = 7.2 Hz, OCH_2), 3.72 (s, 2H, CH_2, cyclic), 3.29 (t, 2H, J = 6.9 Hz, CH_2), 2.92 (t, 2H, J = 6.6 Hz, CH_2CO), 1.27 (t, 3H, J = 7.2 Hz, CH_3); ^{13}C NMR ($CDCl_3$): 175.61 (C=O), 161.25, 157.54 (C-triazole), 156.71, 156.37 (C=N), 131.23, 129.81, 123.43, 122.76, 122.30, 120.51 (Ar–C), 50.17 (CH_2, cyclic), 42.51 (OCH_2), 35.25 (CH_2, CH_2CO), 23.42 (CH_2), 18.32 (CH_3); ESI MS (m/z): 356 (M^+); Anal. calcd. for $C_{16}H_{16}N_6O_2S$: C, 53.92; H, 4.52; N, 23.58. Found: C, 53.97; H, 4.55; N, 23.73.

*6.2.9.4. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-chloro-7H-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazin-3-yl)propan-1-one (**5w**).* Yield: 63%, m.p. 228–229 °C; R_f = 0.47 [benzene:acetone (9:1)]; IR (KBr, cm^{-1}): 3336 (NH), 3012 (CH), 2904 (CH_2), 1666 (C=O), 1608 (C=N), 1569 (C=C), 1342 (N=C–S), 1145 (N–N=C), 759 (CCl); ^{1}H NMR ($CDCl_3$): 12.34 (s, 1H, NH, D_2O exchangeable), 7.68 (d, 1H, J = 7.5 Hz, H-4, benzimidazole), 7.50 (t, 1H, J = 7.2 Hz, H-7, benzimidazole), 7.27 (t, 2H, J = 8.7 Hz, H-5,6, benzimidazole), 3.60 (s, 2H, CH_2, cyclic), 3.05 (t, 2H, J = 6.9 Hz, CH_2), 2.79 (t, 2H, J = 6.9 Hz, CH_2CO); ^{13}C NMR ($CDCl_3$): 173.60 (C=O), 159.73, 159.13 (C-triazole), 156.25, 155.23 (C=N), 132.62, 130.81, 129.88, 128.90, 124.16, 115.60 (Ar–C), 51.79 (CH_2, cyclic), 30.01 (CH_2, CH_2CO), 28.03 (CH_2); ESI MS (m/z): 346 (M^+); Anal. calcd. for $C_{14}H_{11}ClN_6OS$: C, 48.49; H, 3.20; N, 24.23. Found: C, 48.51; H, 3.27; N, 25.87.

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*6.2.10. General method 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 (**5x-5a[1]**)*

A solution of compound **5** (0.003 M) and different types of α-chloro containing carbonyl compounds (0.003 M) in absolute ethanol was allowed to reflux for 3–4 h. After completion the reaction, cooled to room temperature and neutralized with ammonia solution. The solid precipitate so obtained was filtered, washed with water and recrystallized from dichloromethane.

*6.2.10.1. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-methyl-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazol-3-yl) propan-1-one (**5x**).* Yield: 66%; m.p. 190–191 °C; R_f = 0.52 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3344 (NH), 3008 (CH), 2904 (CH_2), 2854 (CH_3), 1670 (C═O), 1612 (C═N), 1566 (C═C), 1385 (N═C–S), 1180 (N–N═C); ^{1}H NMR ($CDCl_3$): 11.91 (s, 1H, NH, D_2O exchangeable), 7.78 (d, 1H, J = 8.1 Hz, H-4, benzimidazole), 7.46 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.34 (t, 2H, J = 7.2 Hz, H-5,6, benzimidazole), 3.29 (t, 2H, J = 6.9 Hz, CH_2), 2.92 (t, 2H, J = 6.9 Hz, CH_2CO), 1.82 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): 172.01 (C═O), 158.23, 156.71 (C-triazole), 155.03 (C═N), 148.10 (C-thiadiazole), 132.72, 132.63, 128.53, 128.01, 123.57, 115.63 (Ar–C), 33.27 (CH_2, CH_2CO), 24.05 (CH_2), 14.83 (CH_3); ESI MS (m/z): 312 (M^+); Anal. calcd. for $C_{14}H_{12}N_6OS$: C, 53.83; H, 3.87; N, 26.91. Found: C, 53.95; H, 3.82; N, 25.97.

*6.2.10.2. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-(3-chloropropyl)-[1,2,4] triazolo[3,4-b][1,3,4] thiadiazol-3-yl)propan-1-one (**5y**).* Yield: 62%; m.p. 234–235 °C; R_f = 0.46 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3325 (NH), 3040 (CH), 2932 (CH_2), 1710 (C═O), 1657 (C═N), 1520 (C═C), 1380 (N═C–S), 1160 (N–N═C), 750 (CCl); ^{1}H NMR (DMSO-d_6): 12.67 (s, 1H, NH, D_2O exchangeable), 7.70 (d, 1H, J = 7.8 Hz, H-4, benzimidazole), 7.51 (t, 1H, J = 7.8 Hz, H-7, benzimidazole), 7.38 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 3.74 (t, 2H, J = 6.9 Hz, CH_2Cl), 3.23 (t, 2H, J = 7.2 Hz, CH_2), 2.90 (t, 2H, J = 7.2 Hz, CH_2CO), 2.51 (t, 2H, J = 7.2 Hz, CH_2, $CH_2CH_2CH_2Cl$), 1.73 (m, 2H, CH_2, CH_2CH_2Cl); ^{13}C NMR (DMSO-d_6): 173.31 (C═O), 160.26, 157.21 (C-triazole), 156.81 (C-thiadiazole), 155.13 (C═N), 130.61, 130.01, 129.53, 128.96, 122.51, 118.13 (Ar–C), 47.32, 27.15, 26.42 ($CH_2CH_2CH_2Cl$), 30.83 (CH_2, CH_2CO), 23.37 (CH_2); ESI MS (m/z): 374 (M^+); Anal. calcd. for $C_{16}H_{15}ClN_6OS$: C, 51.27; H, 4.03; N, 22.42. Found: C, 51.43; H, 4.12; N, 22.45.

