European Journal of Medicinal Chemistry 54 (2012) 855–866

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis of benzimidazoles bearing oxadiazole nucleus as anticancer agents

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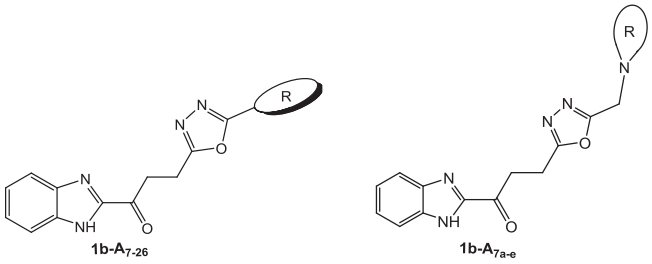
HIGHLIGHTS

- 25 Novel benzimidazoles bearing oxadiazole nucleus were synthesized under microwave irradiation.
- Initial screening of compounds showed significant to good *in vitro* anticancer activity.
- One compound, **1b**–**A_{18}**, emerged as lead compound and screened at five dose level.
- The compound **1b**–**A_{18}** can be used as a template for designing potential anticancer agents.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 November 2011
Received in revised form
15 April 2012
Accepted 18 April 2012
Available online 9 May 2012

Keywords:
Benzimidazole
1,3,4-oxadiazole
Microwave-assisted
Anticancer activity

ABSTRACT

Starting from 4-(1*H*-benzo[*d*]imidazol-2-yl)-4-oxobutanehydrazide (**1**), twenty new 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(1,3,4-oxadiazol-5-substituted derivatives-2-yl)propan-1-ones (**1b**–**A_{7-26}**) were synthesized under microwave irradiation in good yields. Further, compound **1b**–**A_7** was reacted with different secondary amines under microwave irradiation to produce five novel 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(5-(methyl substituted)-1,3,4-oxadiazol-2-yl)propan-1-ones (**1b**–**A_{7a-e}**). The title compounds were screened for their *in vitro* anticancer activity at National Cancer Institute (NCI), USA; at a single dose (10 μM) in NCI 60 cell line panel and results showed significant to good anticancer activity. One compound, **1b**–**A_{18}** (NSC: 759205), 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-(5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl)propan-1-one, emerged as lead compound; it was selected for five-dose level screening and found to have significant growth inhibition activity.

1. Introduction

Cancer is a collection of different life threatening diseases characterized by uncontrolled growth of cells, leading to invasion of surrounding tissue and often spreading to other parts of the body. When it comes to understanding and controlling cancer, scientists are now working from a position of strength – a sturdy foundation of knowledge about cancer built over the past 50 years. There is an urgent need for novel effective drug regimens for the treatment of cancer because the current chemotherapy suffers from a slim therapeutic index, with significant toxicity from effective drug doses or tumor recurrence at low drug doses. Searching of new anticancer agents having heterocyclic nucleus continues worldwide at various laboratories [1–3].

Scientists from worldwide have reported remarkable antitumor/antiproliferative/anticancer [4–13], anti-inflammatory [12], antiviral including anti-HIV [13,14], antibacterial [15–17], antifungal [18,19] and antioxidant [19,20] activities of different benzimidazole derivatives. Similarly, 1,3,4-oxadiazole is a class of heterocyclic compound that have attracted significant interest in medicinal chemistry as they have a wide range of pharmaceutical and pharmacological applications including potential antitumor, antiproliferative or anticancer activities [21–26]. Benzimidazole clubbed with other heterocyclic moieties including the oxadiazole

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doi:10.1016/j.ejmech.2012.04.027

nucleus resulted in compounds with improved biological activities including anticancer activity [10–13,27]. Motivated by the these observations, it was thought worthwhile to prepare new hybrid compounds that comprise both benzimidazole and 1,3,4-oxadiazole ring systems with a view to obtaining promising anticancer agents.

Therefore, several benzimidazoles having 1,3,4-oxadiazole moieties were synthesized and screened for their *in vitro* anticancer activities at National Cancer Institute (NCI), USA. The selected compounds were submitted to NCI and granted NCS codes as NCS: 755135 **(1b–A_7)**, NCS: 755139 **(1b–A_{10})**, 759200 **(Ib–A_{11})**, NCS: 759201 **(Ib–A_{12})**, NCS: 759202 **(Ib–A_{13})**, NCS: 759203 **(Ib–A_{14})**, NCS: 759204 **(Ib–A_{15})**, NCS: 759205 **(Ib–A_{18})**, NCS: 759206 **(Ib–A_{23})**, NCS: 759207 **(Ib–A_{25})**, NCS: 759208 **(Ib–A_{26})**, NCS: 759209 **(Ib–A_{7a})**, NCS: 759210 **(Ib–A_{7b})**, NCS: 759211 **(Ib–A_{7c})**, NCS: 760445 **(Ib–A_{7d})** and NCS: 759212 **(Ib–A_{7e})** for screening their anticancer activity against full NCI 60 cell line panel at single dose (10 μM), among these, the compound **Ib–A_{18}** (NCS: 759205) exhibited significant growth inhibition at a single dose and further selected for anticancer screening at five dose concentration level against full NCI 60 cell panel.

2. Chemistry

The starting material, 4-(1*H*-benzo[*d*]imidazol-2-yl)-4-oxobutanoic acid **(1)**, was synthesized by reacting *o*-phenylene diamine with α-ketoglutaric acid in presence of HCl. Compound (**1**) was treated with hydrazine hydrate through ester intermediate to furnish 4-(1*H*-benzo[*d*]imidazol-2-yl)-4-oxobutanehydrazide **(Ib)**. Different types of acids were reacted with compound **(Ib)** under microwave irradiation to get 1-(1*H*-benzo[*d*]imidazol-2-yl) -3-(1,3,4-oxadiazol-5-substituted-2-yl)propan-1-ones **(Ib–$A_{7–26}$)** (Fig. 1). The purity of compounds was checked by single-spot TLC using toluene: ethyl acetate: formic acid (5:4:1) and benzene: acetone (9:1) solvent systems and spots located under iodine vapors/UV light. The final products were purified by flash chromatography column (hexane/ethyl acetate gradient, 8:2, gradient varied compound to compound). The compound **Ib–A_7** was further reacted with different types of secondary amines in presence of sodium acetate under microwave irradiation to furnish new 1-(1*H*-benzo[*d*] imidazol-2-yl)-3-(5-(methyl substituted)-1,3,4-oxadiazol-2-yl) propan-1-ones **(Ib–$A_{7a–e}$)** (Fig. 2). Spectral data FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectra of the synthesized compounds were recorded and found to be in full agreement with the proposed structures. The elemental analysis results were found to be within ±0.4% of the theoretical values. The substituent (R), irradiation time, NCS code no., yield, melting point, molecular formula and molecular weight of the newly synthesized compounds have been provided in Table 1.

3. Pharmacological evaluation

3.1. In vitro anticancer assay [1,28–31]

The selected compounds among the newly synthesized compounds were submitted for *in vitro* anticancer assay at National Cancer Institute (NCI), USA against full NCI 60 cell lines panel representing full nine human systems as leukemia, melanoma and cancers of lung, colon, brain, breast, ovary, kidney and prostate. Initially, structures of the synthesized compounds are submitted to the NCI. The structures are generally selected for screening based on their ability to add diversity to the NCI small molecule compound collection. The structures with novel heterocyclic ring systems are particularly given attention. The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10 μM. The output from the single dose screen was reported as a mean graph and available for analysis by the COMPARE program. The compounds which exhibit significant growth inhibition at a single dose of 10 μM are evaluated against the 60 cell panel at five concentration levels.

4. Results and discussion

4.1. Chemistry

Overall twenty-eight compounds were prepared as outlined in Figs. 1, 2. The structures assigned to the compounds were supported by the results of elemental analysis as well as IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data results. In general, IR value showed peaks at around 3424 (N–H, NH_2), 3350 (N–H, NH), 3020(C–H, Ar–H, aromatic hydrogen), 2950, 2860 (C–H, CH_2), 1720 (C=O), 1674 (C=N), 1562, 1504, 1485 (C=C, ring stretch), 1308 (N–N=C), 1164 (C–O–C, asymmetric), 1028 (C–O–C, symmetric), 834 (C–N, aliphatic stretch). ^{1}H NMR spectra peak of the respective protons of the synthesized compounds verified on the basis of their chemical shifts (δ), multiplicities, and coupling constants (*J*). All the compounds showed two triplets at around δ 2.8 and 3.3 which could be accounted for two methylene groups (–CH_2–CH_2–), doublet, triplet at around δ 7.6, 7.5 and 7.3 indicative of benzimidazole hydrogen and a singlet at around δ 11.9 indicative of ring H–N (D_2O exchangeable). Other peaks were observed at appropriate δ values supporting the structure. ^{13}C NMR spectra peaks at δ 175.5 for carbonyl group, δ 162.7, 159.6 indicative of oxadiazole carbon, δ 154.96 for C=N and δ 138.27, 132.21, 130.19, 128.57, 124.65, 123.64 indicative of Ar–C (aromatic carbon), δ 38.7, 27.4 accounted for two methylene groups (–CH_2–CH_2–). The mass spectra (ESI-MS) showed the presence of peak at definite *m/z* value in accordance to the molecular formula. In case of aryl groups having chloro-substituent(s), the molecular ion peak appeared as cluster of peaks. The elemental analysis results were found to be within ±0.4% deviation from the theoretical values.

4.2. Pharmacological assay (in vitro anticancer activity)

4.2.1. Primary at a single dose (10 μM) full NCI 60 cell panel

All the selected compounds (16 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 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. Results for each compound were reported as a mean graph of the percent growth of the treated cells and obtained result mentioned in Table 2.There after obtaining the results for one dose assay, analysis of historical Developmental Therapeutics Program (DTP) was performed and compound **Ib–A_{18}** (NCS: 759205) satisfied pre-determined threshold inhibition criteria and selected for NCI full panel five dose assay.

