Design, synthesis, and *in-vitro* antiproliferative effect of some novel 1,3,4-oxadiazole derivatives bearing benzimidazole nucleus

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Background and objectives
Development of a novel compound containing a heterocyclic nucleus as an anticancer therapeutic agent is the most important focal point in medicinal chemistry. Programmed cell death or apoptosis is a fundamental phenomenon and plays a central role in immune regulation, embryogenesis, and general tissue homeostasis. Therefore, identification of novel potent, selective, and less toxic anticancer agents is one of the most crucial concerns.

Materials and methods
A series of 4-{1-[(4-acetyl-5-(substituted)-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile **6 (I–IV)** and novel 4-{5-substituted-1-[(4,5-disubstituted)-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile **7 (V–XXXIII)** derivatives were synthesized.

Results and conclusion
Nine of them were selected by the Department of Biotechnology Drug Discovery R&D, Century Pharmaceuticals Ltd, for evaluation of their *in-vitro* anticancer activity. Three of the investigated compounds, **7.X, 7.XIX**, and **7.XXIV**, displayed *in-vitro* anticancer activity in the primary assay. These compounds were selected for a full anticancer screening against a three-cell panel MTT assay, and they showed a nonselective broad spectrum antiproliferative activity against L929, HCT15, and Hep2 cancer cell lines. Compound **7.XIX** showed antiproliferative activity, which can be comparable to that of 5-fluorouracil, methotrexate, and daunorubicin, and this compound has been identified as a promising lead compound.

Keywords:
anticancer activity, daunorubicin, 5-fluorouracil, methotrexate, MTT assay, 1,3,4-oxadiazoles

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Introduction

Chemists over the years have drawn attention to the abundant active molecules that contain various heteroatoms because of their biological significance [1,2]. Oxadiazole derivatives play a significant role in various pharmaceutical formulations [3,4]. Novel therapeutics include small molecular agents, such as compounds having the 1,3,4-oxadiazole ring, which has significant biological properties in the form of anti-inflammatory [5], antibacterial [6], antitubercular [7], antiviral [8], and anticancer [9–12] activities. Therefore, identification of novel potent, selective, and less toxic anticancer agents is one of the most important challenges facing the health sector [13]. Benzimidazole derivatives are well known for their anti-inflammatory activity and more recently it has been discovered to have anticancer effect. Therefore, the present work aimed to incorporate the benzimidazole moiety with 1,3,4-oxadiazole to study their anticancer activity against HCT15 (colon cancer), Hep2 human cancer cell lines, and L929 (connective tissue mouse) control cell using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay.

All cell lines were supplied by National Center for Cell Sciences (NCCS, Pune, India). These cell lines were grown and maintained using suitable (Dulbecco's Modified Eagle Media (DMEM)) media and were grown in culture medium supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin–streptomycin antibiotic solution. Cells were seeded in 25 cm^2 tissue culture flasks (Falcon, Thermo Fisher Scientific, Waltham, Massachusetts, USA), at 2.5×10^5 cells/flask in a total volume of 5 ml. When confluent, all cells were trypsinized (using trypsin-EDTA; HiMedia, Mumbai, India) and seeded in 96-well plates.

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Materials and methods

Chemistry

Melting points were determined using the open capillary method and were uncorrected. The IR spectra (in KBr) were recorded on a Shimadzu IR Affinity-1 spectrophotometer (Rydalmere 2116 Sydney, NSW, Australia). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Perkin-Elmer EM (940 Winter St. Waltham, Massachusetts, USA) 300 MHz spectrometer using tetramethylsilane (TMS) as internal standard. The mass spectra were recorded on a Jeol JMS-D 300 spectrometer (Jeol, 11 Dearborn Road Peabody, MA 01960, USA) operating at 70 eV. Purity of the compounds was checked using TLC silica coated plates obtained from Merck (2000 Galloping Hill Road Kenilworth, NJ 07033, USA).

General procedure for the preparation of 4-{1-[(4-acetyl-5-(substituted)-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile 6 (I–IV)

A mixture of imine intermediate **4 (I–IV)** (0.01 mol) and excess of acetic anhydride (10 ml) were refluxed for 3–4 h. The acetic anhydride was distilled off and the residue was poured on to crushed ice. The solid thus obtained was collected by filtration, washed with water, and recrystallized using ethanol. The purity of the product was confirmed by a single spot on TLC plate.

