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Synthesis, anticancer evaluation and molecular docking studies of bis(indolyl) triazinones, Nortopsentin analogs

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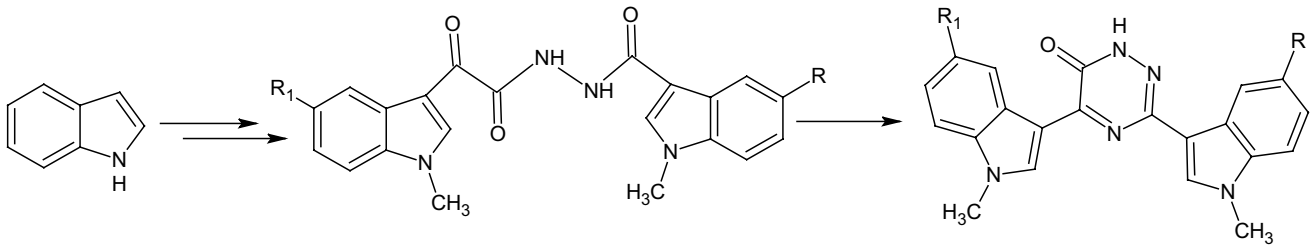
Abstract

A new series of bis indolyl tri keto diazo compounds and 3,5-bis(3′-indolyl) triazinones were designed and synthesized as anticancer agents. Their anticancer activity was screened in vitro towards four different human cancer cell lines like HeLa, MCF-7, MDA-MB-231 and A549 cell lines. Among them, compounds **17a and 17b** showed potent cytotoxicity with inhibition (IC_{50}) values of 4.6 and 1.3 μM on Human Cervical cancer cell line, respectively. The in silico simulation studies using ADT 1.5.6 tools revealed unique π–π interactions of indole ring of compound **17b** with colchicines active site residue Tyr312 could be a valid reason behind its maximum potency when compared to remaining compounds in responsible of its higher activity.

Graphical Abstract



Keywords Nortopsentins · Triazinones · Diazo tri keto compounds · Anti-tumor activity · Colchicine

Introduction

Over the past decade, a large number of marine alkaloids with unique chemical structure such as Topsentins, Dragmacidins, Coscinamides and Nortopsentins were isolated from different marine sponge metabolites, which exhibit potent biological activities including anti-tumor, antiviral, anti-inflammatory, antimicrobial, and HIV inhibitory activities (Bao et al. 2005; Bokesch et al. 2000; Casapullo et al. 2000; Gul and Hamann 2005; Gunasekera et al. 1988; Shin et al. 1999; Sun et al. 1990). Nortopsentins A–C and its analogue D having a 2,4-bis (3′-indolyl)imidazole structural skeleton (Fig. 1), exhibited in vitro anti-tumor activity against P_{338}cells (IC_{50}, 4.5–20.7 μM) and also antifungal activity against Bacillus subtilis and Candida albicans. The

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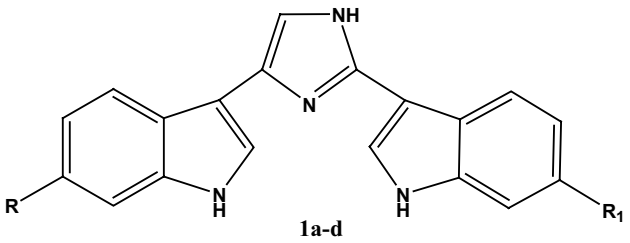


1a-d

Nortopsentin A	$R = R_1 = Br$
Nortopsentin B	$R = H, R_1 = Br$
Nortopsentin C	$R = Br, R_1 = H$
Nortopsentin D	$R = R_1 = H$

Fig. 1 Basic structure of nortopsentin derivatives

N-methyl derivatives of Nortopsentins showed remarkable improvement in P_{338} activity than the cytotoxic activities of the parent compounds (IC_{50}, 0.8–2.1 μM) (Alvarez et al. 1991; Kawasaki et al. 1996; Sakem and Sun 1991).