*6.2.10.3. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-(2-chloroethyl)-[1,2,4] triazolo[3,4-b][1,3,4] thiadiazole-3-yl)propan-1-one (**5z**).* Yield: 58%; m.p. 217–218 °C; R_f = 0.41 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3312 (NH), 3020 (CH), 2936 (CH_2), 1704 (C═O), 1645 (C═N), 1563 (C═C), 1383 (N═C–S), 1174 (N–N═C), 770 (CCl); ^{1}H NMR (DMSO-d_6): 12.13 (s, 1H, NH, D_2O exchangeable), 7.80 (d, 1H, J = 7.8 Hz, H-4, benzimidazole), 7.63 (t, 1H, J = 7.8 Hz, H-7, benzimidazole), 7.44 (t, 2H, J = 7.8 Hz, H-5,6, benzimidazole), 3.75 (t, 2H, J = 6.9 Hz, CH_2Cl), 3.26 (t, 2H, J = 6.6 Hz, CH_2), 2.84 (t, 2H, J = 6.9 Hz, CH_2CO), 2.54 (t, 2H, J = 7.2 Hz, CH_2CH_2Cl); ^{13}C NMR (DMSO-d_6): 172.67 (C═O), 161.32, 158.41 (C-triazole), 157.92 (C-thiadiazole), 155.01 (C═N), 131.52, 130.23, 128.94, 128.12, 120.75, 119.38 (Ar–C), 50.43, 36.23 (CH_2CH_2Cl), 33.27 (CH_2, CH_2CO), 24.15 (CH_2); ESI MS (m/z): 360 (M^+); Anal. calcd. for $C_{15}H_{13}ClN_6OS$: C, 49.93; H, 3.63; N, 23.29. Found: C, 49.97; H, 3.75; N, 23.37.

*6.2.10.4. 1-(1H-Benzo[d]imidazol-2-yl)-3-(6-ethoxy-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazol-3-yl) propan-1-one (**5a[1]**).* Yield: 61%; m.p. 208–209 °C; R_f = 0.47 [toluene:ethyl acetate:formic acid (5:4:1)]; IR (KBr, cm^{-1}): 3327 (NH), 3010 (CH), 2950 (CH_2), 1713 (C═O), 1657 (C═N), 1547 (C═C), 1387 (N═C–S), 1168 (N–N═C); ^{1}H NMR (DMSO-d_6): 12.06 (s, 1H, NH, D_2O exchangeable), 7.81 (d, 1H, J = 8.1 Hz, H-4, benzimidazole), 7.51 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.32 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 3.97 (q, 2H, J = 7.2 Hz, OCH_2), 3.32 (t, 2H, J = 7.2 Hz, CH_2), 2.81 (t, 2H, J = 6.9 Hz, CH_2CO), 1.36 (t, 3H, J = 6.9 Hz, CH_3); ^{13}C NMR (DMSO-d_6): 173.61 (C═O), 165.27, 163.45 (C-triazole), 160.72 (C-thiadiazole), 156.17 (C═N), 131.35, 130.17, 127.97, 126.87, 120.72, 115.64 (Ar–C), 55.63 (OCH_2), 35.41 (CH_2, CH_2CO), 25.61 (CH_2), 16.23 (CH_3); ESI MS (m/z): 360 (M^+); Anal. calcd. for $C_{15}H_{14}N_6O_2S$: C, 52.62; H, 4.12; N, 24.55. Found: C, 52.72; H, 4.26; N, 24.85.

6.3. Anticancer screening

The human tumor cell lines were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. After cell inoculation, the microtitre plates incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of tested compounds. After 24 h, two plates of each cell line fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of sample addition (Tz). The sample solubilized in DMSO at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of compounds addition, an aliquot of frozen concentrate was thawed and diluted to twice, the desired final maximum test concentration with complete medium containing 50 μg/mL gentamicin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 μL of these different sample dilutions added to the appropriate microtitre wells already containing 100 μL of medium, resulting in the required final sample concentrations. The tested compounds addition, the plates was incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. Cells fixed *in situ* by the gentle addition of 50 μL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant discarded and plates washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid added to each well and plates incubated for 10 min at RT. Bound stain subsequently solubilized with 10 mM trizma base and the absorbance was read on an automated plate reader at a wavelength of 515 nm. Using the seven absorbance measurements [time zero, (Tz), control growth (C) and test growth in the presence of sample at the five concentration levels (Ti)]. Percentage growth inhibition calculated as:

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

Three dose response parameters (GI_{50}, TGI and LC_{50}) were calculated for each experimental agent. Growth inhibition of 50% (GI_{50}) calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$ (which was the drug concentration resulting in a 50% reduction in the net protein increase), Total growth inhibition (TGI) calculated from Ti = Tz (concentration at which the total growth inhibition is 100%) and LC_{50}calculated from $[(Ti - Tz)/Tz] \times 100 = -50$ (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of the cells. Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested [39–42]. The logs molar concentration also calculated of individual GI_{50}, TGI and $LC_{50}$and represented as $\log_{10}$ GI_{50}, $\log_{10}$ TGI and $\log_{10}$ LC_{50} respectively. The lowest values of response parameter were obtained with the most sensitive cell lines.

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