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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and anticancer activity study of indolyl hydrazide–hydrazones

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Abstract A series of *N*′-((1-(substituted)-1*H*-indol-3-yl)methylene)hydrazides were synthesized and evaluated for their in vitro antiproliferative activities against various cancer cell lines. Formation of indole hydrazide–hydrazones was accomplished by the reaction of indole 3-carboxaldehyde with aryl/alkyl hydrazides in the presence of acetic acid. Out of synthesized twenty-two compounds, some of the analogs exhibited specificity toward breast (**18b**, **18d**, **18f** and **18j)** and prostate (**18t** and **18v**) cancer cells. Among the prepared derivatives, compounds **18b, 18d** and **18j** were most cytotoxic (IC_{50} = 0.9, 0.4 and 0.8 μM, respectively) against the screened cancer cell lines. Exposure of PC3 cells to either **18d** or **18j** resulted in increased levels of cleaved PARP1, indicating that indolyl hydrazide–hydrazones induce apoptosis in PC3 cells.

Keywords Antiproliferative activity · Indole hydrazide–hydrazones · MTT assay

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Introduction

Indole is an important pharmacophore found in a large number of molecules with significant biological activity including anti-inflammatory, anticancer, antinociceptive, antipsychotic, antileishmanial and antiviral activities (Adam *et al*., 2010; Choppara *et al*., 2015; Gupta *et al*., 2007; Zhang *et al*., 2015). Since indole scaffolds have shown promising results in anticancer drug discovery research, they have constantly garnered the attention of researchers (El-Sayed *et al*., 2014; Kamal *et al*., 2012; Panathur *et al*., 2013; Singh *et al*., 2009). Literature survey revealed that in the recent past, several indole-based synthetic as well as natural products exhibited potent anticancer activity. For example, natural products camalexin (**1**), labradorins (**2**), nortopsentins (**3**), topsentins (**4**) and meridianin (**5**), isolated from *Arabidopsis thaliana, Pseudomonas syringae pv. Coronafaciens, Spongosorites ruetzleri, Topsentia genitrix* and *Aplidium meridianum,* respectively, are found to be effective against various cancer cell lines (Diana *et al*., 2010; Kumar *et al*., 2014; Pettit *et al*., 2002). Recently, many indole-containing small molecules have been identified as potent anticancer agents. The 3-aroylindole (**6**) and indibulin (**7**) are endowed with interesting anticancer activity via modulation of tubulin polymerization in clinical trials (Brancale and Silvestri, 2007; Wienecke and Bacher, 2009). Similarly, 2-aryl-4-benzoylimidazole (**8**) showed anticancer activity through the inhibition of tubulin polymerization with typical IC_{50} values around 3.8 nm (Chen *et al*., 2012). Moreover, Silvestri and coworkers described 2-heteroarylthioindole (**9**) as tubulin-targeting anticancer agent with IC_{50} = 1.0 nM in MCF-7 cells (Fig. 1) (La Regina *et al*., 2012).

On the other hand, hydrazide–hydrazones have been received significant attention due to their diverse biological

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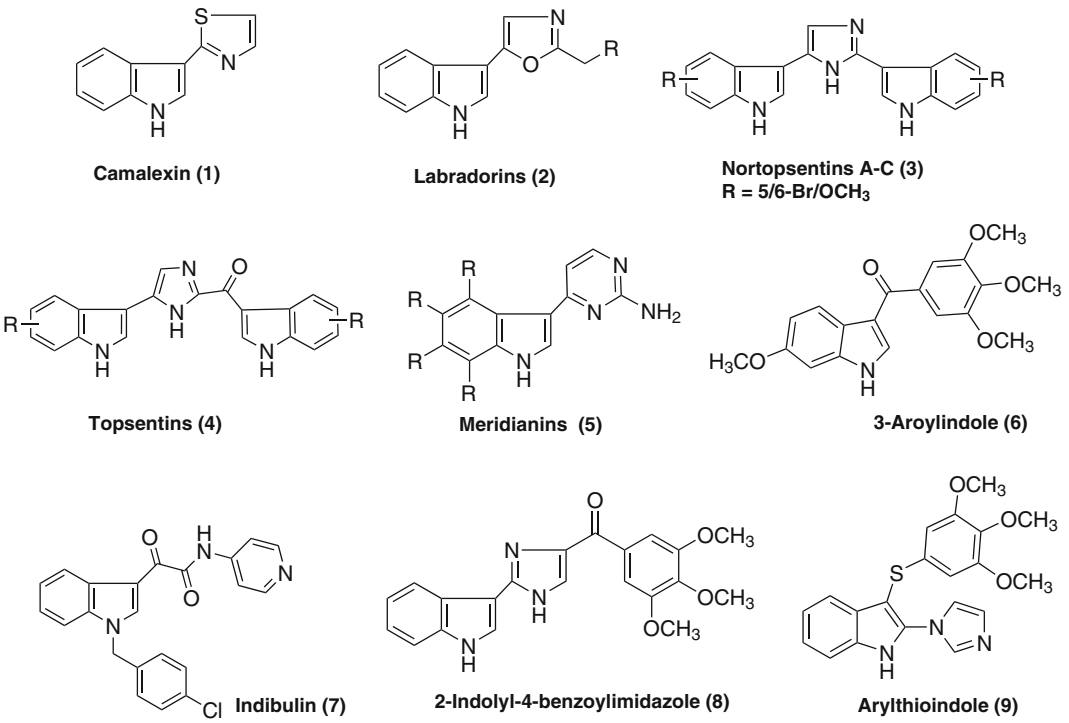


Fig. 1 Representative indole-based anticancer agents

properties including anticancer, antimicrobial, anticonvulsant, anti-inflammatory, antimalarial and antitubercular (Nasr *et al*., 2014). Literature survey revealed that the hydrazide–hydrazone moiety plays a crucial role in anticancer activity of nitrogen-containing heterocycles (Salum *et al*., 2015).

In the recent past, several functionalized indoles were found to be effective against various cancer cells. The *N*-4-chlorobenzyl-3-formylindole, oncrasin-1 (**10**) (Wu *et al*., 2011), was found to be effective against K-Ras mutant cancer cells. Our studies on the conversion of indole to indolyl chalcones (**11**) (Kumar *et al*., 2010a) and bis(indolyl)hydrazide–hydrazones (**13**) (Kumar *et al*., 2012b) showed promising activity against a panel of cancer cell lines. Also, Sato et al. isolated rhopaladins A ~ D (**12**) from marine tunicate *Rhopalaea sp.* with inhibitory activity against CDK4 (IC_{50} = 12.5 μg/mL) and c-erbβ-2 (IC_{50} = 7.4 μg/mL) kinases (Sato *et al*., 1998). Recently, we have also synthesized several indole-based small molecules as promising anticancer agents. For example, 5-(3′-indolyl)-1,3,4-thiadiazoles (Kumar *et al*., 2010b), 5-(3′-indolyl)-1,3,4-oxadiazoles (Kumar *et al*., 2009), indolyl-1,2,4-triazoles (Kumar *et al*., 2011), 2-arylamino(indolyl)-1,3,4-thiadiazoles (Kumar *et al*., 2012a), 2-arylamino-5-(3′-indolyl)-1,3,4-oxadiazoles (Tantak *et al*., 2013), α-cyano bis(indolyl)chalcones (Kumar *et al*., 2014). Keeping in view the aforementioned anticancer potency of functionalized indoles and hydrazide–hydrazones, we have designed and synthesized indolyl hydrazide–hydrazones by tethering indole with hydrazide–hydrazone moiety into a single molecule (Fig. 2).

