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Benzimidazole bearing oxadiazole and triazolo-thiadiazoles nucleus: Design and synthesis as anticancer agents

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ABSTRACT

Two new series of benzimidazole bearing oxadiazole[1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(5-substituted-1,3,4-oxadiazol-2-yl)propan-1-ones (**4a–l**)] and triazolo-thiadiazoles[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 (**7a–e**)] have been synthesized successfully from4-(1*H*-benzo[*d*]imidazol-2-yl)-4-oxobutanehydrazide (**3**) with an aim to produce promising anticancer agents. In vitro anticancer activities of synthesized compounds were screened at the National Cancer Institute (NCI), USA, according to their applied protocol against full NCI 60 human cell lines panel; results showed good to remarkable anticancer activity. Among them, compound (**4j**, NCS: 761980) exhibited significant growth inhibition and further screened at 10-fold dilutions of five different concentrations (0.01, 0.1, 1, 10 and 100 μM) with GI_{50} values ranging from 0.49 to 48.0 μM and found superior for the non-small cell lung cancer cell lines like HOP-92 (GI_{50} 0.49, TGI 19.9,LC_{50} >100 and $Log_{10}GI_{50}$ −6.30, $Log_{10}TGI$ −4.70, $Log_{10}LC_{50}$ >−4.00).

The searching of a new agent for the treatment of cancer is an important tool of medicinal chemistry. It is a disease of cell cycle, in which abnormal cells divide mitosis without control and being one of the major health problems in the world from decades.[1,2] A lot of chemical classes of heterocyclic and fused heterocyclic compounds have been identified through molecular biology, empirical screening and rational drug development for evaluation of anticancer agents during the past decades. In terms of searching, it could be considered that the benzimidazole heterocyclics are great importance in their biological as well as synthetic approach of medicinal chemistry. From worldwide reported literature, the various substituted derivatives of benzimidazole nucleus showed remarkable biological activity as antitumor/antiproliferative/anticanceractivity,[3–12] antimicrobial including anti-HIV,[13–16] antioxidant[17] and cysticidal activities.[18] Similarly, oxadiazole and triazolo-thiadiazoles are a class of heterocyclic's, which have attracted significant interest in medicinal chemistry as they have a wide range of pharmaceutical and biological activities including antitumor and antimicrobial activities.[19–25]Here light on some rationally designed targeted compounds with biologically active antitumor agents having benzimidazole and other heterocyclic moiety, for example Treanda (bendamustine hydrochloride), made up by Cephalon Inc. USA and comprises by mechlorethamine group and benzimidazole heterocyclic ring with a butyric acid substituent, chemical, 1*H*-benzimidazole-2-butanoicacid-5-[bis(2-chloroethyl)amino]-1methyl- monohydrochloride, a rationally designed purine analog and alkylation hybrid of chlorambucil chemotherapeutic drug shown in Figure 1. 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).[26–28] The chemically drawn of newly prepared targeted compound as rationally designed with bendamustine along with chlorambucil, represented in Figure 1.

In view of these points, it was thought worthwhile to prepare a new type of hybrid that clubbed both of benzimidazole as well as oxadiazole/(1,2,4)triazolo(3,4-*b*)(1,3,4)thiadiazole ring systems with a view to produce promising anticancer agents. Therefore, several hybrids were synthesized and screened for their in vitro anticancer activities at Development Therapeutic Program, National Cancer Institute (NCI), Chemotherapeutic Research division, USA, against full NCI 60 cell line panel according their applied protocol. The selected compounds were submitted to NCI and granted NCS codes shown in Table 1.

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 4 N HCl. 4-(1*H*-benzo[*d*]imidazol-2-yl)-4-oxobutanehydrazide (**3**) was obtained by treating the compound (**1**) with hydrazine hydrate through an ethyl–ester intermediate (**2**). The compound **3** was treated with CS_2/KOH using ethanol as