General procedure for the preparation of novel 4-{5-substituted-1-[(4,5-disubstituted)-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile 7 (V–XXXIII)

To a solution of imine intermediate **5 (V–XXXIII)** (0.01 mol) in ethanol (15 ml), chloramine-T (0.01 mol) was added. The reaction mixture was exposed to microwave at 300 W intermittently at 30 s intervals for specified time. After complete conversion, as indicated by TLC, the reaction mixture was cooled and digested with cold water.

6.I: 4-{1-[(4-acetyl-5-(3-chlorophenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile

IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2850 (C–H), 2210 (C≡N), 1642 (C=O), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 733 (C–Cl).

^{1}H NMR ($CDCl_3$) δ ppm 3.833 (s, 2H, –CH_2), 2.021 (s, 3H, –CH_3), 6.613–8.633 (m, 12H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 23, 47, 74, 110, 113, 115, 116, 118, 125, 126, 127, 128, 130, 133, 134, 135, 139, 140, 142, 143, 153, 155, 169; MS: *m/z* 501 (M^+).

6.II: 4-{1-[(4-acetyl-5-(2-chlorophenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile

IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2850 (C–H), 2210 (C≡N), 1642 (C=O), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 733 (C–Cl).

^{1}H NMR ($CDCl_3$) δ ppm 3.833 (s, 2H, –CH_2), 2.021 (s, 3H, –CH_3), 6.781–8.458 (m, 12H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 23, 47, 74, 110, 113, 115, 116, 118, 125, 126, 127, 128, 130, 133, 134, 135, 139, 140, 142, 143, 153, 155, 169; MS: *m/z* 501 (M^+).

6.III: 4-{1-[(4-acetyl-5-(benzene-1,4-diol)-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile

IR (KBr, cm^{-1}) 3655 (O–H), 3062 (Ar–C–H), 2850 (C–H), 2210 (C≡N), 1642 (C=O), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C).

^{1}H NMR ($CDCl_3$) δ ppm 3.833 (s, 2H, –CH_2), 2.021 (s, 3H, –CH_3), 6.133–8.458 (m, 11H, aromatic protons), 8.866 (s, 2H, OH), ^{13}C NMR ($CDCl_3$) δ ppm 23, 47, 74, 110, 113, 115, 116, 118, 125, 126, 127, 128, 130, 133, 134, 135, 139, 140, 142, 143, 153, 155, 169; MS: *m/z* 498 (M^+).

6.IV: 4-{1-[(4-acetyl-5-(3-bromophenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile

IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2850 (C–H), 2210 (C≡N), 1642 (C=O), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 575 (C–Br).

^{1}H NMR ($CDCl_3$) δ ppm 3.833 (s, 2H, –CH_2), 2.021 (s, 3H, –CH_3), 6.511–8.458 (m, 12H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 23, 47, 74, 110, 113, 115, 116, 118, 125, 126, 127, 128, 130, 133, 134, 135, 139, 140, 142, 143, 153, 155, 169; MS: *m/z* 545, 547 (M^+).

7.V: 4-{1-[(5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile

IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 733 (C–Cl).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, –CH_2), 7.361–8.437 (m, 11H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 49, 112, 114, 119, 120, 126, 129, 130, 131, 132, 134, 136, 141, 144, 145, 156, 166, 167; MS: *m/z* 457 (M^+).

7.VI: 4-{1-[(5-(3-chlorophenyl)-1,3,4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile

IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 733 (C–Cl).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 7.332–8.437 (m, 11H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 49, 112, 114, 119, 120, 127, 128, 129, 131, 133, 134, 136, 141, 144, 145, 158, 165, 167; MS: *m/z* 457 (M^+).

7.VII: 4-{1-[(5-(2-aniline)-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile

IR (KBr, cm^{-1}) 3265 (N–H), 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 733 (C–Cl).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 6.456–8.437 (m, 11H, aromatic protons), 3.189 (s, 2H, NH_2), ^{13}C NMR ($CDCl_3$) δ ppm 49, 112, 114, 115, 118, 119, 120, 122, 126, 130, 131, 134, 137, 141, 144, 145, 156, 164, 167; MS: *m/z* 437 (M^+).

7.VIII: 4-{1-[(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile

IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 733 (C–Cl).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 7.362–8.43 (m, 11H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 49, 112, 114, 119, 120, 127, 129, 130, 131, 134, 136, 137, 141, 145, 156, 164, 167; MS: *m/z* 457 (M^+).