Taking cue from the literature, we have attempted to synthesize a series of hydrazide–hydrazones that derive from the reaction of indole 3-carboxaldehyde (**16**) with various aromatic/aliphatic acid hydrazides (**17**) (Scheme 1). Initially, *N*-alkyl indoles **15** were prepared from the reaction of indole (**14**) with alkyl halides in the presence of a base (Kumar *et al*., 2012c). Subsequently, treatment of **15** with phosphorus oxychloride in dimethylformamide led to 3-carboxaldehyde (**16**) (James and Snyder, 1959). On the other hand, aromatic/aliphatic acid hydrazides **17** were prepared by following the literature procedures (Palace-Berl *et al*., 2013; Sadek *et al*., 2014). Synthesized hydrazide–hydrazones **18a–v** were studied for their *in vitro* antiproliferative activities in different cancer cell lines: prostate (PC3, DU145 and LnCaP), breast (MCF-7 and MDA-MB-231) and pancreas (PaCa2). Also, in an attempt to study the role of indolyl *N*–H, we substituted the proton at first position of indole ring with methyl (**18m–q**) and *p*-chlorobenzyl moieties (**18r–v**).

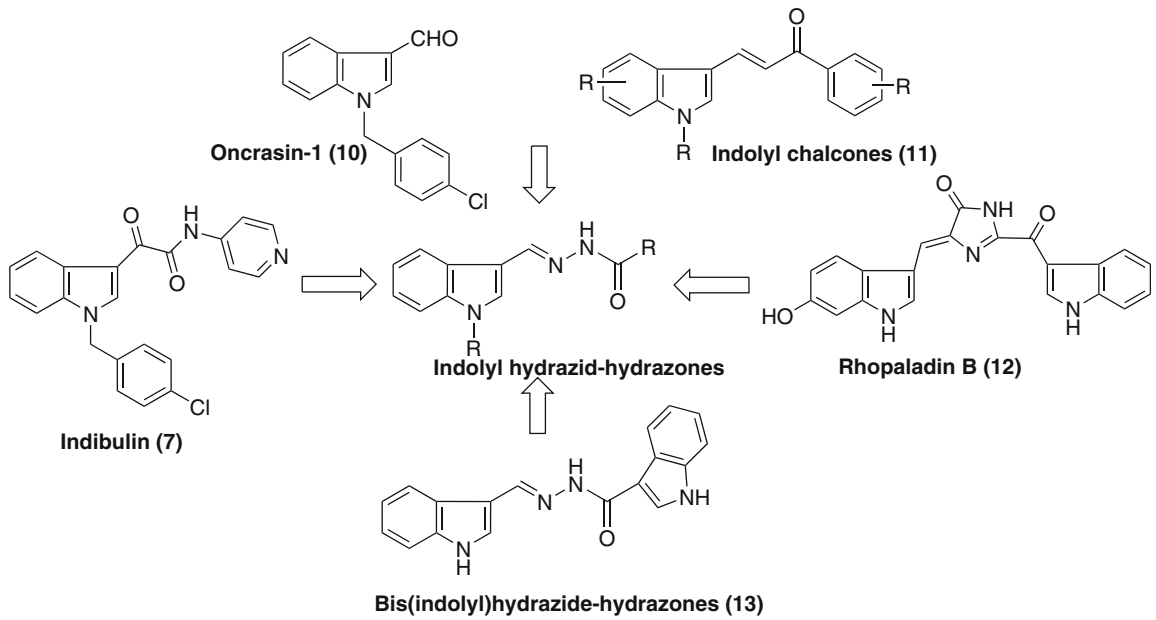
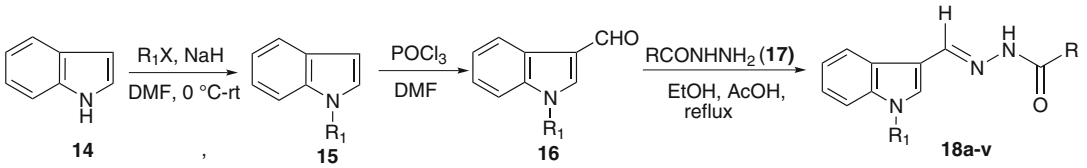


Fig. 2 Rational design for indolyl hydrazide–hydrazones



Scheme 1 Synthesis of a library of indolyl hydrazide–hydrazones **18a–v**

Materials and methods

Chemistry

Infrared (IR) spectra were recorded on a Jasco FTIR-4100 spectrometer in the wave number range of 4000–400 cm^{-1}. Nuclear magnetic resonance (^1H and ^{13}C) spectra were recorded on a Bruker AM-400 MHz spectrometer using $CDCl_3$ or DMSO-d_6 as solvent and tetramethylsilane as an internal standard. The chemical shifts are expressed in δ values and the following abbreviations are used: *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet) and *m* (multiplet). Purity of the crude compounds was qualitatively analyzed by thin-layer chromatography (TLC). Silica gel column chromatography was performed using Merck® silica gel (60–120 mesh). All the reagents and solvents were purchased from Spectrochem® or Sigma-Aldrich®.

*General procedure for the synthesis of N′-((1-(substituted)-1H-indol-3-yl)methylene)hydrazides (**18a–v**)*

Indole-3-aldehyde **16** (1 mmol) and the corresponding alkyl/aryl acid hydrazide **17** (1.05 mmol) were refluxed in ethanol (5 mL) in the presence of glacial acetic acid (0.3 mL) for 4 h. On cooling the reaction mixture to room temperature, the crude product was precipitated, filtered and dried. Further recrystallization of the crude products in ethanol allowed to obtain pure products in 85–95 % yields.

*N′-((1H-Indol-3-yl)methylene)acetohydrazide (**18a**)* White solid, yield 85 %; FTIR (KBr) νmax 3210, 3101, 1605, 1574, 1444, 1354 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 2.12$ (s, 3H, CH_3), 7.31–7.34 (m, 2H, Ar–H), 7.42 (d, 1H, $J = 7.3$ Hz, Ar–H), 7.49 (d, 1H, $J = 2.6$ Hz, Ar–H), 7.96 (s, 1H, azomethine-CH), 8.09 (d, 1H, $J = 7.3$ Hz, Ar–H), 9.69 (s, 1H, hydrazine-NH), 11.27 (brs, 1H, NH); ^{13}C NMR ($CDCl_3$, 101 MHz) $\delta = 171.73$ (CONH), 155.22 (C=NH), 137.50 (C, C-8), 131.16 (C, C-9), 125.08 (CH, C-2), 122.80 (CH, C-6), 122.47 (CH, C-4), 120.71 (CH, C-5), 112.55 (CH, C-7), 111.99 (C, C-3), 20.73 (CH_3); LCMS m/z (pos) 201.1 $C_{11}H_{11}N_3O$ (calcd 201.0); HRMS m/z (pos); 201.0910 $C_{11}H_{11}N_3O$ (calcd. 201.0902).