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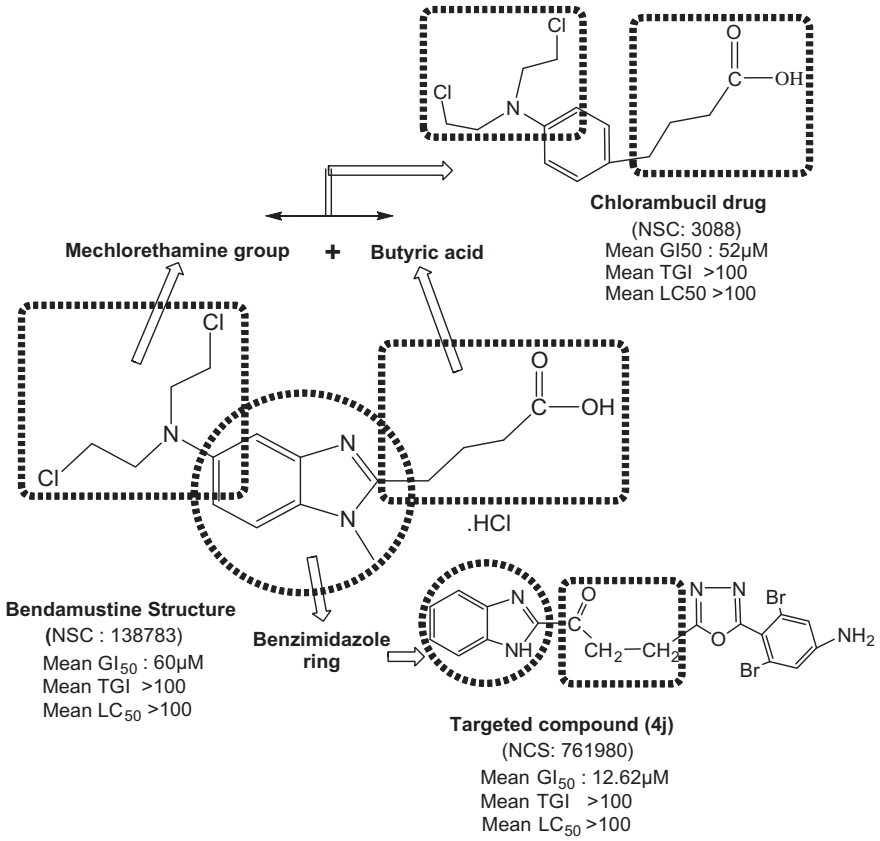


Figure 1. Structure of biologically active agent of benzimidazoleheterocyclic'sas anticancer and rationally designed template for targeted compound.

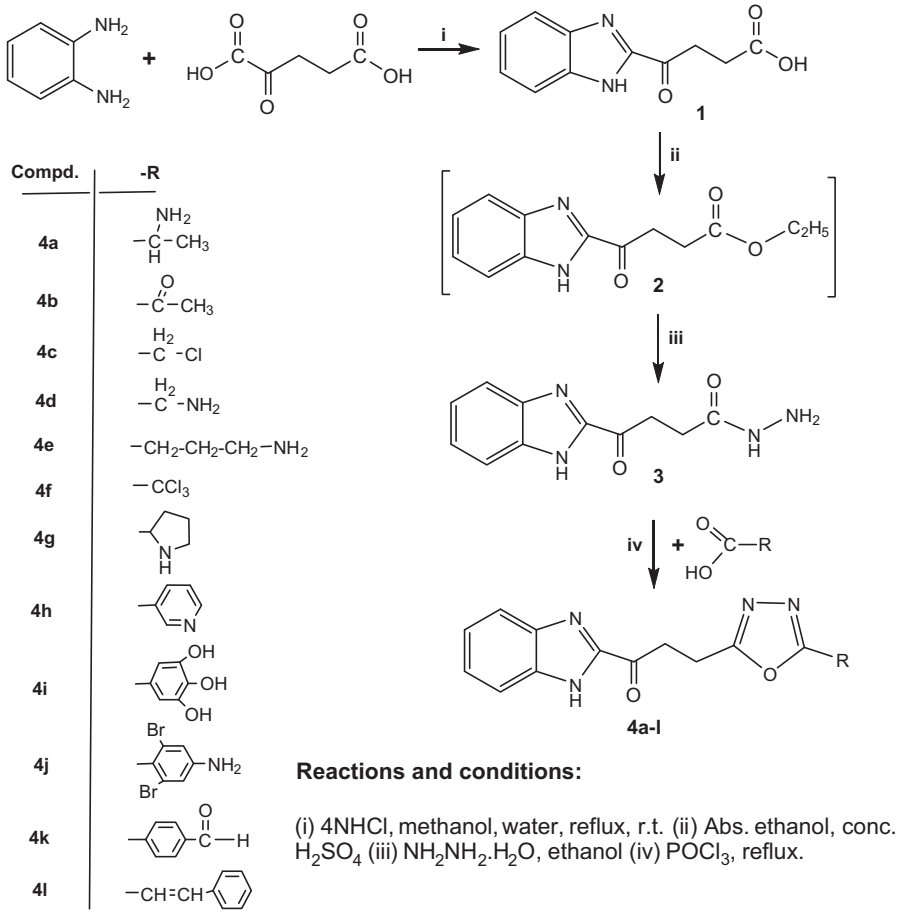
Table 1
NSC: code, sensitivity, growth percent, mean growth percent of NCI cancer cell lines treated with synthesized compounds (10 μM)