7.IX: 4-{1-[(5-(3-bromophenyl)-1,3,4-oxadiazol-2-yl) methyl]-5-chloro-1*H*-benzimidazol-2-yl}benzonitrile

IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1331 (C–N), 1242 (C–O–C), 733 (C–Cl), 575 (C–Br).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 7.137–8.11 (m, 11H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 49, 112, 114, 119, 123, 124, 125, 128, 130, 131, 132, 134, 137, 144, 158, 164, 167; MS: *m/z* 491, 493 (M^+).

7.X: 4-{1-[(5-(3,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile

IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 733 (C–Cl).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 7.36–8.43 (m, 10H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 49, 112, 114, 119, 120, 125, 128, 131, 132, 133, 134, 136, 141, 144, 145, 158, 165, 167; MS: *m/z* 491 (M^+).

7.XI: 4-{1-[(5-(benzene-1,4-diol)-1,3,4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile

IR (KBr, cm^{-1}) 3655 (O–H), 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 733 (C–Cl).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 6.308–8.43 (m, 10H, aromatic protons), 6.433 (s, 2H, OH), ^{13}C NMR ($CDCl_3$) δ ppm 49, 101, 112, 113, 114, 119, 120, 122, 131, 134, 136, 141, 144, 145, 148, 149, 158, 167; MS: *m/z* 454 (M^+).

7.XII: 4-{1-[(5-(3-bromophenyl)-1,3,4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile

IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 733 (C–Cl), 575 (C–Br).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 7.23–8.43 (m, 11H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 49, 112, 114, 119, 120, 125, 128, 131, 132, 134, 136, 141, 144, 145, 158, 164, 167; MS: *m/z* 501, 503 (M^+).

7.XIII: 4-{1-[(5-(4-bromophenyl)-1,3,4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile

IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 733 (C–Cl), 575 (C–Br).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 7.36–8.43 (m, 11H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 49, 112, 114, 119, 120, 124, 125, 130, 131, 133, 134, 136, 141, 144, 145, 158, 164, 167; MS: *m/z* 501, 503 (M^+).

7.XIV: 4-{1-[(5-(3-(bromomethyl)phenyl)-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile

IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2850 (C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 575 (C–Br).

^{1}H NMR ($CDCl_3$) δ ppm 4.49–5.12 (s, 4H, $–CH_2$), 7.33–8.43 (m, 11H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 33, 49, 112, 114, 119, 120, 126, 129, 130, 131, 132, 134, 135, 136, 140, 141, 144, 145, 158, 164, 167; MS: *m/z* 515, 517 (M^+).

7.XV: 4-{1-[(5-(2-methylphenyl)-1,3,4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile
IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2850 (C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 733 (C–Cl).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 2.36 (s, 3H, $–CH_3$), 7.36–8.43 (m, 11H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 19, 49, 112, 119, 120, 124, 126, 129, 130, 131, 134, 136, 141, 144, 145, 158, 164, 167; MS: *m/z* 436 (M^+).

7.XVI: 4-{1-[(5-(2-methoxyphenyl)-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile
IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2850 (C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 733 (C–Cl).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 3.87 (s, 3H, $–CH_3$), 7.23–8.43 (m, 11H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 49, 55, 103, 112, 114, 116, 119, 120, 122, 127, 131, 133, 134, 136, 141, 144, 145, 156, 158, 167, 168; MS: *m/z* 452 (M^+).

7.XVII: 4-{1-[(5-(2-bromo-6-nitrophenyl)-1,3,4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile
IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 575 (C–Br).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 7.36–8.43 (m, 10H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 49, 112, 114, 118, 119, 120, 127, 131, 133, 134, 136, 138, 141, 144, 145, 158, 163, 167; MS: *m/z* 546, 548 (M^+).

7.XVIII: 4-{1-[(5-(4-bromo-3-(1,3,4-oxadiazol-2-yl)phenol) methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile
IR (KBr, cm^{-1}) 3655 (O–H), 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 575 (C–Br).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 6.87–8.43 (m, 10H, aromatic protons), 6.84 (s, 1H, OH), ^{13}C NMR ($CDCl_3$) δ ppm 49, 108, 112, 113, 114, 115, 119, 120, 131, 132, 134, 135, 136, 141, 144, 145, 157, 158, 163, 167; MS: *m/z* 517, 519 (M^+).

7.XIX: 4-{1-[(5-(4,5-difluoro-2-(1,3,4-oxadiazol-2-yl)phenol)methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile
IR (KBr, cm^{-1}) 3655 (O–H), 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1356 (C–F), 1352 (N=O), 1331 (C–N), 1242 (C–O–C).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 6.84–8.43 (m, 9H, aromatic protons), 4.71 (s, 1H, OH), ^{13}C NMR ($CDCl_3$) δ ppm 49, 101, 110, 112, 114, 116, 119, 120, 131, 134, 136, 141, 144, 145, 151, 155, 158, 167, 168; MS: *m/z* 474 (M^+).