4.2.2. In vitro 5 dose full NCI 60 cell panel assay

In the second step, the selected compounds are evaluated against all the 60 cell lines, representing nine tumor subpanels, at 10-fold dilutions of five different concentrations (0.01, 0.1, 1, 10 and 100 μM). The result are given in three calculated response parameters (GI_{50}, TGI and LC_{50}) for each cell line from log concentration vs % growth inhibition curves. 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.

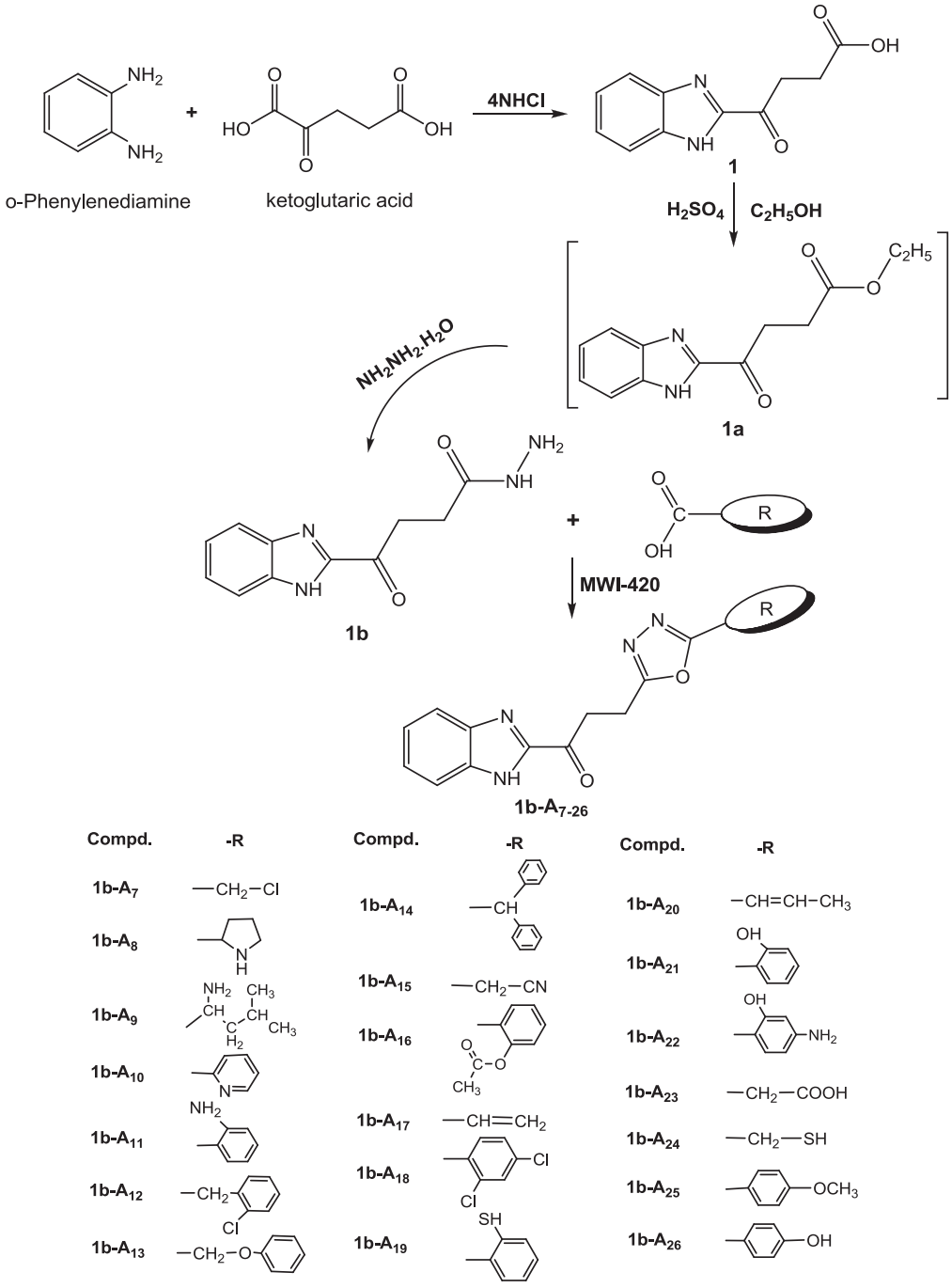


Fig. 1. Protocol for synthesis of title compounds **1b**–$\mathbf{A_{7–26}}$.

The compound **Ib**–$\mathbf{A_{18}}$ (NCS: 759205) exhibited remarkable anticancer activity against most of the tested cell lines with GI_{50} values between 0.79 and 17.8 μM (Table 3). With regard to the sensitivity against some individual cell lines, the compound showed high activity against MDA-MB-468 (Breast Cancer), A498 (Renal Cancer) and HOP-92 (Non-Small Cell Lung Cancer) with GI_{50} 0.797, 1.53 and 1.77 and less sensitivity against SNB-75 (CNS Cancer), DU-145 (Prostate Cancer) and SNB-19 (CNS Cancer) with GI_{50} 18.7, 17.8 and 17.4 μM, respectively. Obtained data revealed an obvious sensitivity profile toward Melanoma subpanel (GI_{50} value ranging from 2.00 to 13.7 μM), least for MDA-MB-435 (GI_{50}, 2.00 μM) and maximum for UACC-257 (GI_{50}, 13.7 μM) cell line. All the tested Leukemia cancer cell lines were found to be sensitive with not more than 4.86 μM concentrations of the tested compound. The highest growth inhibitory activity was observed against the breast cancer MDA-MB-468 cancer cell line with GI_{50} less than 1 μM (0.797). The

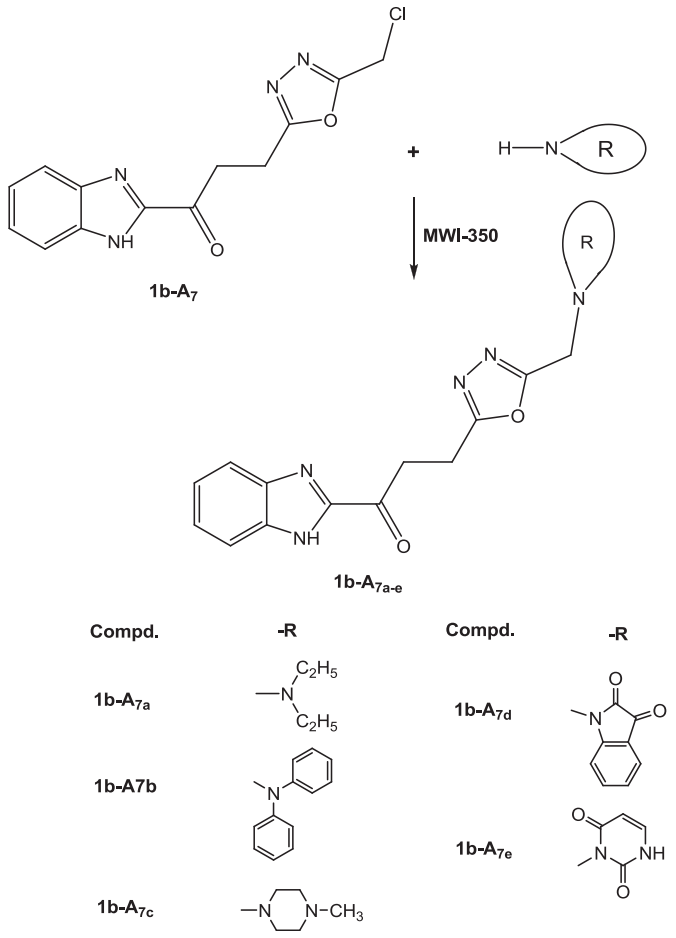


Fig. 2. Protocol for synthesis of title compounds **1b**–$\mathbf{A_{7a-e}}$.

all remaining subpanel cell line showed maximum sensitive toward tested compound with not more than 16.4 μM concentrations. The detailed results are mentioned in Table 3. 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 toward the test agent) by their individual subpanel MID[b] (the average sensitivity of all cell lines of a particular subpanel toward the test agent). Ratios between 3 and 6 refer to moderate selectivity; ratio greater than 6 indicates high selectivity toward the corresponding cell line, while compounds not meeting either of these criteria rated as non-selective. As per this criterion, compound in the study found to be mild selective toward Leukemia cancer subpanel.

An analysis of results (structure activity relationship) indicated that that electron donating groups like ($-NH_2$, 2-chloro and 2,4-dichloro) on the phenyl ring at position 5 of oxadiazole moiety have high influence on anticancer activity and groups like $-N(C_2H_5)_2$, $-O-C_6H_5$ on methyl at position 5 of the oxadiazole ring also resulted in compounds with improved anticancer activity. Electron withdrawing groups like −COOH and −C=O did not show remarkable anticancer activity. Further studies to acquire more information about quantitative structure activity relationship (QSAR) of the compounds are in progress in our laboratory.

5. Experimental protocols

5.1. Chemistry

All the reagents and solvents were obtained from S.D. Fine chemicals or E. Merck Ltd. Melting points were determined 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. Microwave irradiation of reactions was done in a scientific microwave synthesizer (model No. CATA-R, Catalyst systems, India). ^{1}H NMR and ^{13}C NMR spectra of the synthetic compounds were recorded on Bruker spectropsin DPX-300 MHz in DMSO-d_6/$CDCl_3$; chemical shift (δ) values reported in parts per million (ppm) using tetramethylsilane as internal reference. 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*. IR spectra were recorded on FT/IR (Jasco), model no.410 using KBr pellets of the compounds. Elemental analyses were performed on a Perkin–Elmer 240 analyzer and found to be in the range of ±0.4% for each element analyzed (C, H, N).

5.1.1. 4-(1H-Benzo[d]imidazol-2-yl)-4-oxobutanoic acid (**1**)

A solution of *o*-phenylene diamine (0.01 mol), α-ketoglutaric acid (equimolar; 0.01 mol) in methanol/water mixture (1:1) and HCl (4N; 5 mL) was refluxed for 6 h and then left to cool to room temperature. The NaOH solution (10% w/v) 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). 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. ESI-MS (*m/z*): 218 (M^+).