Compd.	NSC: Code	The most sensitive cell line	Growth % of the most sensitive cell line	Range of growth, (%)	Mean	Range	Activity
4i	761979/1	UO-31 (Renal cancer)	62.25	62.25–125.99	97.16	63.74	Active
4j	761980/1	MDA-MB-435 (Melanoma)	5.71	5.71–104.44	70.36	98.73	Active
4l	761981/1	K-562 (Leukemia)	24.57	24.57–116.54	87.22	91.97	Active
7c	761984/1	K-562 (Leukemia)	8.30	8.30–116.26	96.71	110.46	Active
7d	761985/1	K-562 (Leukemia)	7.05	7.05–124.02	95.24	116.97	Active
7e	761986/1	K-562 (Leukemia)	2.17	2.17–118.42	85.93	116.25	Active

The tested compounds, which showed growth inhibition ⩽32% is called as active for that particular cell lines. Percent cell growth reduction following 48 h incubation with test compounds (used sulphorhodamine B procedure).

solvent to get 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(5-mercapto-1,3,4-oxadiazol-2-yl)propan-1-one (**5**) and 3-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)-1-(1*H*-benzo[*d*]imidazol-2-yl)propan-1-one (**6**) was obtained by reacting hydrazine hydrate with compound (**4**). After that different types of aromatic/aliphatic acids were reacted with compound **3** and **5** in presence of $POCl_3$ to produce newly 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(5-substituted-1,3,4-oxadiazol-2-yl)propan-1-ones (**4a-l**) (Scheme 1) 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 (**7a–e**), respectively (Scheme 2). The purity of compounds was checked by single-spot TLC using T/E/F (toluene/ethyl-acetate/formic acid, 5:4:1) and benzene/acetone (9:1) as solvent systems and spots located under iodine vapors/UV light and sharp melting point. The final products were purified by recrystallization with suitable solvent. The structures assigned to all the synthesized compounds were supported by the results of elemental analysis as well as IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data and found in full agreement with the proposed structures.

Melting points were determined in open capillary tubes and are uncorrected. Thin-layer chromatography (TLC) was carried out to monitor the progress of the reactions using silica gel G (Merck No. 5554) as stationary phase and solvent systems—toluene/ethyl acetate/formic acid (5:4:1) and benzene/acetone (9:1). The spots were located by exposure to iodine vapors or under UV-light. IR spectra were measured as potassium bromide pellets using Perkin–Elmer 1725X spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Brukerspectropsin DPX-300 MHz in $CDCl_3$/DMSO-d_6 using tetramethylsilane as internal reference; chemical shift (δ) values are reported in parts per million (ppm). The splitting pattern abbreviations are as follows: s, singlet; bs, broad singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet. Mass spectra were recorded on LCMS/MS (Perkin–Elmer and LABINDIA, Applied Biosystem) model no. API 3000 presented as *m/z*. Elemental analyses were performed on Perkin–Elmer 240 analyzer and was in range of ±0.4% for each element analyzed (C, Hand N). Dry solvents were used throughout.