Already various analogs of Nortopsentins, in which the imidazole moiety was replaced by thiazole (Gu et al. 1999; Parrino et al. 2017; Spano et al. 2016), thiophene (Diana et al. 2007a), pyrazole (Diana et al. 2007b), furan-isoxazole (Diana et al. 2010), pyrrole (Carbone et al. 2013), and Pyridine (Xiong et al. 2001) were synthesized (Fig. 2) and reported their strong inhibitory activity against tumor cancer cell lines (GI_{50} < 0.01–89.4 μM). Moreover, the structural manipulation of natural Nortopsentins, beside the heterocyclic spacer, was further extended to one or two indole units and led to phenyl thiazolyl indoles and phenyl thiazolyl aza indoles (Diana et al. 2011), Indolyl-thiazolyl-pyrrolo[2,3-*c*] pyridines (Carbone et al. 2015), 3-[4-(1*H*-Indol-3-yl)-1,3-thiazol-2-yl]-1*H*-pyrrolo[2,3-*b*]pyridines (Parrino et al. 2015) and 1*H*-pyrrolo[2,3-b]pyridine (Diana et al. 2013) and these compounds showed antiproliferative activity against a wide range of human tumor cell lines in the micromolar – submicromolar range.

Previously, we designed and synthesized different heterocyclic systems with potent antitumor activities against a panel of various human cancer cell lines (Agarwal et al. 2016; Hatti et al. 2015a, b; Reddy et al. 2016a; b; Sreenivasulu et al. 2017; Madhavi et al. 2016; Madhavi et al. 2017a, b; Ahsan et al. 2015). Due to the potent antitumor activities of above molecules, we prompted to synthesize the Bis(indolyl)-triazinones, new analogs of Nortopsentins. Thus 1,2,5 tri keto 3,4-diazo moieties appeared as important scaffolds for the synthesis of bis(indolyl) triazinone analogues. The anti-tumor activities of these derivatives were evaluated in vitro towards four different human cancer cell lines like HeLa, MCF-7, MDA-MB-231 and A549 cell lines. The indole derivatives act as potent anti-proliferative agents with the functioning of tubulin polymerization (Alvarez et al. 2013; Kumar et al. 2013; Tunbridge et al. 2013). In this regard, several indole and indazolyl hydrazide analogs were also successfully studied for their essential tubulin interfering properties at colchicine site using molecular docking predictive tools (Sreenivasulu et al. 2017). The binding mode exploration of current ligands along with Nortopsentin analogs was studied using Schrodinger's LLC and Autodock tools ADT 1.5.6. The estimated results through ADT 1.5.6 were found to be relevant with in vitro biological data and are compiled in results.

Results and discussion

Chemistry

The total synthesis of bis(indolyl)triazinones were shown in Scheme 1 and 2. Indole and 5-bromo indole **9 a–b** derivatives undergo reaction with $POCl_3$ and DMF solvent by Vilsmeir–Haack reaction (James and Snyder 1959; Ishiyama et al. 2012), then the corresponding indole-3-carboxaldehydes **10 a–b** were obtained in good yield. These aldehyde derivatives oxidized with a mixture of potassium permanganate and acetone solvent yielded the carboxylic acid derivatives **11 a–b** (Papayan and Galstyan 1976) which undergo further reflux with methanol and conc. H_2SO_4 over a period of 6–7 h afforded the ester compounds **12a–b** (Whiting and Hof 2012). The methyl protection of these ester compounds would be achieved with dimethyl carbonate and K_2CO_3 followed by reflux in DMF over a period of 4 h (Jiang et al. 2001). The N – Methyl indolyl ester treated with hydrazine hydrate in ethanol solvent under reflux conditions over 8 – 10 hours time period then gave the corresponding carbo hydrazide derivatives **14 a–b** in good yields (see Scheme 1).