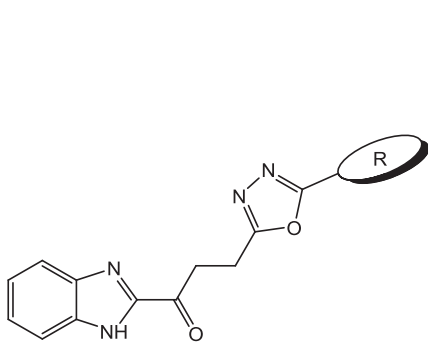
5.1.2. Ethyl- 4-(1H-benzo[d]imidazol-2-yl)-4-oxobutanoate (***1a***)

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), 20.31(CH_3). 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. ESI-MS (*m/z*): 246 (M^+).

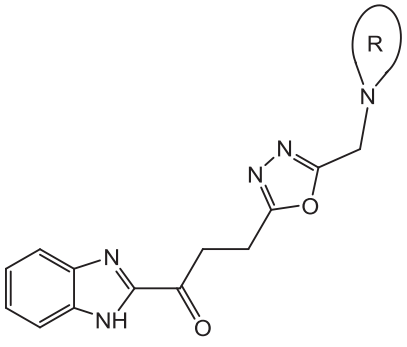
5.1.3. 4-(1H-Benzo[d]imidazol-2-yl)-4-oxobutane hydrazide (***1b***)

A solution of compound (**Ia**, 0.01 mol) and hydrazine hydrate (0.015 mol) in ethanol (25 mL) 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.

Table 1
Substituted value (−R), NCS code, yield, R_f-value, melting point, molecular formula/mol. weight and eluent mixture ratio of title compounds **(1b−A$_{7-26}$)** and **(1b−A$_{7a-e}$)**



1b-A$_{7-26}$



1b-A$_{7a-e}$

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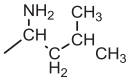
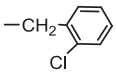
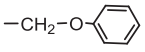
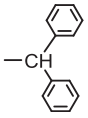
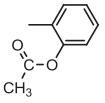
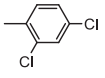
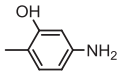
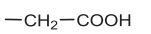
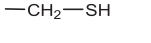
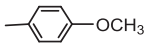
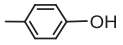
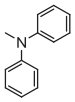
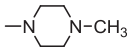
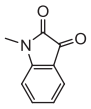
Compd. No.	−R	NCS code[a]	Yield	R_{f-} value	Melting point (°C)	Mol. Formula/Mol. weight	Eluent mixture ratio[b]
1b−A$_7$	—CH_2—Cl	755135	85	0.65	223-224	$C_{13}H_{11}ClN_4O_2$/290	9:1
1b−A$_8$		ns	72	0.61	212-213	$C_{16}H_{17}N_5O_2$/311	8:2
1b−A$_9$		ns	82	0.73	233-235	$C_{17}H_{21}N_5O_2$/327	8:2
1b−A$_{10}$		755139	85	0.56	203-204	$C_{17}H_{13}N_5O_2$/319	8:2
1b−A$_{11}$		759200	90	0.58	199-201	$C_{18}H_{15}N_5O_2$/333	8:2
1b−A$_{12}$		759201	87	0.56	240-242	$C_{19}H_{15}ClN_4O_2$/366	9:1
1b−A$_{13}$		759202	82	0.58	233-234	$C_{19}H_{16}N_4O_3$/348	8:2
1b−A$_{14}$		759203	70	0.43	246-247	$C_{25}H_{20}N_4O_2$/408	7:3
1b−A$_{15}$	—CH_2—CN	759204	80	0.64	217-218	$C_{14}H_{11}N_5O_2$/281	9:1
1b−A$_{16}$		ns	75	0.55	221-222	$C_{20}H_{16}N_4O_4$/376	9:1
1b−A$_{17}$	—CH=CH_2	ns	72	0.51	211-213	$C_{14}H_{12}N_4O_2$/268	8:2
1b−A$_{18}$		759205	94	0.53	227-228	$C_{18}H_{12}Cl_2N_4O_2$/387	9:1
1b−A$_{19}$		ns	85	0.52	217-219	$C_{18}H_{14}N_4O_2S$/350	8:2
1b−A$_{20}$	−CH=CH−CH_3	ns	83	0.60	241-242	$C_{15}H_{14}N_4O_2$/282	7:3
1b−A$_{21}$		ns	87	0.50	209-210	$C_{18}H_{14}N_4O_3$/334	8:2

(*continued on next page*)

Table 1 (*continued*)

Compd. No.	−R	NCS code[a]	Yield	R_{f-} value	Melting point (°C)	Mol. Formula/Mol. weight	Eluent mixture ratio[b]
1b−A$_{22}$		ns	92	0.62	218-219	$C_{18}H_{15}N_5O_3$/349	8:2
1b−A$_{23}$		759206	78	0.75	224-225	$C_{14}H_{12}N_4O_4$/300	9:1
1b−A$_{24}$		ns	82	0.61	213-214	$C_{13}H_{12}N_4O_2S$/288	8:2
1b−A$_{25}$		759207	92	0.67	231-232	$C_{19}H_{16}N_4O_3$/348	8:2
1b−A$_{26}$		759208	84	0.63	219-220	$C_{18}H_{14}N_4O_3$/334	8:2
1b−A$_{7a}$		759209	78	0.49	242-243	$C_{17}H_{21}N_5O_2$/327	7:3
1b−A$_{7b}$		759210	80	0.45	243-244	$C_{25}H_{21}N_5O_2$/423	7:3
1b−A$_{7c}$		759211	78	0.59	225-226	$C_{18}H_{22}N_6O_2$/354	7:3
1b−A$_{7d}$		760445	72	0.68	240-241	$C_{21}H_{15}N_5O_4$/401	8:2
1b−A$_{7e}$		759212	86	0.66	237-238	$C_{17}H_{14}N_6O_4$/366	8:2

[a] Some compounds were not selected (ns) for anticancer activity by the NCI.
[b] Hexane and ethyl acetate mixture was used as eluent mixture.

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). ^{1}H 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, 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). 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. ESI-MS (*m/z*): 232 (M^+).

5.1.4. General Procedure for synthesis of 1-(1H-Benzo[d]imidazol-2-yl)-3-(1,3,4-oxadiazol-5 -substituted-2-yl) propan-1-one (**1b–A$_{7–26}$**)

A mixture of 4-(1*H*-benzo[*d*]imidazol-2-yl)-4-oxobutanehydrazide (**1b**) (0.001 mol), an aliphatic/aromatic acid (equimolar, 0.001 mol) and $POCl_3$ (5 mL) was placed into a microwave reaction vessel equipped with a magnetic stir bar. The reaction vessel was placed into the scientific microwave synthesizer and irradiated at a power level of 6 (60%, 420 W) for 6–13 min. After completion of the reaction, checked by single-spot TLC using the solvent systems; toluene: ethyl acetate: formic acid (5:4:1) or benzene: acetone (9:1), the reaction mixture was cooled and poured slowly onto crushed ice, neutralized with sodium bicarbonate solution. A solid mass precipitated out, which was filtered and washed with excess quantity of water to remove the inorganic component. The product was purified by flash chromatography (hexane/ethyl acetate gradient, 8:2, concentration of gradient varied compound to compound) to get pure compound **(1b–A$_{7–26}$)**.

5.1.4.1. 1-(1H-Benzo[d]imidazol-2-yl)-3-(5-(chloromethyl)-1,3,4-oxadiazol-2-yl)propan-1-one (**1b–A$_7$**).

IR (KBr, cm^{-1}): 3350 (N–H, NH), 3020 (C–H, Ar–H), 2935(C–H, CH_2), 1710 (C=O), 1682 (C=N), 1574 (C=C), 1304 (N–N=C), 1164 (C–O–C asymmetric), 1028 (C–O–C, symmetric), 714 (C–Cl). ^{1}H NMR ($CDCl_3$): 12.51 (s, 1H, NH, D_2O exchangeable), 7.80 (d, 1H, J = 7.8 Hz, H-4, benzimidazole), 7.48 (t, 1H, J = 7.2 Hz, H-7, benzimidazole), 7.33(t, 2H, J = 5.4 Hz, H-5,6, benzimidazole), 3.29 (t, 2H, J = 6.9 Hz, CH_2), 2.90 (t, 2H, J = 6.6 Hz, CH_2), 2.66 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): 169.45 (C=O), 157.21, 155.36 (2C, oxadiazole), 153.67 (C=N), 135.15, 133.21, 131.13, 127.53, 125.10, 125.84 (Ar–C), 50.01 (CH_2, CH_2Cl), 38.75 (CH_2, CH_2CO), 27.41 (CH_2). Anal. calcd./Found: [C, 53.71/53.62; H, 3.81/3.83; N, 19.27/19.41]. ESI-MS (*m/z*): 290 (M^+).

5.1.4.2. 1-(1H-Benzo[d]imidazol-2-yl)-3-(5-(pyrrolidin-2-yl)-1,3,4-oxadiazol-2-yl)propan-1-one (**1b–A$_8$**).

IR (KBr, cm^{-1}): 3365 (N–H, NH), 3030 (C–H, Ar–H), 2960 (C–H, CH_2), 2824 (C–H, cyclic CH_2), 1690 (C=O), 1615 (C=N), 1542 (C=C), 1305 (N–N=C), 1130

Table 2
In vitro anticancer testing results of compound **Ib**–**A_{18}** (NCS: 759205).