7.XX: 4-{1-[(5-(3,5-dichlorophenyl)-1,3,4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile
IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 733 (C–Cl).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 7.36–8.43 (m, 9H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 49, 112, 114, 119, 120, 127, 129, 131, 134, 135, 136, 139, 141, 144, 145, 158, 164, 167; MS: *m/z* 491 (M^+).

7.XXI: 4-{1-[(5-(2-chloro-4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile
IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 733 (C–Cl).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 7.36–8.43 (m, 10H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 49, 112, 114, 119, 120, 124, 125, 129, 131, 132, 133, 134, 136, 141, 144, 145, 149, 158, 166, 167; MS: *m/z* 546 (M^+).

7.XXII: 4-{1-[(5-(2-bromo-4-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile
IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1356 (C–F), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 575 (C–Br).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 7.36–8.43 (m, 10H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 49, 112, 114, 119, 120, 124, 126, 127, 128, 131, 134, 135, 136, 141, 145, 158, 164, 167; MS: *m/z* 569, 571 (M^+).

7.XXIII: 4-{1-[(5-(2-chloro-3-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile
IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 733 (C–Cl).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 7.36–8.43 (m, 10H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 49, 112, 114, 118, 119, 120, 126, 129, 130, 131, 134, 135, 136, 141, 144, 145, 151, 158, 167, 168; MS: *m/z* 502 (M^+).

7.XXIV: 4-{1-[(5-(2-chloro-4,5-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile

IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C), 733 (C–Cl).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 3.77 (s, 6H, $–CH_3$), 7.36–8.43 (m, 9H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 49, 56, 112, 113, 114, 117, 118, 119, 120, 127, 131, 134, 136, 144, 145, 151, 158, 165, 167; MS: *m/z* 517 (M^+).

7.XXV: 4-{1-[(5-(2-ethoxy-4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile

IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2849 (C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1305 (C–C), 1242 (C–O–C).

^{1}H NMR ($CDCl_3$) δ ppm 4.148–5.126 (s, 4H, $–CH_2$), 1.45–3.82 (s, 6H, $–CH_3$), 6.62–8.43 (m, 10H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 15, 49, 56, 64, 97, 105, 108, 112, 114, 119, 120, 127, 131, 134, 136, 141, 144, 145, 157, 158, 164, 166, 167; MS: *m/z* 496 (M^+).

7.XXVI: 4-{1-[(5-[2-(1,3-dioxolan-2-ylmethoxy)phenyl]-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile

IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2849 (C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C).

^{1}H NMR ($CDCl_3$) δ ppm 3.98–5.126 (s, 4H, $–CH_2$), 3.83–5.22 (m, 5H, 1,3-dioxolane protons), 7.16–8.43 (m, 11H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 49, 64, 69, 101, 103, 112, 114, 115, 119, 120, 122, 126, 131, 133, 134, 136, 141, 144, 145, 155, 158, 167, 168; MS: *m/z* 524 (M^+).

7.XXVII: 4-{1-[(5-[3-(3-bromopropoxy)phenyl]-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile

IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2849 (C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1305 (C–C), 1242 (C–O–C), 575 (C–Br).

^{1}H NMR ($CDCl_3$) δ ppm 2.138–5.126 (s, 8H, $–CH_2$), 7.095–8.43 (m, 11H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 31, 32, 49, 67, 108, 112, 114, 119, 120, 123, 125, 129, 130, 131, 134, 136, 141, 145, 158, 163, 167; MS: *m/z* 559, 561 (M^+).

7.XXVIII: 4-{1-[(5-[3-ethoxy-4-(prop-2-yn-1-yloxy) phenyl]-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile

IR (KBr, cm^{-1}) 3062 (Ar–C–H), 2849 (C–H), 2119 (C≡C), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1305 (C–C), 1242 (C–O–C).

^{1}H NMR ($CDCl_3$) δ ppm 2.41 (s, 1H, –CH), 4.09–5.126 (s, 6H, $–CH_2$), 1.488 (s, 3H, $–CH_3$), 6.87–8.43 (m, 10H, aromatic protons), ^{13}C NMR ($CDCl_3$) δ ppm 15, 49, 57, 65, 76, 79, 110, 111, 112, 114, 119, 120, 123, 131, 134, 136, 141, 144, 145, 149, 157, 158, 163, 167; MS: *m/z* 520 (M^+).