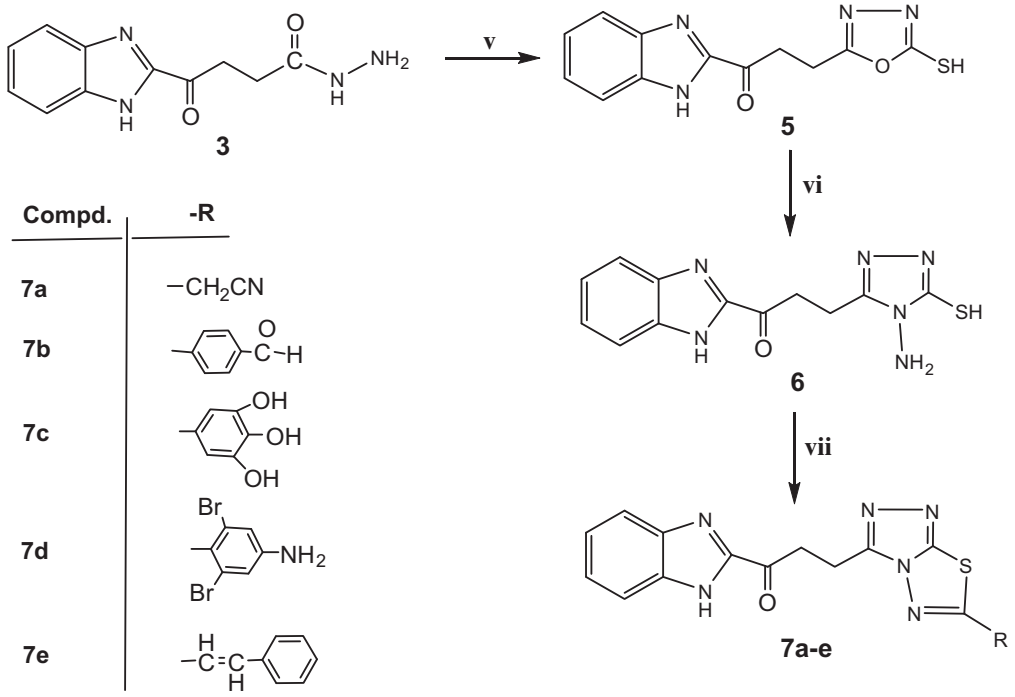


Scheme 1. Synthetic pathway for compounds (**4a–l**).

The newly compounds were prepared as outlined in Schemes 1 and 2. In general, IR spectra of the compounds showed peaks at 3341 and 2599 cm^{-1} for NH and SH, respectively. In ^1H NMR spectra, there appeared a singlet at around δ 12.1 indicative of ring H–N and another singlet at δ13.4 for S–H, both disappeared by addition of D_2O as confirmation for these groups. The appeared peak in IR spectra at around 1716 and 1648 cm^{-1} accounted for C=O and C=N. The chemical shift in ^{13}C NMR spectra at δ 174.3 and 155.8 could be accounted for C=O and C=N. The characteristic peaks at around 1384 and 1263 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 ^1H NMR spectra at around δ 3.3 (J = 7.2 Hz) and δ 2.8 (J = 6.9 Hz) and ^{13}C NMR spectra showed at around δ 30.3 and 28.1 which could be accounted for two methylene groups (–CH_2-CH_2–) forming a linker chain through which benzimidazole nuclei attached with oxadiazole/triazole/thiadiazole ring, made up the complete back bone of synthetic compounds. The presence of doublet, triplet at around δ 7.7, 7.4 and 7.2 (7.8, 7.5, 7.5 Hz) indicated benzimidazole hydrogen. The signals in ^{13}C NMR spectra which appeared at around δ 172.8 could be for thiadiazole carbon ring and other signals at δ 160.7, 159.8 indicative of triazole carbon ring. Other peaks were observed at appropriate δ values supporting the structure. The mass spectra (ESI MS) showed the 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. The elemental analysis results were within ±0.4% deviation from the theoretical values.

All the selected compounds (14 in no.) were submitted to National Cancer Institute (NCI), USA for evaluating their in vitro anticancer activity at single dose (10 μM) 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 (10 μM) and the culture incubated for 48 h. End point determinations made with a protein binding dye, sulforhodamine B.[31] Results for each compound were reported as mean graph of the percent growth of the treated cells when compared with untreated control cells.[32,33] The compounds which reduced the growth of the cell lines to 32% or less (negative number indicate kills) is considered in vitro activity shown in Table 1.[34] Compound (**4j**, NCS: 761980)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 versus % growth inhibition curves on nine cancer disease. 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 over all cell lines.