Panel	Cell line	Developmental therapeutics program one dose mean graph value (10 μM)	
		Growth percent	Growth inhibition percent
Leukemia	CCRF-CEM	47.29	52.71
	HL-60(TB)	−33.81[a]	133.81
	K-562	2.22	97.78
	MOLT-4	20.71	79.29
	RPMI-8226	30.87	69.13
Non-small cell lung cancer	A549/ATCC	69.67	30.33
	EKVX	60.96	39.04
	HOP-62	71.65	28.35
	HOP-92	6.67	93.33
	NCI-H226	83.71	16.29
	NCI-H23	61.94	38.06
	NCI-H322M	75.97	24.03
	NCI-H460	77.33	22.67
	NCI-H522	49.76	50.24
Colon cancer	COLO 205	62.65	37.35
	HCC-2998	65.88	34.12
	HCT-116	22.07	77.93
	HCT-15	81.50	18.50
	HT29	9.39	90.61
	KM12	28.69	71.31
	SW-620	35.81	64.19
CNS cancer	SF-268	68.09	31.91
	SF-295	59.47	40.53
	SF-539	95.24	4.76
	SNB-19	75.09	24.91
	SNB-75	63.36	36.64
	U251	52.50	47.50
Melanoma	LOX IMVI	55.09	44.91
	MALME-3M	79.33	20.67
	M14	55.79	44.21
	MDA-MB-435	−0.53[a]	100.53
	SK-MEL-2	59.44	40.56
	SK-MEL-28	82.67	17.33
	SK-MEL-5	66.98	33.02
	UACC-257	78.62	21.38
	UACC-62	42.34	57.66
Ovarian cancer	IGROV1	57.56	42.44
	OVCAR-3	66.38	33.62
	OVCAR-4	58.75	41.25
	OVCAR-5	81.15	18.85
	OVCAR-8	57.16	42.84
	NCI/ADR-RES	63.29	36.71
	SK-OV-3	64.83	35.17
Renal cancer	786-0	73.82	26.18
	A498	58.42	41.58
	ACHN	81.30	18.70
	CAKI-1	59.83	40.17
	RXF 393	81.55	18.45
	SN12C	61.18	38.82
	TK-10	63.99	36.01
	UO-31	52.05	47.95
Prostate cancer	PC-3	53.14	46.86
	DU-145	82.16	17.84
Breast cancer	MCF7	44.72	55.28
	MDA-MB-231 /ATCC	38.32	61.68
	HS 578T	82.33	17.67
	BT-549	56.35	43.65
	T-47D	51.96	48.04
	MDA-MB-468	−2.19[a]	102.19
	Mean	**55.13**	

[a] Negative (−) indicates the cell kill.

(C—O—C, asymmetric), 1018 (C—O—C, symmetric). ^{1}H NMR ($CDCl_3$): 12.77 (s, 1H, NH, benzimidazole, D_2O exchangeable), 7.78 (d, 1H, J = 7.5 Hz, H-4, benzimidazole), 7.51 (t, 1H, J = 7.2 Hz, H-7, benzimidazole), 7.30 (t, 2H, J = 8.7 Hz, H-5,6, benzimidazole), 3.92 (s, 1H, NH pyrrolidine, D_2O exchangeable), 3.01 (t, 2H, J = 6.9 Hz, CH_2), 2.74 (t, 2H, J = 6.9 Hz, CH_2), 2.30–1.19 (m, 7H, pyrrolidine). ^{13}C NMR ($CDCl_3$): 172.61 (C═O), 161.01, 158.63 (2C oxadiazole), 155.21 (C═N), 132.17, 131.83, 131.03, 127.03, 124.71, 123.61 (Ar—C), 55.07, 53.17, 50.13, 44.51 (4C pyrrolidine), 38.07 (CH_2, CH_2CO), 30.41 (CH_2). Anal. calcd./Found: [C, 61.72/61.83; H, 5.50/5.54; N, 22.49/22.37]. ESI-MS (m/z): 311 (M^+).

*5.1.4.3. 3-(5-(1-Amino-3-methylbutyl)-1,3,4-oxadiazol-2-yl)-1-(1H-benzo[d]imidazol-2-yl) propan-1-one (**1b**–**A_9**).* IR (KBr, cm^{-1}): 3484 (N—H, NH_2), 3324 (N—H, NH), 3016 (C—H, Ar—H), 2952 (C—H, CH_2), 1712 (C═O), 1635 (C═N), 1562 (C═C), 1328 (N—N═C), 1176 (C—O—C, asymmetric), 1020 (C—O—C, symmetric). ^{1}H NMR ($CDCl_3$): 12.71 (s, 1H, NH, D_2O exchangeable), 8.37 (s, 2H, NH_2, D_2O exchangeable), 7.60 (d, 1H, J = 7.5 Hz, H-4, benzimidazole), 7.41 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.28 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 4.16 (t, 1H, J = 7.2, C—H), 3.31 (t, 2H, J = 6.9 Hz, CH_2), 2.87 (t, 2H, J = 6.9 Hz, CH_2), 1.49 (dd, 2H, J = 5.4 Hz, J = 5.7 Hz, CH_2), 1.36 (q, 1H, J = 5.4 Hz, CH), 1.05 (d, 6H, J = 1.2 Hz, 2xCH_3). ^{13}C NMR ($CDCl_3$): 177.41 (C═O), 162.13, 161.51 (2C oxadiazole), 154.97 (C═N), 131.73, 131.01, 128.31, 127.07, 125.41, 124.37 (Ar—C), 56.07 (CH, CH—NH_2), 49.17 (CH_2, CH_2CH), 36.73 (CH_2, CH_2CO), 31.19 (CH, $CHCH_3$), 27.41 (CH_2), 25.61 (CH_3, $CHCH_3$). Anal. calcd./Found: [C, 62.37/61.79; H, 6.47/6.41; N, 21.39/21.36]. ESI-MS (m/z): 327(M^+).

*5.1.4.4. 1-(1H-Benzo[d]imidazol-2-yl)-3-(5-(pyridin-2-yl)-1,3,4-oxadiazol-2-yl)propan-1-one (**1b**–**A_{10}**).* IR (KBr, cm^{-1}): 3374 (N—H, NH), 3042 (C—H, Ar—H), 2934(C—H, CH_2), 1720 (C═O), 1625 (C═N), 1560 (C═C), 1322 (N—N═C), 1152 (C—O—C, asymmetric), 988 (C—O—C, symmetric). ^{1}H NMR ($CDCl_3$): 12.57 (s, 1H, NH, D_2O exchangeable), 8.24–7.82 (m, 4H, pyridine), 7.70 (d, 1H, J = 7.5 Hz, H-4, benzimidazole), 7.45 (t, 1H, J = 7.8 Hz, H-7, benzimidazole), 7.21 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 3.17 (t, 2H, J = 7.2 Hz, CH_2), 2.69 (t, 2H, J = 7.2 Hz, CH_2). ^{13}C NMR ($CDCl_3$): 175.63 (C═O), 165.37, 163.71 (2C oxadiazole), 153.43 (C═N), 138.23, 138.03, 134.17, 133.71, 131.83 (5C pyridine), 130.97, 130.07, 128.50, 127.61, 125.47, 124.93 (Ar—C), 33.43(CH_2, CH_2CO), 26.31(CH_2). Anal. calcd./Found: [C, 63.94/63.81; H, 4.10/4.17; N, 21.93/21.95]. ESI-MS (m/z): 319 (M^+).

*5.1.4.5. 3-(5-(2-Aminophenyl)-1,3,4-oxadiazol-2-yl)-1-(1H-benzo[d] imidazol-2-yl)propan-1one (**1b**–**A_{11}**).* IR (KBr, cm^{-1}): 3550 (N—H, NH_2), 3340 (N—H, NH), 3110 (C—H, Ar—H), 2970 (C—H, CH_2), 1710 (C═O), 1660 (C═N), 1564 (C═C), 1314 (N—N═C), 1132 (C—O—C, asymmetric), 1038 (C—O—C, symmetric). ^{1}H NMR (DMSO-d_6): 12.37 (s, 1H, NH, D_2O exchangeable), 8.21 (s, 2H, NH_2), 7.74 (d, 1H, J = 8.1.Hz, H-4, benzimidazole), 7.54 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.41 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 7.32–7.02 (m, 4H, phenyl), 2.91 (t, 2H, J = 7.2 Hz, CH_2), 2.37 (t, 2H, J = 6.9 Hz, CH_2). ^{13}C NMR (DMSO-d_6): 179.13 (C═O), 164.83, 161.21 (2C oxadiazole), 155.71 (C═N), 138.01, 132.61, 131.13, 130.97, 130.11, 129.51, 128.57, 127.63, 122.47, 121.93, 119.27 (Ar—C), 40.75 (CH_2, CH_2CO), 25.81(CH_2). Anal. calcd./Found: [C, 64.86/64.78; H, 4.54/4.52; N, 21.01/21.13]. ESI-MS (m/z): 333(M^+).

*5.1.4.6. 1-(1H-Benzo[d]imidazol-2-yl)-3-(5-(2-chlorobenzyl)-1,3,4-oxadiazol-2-yl)propan-1-one (**1b**–**A_{12}**).* IR (KBr, cm^{-1}): 3326 (N—H), 3050 (C—H, Ar—H), 2890 (C—H, CH_2), 1724 (C═O), 1630 (C═N), 1506 (C═C), 1306 (N—N═C), 1150 (C—O—C, asymmetric), 1074 (C—O—C, symmetric), 720 (C—Cl). ^{1}H NMR (DMSO-d_6): 12.58 (s, 1H, NH, D_2O exchangeable), 7.81 (d, 1H, J = 8.7 Hz, H-4, benzimidazole), 7.51 (t, 1H, J = 7.2 Hz, H-7, benzimidazole), 7.48 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 7.29–7.09 (m, 4H, phenyl), 3.71 (bs, 2H, CH_2), 3.01(t, 2H, J = 6.9 Hz, CH_2), 2.71 (t, 2H, J = 6.9 Hz, CH_2). ^{13}C NMR (DMSO-d_6): 177.56 (C═O), 162.72, 159.86 (2C oxadiazole), 156.39 (C═N), 136.28, 135.59, 133.92, 129.45, 128.41, 125.74, 124.99, 123.63, 122.13, 121.65, 120.91, 118.76 (Ar—C), 35.83(CH_2, CH_2CO),

Table 3
Calculated values of GI_{50}, TGI and LC_{50} of the cell lines, NCI: full cell lines, of compound **Ib–A_{18}** (NCS: 759205).