7.XXIX: 4-{1-[(5-[2,3-dimethoxy-6-(1,3,4-oxadiazol-2-yl)phenol]methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile

IR (KBr, cm^{-1}) 3655 (O–H), 3062 (Ar–C–H), 2849 (C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 3.75–3.85 (s, 6H, $–CH_3$), 6.70–8.47 (m, 9H, aromatic protons), 5.44 (s, 1H, OH), ^{13}C NMR ($CDCl_3$) δ ppm 49, 57, 60, 95, 107, 112, 114, 119, 120, 121, 131, 134, 136, 141, 142, 144, 145, 149, 157, 158, 167; MS: *m/z* 498 (M^+).

7.XXX: 4-{1-[(5-[2,4-dinitro-6-(1,3,4-oxadiazol-2-yl)phenol]methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile

IR (KBr, cm^{-1}) 3655 (O–H), 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 7.36–9.14 (m, 9H, aromatic protons), 11.26 (s, 1H, OH), ^{13}C NMR ($CDCl_3$) δ ppm 49, 103, 112, 114, 119, 124, 131, 134, 136, 139, 140, 141, 144, 145, 154, 158, 167, 172; MS: *m/z* 528 (M^+).

7.XXXI: 4-{1-[(5-[2,4-di-*tert*-butyl-6-(1,3,4-oxadiazol-2-yl)phenol]methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile

IR (KBr, cm^{-1}) 3655 (O–H), 3062 (Ar–C–H), 2849 (C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 1.31–1.38 (s, 18H, $–CH_3$), 7.28–8.43 (m, 9H, aromatic protons), 5.42 (s, 1H, OH), ^{13}C NMR ($CDCl_3$) δ ppm 30, 31, 34, 37, 49, 109, 112, 114, 119, 120, 126, 127, 131, 134, 136, 140, 141, 144, 145, 157, 158, 167, 170; MS: *m/z* 551 (M^+).

7.XXXII: 4-{1-[(5-[3-(1,3,4-oxadiazol-2-yl)-2-prop-2-en-1-ylphenol]methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile

IR (KBr, cm^{-1}) 3655 (O–H), 3075 (C=C), 3062 (Ar–C–H), 3012 (C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C).

^{1}H NMR ($CDCl_3$) δ ppm 5–5.81 (s, 3H, prop-1-ene protons), 3.33–5.126 (s, 4H, $–CH_2$), 6.76–8.43 (m, 10H, aromatic protons), 4.7 (s, 1H, OH), ^{13}C NMR ($CDCl_3$) δ ppm 28, 49, 112, 114, 115, 116, 118, 119, 120, 123, 125, 131, 134, 136, 141, 144, 145, 158, 159, 162, 167; MS: *m/z* 496 (M^+).

7.XXXIII: 4-{1-[(5-[3-(1*H*-pyrazol-3-yl)phenyl]-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile

IR (KBr, cm^{-1}) 3265 (N–H), 3062 (Ar–C–H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C–N), 1242 (C–O–C).

^{1}H NMR ($CDCl_3$) δ ppm 5.126 (s, 2H, $–CH_2$), 6.62–9.54 (m, 13H, aromatic protons), 13.8(s, 1H NH), ^{13}C NMR ($CDCl_3$) δ ppm 49, 102, 112, 114, 119, 120, 126, 127, 128, 130, 131, 132, 134, 135, 136, 141, 144, 145, 149, 158, 164, 167; MS: *m/z* 488 (M^+).

In-vitro antiproliferative screening

MTT assay

The 33 compounds synthesized were screened for anticancer activity using the MTT assay technique according to the reported method [14–16]. To check the viability and antiproliferative activity of the compound, the aforementioned assays were performed. For assaying cytotoxicity (e.g. anticancer effect) in *in-vitro* cell culture experiments, generally the MTT assay is used. The MTT reduction assay is one of the most frequently used methods for measuring cell proliferation and cytotoxicity. The intensity of color (measured spectrophotometrically) of the MTT formazan produced by living, metabolically active cells is proportional to the number of live cells present. Cell viability was defined as the ratio (expressed as a percentage) of absorbance of treated cells to untreated cells. Values given represent the mean ± SDs of three independent experiments carried out in triplicate. The GI_{50} values were calculated as the concentration of test sample resulting in a 50% reduction of absorbance compared with untreated cells.