*N′-((1H-Indol-3-yl)methylene)-2,2,2-trifluoroacetohydrazide (**18b**)* White solid, yield 87 %; FTIR (KBr) νmax 3214, 3107, 1608, 1577, 1454, 1360 cm^{-1}; ^1H NMR ($CDCl_3$,

400 MHz): $\delta = 7.26–7.29$ (m, 2H, Ar–H), 7.47 (d, 1H, $J = 7.2$ Hz, Ar–H), 7.52 (d, 1H, $J = 2.4$ Hz, Ar–H), 7.99 (d, 1H, $J = 7.2$ Hz, Ar–H), 8.02 (s, 1H, azomethine-CH), 9.69 (s, 1H, hydrazine-NH), 11.32 (brs, 1H, NH); ^{13}C NMR ($CDCl_3$, 101 MHz): $\delta = 154.81$ (CONH), 134.90 (C, C-8), 134.39 (C, C-2), 131.42 (C, C-9), 127.19 (CH, C-6), 121.26 (CH, C-4), 120.32 (CH, C-5), 114.13 (CF_3, q), 113.26 (CH, C-7), 105.72 (C, C-3); HRMS m/z (pos): 256.0912 $C_{11}H_9F_3N_3O$ (calcd 256.0698).

N′-((1H-Indol-3-yl)methylene)benzohydrazide (***18c***) White solid, yield 89 %, mp 239–241 °C; FTIR (KBr) νmax 3209, 3040, 1605, 1574, 1443, 1358, 1319, 1250 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.16–7.18$ (m, 5H, Ar–H), 7.27–7.30 (m, 2H, Ar–H), 7.38 (d, 1H, $J = 7.4$ Hz, Ar–H), 7.50 (d, 1H, $J = 2.4$ Hz, Ar–H), 8.05 (s, 1H, azomethine-CH), 8.09 (d, 1H, $J = 7.4$ Hz, Ar–H), 9.71 (s, 1H, hydrazine-NH), 11.29 (brs, 1H, NH); ^{13}C NMR (DMSO-d_6, 101 MHz): $\delta = 163.01$ (CONH), 145.44 (C=NH), 137.51 (CH, C-8), 134.54 (C, C-9), 131.79 (C, C-1′), 130.81 (CH, C-4′), 128.87 (CH, C-3′, C-5′), 127.95 (CH, C-2′, C-6′), 124.83 (CH, C-2), 123.11 (CH, C-5), 122.52 (CH, C-6), 120.87 (CH, C-4), 112.29 (CH, C-7), 112.20 (C, C-3); HRMS m/z (pos): 264.1421 $C_{16}H_{14}N_3O$ (calcd 264.1137).

N′-((1H-Indol-3-yl)methylene)-4-chlorobenzohydrazide (***18d***) White solid, yield 87 %, mp 226–227 °C; FTIR (KBr) νmax 3340, 3194, 1605, 1558, 1450, 1358, 1103, 758 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.12–7.14$ (m, 4H, Ar–H), 7.29–7.32 (m, 2H, Ar–H), 7.38 (d, 1H, $J = 7.2$ Hz, Ar–H), 7.49 (d, 1H, $J = 2.4$ Hz, Ar–H), 8.02 (s, 1H, azomethine-CH), 8.07 (d, 1H, $J = 7.2$ Hz, Ar–H), 9.79 (s, 1H, hydrazine-NH), 11.35 (brs, 1H, NH); ^{13}C NMR (DMSO-d_6, 101 MHz): $\delta = 161.87$ (CONH), 145.75 (C=NH), 137.51 (C, C-8), 136.58 (C, C-4′), 133.23 (C, C-1′), 130.99 (C, C-9), 129.88 (CH, C-3′, C-5′), 128.96 (CH, C-2′, C-6′), 124.80 (CH, C-2), 123.12 (CH, C-5), 122.49 (CH, C-6), 120.90 (CH, C-4), 112.30 (CH, C-7), 112.12 (C, C-3); HRMS m/z (pos): 298.1202 $C_{16}H_{13}ClN_3O$ (calcd 298.0747).

N′-((1H-Indol-3-yl)methylene)-4-methoxybenzohydrazide (***18e***) White solid, yield 92 %, mp 253–255 °C; FTIR (KBr) νmax 3240, 3115, 1605, 1558, 1512, 1450, 1358, 1296, 1265 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.62$ (s, 3H, OCH_3), 7.18–7.21 (m, 4H, Ar–H), 7.34–7.36 (m, 2H, Ar–H), 7.48 (d, 1H, $J = 7.4$ Hz, Ar–H), 7.54 (d, 1H, $J = 2.3$ Hz, Ar–H), 8.00 (d, 1H, $J = 7.4$ Hz, Ar–H), 8.10 (s, 1H, azomethine-CH), 9.79 (s, 1H, hydrazine-NH), 11.26 (brs, 1H, NH); ^{13}C NMR (DMSO-d_6, 101 MHz): $\delta = 162.44$ (CONH), 162.16 (C, C-4′), 144.88 (C=NH), 137.49 (C, C-8), 130.55 (C, C-9), 129.78 (CH, C-2′, C-6′), 126.57 (C, C-1′), 124.84 (CH, C-2), 123.06 (CH, C-5), 122.52 (CH, C-6), 120.80 (CH, C-4), 114.10 (CH, C-3′, C-5′), 112.29 (CH, C-7), 112.26 (C, C-3), 55.86 (CH_3, OCH_3); HRMS m/z (pos): 294.1204 $C_{17}H_{16}N_3O_2$ (calcd 294.1243).

N′-((1H-Indol-3-yl)methylene)-2-phenylacetohydrazide (***18f***) White solid, yield 89 %; ^{1}H NMR ($CDCl_3$400, MHz): $\delta = 4.24$ (s, 2H, CH_2, Ar–H), 7.19–7.21 (m, 5H, Ar–H), 7.32–7.34 (m, 2H, Ar–H), 7.44 (d, 1H, $J = 7.2$ Hz, Ar–H), 7.52 (d, 1H, $J = 2.6$ Hz, Ar–H), 8.02 (d, 1H, $J = 7.2$ Hz, Ar–H), 8.10 (s, 1H, azomethine-CH), 9.74 (s, 1H, hydrazine-NH), 11.35 (brs, 1H, NH); ^{13}C NMR ($CDCl_3$, 101 MHz): $\delta = 168.61$ (CONH), 134.84 (C=NH), 134.81 (C, C-8), 134.54 (C, C-1′), 133.72 (C, C-9), 129.45 (CH, C-2′, C-6′), 129.18 (CH, C-3′, C-5′), 128.42 (CH, C-4′), 127.16 (CH, C-2), 121.39 (CH, C-5), 120.77 (CH, C-6), 118.42 (CH, C-4), 113.45 (CH, C-7), 105.72 (C, C-3), 40.81 (CH_2, CH_2Ph); LCMS m/z (pos): 277.2 $C_{17}H_{15}N_3O$: (calcd 277.1); HRMS m/z (pos): 277.1231 $C_{17}H_{15}N_3O$ (calcd 277.1221).