Panel	Cell line	GI_{50}		TGI (10^{-5}M)	LC_{50} (10^{-4}M)
		Concentration per cell line (10^{-6}M)	Subpanel MID[b]		
Leukemia	CCRF-CEM	4.86	3.48	6.60	1.00
	HL-60(TB)	2.23		1.93	1.00
	MOLT-4	4.07		2.58	1.00
	RPMI-8226	3.61		2.33	1.00
	SR	2.63		1.52	1.00
Non-small cell lung cancer	A549/ATCC	10.4	5.74	10.0	1.00
	EKVX	5.48		10.0	1.00
	HOP-62	7.67		4.13	1.00
	HOP-92	1.77		1.58	1.00
	NCI-H226	6.66		3.12	1.00
	NCI-H23	3.23		6.32	1.00
	NCI-H460	4.99		2.86	1.00
Colon cancer	COLO 205	2.66	4.00	0.708	1.00
	HCC-2998	6.52		3.23	1.00
	HCT-116	3.33		1.35	0.525
	HCT-15	5.13		4.86	1.00
	HT29	3.77		5.51	1.00
	KM12	3.16		1.10	1.00
	SW-620	3.46		1.63	0.743
CNS cancer	SF-268	7.30	11.79	4.66	1.00
	SF-295	5.98		4.29	1.00
	SF-539	10.5		3.96	1.00
	SNB-19	17.4		10.0	1.00
	SNB-75	18.7		10.0	1.00
	U251	10.9		7.76	1.00
Melanoma	LOX IMVI	4.03	7.18	3.98	1.00
	MALME-3M	13.0		5.26	1.00
	M14	5.86		3.15	1.00
	MDA-MB-435	2.00		0.477	1.00
	SK-MEL-28	12.7		4.27	1.00
	SK-MEL-5	2.88		1.49	0.646
	UACC-257	13.7		4.44	1.00
	UACC-62	3.34		2.78	1.00
Ovarian cancer	IGROV1	5.26	6.28	3.91	1.00
	OVCAR-3	5.63		2.48	0.907
	OVCAR-4	5.93		10.0	1.00
	OVCAR-5	7.82		10.0	1.00
	OVCAR-8	9.26		10.0	1.00
	NCI/ADR-RES	5.47		10.0	1.00
	SK-OV-3	4.60		3.05	1.00
Renal cancer	786-0	10.7	7.65	4.55	1.00
	A498	1.53		1.01	1.00
	ACHN	11.9		10.0	1.00
	CAKI-1	3.49		4.11	1.00
	RXF 393	4.30		4.97	1.00
	SN12C	6.22		5.69	1.00
	TK-10	16.4		10.0	1.00
	UO-31	6.71		3.29	1.00
Prostate cancer	PC-3	3.71	10.75	10.0	1.00
	DU-145	17.8		10.0	1.00
Breast cancer	MCF7	4.57	4.77	10.0	1.00
	MDA-MB-231/ATCC	2.68		4.26	1.00
	HS 578T	9.71		9.39	1.00
	BT-549	7.54		9.25	1.00
	T-47D	3.34		6.00	1.00
	MDA-MB-468	0.797		1.22	1.00
MID[a]		**6.59**			

[a] Mean graph midpoint (arithmetical mean value of treated cancer cell lines) representing the average sensitivity of all cell lines (full panel) toward the test agent in μM.
[b] Average sensitivity of all cell lines of a particular subpanel toward the test agent in μM.

27.21 (CH_2, oxadiazole), 24.86 (CH_2). Anal. calcd./Found: [C, 62.21/62.23; H, 4.12/4.17; N, 15.27/15.27]. ESI-MS (*m/z*): 366 (M^+).

*5.1.4.7. 1-(1H-Benzo[d]imidazol-2-yl)-3-(5-(phenoxymethyl)-1,3,4-oxadiazol-2-yl)propan-1-one (**1b–A_{13}**).* IR (KBr, cm^{-1}): 3377 (N–H), 3029 (C–H, Ar–H), 2999(C–H, CH_2), 1728 (C=O), 1600 (C=N), 1562 (C=C), 1384 (N–N=C), 1228 (C–O–C, asymmetric), 1078 (C–O–C, symmetric). ^{1}H NMR (DMSO-d_6): 12.41 (s, 1H, NH, D_2O exchangeable), 7.80 (d, 1H, J = 7.5.Hz, H-4 benzimidazole), 7.50 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.47 (t, 2H, J = 7.2 Hz, H-5,6, benzimidazole), 7.40–6.95 (m, 5H, phenyl), 3.76 (s, 2H, CH_2), 3.10 (t, 2H, J = 7.2 Hz, CH_2), 2.67 (t, 2H, J = 7.5 Hz, CH_2). ^{13}C NMR (DMSO-d_6): 171.53 (C=O), 164.02, 161.93 (2C oxadiazole), 158.15 (C=N), 147.31, 143.12, 140.32, 133.18, 130.15, 128.51, 126.23, 124.46, 123.72, 122.13, 121.89, 120.57 (Ar–C), 65.34 (CH_2, CH_2O), 36.92 (CH_2, CH_2CO), 25.57 (CH_2). Anal. calcd./Found: [C, 65.51/65.57; H, 4.63/4.51; N, 16.08/16.04]. ESI-MS (*m/z*): 348 (M^+).

*5.1.4.8. 3-(5-Benzhydryl-1,3,4-oxadiazol-2-yl)-1-(1H-benzo[d]imidazol-2-yl)propan-1-one (**1b–A_{14}**).* IR (KBr, cm^{-1}): 3328 (N–H), 3056

(C–H, Ar–H), 2966(C–H, CH_2), 1695 (C=O), 1608 (C=N), 1512 (C=C), 1350 (N–N=C), 1203 (C–O–C, asymmetric), 1089 (C–O–C, symmetric). ^{1}H NMR (DMSO-d_6): 12.45 (s, 1H, NH, D_2O exchangeable), 7.73 (d, 1H, J = 7.8 Hz, H-4, benzimidazole), 7.50 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.32 (t, 2H, J = 7.2 Hz, H-5,6 benzimidazole), 7.24–6.73 (m, 10H, phenyl), 4.42 (bs, H, CH), 3.08 (t, 2H, J = 6.9 Hz, CH_2), 2.73 (t, 2H, J = 6.9 Hz, CH_2). ^{13}C NMR (DMSO-d_6): 175.15 (C=O), 162.72, 161.17 (2C oxadiazole), 156.45 (C=N), 144.73, 143.75, 142.53, 137.26, 134.13, 130.41, 129.21, 126.87, 125.42, 123.47, 122.37, 121.89, 121.13, 120.57, 118.46, 118.23, 116.76 (Ar–C), 49.27 (CH_2, CH_2, biphenyl), 40.12 (CH_2, CH_2O), 26.13 (CH_2, CH_2CO). Anal. calcd./Found: [C, 73.51/73.53; H, 4.94/4.81; N, 13.72/13.78]. ESI-MS (m/z): 408 (M^+).

*5.1.4.9. 2-(5-(3-(1H-Benzo[d]imidazol-2-yl)-3-oxopropyl)-1,3,4-oxadiazol-2-yl)acetonitrile (**1b**–**A$_{15}$**).* IR (KBr, cm^{-1}): 3404 (N–H), 3029 (C–H, Ar–H), 2947 (C–H, CH_2), 2270 (CN), 1715 (C=O), 1604 (C=C), 1594 (C=N), 1343 (N–N=C), 1256 (C–O–C, asymmetric), 1083 (C–O–C, symmetric). ^{1}H NMR ($CDCl_3$): 11.91 (s, 1H, NH, D_2O exchangeable), 7.78 (d, 1H, J = 8.1 Hz, H-4, benzimidazole), 7.49 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.32 (t, 2H, J = 3.9 Hz, H-5,6, benzimidazole), 1.82 (s, 2H, CH_2), 3.31 (t, 2H, J = 6.9 Hz, CH_2), 2.92 (t, 2H, J = 6.9 Hz, CH_2). ^{13}C NMR ($CDCl_3$): 175.57 (C=O), 162.72, 159.67 (2C oxadiazole), 154.96 (C=N), 138.27, 132.21, 130.19, 128.57, 124.65, 123.64 (Ar–C), 115.78 (CN), 34.23 (CH_2, CH_2O), 29.32 (CH_2, CH_2), 21.83 (CH_2, CH_2CN). Anal. calcd./Found: [C, 59.78/59.76; H, 3.94/3.68; N, 24.90/24.97]. ESI-MS (m/z): 281(M^+).

*5.1.4.10. 2-(5-(3-(1H-Benzo[d]imidazol-2-yl)-3-oxopropyl)-1,3,4-oxadiazol-2-yl)phenyl acetate (**1b**–**A$_{16}$**).* IR (KBr, cm^{-1}): 3317 (N–H), 3076 (C–H, Ar–H), 2970(C–H, CH_2), 1718 (C=O), 1612 (C=C), 1556 (C=N), 1323 (N–N=C), 1264 (C–O–C, asymmetric), 1104 (C–O–C, symmetric). ^{1}H NMR ($CDCl_3$): 12.15 (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), 7.36 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 7.30–6.68 (m, 4H, phenyl), 2.24 (s, 3H, CH_3), 2.91 (t, 2H, J = 6.9 Hz, CH_2), 2.53 (t, 2H, J = 6.9 Hz, CH_2). ^{13}C NMR ($CDCl_3$): 176.68 (C=O), 169.87 (C=O, $COCH_3$), 162.97, 161.77 (2C oxadiazole), 155.67 (C=N), 150.02, 146.34, 140.13, 138.26, 130.31, 128.25, 125.32, 124.56, 123.72, 123.13, 122.92, 121.43 (Ar–C), 41.25 (CH_2, CH_2CO), 24.15 (CH_2), 17.78 (CH_3). Anal. calcd./Found: [C, 63.82/64.96; H, 4.28/4.16; N, 14.89/14.87]. ESI-MS (m/z): 376 (M^+).