On the basis of the number of cells available in the cell suspension, the cells were diluted to obtain a cell concentration of 5000 cells/well in 100 µl of medium of L929, HCT15, and Hep2 in a 96-well plate. The cultures were suspended in the growth medium containing DMEM medium and 10% fetal bovine serum; the cells were incubated overnight in the incubator at 37°C and 5% CO_2. After 24 h incubation the medium was discarded from the plate. A volume of 20 µl of MTT was added to the control plates and incubated for 3–4 h. The other plates were treated with the diluted samples, 100, 10, 1 µmol/l, and 100 and 10 nmol/l, and control drug 5-fluorouracil, methotrexate, and daunorubicin. The triplicates were maintained for each dilution of every sample to all three cell lines. The cells were checked and observed every 24 h interval for contamination and cellular changes. The 48 h post-treated cells were treated with 20 µl of MTT and incubated for 3–4 h at 37°C. After 3 h incubation, the entire medium was replaced with 200 µl DMSO in the wells. The optical density was determined at 570 nm using the microplate reader.

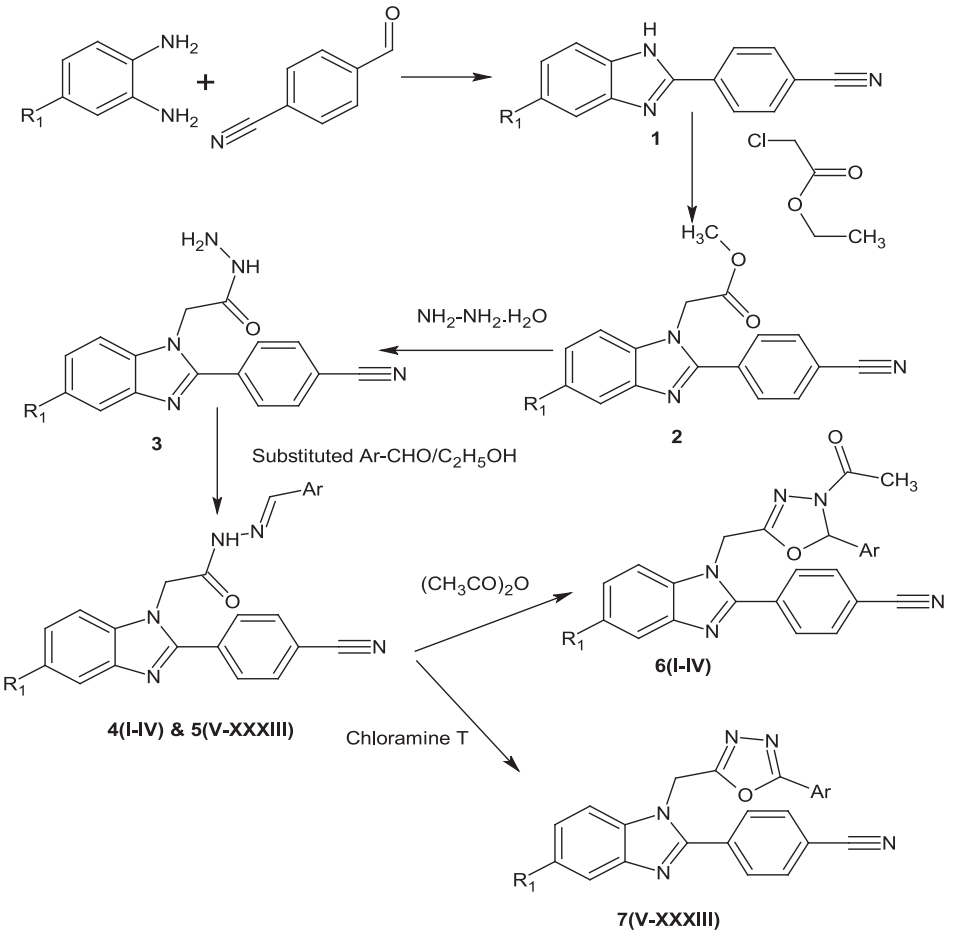
Result and discussion

The reaction sequence of the synthesis of the target compounds is outlined in scheme 1. 4-Chloro and 4-nitro-*O*-phenylenediamine were reacted with appropriately substituted 4-cyanobenzaldehydes in the presence of sodium metabisulfite to furnish the corresponding 2-(4-cyanophenyl)-1H-benzimidazoles **(1).** These substituted 2-(4-cyanophenyl)-1H-benzimidazoles were further treated with ethyl chloroacetate in KOH/DMSO to afford the *N*-alkylated product, (2-(4-cyanophenyl)-benzimidazol-1-yl)-acetic acid ethyl esters **(2).** The reaction between the hydrazine hydrate and the esters **(2)** led to the synthesis of 2-(4-cyanophenyl)-benzimidazol-1-yl)-acetic acid hydrazides **(3).** In addition, when a mixture of 2-(4-cyanophenyl)-benzimidazol-1-yl)-acetic acid hydrazides and respective aldehydes was allowed to react to generate imines intermediates **4 (I–IV)** and **5 (V–XXXIII)**, 4-{1-[(4-acetyl-5-(substituted)-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitriles **6 (I–IV)** were obtained through the reaction of the imine intermediates and excess acetic anhydride (Fig. 1). In addition, the reaction of imine intermediates in ethanol and chloramines-T afforded the products 7 **(V–XXXIII)** (Fig. 1).