N′-((1H-Indol-3-yl)methylene)-3,4-dimethoxybenzohydrazide (***18g***) White solid, yield 90 %; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.62$ (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 7.25–7.27 (m, 3H, Ar–H), 7.38–7.40 (m, 2H, Ar–H), 7.43 (d, 1H, $J = 7.2$ Hz, Ar–H), 7.50 (d, 1H, $J = 2.6$ Hz, Ar–H), 8.04 (s, 1H, azomethine-CH), 8.10 (d, 1H, $J = 7.2$ Hz, Ar–H), 9.68 (s, 1H, hydrazine-NH), 11.35 (brs, 1H, NH); ^{13}C NMR (DMSO-d_6, 101 MHz): $\delta = 166.11$ (CONH), 152.06 (C, C-4′), 148.74 (C, C-3′), 136.49 (C=NH), 128.70 (C, C-8), 126.77 (C, C-9), 125.43 (C, C-1′), 124.07 (CH, C-2), 122.58 (CH, C-5), 121.36 (CH, C-6), 121.32 (CH, C-6′), 121.12 (CH, C-4), 112.41 (CH, C-7), 111.47 (CH, C-2′), 111.09 (CH, C-5′), 108.70 (C, C-3), 56.09 (CH_3, OCH_3), 56.03 (CH_3, OCH_3); HRMS m/z (pos): 324.1321 $C_{18}H_{18}N_3O_3$ (calcd 324.1348).

N′-((1H-Indol-3-yl)methylene)-4-(hydroxy)-3-methoxybenzohydrazide (***18h***) White solid, yield 93 %; FTIR (KBr) νmax 3320, 3154, 1606, 1554, 1358, 1268, 1140 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.78$ (s, 3H, OCH_3), 7.25–7.28 (m, 3H, Ar–H), 7.32–7.35 (m, 2H, Ar–H), 7.44 (d, 1H, $J = 7.5$ Hz, Ar–H), 7.52 (d, 1H, $J = 2.6$ Hz, Ar–H), 8.00 (d, 1H, $J = 7.5$ Hz, Ar–H), 8.09 (s, 1H, azomethine-CH), 9.72 (s, 1H, hydrazine-NH), 11.31 (brs, 1H, NH); ^{13}C NMR ($CDCl_3$, 101 MHz): $\delta = 164.25$ (CONH), 150.12 (C, C-4′), 149.09 (C, C-3′), 134.80 (C=NH), 134.51 (C, C-8), 133.02 (C, C-9), 127.12 (C, C-1′), 126.03 (CH, C-2), 125.00 (CH, C-6′), 121.37 (CH, C-5), 120.72 (CH, C-6), 118.39 (CH, C-4), 114.37 (CH, C-2′), 113.21 (CH, C-7), 112.42 (CH, C-5′), 105.68 (C, C-3), 56.80 (OCH_3); HRMS m/z (pos): 310.1231 $C_{17}H_{16}N_3O_3$ (calcd 310.1192).

N′-((1H-Indol-3-yl)methylene)-4-(benzyloxy)-3-methoxybenzohydrazide (***18i***) White solid, yield 87 %; ^{1}H NMR

($CDCl_3$, 400 MHz): $\delta = 3.60$ (s, 3H, OCH_3), 5.10 (s, 2H, CH_2), 7.19–7.22 (m, 3H), 7.29–7.34 (m, 2H), 7.36–7.39 (m, 5H, ArH), 7.42 (d, 1H, $J = 7.3$ Hz), 7.49 (d, 1H, $J = 2.4$ Hz), 8.07 (s, 1H, azomethine-CH), 8.10 (d, 1H, $J = 7.3$ Hz), 9.69 (s, 1H, hydrazine-NH), 11.29 (brs, 1H, NH); ^{13}C NMR ($CDCl_3$, 101 MHz): $\delta = 167.91$ (CONH), 155.32 (C, C-4′), 153.91 (C, C-3′), 149.59 (C, C=NH), 142.00 (C, C-8), 141.39 (C, C-9), 133.54 (C, C-1″), 133.37 (CH, C-3″, C-5″), 132.82 (CH, C-2″, C-6″), 132.27 (CH, C-4″), 131.86 (C, C-1′), 129.64 (CH, C-2), 127.55 (CH, C-5), 127.05 (CH, C-6), 125.49 (CH, C-4), 125.45 (CH, C-6′), 117.42 (CH, C-7), 116.96 (CH, C-4), 116.52 (CH, C-6′), 116.36 (C, C-3), 75.34 (CH_2, OCH_2Ph), 60.83 (CH_3, OCH_3); HRMS m/z (pos): 400.1621 $C_{24}H_{22}N_3O_3$ (calcd 400.1661).

N′-((1H-Indol-3-yl)methylene)-3,4,5-trimethoxybenzohydrazide (***18j***) White solid, yield 95 %, mp 231–232 °C; FTIR (KBr) νmax 3271, 3163, 2932, 1612, 1582, 1551, 1458, 1335, 1234, 1126, 764 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.62$ (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 7.28 (s, 1H, Ar–H), 7.32 (s, 1H, Ar–H), 7.36–7.39 (m, 2H, Ar–H), 7.45 (d, 1H, $J = 7.4$ Hz, Ar–H), 7.54 (d, 1H, $J = 2.4$ Hz, Ar–H), 7.99 (d, 1H, $J = 7.4$ Hz, Ar–H), 8.08 (s, 1H, azomethine-CH), 9.72 (s, 1H, hydrazine-NH),; ^{13}C NMR (DMSO-d_6, 101 MHz): $\delta = 162.44$ (CONH), 153.13 (2C, C-3′, C-5′), 145.54 (C, C-4′), 140.52 (C, C=NH), 137.51 (C, C-8), 130.75 (C, C-9), 129.69 (CH, C-2), 124.82 (CH, C-5), 123.11 (CH, C-6) 122.45 (CH, C-4), 120.87 (CH, C-1′), 112.31 (CH, C-7), 112.15 (C, C-2′, C-6′), 105.48 (C, C-3), 60.58 (CH_3, OCH_3), 56.53 (CH_3, $2OCH_3$); LCMS m/z (pos): 353.2 $C_{19}H_{19}N_3O_4$ (calcd 353.1); HRMS m/z (pos): 400.1621 $C_{24}H_{22}N_3O_3$ (calcd 400.1661).

N′-((1H-Indol-3-yl)methylene)isonicotinohydrazide (***18k***) White solid, yield 91 %, mp 208–210 °C; FTIR (KBr) νmax 3340, 3194, 1605, 1558, 1450, 1358, 1103, 756 cm^{-1}; ^{1}H NMR ($CDCl_3$,400 MHz): $\delta = 7.21$–7.23 (m, 2H, Ar–H), 7.27–7.29 (m, 4H, Ar–H), 7.41 (d, 1H, $J = 7.2$ Hz, Ar–H), 7.49 (d, 1H, $J = 2.3$ Hz, Ar–H), 7.94 (d, 1H, $J = 7.2$ Hz, Ar–H), 8.05 (s, 1H, azomethine-CH), 9.63 (s, 1H, hydrazine-NH); ^{13}C NMR (DMSO-d_6, 101 MHz,): $\delta = 161.31$(CONH), 150.70 (CH, C-3′, C-5′), 149.90 (C, C-1′), 146.60 (C, C=NH), 141.54 (C, C-8), 137.53 (C, C-9), 131.39 (CH, C-2), 124.77 (CH, C-2′, C-6′), 123.35 (CH, C-5), 123.18 (CH, C-6), 122.45 (CH, C-4), 112.34 (CH, C-7), 111.96 (C, C-3); HRMS m/z (pos): 265.1092 $C_{15}H_{13}N_4O$ (calcd. 265.1089).