*5.1.4.11. 1-(1H-Benzo[d]imidazol-2-yl)-3-(5-vinyl-1,3,4-oxadiazol-2-yl)propan-1-one (**1b**–**A$_{17}$**).* IR (KBr, cm^{-1}): 3356 (N–H), 3089 (C–H, Ar–H), 2983 (C–H, H_2C=CH_2), 2945 (C–H, CH_2), 1709 (C=O), 1643 (C=C), 1503 (C=N), 1299 (N–N=C), 1205 (C–O–C, asymmetric), 1076 (C–O–C, symmetric). ^{1}H NMR ($CDCl_3$): 11.94 (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), 7.30 (t, 2H, J = 6.3 Hz, H-5,6, benzimidazole), 4.21 (t, J = 7.2 Hz, H, CH), 4.14 (d, J = 7.2 Hz, 2H, CH_2), 3.32(t, 2H, J = 7.2 Hz, CH_2), 2.91 (t, 2H, J = 5.7 Hz, CH_2). ^{13}C NMR ($CDCl_3$): 177.58 (C=O), 163.72, 159.83 (2C oxadiazole), 156.32 (C=N), 133.99, 132.28, 129.59, 128.45 (Ar–C), 122.92, 116.85 (2C, CH_2=CH_2), 33.17 (CH_2, CH_2CO), 22.27 (CH_2). Anal. calcd./Found: [C, 62.68/62.63; H, 4.51/5.03; N, 20.88/20.56]. ESI-MS (m/z): 268 (M^+).

*5.1.4.12. 1-(1H-Benzo[d]imidazol-2-yl)-3-(5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl)propan-1-one (**1b**–**A$_{18}$**).* IR (KBr, cm^{-1}): 3336 (N–H), 3093 (C–H, Ar–H), 2900 (C–H, CH_2), 1685 (C=O), 1654 (C=N), 1627 (C=C), 1373 (N–N=C), 1161 (C–O–C, asymmetric), 1060 (C–O–C, symmetric), 763 (C–Cl). ^{1}H NMR (DMSO-d_6): 11.99 (s, 1H, NH, D_2O exchangeable), 7.87 (d, 1H, J = 8.1.Hz, H-4, benzimidazole), 7.63 (t, 1H, J = 7.2 Hz, H-7, benzimidazole), 7.48 (t, 2H, J = 7.5 Hz, H-5,6 benzimidazole), 7.37–7.01 (m, 3H, phenyl), 3.17 (t, 2H, J = 6.9 Hz, CH_2), 2.66 (t, 2H, J = 6.9 Hz, CH_2). ^{13}C NMR (DMSO-d_6): 174.45 (C=O), 163.23, 160.13 (2C oxadiazole), 154.35 (C=N), 138.24, 135.45, 133.21, 131.76, 128.51, 124.15, 123.63, 122.97, 122.05, 121.67, 120.53, 118.94 (Ar–C), 33.76 (CH_2, CH_2CO), 24.57 (CH_2). Anal. calcd./Found: [C, 55.83/55.86; H, 3.12/3.37; N, 14.47/14.63]. ESI-MS (m/z): 387 (M^+).

*5.1.4.13. 1-(1H-Benzo[d]imidazol-2-yl)-3-(5-(2-mercaptophenyl)-1,3,4-oxadiazol-2-yl)propan-1-one (**1b**–**A$_{19}$**).* IR (KBr, cm^{-1}): 3344 (N–H), 3028 (C–H, Ar–H), 2925 (C–H, CH_2), 2570 (S–H), 1703 (C=O), 1613 (C=N), 1602 (C=C), 1336 (N–N=C), 1128 (C–O–C, asymmetric), 1056 (C–O–C, symmetric). ^{1}H NMR (DMSO-d_6): 12.07 (s, 1H, NH, D_2O exchangeable), 7.78 (d, 1H, J = 7.8.Hz, H-4, benzimidazole), 7.54 (t, 1H, J = 7.5 Hz, H-7, benzimidazole), 7.32 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 7.32–6.92 (m, 4H, phenyl), 3.37 (s, 1H, SH), 2.89 (t, 2H, J = 7.2 Hz, CH_2), 2.61 (t, 2H, J = 6.9 Hz, CH_2). ^{13}C NMR (DMSO-d_6): 169.85 (C=O), 160.89, 160.02 (2C oxadiazole), 153.74 (C=N), 134.53, 133.21, 130.66, 129.43, 127.74, 125.47, 124.97, 124.13, 122.91, 121.78, 120.63, 119.71 (Ar–C), 37.14 (CH_2, CH_2CO), 26.28 (CH_2). Anal. calcd./Found: [C, 61.70/61.75; H, 4.03/4.16; N, 15.99/15.95]. ESI-MS (m/z): 350(M^+).

*5.1.4.14. 1-(1H-Benzo[d]imidazol-2-yl)-3-(5-(prop-1-enyl)-1,3,4-oxadiazol-2-yl)propan-1-one (**1b**–**A$_{20}$**).* IR (KBr,cm^{-1}): 3350 (N–H), 3070 (C–H, Ar–H), 3040 (C–H, aliphatic-H), 3010 (C–H, CH_2=CH_2), 2899 (C–H, CH_2), 1710 (C=O), 1657 (C=N), 1596 (C=C), 1290 (N–N–C), 1145 (C–O–C, asymmetric), 1014 (C–O–C, symmetric). ^{1}H NMR ($CDCl_3$): 12.06 (s, 1H, NH, D_2O exchangeable), 7.73 (d, 1H, J = 8.1.Hz, H-4, benzimidazole), 7.40 (t, 1H, J = 6.6 Hz, H-7, benzimidazole), 7.27 (t, 2H, J = 8.7 Hz, H-5,6, benzimidazole), 4.18 (d, 1H, J = 7.2 Hz, CH), 4.13 (m, 1H, CH), 1.28 (t, 3H, J = 7.2 Hz, CH_3), 3.23 (t, 2H, J = 7.2 Hz, CH_2), 2.85 (t, 2H, J = 6.6 Hz, CH_2). ^{13}C NMR ($CDCl_3$): 170.37 (C=O), 163.91, 162.27 (2C oxadiazole), 156.15 (C=N), 133.14, 132.71, 129.23, 128.63, 127.56, 124.31, 123.97, 122.76 (Ar–C), 34.65 (CH_2, CH_2CO), 23.17 (CH_2), 15.09 (CH_3). Anal. calcd./Found: [C, 63.82/64.02; H, 5.00/5.04; N, 19.85/19.88]. ESI-MS (m/z): 282 (M^+).

*5.1.4.15. 1-(1H-Benzo[d]imidazol-2-yl)-3-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)propan-1-one (**1b**–**A$_{21}$**).* IR (KBr, cm^{-1}): 3396 (O–H), 3310 (N–H), 3105 (C–H, Ar–H), 2891 (C–H, CH_2), 1700 (C=O), 1640 (C=N), 1610 (C=C), 1297 (N–N=C), 1204 (C–O–C, asymmetric), 1040 (C–O–C, symmetric). ^{1}H NMR (DMSO-d_6): 11.87 (s, 1H, NH, D_2O exchangeable), 7.92 (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 = 7.5 Hz, H-5,6, benzimidazole), 7.27-7.01 (m, 4H, phenyl), 6.51 (s, 1H, OH), 3.01 (t, 2H, J = 6.9 Hz, CH_2), 2.73 (t, 2H, J = 7.2 Hz, CH_2). ^{13}C NMR (DMSO-d_6): 176.23(C=O), 166.41, 162.92(2C oxadiazole), 157.31(C=N), 148.12, 130.15, 129.32, 128.74, 126.51, 124.67, 123.87, 123.05, 122.78, 121.57, 120.43 (Ar-C), 36.71(CH_2, CH_2CO), 24.15(CH_2). Anal. calcd./Found: [C, 64.66/64.63; H, 4.22/4.31; N, 16.76/16.79]. ESI-MS (m/z): 334 (M^+).

*5.1.4.16. 3-(5-(4-Amino-2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-1-(1H-benzo[d]imidazol-2-yl) propan-1-one (**1b**–**A$_{22}$**).* IR (KBr, cm^{-1}): 3452 (N–H, NH_2), 3390 (O–H), 3305 (N–H, NH), 3055 (C–H, Ar–H), 2931 (C–H, CH_2), 1790 (C=O), 1654 (C=N), 1570 (C=C), 1317 (N–N=C), 1140 (C–O–C, asymmetric), 1015 (C–O–C, symmetric). ^{1}H NMR (DMSO-d_6): 12.09 (s, 1H, NH, D_2O exchangeable), 7.78 (d, 1H, J = 7.8 Hz, H-4, benzimidazole), 7.51 (t, 1H, J = 7.5 Hz, H-7 benzimidazole), 7.30 (t, 2H, J = 7.5 Hz, H-5,6, benzimidazole), 7.24–6.81 (m, 3H, phenyl), 6.71 (s, 2H, NH_2), 6.03 (s, 1H, OH), 3.11 (t, 2H, J = 7.2 Hz, CH_2), 2.57 (t, 2H, J = 7.2 Hz, CH_2). ^{13}C NMR

(DMSO-d_6): 177.31 (C=O), 163.23, 162.12 (2C oxadiazole), 153.17 (C=N), 150.13, 147.32, 146.63, 130.14, 127.14, 126.53, 123.75, 122.78, 122.17, 121.53, 120.37, 119.63 (Ar–C), 38.85 (CH_2, CH_2CO), 27.53(CH_2). Anal. calcd./Found: [C, 61.89/61.85; H, 4.33/4.41; N, 20.05/21.03]. ESI-MS (*m/z*): 349 (M^+).