The title compound (**4j**, NCS: 761980) under investigation exhibited remarkable anticancer activity against all the tested cell



Scheme 2. Synthetic pathway for compounds (**7a-e**).

lines representing nine different subpanels with GI_{50} values between 0.49 and 48.0 μM except HCT-15 (GI_{50} >100) under sensitive range an outstanding activity (Table 2). With regard to the sensitivity against some individual cell lines the compound showed high activity against Non-Small Cell Lung Cancer cell lines like HOP-92 (GI_{50} 0.49, TGI 19.9, LC_{50} >100 and $Log_{10}GI_{50}$ −6.30, $Log_{10}TGI$ −4.70, $Log_{10}LC_{50}$ >−4.00). Obtained data revealed an obvious sensitivity profile towards Ovarian Cancer subpanel (GI_{50} value ranging from 2.60 to 29.4 μM), least for OVCAR-3and maximum for OVCAR-5 cell line. The compound proved to be sensitive towards all the tested Leukemia cancer cell lines with not more than 4.94 μM concentrations. All the tested Prostate cancer cell lines were sensitive with not more than GI_{50} 5.40 μM concentrations of the tested compound. The highest growth inhibitory activity was observed against the HOP-92 cell line of Non-Small Cell Lung Cancer with GI_{50} value 0.49 μM and minimum growth inhibitory activity against ACHN cell line of Renal Cancer with GI_{50} value 48.0 μM. The all remaining subpanel cell line showed maximum sensitive towards tested compound with not more than 36.5 μM concentrations (Table 2). Over all the GI_{50} values of the screening process resulted in a sensitive range inferior of 48.0 μM denoting an outstanding activity and the values of LC_{50} are in most of the cell lines >100 μM except COLO 205 (80.1 μM) and MDA-MB-435 (8.83 μM) cell line (Table 2).The log molar concentration of the resulted screening as log GI_{50} ranged from −6.30 to −4.32 except HCT-15(>−4.00), minimum concentration for HOP-92 cell line (−6.30) of Non-Small Cell Lung Cancer and maximum for ACHN cell line (−4.32 μM) of Renal Cancer, log TGI value for most cell lines showed more than >−4.00 and mostly cell lines showed log LC_{50} >−4.00 except COLO 205 (−4.10 μM) and MDA-MB-435 (−5.05 μM) cell line. A mean graph midpoint (MG-MID) calculated for each of parameters, giving as log GI_{50} (−5.13), log TGI (−4.23) and log LC_{50} (−4.02) and insensitive cell lines are also included with the highest concentration (Table 3). The criterion for selectivity of a compound depends upon a ratio called as selective index, which is 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.[35] As per this criterion, compound **4j**, in the study was found to be moderate selective towards Leukemia cancer subpanel with selective index 3.23.

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 micro titer 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 micro titer wells already containing 100 μL of medium, resulting in the required final sample concentrations.[31] The tested compounds

Table 2
Calculated values of GI_{50}, TGI, LC_{50} of the cell lines, NCI: full cell lines panel, MG-MID and selectivity index of the compound (**4j**, NCS: 761980/1)

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	3.81				>100	>100
	HL-60(TB)	3.44	23.49	3.91	3.23	>100	>100
	K-562	2.89				>100	>100
	MOLT-4	4.94				>100	>100
	RPMI-8226	4.21				>100	>100
	SR	4.20				>100	>100
Non-small cell lung cancer	A549/ATCC	27.6				>100	>100
	EKVX	23.1				>100	>100
	HOP-62	23.7	115.799	12.86	0.98	>100	>100
	HOP-92	0.499				19.9	>100
	NCI-H226	22.6				>100	>100
	NCI-H23	3.26				36.4	>100
	NCI-H322M	8.15				>100	>100
	NCI-H460	3.99				20.1	>100
	NCI-H522	2.90				16.9	>100
Colon cancer	COLO 205	11.8				30.8	80.1
	HCC-2998	11.0				42.5	>100
	HCT-116	8.26		22.46	0.56	>100	>100
	HCT-15	>100	157.25			>100	>100
	HT29	19.2				83.5	>100
	KM12	3.71				18.0	>100
	SW-620	3.28				>100	>100
CNS cancer	SF-268	5.14				81.6	>100
	SF-295	9.78				35.6	>100
	SF-539	17.3		9.79	1.29	60.8	>100
	SNB-19	20.1	58.78			>100	>100
	SNB-75	2.96				20.5	>100
	U251	3.50				>100	>100
Melanoma	LOX IMVI	3.25				46.4	>100
	MALME-3M	2.06				17.2	>100
	M14	17.5		6.90	1.83	55.5	>100
	MDA-MB-435	1.56	62.17			3.71	8.83
	SK-MEL-2	4.30				>100	>100
	SK-MEL-28	6.30				79.0	>100
	SK-MEL-5	14.2				>100	>100
	UACC-257	7.63				>100	>100
	UACC-62	5.37				>100	>100
Ovarian cancer	IGROV1	5.87				62.7	>100
	OVCAR-3	2.60				6.98	>100
	OVCAR-4	5.49		15.69	0.80	78.2	>100
	OVCAR-5	29.4	109.86			>100	>100
	OVCAR-8	21.6				>100	>100
	NCI/ADR-RES	22.6				>100	>100
	SK-OV-3	22.3				80.5	>100
Renal cancer	786-0	21.0				61.7	>100
	A498	0.662				10.5	>100
	ACHN	48.0				>100	>100
	CAKI-1	19.8		21.56	0.58	>100	>100
	RXF 393	13.0	172.522			58.6	>100
	SN12C	29.5				>100	>100
	TK-10	36.5				>100	>100
	UO-31	4.06				>100	>100
Prostate cancer	PC-3	2.90	8.3	4.15	3.04	>100	>100
	DU-145	5.40				53.5	>100
Breast cancer	MCF7	3.06				>100	>100
	MDA-MB-231/ ATCC	10.1				86.8	>100
	HS 578T	3.75	49.06	8.17	1.54	31.7	>100
	BT-549	6.82				>100	>100
	T-47D	23.4				>100	>100
	MDA-MB-468	1.93				6.97	>100
MID[a]	60	12.62	757.231				