Indole derivatives **9 a–b** were reacted with dimethyl carbonate reagent and K_2CO_3 in DMF solvent afforded corresponding N-methyl derivatives **15 a–b** (Jiang et al. 2001). Further, **15 a-b** were treated with oxalyl chloride in diethyl ether at 0–4 °C over 4-h time period then gave N-methyl indole-3-glyoxalyl chloride derivatives **16 a–b** in good yields (Wang et al. 2011) and these crudes were used for next reaction without any purification. Here, the compounds **16a-b** are not purified because these compounds are decomposed in silicagel during chromatography. The reaction of crude **16 a–b** reacted with N-methyl indolyl-3-carbohydrazides **14 a–b** in THF and Et_3N under reflux conditions over a period of 4 h afforded 1,2,5-triketo 3,4-diamino derivatives **17 a–c**. These tri keto diamino derivatives reacted with equal ratio of Glac. AcOH, methanol and ammonium acetate at reflux conditions over 12-h time period then afforded the corresponding triazinone **18 a–c** derivatives in 60 – 65% yield (see Scheme 2).

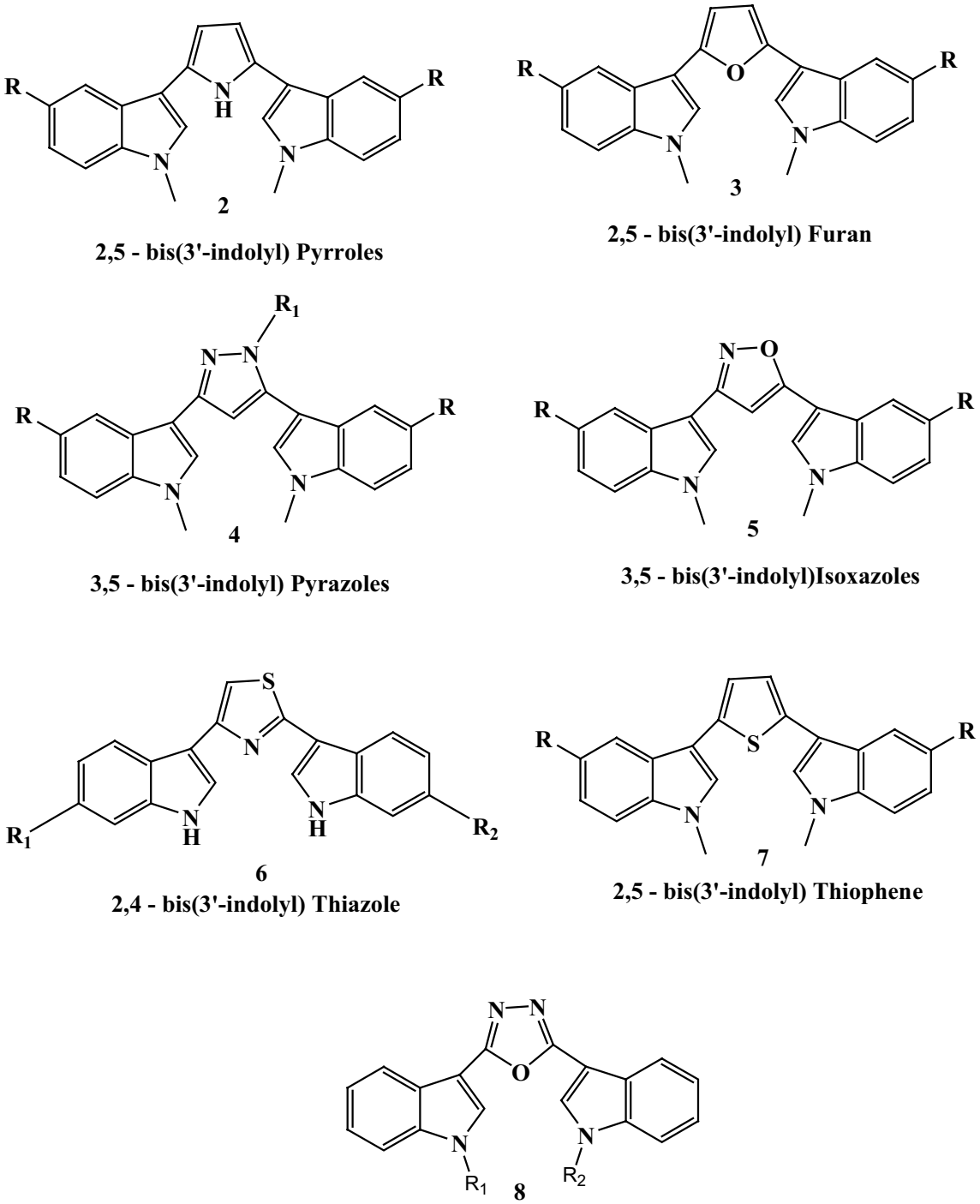
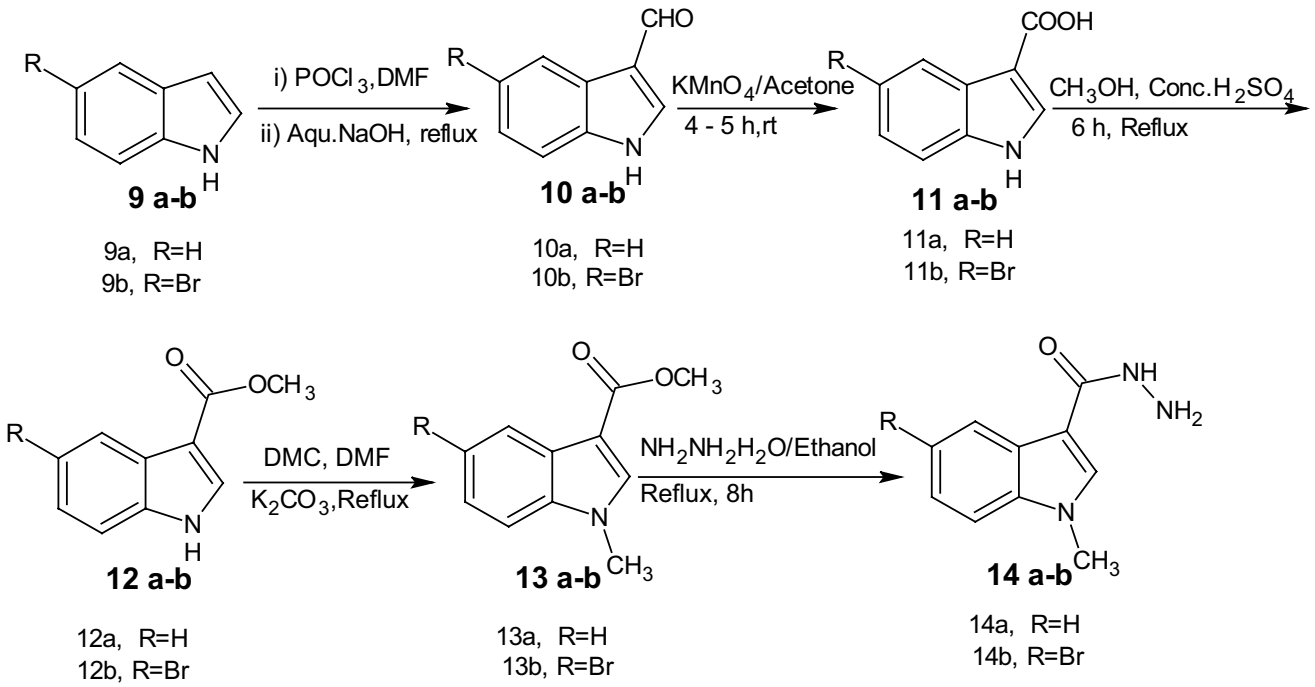


Fig. 2 Structures of different Nortopsentin analogues (2–8)