*5.1.4.17. 2-(5-(3-(1H-Benzo[d]imidazol-2-yl)-3-oxopropyl)-1,3,4-oxadiazol-2-yl)acetic acid (**1b**–**A$_{23}$**).* IR (KBr, cm^{-1}): 3413 (O–H), 3301 (N–H), 3012 (C–H, Ar–H), 2850 (C–H, CH_2), 1728 (C=O), 1670 (C=N), 1608 (C=C), 1384 (N–N=C), 1180 (C–O–C, asymmetric), 1029 (C–O–C, symmetric). ^{1}H NMR (DMSO-d_6): 11.48 (s, 1H, NH, D_2O exchangeable), 10.37 (s, 1H, OH), 7.78 (d, 1H, *J* = 8.1.Hz, H-4, benzimidazole), 7.48 (t, 1H, *J* = 7.5 Hz, H-7, benzimidazole), 7.26 (t, 2H, *J* = 7.5 Hz, H-5,6, benzimidazole), 3.88 (bs, 2H, CH_2), 3.08 (t, 2H, *J* = 6.9 Hz, CH_2), 2.68 (t, 2H, *J* = 6.9 Hz, CH_2). ^{13}C NMR (DMSO-d_6): 178.67 (C=O), 169.43 (C=O, COOH), 165.37, 163.28 (2C oxadiazole), 157.53 (C=N), 129.17, 128.45, 124.31, 123.74, 121.58, 120.78 (Ar–C), 41.13 (CH_2, CH_2CO), 36.23 (CH_2, CH_2COOH), 25.37 (CH_2). Anal. calcd./Found: [C, 56.00/56.03; H, 4.03/4.15; N, 18.66/18.67]. ESI-MS (*m/z*): 300 (M^+).

*5.1.4.18. 1-(1H-Benzo[d]imidazol-2-yl)-3-(5-(mercaptomethyl)-1,3,4-oxadiazol-2-yl)propan-1-one (**1b**–**A$_{24}$**).* IR (KBr, cm^{-1}): 3307 (N–H), 3076 (C–H, Ar–H), 2981 (C–H, CH_2), 2490 (S–H), 1704 (C=O), 1650 (C=N), 1614 (C=C), 1376 (N–N=C), 1192 (C–O–C, asymmetric), 1046 (C–O–C, symmetric). ^{1}H NMR ($CDCl_3$): 11.76 (s, 1H, NH, D_2O exchangeable), 7.78 (d, 1H, *J* = 8.7 Hz, H-4, benzimidazole), 7.49 (t, 1H, *J* = 7.8 Hz, H-7, benzimidazole), 7.33 (t, 2H, *J* = 6.3 Hz, H-5,6, benzimidazole), 4.16 (d, *J* = 7.2 Hz, 2H, CH_2), 3.34 (t, 2H, *J* = 7.2 Hz, CH_2), 2.92 (t, 2H, *J* = 6.9 Hz, CH_2), 1.27 (t, *J* = 7.2 Hz, 1H, HS). ^{13}C NMR ($CDCl_3$): 173.42 (C=O), 160.97, 160.24 (2C oxadiazole), 155.33(C=N), 132.59, 131.92, 129.86, 128.50, 123.39, 116.00 (Ar–C), 51.80 (CH_2, CH_2SH), 29.80(CH_2, CH_2CO), 28.06 (CH_2). Anal. calcd./Found: [C, 54.15/54.16; H, 4.20/4.27; N, 19.43/19.31]. ESI-MS (*m/z*): 288 (M^+).

*5.1.4.19. 1-(1H-Benzo[d]imidazol-2-yl)-3-(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)propan-1-one (**1b**–**A$_{25}$**).* IR (KBr, cm^{-1}): 3386 (N–H), 3082 (C–H, Ar–H), 2974 (C–H, CH_2), 2758 (C–H, CH_3), 1716 (C=O), 1624 (C=N), 1608 (C=C), 1384 (N–N=C), 1161 (C–O–C, asymmetric), 1022 (C–O–C, symmetric). ^{1}H NMR (DMSO-d_6): 11.88 (s, 1H, NH, D_2O exchangeable), 8.30–7.84 (m, 4H, phenyl), 7.70 (d, 1H, *J* = 7.5.Hz, H-4, benzimidazole), 7.48 (t, 1H, *J* = 7.5 Hz, H-7, benzimidazole), 7.27 (t, 2H, *J* = 7.8 Hz, H-5,6, benzimidazole), 4.15 (s, 3H, CH_3), 3.21 (t, 2H, *J* = 6.9 Hz, CH_2), 2.83 (t, 2H, *J* = 6.9 Hz, CH_2). ^{13}C NMR (DMSO-d_6): 176.49 (C=O), 164.73, 161.48 (2C oxadiazole), 158.12 (C=N), 151.32, 133.52, 132.23, 130.63, 129.83, 128.74, 124.53, 123.39, 122.30, 121.43, 120.34 (Ar–C), 45.21 (CH_3, OCH_3), 32.67 (CH_2, CH_2CO), 25.47 (CH_2). Anal. calcd./Found: [C, 65.51/C, 65.61; H, 4.63/4.75; N, 16.08/16.12]. ESI-MS (*m/z*): 348 (M^+).

*5.1.4.20. 1-(1H-benzo[d]imidazol-2-yl)-3-(5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)propan-1-one (**1b**–**A$_{26}$**).* IR (KBr, cm^{-1}): 3420 (O–H), 3325 (N–H), 3043 (C–H, Ar–H), 2936 (C–H, CH_2), 1697 (C=O), 1634 (C=N), 1585 (C=C), 1327 (N–N=C), 1176 (C–O–C, asymmetric), 1058 (C–O–C, symmetric). ^{1}H NMR (DMSO-d_6): 12.05 (s, 1H, NH, D_2O exchangeable), 8.11–7.85 (m, 4H, phenyl), 7.68 (d, 1H, *J* = 7.5.Hz, H-4, benzimidazole), 7.51 (t, 1H, *J* = 7.5 Hz, H-7, benzimidazole), 7.27 (t, 2H, *J* = 8.1 Hz, H-5,6, benzimidazole), 6.71 (s, 1H, OH), 3.09 (t, 2H, *J* = 6.9 Hz, CH_2), 2.75 (t, 2H, *J* = 6.9 Hz, CH_2). ^{13}C NMR (DMSO-d_6): 171.93 (C=O), 162.87, 160.68 (2C oxadiazole), 155.27 (C=N), 150.16, 130.53, 129.53, 128.75, 125.34, 124.31, 123.91, 123.16, 122.70, 121.35, 120.53, 120.05 (Ar–C), 36.21 (CH_2, CH_2CO), 26.43 (CH_2). Anal. calcd./Found: [C, 64.66/64.71; H, 4.22/4.17; N, 16.76/16.89]. ESI-MS (*m/z*): 334 (M^+).

*5.1.5. General procedure for synthesis of 1-(1H-benzo[d]imidazol-2-yl)-3-(5-(methyl substituted)-1,3,4-oxadiazol-2-yl)propan-1-one (**1b**–**A$_{7a-e}$**)*

The suspension of compound (**1b**–**A$_7$**) (0.003 mol) and a secondary amine (equimolar; 0.003 mol) in absolute ethanol (10 mL) was placed into a microwave reaction vessel equipped with a magnetic stir bar, adding sodium acetate (0.001 mol) into microwave reaction vessel equipped with a magnetic stir bar. The reaction vessel was placed into the scientific microwave synthesizer (model No. CATA-R, Catalyst systems, India) and irradiated at a power level of 5 (50%, 350 W) for 8–13 min. The completion of reaction was checked by single-spot TLC by using toluene: ethyl acetate: formic acid (5:4:1) and benzene: acetone (9:1) solvent system. The reaction mixture was cooled, poured onto crushed ice and acidified with glacial acetic acid to yield a solid mass. It was filtered and washed with water to remove the inorganic components. The product was purified by flash chromatography (hexane/ethyl acetate gradient, 8:2, concentration of gradient varied compound to compound) to get pure compound **(1b–A$_{7a-e}$)**.

*5.1.5.1. 1-(1H-Benzo[d]imidazol-2-yl)-3-(5-((diethylamino)methyl)-1,3,4-oxadiazol-2-yl)propan-1-one (**1b**–**A$_{7a}$**).* IR (KBr, cm^{-1}): 3309 (N–H), 3072 (C–H, Ar–H), 2950 (C–H, CH_2), 2873 (C–H, CH_3), 1715 (C=O), 1670 (C=N), 1547 (C=C), 1373 (N–N=C), 1184 (C–O–C, asymmetric), 1077 (C–O–C, symmetric). ^{1}H NMR ($CDCl_3$): 12.04 (s, 1H, NH, D_2O exchangeable), 7.73 (d, 1H, *J* = 7.8.Hz, H-4, benzimidazole), 7.42 (d, 1H, *J* = 11.7 Hz, H-7, benzimidazole), 7.28 (t, 2H, *J* = 8.7 Hz, *J* = 9.6 Hz, H-5,6, benzimidazole), 4.13 (q, 4H, *J* = 5.4 Hz, 2xCH_2), 3.23 (t, 2H, *J* = 6.9 Hz, CH_2), 2.87 (t, 2H, *J* = 6.6 Hz, CH_2), 2.60 (s, 2H, CH_2), 1.25 (t, 6H, *J* = 1.2 Hz, 2xCH_3). ^{13}C NMR ($CDCl_3$): 171.50 (C=O), 165.11, 161.19 (2C oxadiazole), 155.02 (C=N), 132.83, 131.96, 129.86, 128.52, 124.73, 123.48 (Ar–C), 60.24 (CH_2, CH_2N), 30.21 (CH_2, CH_2CO), 28.08 (CH_2), 14.60 (CH_3). Anal. calcd./Found: [C, 62.37/63.87; H, 6.47/6.51; N, 21.39/21.35]. ESI-MS (*m/z*): 327(M^+).

*5.1.5.2. 1-(1H-Benzo[d]imidazol-2-yl)-3-(5-((diphenylamino) methyl)-1,3,4-oxadiazol-2-yl) propan-1-one (**1b**–**A$_{7b}$**).* IR (KBr, cm^{-1}): 3290 (N–H), 3067 (C–H, Ar–H), 2957 (C–H, CH_2), 1690 (C=O), 1654 (C=N), 1583 (C=C), 1287 (N–N=C), 1162 (C–O–C, asymmetric), 1027 (C–O–C, symmetric). ^{1}H NMR (DMSO-d_6): 12.13 (s, 1H, NH, D_2O exchangeable), 7.92 (d, 1H, *J* = 8.7 Hz, H-4, benzimidazole), 7.70 (d, 1H, *J* = 7.8 Hz, H-7, benzimidazole), 7.48 (t, 2H, *J* = 7.5 Hz, H-5,6, benzimidazole), 7.27–6.87 (m, 10H, phenyl), 3.72 (s, 2H, CH_2), 3.16 (t, 2H, *J* = 7.2 Hz, CH_2), 2.54 (t, 2H, *J* = 7.2 Hz, CH_2). ^{13}C NMR (DMSO-d_6): 173.43 (C=O), 163.51, 162.73 (2C oxadiazole), 157.23 (C=N), 150.43, 149.27, 148.03, 133.17, 132.56, 128.12, 127.53, 127.02, 124.35, 123.43, 122.73, 120.47 (Ar–C), 57.48 (CH_2, CH_2N), 33.71 (CH_2, CH_2CO), 25.32 (CH_2). Anal. calcd./Found: [C, 70.91/70.93; H, 5.00/5.07; N, 16.54/16.68]. ESI-MS (*m/z*): 423(M^+).