In-vitro antiproliferative activity

The anticancer activity of all synthesized compounds was evaluated against L929, HCT15, and Hep2 cancer cell lines using the MTT method, and the GI_{50} values of all compounds were calculated. The GI_{50} values of all synthetic test compounds were

Figure 1



Synthetic route for the preparation of the compounds **6 (I–IV)** and **7 (V–XXXIII)**.

found to be between 0.8 and 22 μmol/l. The GI_{50} of these compounds was as good as that of the known anticancer agent 5-fluorouracil, methotrexate, and daunorubicin (GI_{50} value between 0.08 and 33 μmol/l). The new studies disclose that, among the human cancer cell lines experienced, HCT15 cells are to some extent more responsive to all tested compounds compared with L929 and Hep2 cells. Many anticancer drugs are powerful against HeLa, IMR-32, and MCF-7 cells [17]. The anticancer agent 5-fluorouracil, methotrexate, and daunorubicin causes cytotoxicity in L929, HCT15, and Hep2 cells by a similar mechanism.

Results indicate that the anticancer activity or cytotoxicity of derivatives varied with structural modification. Among the synthesized oxadiazole compounds, **7.XIX**, **7.XXIV**, and **7.X** with halogen atom, electronegative, hydroxyl and methoxy group (electron-releasing groups) at 2-OH,4,5-difluoro benzaldehyde derivative, 2-chloro,4,5-dimethoxy benzaldehyde derivative, and 4,5-dichloro benzaldehyde derivative showed the most potent activity compared with other synthesized compounds. From the literature review it has been revealed that strong electronegative atom exchange such as chloro/bromo at the C_5 position of the aromatic ring amplifies the lipophilicity of molecules and is reliable for improved cytotoxicity in MTT model [18]. Similar types of substitutions are present in the compounds **7.XIX**, **7.XXIV**, and **7.X.** We have also observed enhanced cytotoxicity in the molecules presented in Tables 1 and 2.

Conclusion

In the present study, it can be concluded that the cytotoxicity of oxadiazole 33 derivatives varies with structural changes. Compounds **7.X**, **7.XIX**, and **7.XXIV** displayed *in-vitro* anticancer activity in the primary MTT assay. Compound **7.XIX** confirms anticancer activity against L929, HCT15, and Hep2 cancer cell lines, which is comparable to that of 5-fluorouracil, methotrexate, and daunorubicin, and this compound seems to be the most potential one for anticancer activity.

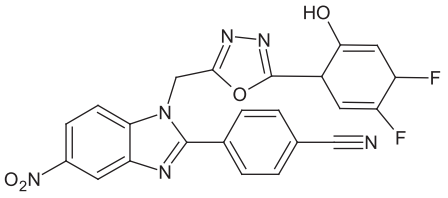
Table 1 Physical data of all synthesized test compounds 6 (I–IV) and 7 (V–XXXIII)