N′-((1H-Indol-3-yl)methylene)nicotinohydrazide (***18l***) White solid, yield 89 %, mp 150–152 °C; FTIR (KBr) νmax 3433, 3209, 1605, 1551, 1450, 1366, 1250, 1119, 795 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.24$–7.26 (m, 2H, Ar–H), 7.29–7.31 (m, 4H, Ar–H), 7.38 (d, 1H, $J = 7.4$ Hz, Ar–H), 7.52 (d, 1H, $J = 2.6$ Hz, Ar–H), 7.97 (d, 1H, $J = 7.4$ Hz, Ar–H), 8.12 (s, 1H, azomethine-CH), 9.68 (s, 1H, hydrazine-NH), indole NH was not observed; ^{13}C NMR (DMSO-d_6,101 MHz): $\delta = 161.46$ (CONH), 152.37 (CH, C-4′), 148.95 (CH, C-2′), 146.04 (C, C=NH), 137.52 (CH, C-2), 135.75 (C, C-8), 131.18 (C, C-9), 130.21 (C, C-1′), 124.79 (CH, C-6′), 124.02 (CH, C-5′), 123.15 (CH, C-5), 122.47 (CH, C-6), 120.95 (CH, C-4), 112.33 (CH, C-7), 112.02 (C, C-3); HRMS m/z (pos): 265.1103 $C_{15}H_{13}N_4O$ (calcd. 265.1089).

N′-((1-Methyl-1H-indol-3-yl)methylene)acetohydrazide (***18m***) White solid, yield 91 %; FTIR (KBr) νmax 3194, 1605, 1566, 1381, 1327, 1196, 748 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz,): $\delta = 2.47$ (s, 3H, CH_3), 3.89 (s, 3H, N-CH_3), 7.27–7.32 (m, 2H, Ar–H), 7.47 (d, 1H, $J = 7.3$ Hz, Ar–H), 7.52 (s, 1H, Ar–H), 7.92 (d, 1H, $J = 7.3$ Hz, Ar–H), 8.08 (s, 1H, azomethine-CH), 9.71 (s, 1H, hydrazine-NH); ^{13}C NMR (DMSO-d_6, 101 MHz): $\delta = 155.14$ (CONH), 138.19 (C, C-8), 135.82 (C, C-9), 125.61 (CH, C-2), 123.20 (CH, C-6), 122.71 (CH, C-4), 121.30 (CH, C-5), 111.56 (CH, C-7), 110.80 (C, C-3), 33.37 (N-CH_3), 20.90 (CH_3); HRMS m/z (pos): 216.1102 $C_{15}H_{13}N_4O$ (calcd. 216.1137).

2,2,2-Trifluoro-N′-((1-methyl-1H-indol-3-yl)methylene)acetohydrazide (***18n***) White solid, yield 94 %; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.91$ (s, 3H, CH_3), 7.24–7.28 (m, 2H, Ar–H), 7.40 (d, 1H, $J = 7.4$ Hz, Ar–H), 7.49 (s, 1H, Ar–H), 8.04 (s, 1H, azomethine-CH), 8.11 (d, 1H, $J = 7.4$ Hz, Ar–H), 9.74 (s, 1H, hydrazine-NH); ^{13}C NMR ($CDCl_3$, 101 MHz): $\delta = 153.97$ (CONH), 137.63 (C, C-8), 134.98 (C, C-9), 125.41 (CH, C-2), 124.58 (CH, C-6), 123.00 (CH, C-4), 122.03 (CH, C-5), 121.21 (C, C-CF_3, q), 110.55 (CH, C-7), 109.72 (C, C-3), 33.15 (CH_3, N-CH_3); HRMS m/z (pos): 270.0921 $C_{12}H_{11}F_3N_3O$ (calcd. 270.0854).

4-(Benzyloxy)-3-methoxy-N′-((1-methyl-1H-indol-3-yl)methylene)benzohydrazide (***18o***) White solid, yield 89 %; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.52$ (s, 3H, OCH_3), 3.89 (s, 3H, CH_3), 5.14 (s, 2H, CH_2), 7.14–7.16 (m, 2H, Ar–H), 7.19–7.23 (m, 2H, Ar–H), 7.27 (d, 1H, $J = 7.4$ Hz, Ar–H), 7.34–7.38 (m, 5H, Ar–H), 7.42 (s, 1H, Ar–H), 7.49 (s, 1H, Ar–H), 8.09 (d, 1H, $J = 7.4$ Hz, Ar–H), 8.12 (s, 1H, azomethine-CH), 9.63 (s, 1H, hydrazine-NH); ^{13}C NMR (DMSO-d_6, 101 MHz): $\delta = 162.44$ (CONH), 150.82 (C, C-4′), 149.09 (C, C-3′), 144.44 (C, C=NH), 137.99 (CH, C-2), 137.19 (C, C-1″), 134.17 (C, C-8), 128.93 (C, C-9), 128.44 (CH, C-3″, C-5″), 128.36 (CH, C-2″, C-6″), 126.89 (CH, C-4″), 125.22 (C, C-1′), 123.14 (CH, C-6), 122.58 (CH, C-5), 121.07 (CH, C-4), 121.05 (CH, C-6′), 113.00 (CH, C-2′), 111.54 (CH, C-5′),

111.26 (CH, C-7), 110.65 (C, C-3), 70.29 (CH_2, –OCH_2–), 56.14 (CH_3, OCH_3), 33.24 (CH_3, N-CH_3); HRMS m/z (pos): 414.1810 $C_{25}H_{24}N_3O_3$ (calcd. 414.1818).

4-Chloro-N′-((1-methyl-1H-indol-3-yl)methylene)benzohydrazide ***(18p)*** Pale yellow solid, yield 90 %, mp 275–277 °C; FTIR (KBr) νmax 3194, 1605, 1565, 1381, 1327, 748, cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.87$ (s, 3H, CH_3), 7.15–7.19 (m, 2H, Ar–H), 7.22–7.25 (m, 2H, Ar–H), 7.31–7.33 (m, 2H, Ar–H), 7.40 (d, 1H, $J = 7.3$ Hz, Ar–H), 7.49 (s, 1H, Ar–H), 7.97 (d, 1H, $J = 7.3$ Hz, Ar–H), 8.09 (s, 1H, azomethine-CH), 9.69 (s, 1H, hydrazine-NH); ^{13}C NMR (DMSO-d_6, 101 MHz): $\delta = 161.83$ (CONH), 145.19 (C=NH), 138.02 (C, C-4′), 136.60 (C, C-8), 134.58 (C, C-9), 133.18 (C, C-1′), 129.86 (CH, C-3′, C-5′), 128.95 (CH, C-2′, C-6′), 125.18 (CH, C-2), 123.18 (C, C-6), 122.58 (CH, C-5), 121.16 (CH, C-4), 111.11 (CH, C-7), 110.68 (C, C-3), 33.27 (CH_3, N-CH_3); HRMS m/z (pos): 312.0913 $C_{17}H_{15}ClN_3O$ (calcd. 312.0904).