*5.1.5.3. 1-(1H-Benzo[d]imidazol-2-yl)-3-(5-((4-methylpiperazin-1-yl)methyl)-1,3,4-oxadiazol-2 yl) propan-1-one (**1b**–**A$_{7c}$**).* IR (KBr, cm^{-1}): 3317 (N–H), 3015 (C–H, Ar–H), 2941 (C–H, CH_2), 2868 (C–H, CH_3), 1711 (C=O), 1645 (C=N), 1576 (C=C), 1363 (N–N=C), 1183 (C–O–C, asymmetric), 1057 (C–O–C, symmetric). ^{1}H NMR (DMSO-d_6): 11.45 (s, 1H, NH, D_2O exchangeable), 7.84 (d, 1H, *J* = 7.5 Hz, H-4, benzimidazole), 7.54 (d, 1H, *J* = 7.5 Hz, H-7, benzimidazole), 7.41 (t, 2H, *J* = 7.8 Hz, H-5,6, benzimidazole), 3.74 (s, 2H, CH_2), 3.59 (t, 4H, *J* = 7.8 Hz, 2xCH_2, piperazine), 3.15 (t, 4H, *J* = 6.9 Hz, 2xCH_2-NCH_3, piperazine), 3.01 (t, 2H, *J* = 7.2 Hz, CH_2),

2.77 (t, 2H, $J = 7.2$ Hz, CH_2), 1.91 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): 175.31 (C=O), 165.32, 163.84 (2C oxadiazole), 156.13 (C=N), 131.52, 128.25, 124.57, 123.61, 123.04, 122.81 (Ar–C), 61.23 (CH_2, CH_2N), 54.27, 54.13, 51.83, 51.09 (4C piperazine), 43.71 (CH_3), 35.02 (CH_2, CH_2CO), 26.51 (CH_2). Anal. calcd./Found: [C, 61.00/61.09; H, 6.26/6.27; N, 23.71/23.83]. ESI-MS (*m/z*): 354 (M^+).

*5.1.5.4. 1-((5-(3-(1H-Benzo[d]imidazol-2-yl)-3-oxopropyl)-1,3,4-oxadiazol-2-yl)methyl)indoline –2,3-dione (**1b–A$_{7d}$**).* IR (KBr, cm^{-1}): 3293 (N–H), 3041 (C–H, Ar–H), 2957 (C–H, CH_2), 1716 (C=O), 1687 (C=N), 1572 (C=C), 1338 (N–N=C), 1168 (C–O–C, asymmetric), 1052 (C–O–C, symmetric). ^1H NMR (DMSO-d_6): 11.95 (s, 1H, NH, D_2O exchangeable), 8.74-7.52 (m, 4H, phenyl), 7.64 (d, 1H, $J = 7.5$.Hz, H-4, benzimidazole), 7.51 (d, 1H, $J = 7.5$ Hz, H-7, benzimidazole), 7.30 (t, 2H, $J = 7.8$ Hz, H-5,6, benzimidazole), 4.36 (s, 2H, CH_2), 3.07 (t, 2H, $J = 7.2$ Hz, CH_2), 2.63 (t, 2H, $J = 7.2$ Hz, CH_2). ^{13}C NMR (DMSO-d_6): 177.65 (C=O, $COCH_2$), 175.23 (CO), 170.31 (C=O, CON), 161.83, 159.75 (2C oxadiazole), 154.97 (C=N), 147.23, 135.76, 133.63, 132.23, 124.35, 124.02, 123.43, 122.73, 122.16, 121.43, 120.73 (Ar–C), 56.89 (CH_2, CH_2N), 34.85 (CH_2, CH_2CO), 26.53 (CH_2). Anal. calcd./Found: [C, 62.84/62.87; H, 3.77/3.73; N, 17.45/17.52]. ESI-MS (*m/z*): 401(M^+).

*5.1.5.5. 3-((5-(3-(1H-Benzo[d]imidazol-2-yl)-3-oxopropyl)-1,3,4-oxadiazol-2-yl)methyl) pyrimi-dine-2,4(1H,3H)-dione (**1b–A$_{7e}$**).* IR (KBr, cm^{-1}): 3345 (N–H), 3028 (C–H, Ar–H), 2911 (C–H, CH_2), 1703 (C=O), 1681 (C=N), 1578 (C=C), 1316 (N–N=C), 1193 (C–O–C, asymmetric), 1034 (C–O–C, symmetric). ^1H NMR (DMSO-d_6): 11.00 (s, 1H, NH, benzimidazole, D_2O exchangeable), 10.81 (s, 1H, NH, D_2O exchangeable), 7.81 (d, 1H, $J = 7.8$ Hz, H-4, benzimidazole), 7.54 (d, 1H, $J = 7.5$ Hz, H-7, benzimidazole), 7.27 (t, 2H, $J = 7.5$ Hz, H-5,6 benzimidazole), 4.32 (s, 2H, CH_2), 3.23 (t, 2H, $J = 7.2$ Hz, CH_2), 2.57 (t, 2H, $J = 6.9$ Hz, CH_2). ^{13}C NMR (DMSO-d_6): 173.51 (C=O), 170.38 (CO, CON), 159.02 (C=O, NCON), 164.75, 162.86 (2C, oxadiazole), 156.46 (C=N), 132.45, 131.64, 129.84, 124.76, 123.43, 122.53, 121.43, 118.83 (Ar–C), 58.96 (CH_2, CH_2N), 38.54 (CH_2, CH_2CO), 28.57 (CH_2). Anal. calcd./Found: [C, 55.74/55.71; H, 3.85/3.85; N, 22.94/22.82]. ESI-MS (*m/z*): 366 (M^+).

5.2. In vitro anticancer activity [1,28–31]

The compounds were evaluated for their *in vitro* anticancer activity at National Cancer Institute (NCI), USA against full NCI 60 cell lines panel representing on full nine human systems as leukemia, melanoma and cancers of lung, colon, brain, breast, ovary, kidney and prostate in accordance with their applied protocol (used SRB assay). Sixteen compounds among the twenty-five newly synthesized compounds were selected for anticancer evaluation. The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10 μM. The output from the single dose screen was reported as a mean graph and available for analysis by the COMPARE program. Compounds that exhibited significant growth inhibition were evaluated against the 60 cell panel at five concentration levels. The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 m ML-glutamine. For a typical screening experiment, cells inoculated into 96 well microtiter plates in 100 μL at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. 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 drug addition (Tz). Experimental drugs solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug 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 drug dilutions added to the appropriate microtiter wells already containing 100 μL of medium, resulting in the required final drug concentrations.

Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay terminated by the addition of cold TCA. 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 was discarded, and the plates washed five times with tap water and then air dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid was added to each well and plates incubated for 10 min at room temperature. After staining, unbound dye removed by washing five times with 1% acetic acid and then the plates air dried. 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. For suspension cells, the methodology was same except that the assay terminated by fixing settled cells at the bottom of the wells by gently adding 50 μL of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C) and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels using the following formula:

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

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

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 (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) calculated from $Ti = Tz$. The LC_{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 cells following treatment was calculated from $[(Ti–Tz)/Tz] \times 100 = -50$. Values were calculated for each of these three parameters, if the level of activity was reached; however, if the effect is not reached or was exceeded, the value for that parameter expressed as greater or less than the maximum or minimum concentration tested.

6. Conclusion

Twenty-five new benzimidazoles bearing 1,3,4-oxadiazole nucleus were successfully synthesized under microwave irradiation in good yields. The compounds were evaluated for their *in vitro* anticancer activity against full NCI 60 cell lines panel. The screening result data obtained from NCI indicated that the compounds **1b–A$_7$**, **1b–A$_{11}$**, **1b–A$_{12}$**, **1b–A$_{13}$**, **1b–A$_{14}$**, **1b–A$_{15}$**, **1b–A$_{23}$**, **1b–A$_{7a}$**, **1b–A$_{7b}$** and **1b–A$_{7c}$** exhibited good percentage growth inhibition of human cancer cell lines, and compound **1b–A$_{18}$** (NSC: 759205), 1-(1*H*-benzo[*d*] imidazol-2-yl)-3-(5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl) propan-1-one, showed maximum growth inhibition and found to be the most active candidate of the series (lead compound). On the basis

of structure-activity relationships, it could be noted that electron donating groups like $-NH_2$, 2-chloro and 2,4-dichloro on the phenyl ring at 5th position of oxadiazole moiety have great influence on anticancer activity and groups like $-N(C_2H_5)_2$, $-O-C_6H_5$ on methyl at 5th position of oxadiazole ring also resulted in compounds with improved anticancer activity. Electron withdrawing groups like −COOH and −C═O did not show remarkable anticancer activity. The most active compound of the series was **1b**–**A_{18}** (2,4-dichloro substituted) therefore, further studies on this compound continue in our research laboratory to acquire more information about QSAR. Finally it is conceivable that further derivatization of these compounds could result in obtaining more selective anticancer agents.

Acknowledgements

We are thankful to UGC, New Delhi, Government of India, for financial assistance in the form of Major Research Project [36-107/2008(SR)]. Thanks are also due to National Cancer Institute (NCI), USA, for *in vitro* anticancer screening of the compounds.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2012.04.027. These data include MOL files and InChIKeys of the most important compounds described in this article.

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