Compound codes	R_1	Ar	Molecular formula	Molecular weight	Yield (%)	Melting point (°C)	R_f value
6.I	$-NO_2$	fx1	$C_{25}H_{17}ClN_6O_4$	500.8	25	273–275	0.731
6.II	$-NO_2$	fx2	$C_{25}H_{17}ClN_6O_4$	500.8	30	270–272	0.727
6.III	$-NO_2$	fx3	$C_{25}H_{18}N_6O_6$	498.4	35	213–215	0.764
6.IV	$-NO_2$	fx4	$C_{25}H_{17}BrN_6O_4$	545.3	32	210–212	0.686
7.V	$-NO_2$	fx5	$C_{23}H_{13}ClN_6O_3$	456.8	30	269–271	0.77
7.VI	$-NO_2$	fx6	$C_{23}H_{13}ClN_6O_3$	456.8	26	274–276	0.729
7.VII	$-NO_2$	fx7	$C_{23}H_{15}N_7O_3$	437.4	20	243–245	0.731
7.VIII	$-NO_2$	fx8	$C_{23}H_{13}ClN_6O_3$	456.8	28	275–277	0.727
7.IX	–Cl	fx9	$C_{23}H_{13}BrClN_5O$	490.7	31	209–211	0.764
7.X	$-NO_2$	fx10	$C_{23}H_{12}Cl_2N_6O_3$	491.2	30	276–278	0.686
7.XI	$-NO_2$	fx11	$C_{23}H_{14}N_6O_5$	454.3	33	211–213	0.77
7.XII	$-NO_2$	fx12	$C_{23}H_{13}BrN_6O_3$	501.2	28	211–213	0.729
7.XIII	$-NO_2$	fx13	$C_{23}H_{13}BrN_6O_3$	501.2	20	183–185	0.731
7.XIV	$-NO_2$	fx14	$C_{24}H_{15}BrN_6O_3$	515.3	26	208–210	0.727
7.XV	$-NO_2$	fx15	$C_{24}H_{16}N_6O_3$	436.4	30	193–195	0.764
7.XVI	$-NO_2$	fx16	$C_{24}H_{16}N_6O_4$	452.4	35	223–225	0.686
7.XVII	$-NO_2$	fx17	$C_{23}H_{12}BrN_7O_5$	546.2	23	256–258	0.77
7.XVIII	$-NO_2$	fx18	$C_{23}H_{13}BrN_6O_4$	517.2	31	243–245	0.729
7.XIX	$-NO_2$	fx19	$C_{23}H_{12}F_2N_6O_4$	474.3	20	254–256	0.731
7.XX	$-NO_2$	fx20	$C_{23}H_{12}Cl_2N_6O_3$	491.2	36	285–287	0.727
7.XXI	$-NO_2$	fx21	$C_{23}H_{12}ClN_7O_5$	501.8	33	276–278	0.764
7.XXII	$-NO_2$	fx22	$C_{24}H_{12}BrF_3N_6O_3$	569.2	25	252–254	0.686
7.XXIII	$-NO_2$	fx23	$C_{23}H_{12}ClN_7O_5$	501.8	31	263–265	0.77
7.XXIV	$-NO_2$	fx24	$C_{25}H_{17}ClN_6O_5$	516.8	30	224–226	0.729
7.XXV	$-NO_2$	fx25	$C_{26}H_{20}N_6O_5$	496.4	19	214–216	0.731
7.XXVI	$-NO_2$	fx26	$C_{27}H_{20}N_6O_6$	524.4	20	219–221	0.727
7.XXVII	$-NO_2$	fx27	$C_{26}H_{19}BrN_6O_4$	559.3	25	223–225	0.764
7.XXVIII	$-NO_2$	fx28	$C_{28}H_{20}N_6O_5$	520.4	23	222–224	0.686
7.XXIX	$-NO_2$	fx29	$C_{25}H_{18}N_6O_6$	498.4	19	212–214	0.77
7.XXX	$-NO_2$	fx30	$C_{23}H_{12}N_8O_8$	528.3	34	262–264	0.729
7.XXXI	$-NO_2$	fx31	$C_{31}H_{30}N_6O_4$	550.6	19	221–223	0.731
7.XXXII	$-NO_2$	fx32	$C_{26}H_{18}N_6O_4$	478.4	36	226–228	0.727
7.XXXIII	$-NO_2$	fx33	$C_{26}H_{16}N_8O_3$	488.4	37	222–224	0.764

Table 2 Antiproliferative activity of synthesized compounds against HCT15, Hep2, and L929 cancer cells using the MTT assay

Compound codes	R_1	Ar	GI_{50} (μmol/l) (HCT15)	GI_{50} (μmol/l) (Hep2)	GI_{50} (μmol/l) (L929)
7.VIII	$-NO_2$	fx34	25	35	80
7.IX	–Cl	fx35	60	>100	40
7.X	$-NO_2$	fx36	8	15	68
7.XIX	$-NO_2$	fx37	0.8	9	22
7.XX	$-NO_2$	fx38	>100	>100	>100
7.XXII	$-NO_2$	fx39	>100	>100	60
7.XXIV	$-NO_2$	fx40	6.5	50	>100
7.XXV	$-NO_2$	fx41	40	>100	>100
7.XXIX	$-NO_2$	fx42	60	>100	>100
5-Fluorouracil			30	33	25
Methotrexate			0.9	0.08	4
Daunorubicin			5	4	0.2

Values are expressed as means ($n = 4$);
MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide.



7(XIXX)

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Conflicts of interest

There are no conflicts of interest.

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