MID = mean graph midpoint, arithmetical mean value of treated cancer cell lines. Full panel (MID[a])—the average sensitivity of all cell lines towards the test agent in μM, Subpanel (MID[b])—the average sensitivity of all cell lines of a particular subpanel towards the test agent in μM.

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 (100μL) 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

Table 3
Values of the log molar concentration of response parameter ($Log_{10}GI_{50}$, $Log_{10}TG$ and $Log_{10}LC_{50}$) of test compound (**4j,** NCS: 761980/1)

Cancer disease	Used cell lines	Log_{10} GI_{50}	Log_{10} TGI	Log_{10} LC_{50}
Leukemia	CCRF-CEM	−5.42	>−4.00	>−4.00
	HL-60 (TB)	−5.46	>−4.00	>−4.00
	K-562	−5.54	>−4.00	>−4.00
	MOLT-4	−5.31	>−4.00	>−4.00
	RPMI-8226	−5.38	>−4.00	>−4.00
	SR	−5.38	>−4.00	>−4.00
Non-small cell lung cancer	A549/ATCC	−4.56	>−4.00	>−4.00
	EKVX	−4.64	>−4.00	>−4.00
	HOP-62	−4.63	>−4.00	>−4.00
	HOP-92	−6.30	−4.70	>−4.00
	NCI-H226	−4.65	>−4.00	>−4.00
	NCI-H23	−5.49	−4.44	>−4.00
	NCI-H322M	−5.09	>−4.00	>−4.00
	NCI-H460	−5.40	−4.70	>−4.00
	NCI-H522	−5.54	−4.77	>−4.00
Colon cancer	COLO 205	−4.93	−4.51	−4.10
	HCC-2998	−4.96	−4.37	>−4.00
	HCT-116	−5.08	>−4.00	>−4.00
	HCT-15	>−4.00	>−4.00	>−4.00
	HT29	−4.72	−4.08	>−4.00
	KM12	−5.43	−4.74	>−4.00
	SW-620	−5.48	>−4.00	>−4.00
CNS cancer	SF-268	−5.29	−4.09	>−4.00
	SF-295	−5.01	−4.45	>−4.00
	SF-539	−4.76	−4.22	>−4.00
	SNB-19	−4.70	>−4.00	>−4.00
	SNB-75	−5.53	−4.69	>−4.00
	U251	−5.46	v4.00	>−4.00
Melanoma	LOX IMVI	−5.49	−4.33	>−4.00
	MALME-3M	−5.69	−4.76	>−4.00
	M14	−4.76	−4.26	>−4.00
	MDA-MB-435	−5.81	−5.43	-5.05
	SK-MEL-2	−5.37	>−4.00	>−4.00
	SK-MEL-28	−5.20	−4.10	>−4.00
	SK-MEL-5	−4.85	>−4.00	>−4.00
	UACC-257	−5.12	>−4.00	>−4.00
	UACC-62	−5.27	>−4.00	>−4.00
Ovarian cancer	IGROV1	−5.23	−4.20	>−4.00
	OVCAR-3	−5.58	−5.16	>−4.00
	OVCAR-4	−5.26	−4.11	>−4.00
	OVCAR-5	−4.53	>−4.00	>−4.00
	OVCAR-8	−4.67	>−4.00	>−4.00
	NCI/ADR-RES	−4.65	>−4.00	>−4.00
	SK-OV-3	−4.65	−4.09	>−4.00
Renal cancer	786-0	−4.68	-4.21	>−4.00
	A498	−6.18	−4.98	>−4.00
	ACHN	−4.32	>−4.00	>−4.00
	CAKI-1	−4.70	>−4.00	>−4.00
	RXF 393	−4.89	−4.23	>−4.00
	SN12C	−4.53	>−4.00	>−4.00
	TK-10	−4.44	>−4.00	>−4.00
	UO-31	−5.39	>−4.00	>−4.00
Prostate cancer	PC-3	−5.51	>−4.00	>−4.00
	DU-145	−5.27	−4.27	>−4.00
Breast cancer	MCF7	−5.51	>−>−4.00	>−4.00
	MDA-MB-231/ATCC	−4.99	−4.06	>−4.00
	HS 578T	−5.43	−4.50	>−4.00
	BT-549	−5.17	>−4.00	>−4.00
	T-47D	−4.63	>−4.00	>−4.00
	MDA-MB-468	−5.71	−5.16	>−4.00
MID		−5.13	−4.23	−4.02