3,4,5-Trimethoxy-N′-((1-methyl-1H-indol-3-yl)methylene)benzohydrazide ***(18q)*** Pale yellow solid, yield 95 %, mp 224–226 °C; FTIR (KBr) νmax 3047, 1582, 1504, 1466, 1335, 1126, 748 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.68$ (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.83 (s, 3H, CH_3), 7.27 (s, 1H, Ar–H), 7.30 (s, 1H, Ar–H), 7.42–7.45 (m, 2H, Ar–H), 7.52 (s, 1H, Ar–H), 7.72–7.79 (m, 2H, Ar–H), 7.99 (s, 1H, azomethine-CH), 9.77 (s, 1H, hydrazine-NH); ^{13}C NMR (DMSO-d_6, 101 MHz): $\delta = 162.38$ (CONH), 153.12 (C, C-3′, C-5′), 144.92 (C, C-4′), 140.50 (C, C=NH), 138.01 (C, C-8), 134.32 (C, C-9), 129.65 (C, C-1′), 125.20 (CH, C-2), 123.17 (CH, C-6), 122.53 (CH, C-5), 121.12 (CH, C-4), 111.14 (CH, C-7), 110.69 (C, C-3), 105.44 (CH, C-2′, C-6′), 60.58 (OCH_3), 56.53 (CH_3, 2OCH_3), 33.26 (CH_3, N-CH_3); LCMS m/z (pos): 367.1 $C_{20}H_{21}N_3O_4$ (calcd. 367.1); HRMS m/z (pos): 367.1542 $C_{20}H_{21}N_3O_4$ (calcd. 367.1532).

N′-((1-(4-Chlorobenzyl)-1H-indol-3-yl)methylene)acetohydrazide ***(18r)*** Pale yellow solid, yield 92 %; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 2.53$ (s, 3H, CH_3), 5.08 (s, 2H, CH_2), 7.19–7.22 (m, 4H, Ar–H), 7.35–7.37 (m, 2H, Ar–H), 7.43 (d, 1H, $J = 7.2$ Hz, Ar–H), 7.49 (s, 1H, Ar–H), 7.99 (d, 1H, $J = 7.2$ Hz, Ar–H), 8.03 (s, 1H, azomethine-CH), 9.69 (s, 1H, hydrazine-NH); ^{13}C NMR (DMSO-d_6, 101 MHz): $\delta = 155.04$ (CONH), 137.34 (CH, C-2), 136.41 (C, C=NH), 134.62 (C, C-8), 132.94 (C, C-4′), 129.13 (C, C-9), 128.96 (C, C-1′), 125.97 (CH, C-3′, C-5′), 123.33 (CH, C-2′, C-6′), 122.92 (CH, C-6), 121.44 (CH, C-5), 121.39 (CH, C-4), 112.35 (CH, C-7), 110.79 (C, C-3), 49.39 (CH_2, -N-CH_2-), 23.05 (CH_3, CH_3); HRMS m/z (pos): 326.1103 $C_{18}H_{17}ClN_3O$ (calcd. 326.1060).

N′-((1-(4-chlorobenzyl)-1H-indol-3-yl)methylene)-2,2,2-trifluoroacetohydrazide ***(18s)*** Pale yellow solid, yield 92 %; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 5.11$ (s, 2H, CH_2), 7.22–7.25 (m, 4H, Ar–H), 7.38–7.40 (m, 2H, Ar–H), 7.50 (s, 1H, Ar–H), 7.53 (d, 1H, $J = 7.4$ Hz, Ar–H), 7.98 (s, 1H, azomethine-CH), 8.02 (d, 1H, $J = 7.4$ Hz, Ar–H), 9.76 (s, 1H, hydrazine-NH); ^{13}C NMR ($CDCl_3$, 101 MHz): $\delta = 154.65$ (CONH), 141.09 (C, C=NH), 136.90 (C, C-1′), 135.52 (C, C-8), 132.68 (C, C-9), 132.07 (CH, C-2), 130.00 (CH, C-3′, C-5′), 129.97 (CH, C-2′, C-6′), 128.94 (C, C-4′), 128.39 (CH, C-6), 123.34 (CH, C-5), 122.94 (CH, C-4), 120.52 (CH, C-7), 114.09 (C, C-CF_3, q), 108.62 (C, C-3), 53.04 (CH_2, –NCH_2–); HRMS m/z (pos): 380.0714 $C_{18}H_{14}ClF_3N_3O$ (calcd. 380.0777).

N′-((1-(4-Chlorobenzyl)-1H-indol-3-yl)methylene)-4-(benzyloxy)-3-methoxybenzohydrazide ***(18t)*** Pale yellow solid, yield 89 %; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.69$ (s, 3H, OCH_3), 5.10 (s, 2H, CH_2), 5.16 (s, 2H, CH_2), 7.16–7.18 (m, 4H, Ar–H), 7.21–7.24 (m, 5H, Ar–H), 7.27–7.30 (m, 2H, Ar–H), 7.35–7.37 (m, 2H, Ar–H), 7.41 (s, 1H, Ar–H), 7.46 (d, 1H, $J = 7.3$ Hz, Ar–H), 7.51 (s, 1H, Ar–H), 7.99 (d, 1H, $J = 7.3$ Hz, Ar–H), 8.07 (s, 1H, azomethine-CH), 9.72 (s, 1H, hydrazine-NH); ^{13}C NMR (DMSO-d_6, 101 MHz): $\delta = 162.50$ (CONH), 150.85 (C, C-4′), 149.11 (C, C-3′), 144.24 (C, C=NH), 137.23 (CH, C-2), 137.19 (C, C-1″), 137.10 (C, C-1‴), 133.45 (C, C-8), 132.63 (C, C-9), 129.48 (CH, C-2‴, C-6‴), 129.09 (CH, C-3″, C-5″), 128.93 (CH, C-3‴, C-5‴), 128.44 (C, C-4‴), 128.36 (CH, C-2″, C-6″), 126.86 (CH, C-4″), 125.56 (CH, C-6), 123.38 (CH, C-5), 122.74 (CH, C-4), 121.30 (CH, C-6′), 121.06 (CH, C-2′), 113.00 (CH, C-2′), 112.08 (CH, C-5′), 111.59 (C, C-7), 111.08 (C, C-3), 70.29 (CH_2, –OCH_2–), 56.14 (CH_3, OCH_3), 49.05 (CH_2, –NCH_2–); HRMS m/z (pos): 524.1720 $C_{31}H_{27}ClN_3O_3$ (calcd. 524.1741).