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:

$$[(Ti - Tz)/(C - Tz)] \times 100 \text{for concentrations for which} Ti > / = Tz$$

$$[(Ti - Tz)/Tz] \times 100 \text{for concentrations for which} Ti < Tz$$

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.[31,32] 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.

On the basis of structure activity relationships, it could be concluded that benzimidazole bearing oxadiazole ring were found to have better anticancer activity than those of benzimidazole bearing triazolo-thiadiazole nucleus. The anticancer activity was influenced by the presence of electron withdrawing group like bromo on *ortho*, *meta* or *para* position of aromatic ring. As obtained result, the compound (**4j**) substituted by bromo group on *ortho* position (2,6-dibromo) of phenyl ring, chemically as 3-(5-(4-amino-2,6-dibromophenyl)-1,3,4-oxadiazol-2-yl)-1-(1*H*-benzo[*d*]imidazol-2-yl)propan-1-one increases the sensitivity of cell line (70.36%) and similarly group substituted on *ortho* position (2,6-dibromo)of compound (**7d**),chemically as3-(6-(4-amino-2,6-dibromophenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-1-(1*H*-benzo[*d*] imid azol-2-yl)propan-1-one decrease the sensitivity of cell line (95.24%). On the other hand the electron releasing group like hydroxyl group attached with phenyl ring decrease the sensitivity (96.71%) and (97.16%) as exhibited by compound (**7c**), 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(6-(3,4,5-trihydroxyphenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)propan-1-one and compound (**4i**),1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(5-(3,4,5-trihydroxyphenyl)-1,3,4-oxadiazol-2-yl)propan-1-one, respectively. Finally, unsubstitution phenyl rings observed in compounds **4l** and **7e** showed the sensitivity of cell line (87.22%) and (85.93%), respectively.

Two series of benzimidazole bearing [1,3,4]oxadiazole and [1,2,4]triazolo[3,4-*b*] [1,3,4] thiadiazole moieties comprising of 20 new compounds were successfully synthesized and among them 14 compounds were selected and evaluated for their in vitro anticancer screening at the NCI, USA. Compounds showed good to remarkable and broad-spectrum anticancer activity. The compound (**4j**), namely 3-(5-(4-amino-2,6-dibromophenyl)-1,3,4-oxadiazol-2-yl)-1-(1*H*-benzo[*d*]imidazol-2-yl)propan-1-one emerged as lead compound with broad spectrum of anticancer activities on tumor cell lines (MG-MID 12.62 of GI_{50} and −5.13, −4.23, −4.02 value of $\text{Log}_{10}GI_{50,}$ $\text{Log}_{10}TGI$, $\text{Log}_{10}LC_{50}$, respectively). Based on these observations, it could be subject of further investigations for searching potential antitumor agents. Finally it's conceivable that further derivatization of such compounds can be serve as novel templates for anticancer chemotherapy and could be possibly lead to more active molecules in the field of cancer chemotherapy.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.07.038.

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