N′-((1-(4-Chlorobenzyl)-1H-indol-3-yl)methylene)-4-chlorobenzohydrazide ***(18u)*** Pale yellow solid, yield 92 %; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 5.18$ (s, 2H, CH_2), 7.21–7.24 (m, 4H, Ar–H), 7.32–7.35 (m, 4H, Ar–H), 7.38 (d, 1H, $J = 7.2$ Hz, Ar–H), 7.40–7.43 (m, 2H, Ar–H), 7.52 (s, 1H, Ar–H), 8.03 (d, 1H, $J = 7.2$ Hz, Ar–H), 8.08 (s, 1H, azomethine-CH), 9.65 (s, 1H, hydrazine-NH); ^{13}C NMR ($CDCl_3$, 101 MHz): $\delta = 166.85$ (CONH), 149.60 (C, C=NH), 141.91 (C, C-4′), 141.68 (CH, C-2), 141.03 (C, C-4″), 137.77 (C, C-8), 137.69 (C, C-9), 137.61 (CH, C-2″, C-6″), 134.37 (C, C-1′), 133.70 (C-1″), 133.68 (CH, C-2′, C-6′), 133.37 (CH, C-3″, C-5″), 130.37 (CH, C-6), 128.06 (CH, C-3′, C-5′), 127.57 (CH, C-5), 126.01 (CH, C-4), 116.87 (CH, C-7), 115.29 (C, C-3), 54.14 (CH_2, –NCH_2–); HRMS m/z (pos): 422.0935 $C_{23}H_{18}Cl_2N_3O$ (calcd. 422.0827).

*N′-((1-(4-Chlorobenzyl)-1H-indol-3-yl)methylene)-3,4,5-trimethoxybenzohydrazide (**18v**)* Pale yellow solid, yield 94 %; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.62$ (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 5.14 (s, 2H, CH_2), 7.19–7.20 (m, 4H, Ar–H), 7.24 (s, 1H, Ar–H), 7.28 (s, 1H, Ar–H), 7.32–7.34 (m, 2H, Ar–H), 7.42 (d, 1H, $J =$ 7.4 Hz, Ar–H), 7.49 (s, 1H, Ar–H), 8.02 (s, 1H, azomethine-CH), 8.10 (d, 1H, $J = 7.4$ Hz, Ar–H), 9.68 (s, 1H, hydrazine-NH); ^{13}C NMR ($CDCl_3$, 101 MHz): $\delta = 167.73$ (CONH), 157.68 (C, C-3′, C-5′), 149.16 (C, C-4′), 145.29 (C=NH), 141.85 (CH, C-2), 140.65 (C, C-8), 137.95 (C, C-9), 136.92 (C, C-1″), 134.12 (C, C-4″), 133.67 (CH, C-2″, C-6″), 133.47 (CH, C-3″, C-5″), 130.45 (C, C-1′), 128.06 (CH, C-6), 127.48 (CH, C-5), 126.02 (CH, C-4), 116.98 (CH, C-7), 115.06 (C, C-3), 110.10 (CH, C-2′, C-6′), 65.38 (2CH3, OCH_3), 61.08 (CH_3, OCH_3), 54.28 (CH_2, NCH_2); HRMS m/z (pos): 478.1518 $C_{26}H_{25}ClN_3O_4$ (calcd. 478.1534).

Antiproliferative activity of indolyl hydrazide–hydrazones

Biology

Cell lines and culture conditions

The human cancer cell lines for screening were cultured in RPMI 1640 medium containing 5 % fetal bovine serum. Cells were seeded in 96-well microtiter plate, at an expected target cell density of 5000–10,000 cells per well, based on cell growth. Inoculates were allowed to pre-incubate for 12 h at 37 °C for stabilization. Test compounds were evaluated at different concentrations ranging from 1 mM to 100 nM. Incubation lasted for 36 h in 5 % CO_2 atmosphere. The anticancer activity was determined for each cell line using formazan dye (MTT) conversion (assay).

Cleaved PARP1 assay

PC3 cells plated on polylysine-coated coverslips were treated with compounds **18d** and **18j** (10 mM) for 48 h. The cells were lysed, separated on SDS-PAGE, transferred to PVDF membrane and analyzed using cleaved PARP1 and actin antibodies. Antibodies against PARP1 (sc-56196) and actin (sc-8432) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Results and discussion

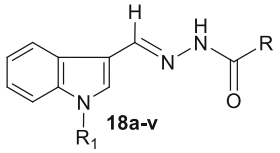
In the present study, we have synthesized a series of twenty-two indolyl hydrazide–hydrazone derivatives as shown in scheme 1. Compounds **15** and **16** were prepared according to the literature procedures (James and Snyder, 1959; Knaack *et al*., 2001; Roy *et al*., 2006; Yu *et al*., 2008). The condensation of aldehydes **16** with hydrazides **17** was achieved in the presence of acetic acid in refluxing ethanol. The products **18a–v** were obtained in good to excellent yields. All the synthesized molecules were characterized by using IR, NMR (^{1}H and ^{13}C) and LC/HRMS.

We have investigated the cytotoxic effects of the synthesized compounds **18a–v** against various cancer cell lines: prostate (PC3, DU145 and LnCaP), breast (MCF-7 and MDA-MB-231) and pancreas (PaCa2). A tentative comparison of the structure of compound and its activity was helpful to understand the structure–activity relationships (SAR). The cytotoxicity results of **18a–v** are summarized in Table 1. Some of the compounds decreased cell viability significantly as established by colorimetric MTT mitochondrial assay with IC_{50} values ranging from 100 nM to 1 mM concentration. In addition, some compounds exhibited more specificity toward one cell type versus the others.

Initial screening was carried out for indolyl hydrazide–hydrazones (**18a–l**) with free *N*–H indole ring. Compounds **18a** and **18b** with alkyl substituents including methyl and trifluoromethyl exhibited remarkable antiproliferative activity with IC_{50} values of 0.97 and 0.99 μM against PaCa2 and MCF-7, respectively. Further, we synthesized derivatives (**18c–l**) of indolyl hydrazide–hydrazones with substituted phenyl ring. Compound **18c** with a phenyl ring was found to be moderately cytotoxic against the tested cancer cell lines. Introduction of a chloro substituent at para position of phenyl ring led to compound **18d** with significantly enhanced activity. Compound **18d** ($IC_{50} = 0.42$) was found to be 173-fold selectively cytotoxic against MCF-7 cells when compared to **18c**. Replacement of a chloro group in **18d** by a methoxy group resulted in derivative **18e** with reduced cytotoxicity against the tested cell lines. Replacement of the phenyl group in compound **18c** with benzyl group led to compound **18f** with increased cytotoxicity ($IC_{50} = 6.94$ μM), when compared to its homologue **18c** ($IC_{50} = 73.22$ μM), against MCF-7 cell line. Introduction of an additional methoxy group in **18e** resulted in compound **18g** with improved cytotoxicity against MCF-7 cell line. Compound **18h** bearing 4-hydroxy-3-methoxyphenyl moiety exhibited moderate activity against MCF-7 and PaCa2 cells. Protection of hydroxy group as in compound **18h** by a benzyl group gave compound **18i** showed improved activity against all the tested cancer cell lines, except DU-145 and MDA-MB-231 cells. Also, compound **18i** was selectively cytotoxic to PaCa2 cells with an IC_{50} of 0.89 μM.

In view of beneficial cytotoxic effects of 3,4,5-trimethoxyphenyl fragment in antimitotic agents known

Table 1 In vitro cytotoxicity data of indolyl hydrazide–hydrazones



18a–v	R_1	R	Cytotoxicity (IC_{50} μM)[a]					
			PC3	DU145	LnCaP	MDA-MB-231	MCF-7	PaCa2
18a	H	CH_3	89.65	$>10^3$	$>10^3$	999.8	181.9	**0.97**
18b	H	CF_3	6.33	122.4	17.96	799.9	**0.99**	**4.06**
18c	H	C_6H_5	95.83	$>10^3$	$>10^3$	$>10^3$	73.22	236.9
18d	H	4-ClC_6H_4	15.34	491.6	160.9	563.5	**0.42**	**2.27**
18e	H	4-$CH_3OC_6H_4$	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$
18f	H	$CH_2C_6H_5$	$>10^3$	115.1	51.63	300.8	**6.94**	91.18
18g	H	3,4-$(CH_3O)_2C_6H_3$	$>10^3$	$>10^3$	758.5	$>10^3$	22.16	24.82
18h	H	4-OH-3-$CH_3OC_6H_3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$	21.88	58.88
18i	H	4-BnO-3-$CH_3OC_6H_3$	**2.54**	155.6	**9.16**	$>10^3$	**2.34**	**0.89**
18j	H	3,4,5-$(CH_3O)_3C_6H_2$	**3.89**	170.1	**5.31**	$>10^3$	**0.87**	**6.62**
18k	H	4-Pyridyl	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$	$>10^3$
18l	H	3-Pyridyl	$>10^3$	$>10^3$	19.53	718	535.6	$>10^3$
18m	CH_3	CH_3	80.9	74.7	44.9	148.1	91.3	40.7
18n	CH_3	CF_3	150.8	121.2	48.7	166.7	34.1	88.2
18o	CH_3	4-BnO-3-$CH_3OC_6H_3$	174.1	40.7	55.5	36.9	200.6	74.2
18p	CH_3	4-ClC_6H_4	251.4	165.2	92.8	264.1	37.4	106.2
18q	CH_3	3,4,5-$(CH_3O)_3C_6H_2$	315.1	44.8	54.6	104.9	305.2	512.4
18r	4-$ClC_6H_4CH_2$	CH_3	55.4	88.6	76.3	39.8	28.7	40.4
18s	4-$ClC_6H_4CH_2$	CF_3	416.2	242.3	78.3	205.3	179.6	320
18t	4-$ClC_6H_4CH_2$	4-BnO-3-$CH_3OC_6H_3$	149.3	18.1	**5.9**	89.8	13.6	11.8
18u	4-$ClC_6H_4CH_2$	4-ClC_6H_4	91.4	82.9	45.5	234	25.7	52.1
18v	4-$ClC_6H_4CH_2$	3,4,5-$(CH_3O)_3C_6H_2$	59.9	12.6	**6.8**	40.9	113	12.4

[a] Data obtained for each compound were analyzed in triplicates, and IC_{50} values given in micromolar concentrations were obtained using a dose–response curve by nonlinear regression using a curve fitting program, GraphPad Prism 5.0

* Bold values indicate $IC_{50} > 10$ μM

(Negi *et al*., 2015), compound **18j** was synthesized with significant cytotoxicity when compared to parent compound **18c**. Also, compound **18j** showed 83-fold selective cytotoxicity against breast cancer cells (MCF-7; $IC_{50} = 0.87$ μM). Introduction of heteroaryl moieties, e.g., 4-pyridyl and 3-pyridyl (compounds **18k** and **18l**), resulted in complete loss of cytotoxicity against all the tested cancer cell lines, except in LnCaP (compound **18l**).

Based on our initial anticancer activity data for indolyl hydrazide–hydrazones and importance of 4-chlorobenzyl group in indibulin (**9**) and oncrasin-1 (**10**), we were interested to study the effect of this substituent on nitrogen atom of indole ring. Therefore, we synthesized another series of indolyl hydrazide–hydrazones possessing *N*-methyl indole (compounds **18m–q)** and *N*-(4-chlorobenzyl)indole (compounds **18r–v**). The cytotoxic activities of compounds **18m–q** were found to be moderate. Hydrazide–hydrazones (compounds **18r–v**) with *N*-(4-chlorobenzyl)indole moiety demonstrated low cytotoxicity, except compounds **18t** and **18v** which displayed selective cytotoxicity against LnCaP cancer cell line with IC_{50} values of 5.9 and 6.8 μM, respectively. Thus, it demonstrates a

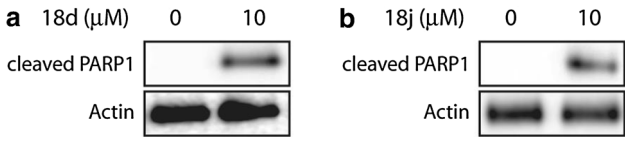


Fig. 3 Exposure of PC3 cells to **18d** and **18j** for cleavage of PARP1 level

diverse mechanism of action for this library of compounds **18a–v**.

Anticancer screening results showed that compounds **18a–v** affect the cell proliferation (Table 1) at concentrations ranging from 100 nM to 1 mM. We observed that the newly synthesized indolyl hydrazide–hydrazones **18** exhibited improved cytotoxicity when compared to reported bis(indolyl)hydrazide–hydrazones (Kumar *et al.*, 2012c). The IC_{50} values were calculated based on the MTT assay. Most of the derivatives showed IC_{50} values <100 μM. At present, a molecular target responsible for the observed cytotoxicity of this series of *N*′-((1-(substituted)-1*H*-indol-3-yl)methylene)hydrazides has not been identified and a reasonable explanation of SAR described above is not yet possible. These results suggest that the antiproliferative activity depends both on the substitution on indole nitrogen atom and hydrazide moiety.

We next examined whether these compounds induce cytotoxicity by inducing apoptosis in cancer cell lines. As compounds **18d** and **18j** exhibited high potency in PC3 cells (IC_{50} = 15.34 and 3.8 μM, respectively), these cells were treated with either **18d** or **18j** for 48 h, and cleaved PARP1 levels analyzed using immunoblotting. As shown in Fig. 3, exposure of PC3 cells to either **18d** or **18j** resulted in increased levels of cleaved PARP1, thereby confirming that this series of compounds indeed induces apoptosis in PC3 cells.

Conclusion

In summary, the synthetic protocol described herein provides an excellent route for the transformation of indole to indolyl hydrazides using easily available starting materials. The methodology has a broad scope with respect to variation in the indole ring or hydrazide moiety. Overall, the cytotoxicity results presented in Table 1 showed that compounds **18a** and **18i** exhibited good activity against pancreatic cell line (PaCa2). The compound **18d** is the most active, and compounds **18b** and **18j** exhibited relatively comparable activity against breast cancer cell line (MCF-7). Further, derivatization of the indolyl hydrazide–hydrazones to increase the efficacy and to study the structure–activity relationship associated with the nature and position of the substituents on indole and hydrazide moieties is in progress. Also, compounds *N*′-((1*H*-indol-3-yl)methylene)acetohydrazide **17a**, *N*′-((1*H*-indol-3-yl)methylene)-4-chlorobenzohydrazide **17d** and *N*′-((1*H*-indol-3-yl)methylene)-4-(benzyloxy)-3-methoxybenzohydrazide **17i** are proved to be potential leads for further cytotoxic activity studies.

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