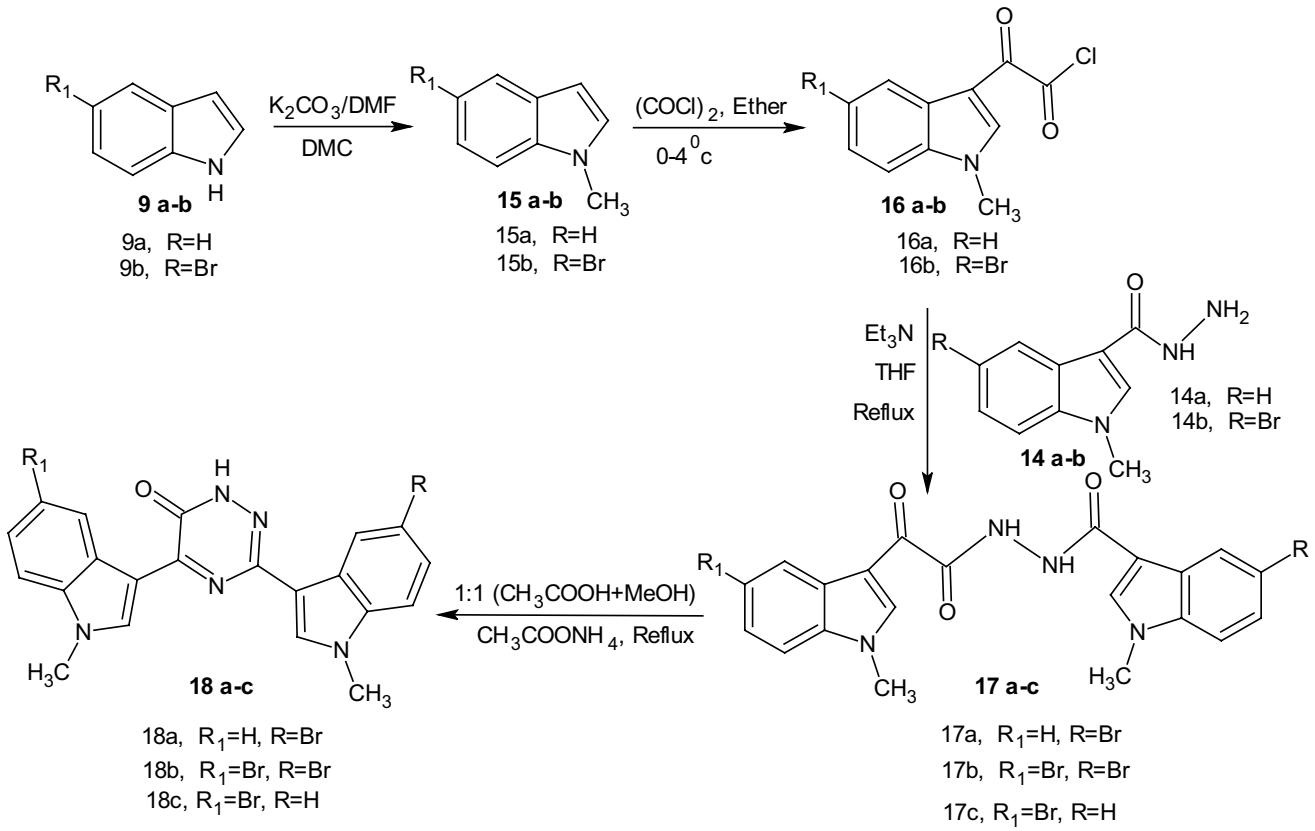
Biological evaluation

In vitro anticancer evaluation

The six synthesized compounds were allowed for the screening of anticancer activity by MTT assay method along four different human cancer cell lines viz. Human breast adenocarcinoma cells (MDA-MB-231 and MCF-7), Cervical cancer cells (HeLa), Human alveolar adenocarcinoma cells (A549) and Normal human embryonic kidney cells (HEK-293). These compounds showed their IC_{50} values in between 1.3 and 51.9 μM (Table 1). Among the series of ten compounds,



Scheme 1 Synthesis of substituted N-methyl indole-3-carbohydrazides



Scheme 2 Synthesis of Bis(indolyl)triazinones, analogues of Nortopsentin derivatives

the two compounds **17a and 17b** showed better cytotoxicity with IC_{50} values of 4.6 and 1.3 µM in Human Cervical cancer cell line, respectively. The compounds **17a**, **17c**, **18a** and **18c** showed their cytotoxicity on specific cancer cell lines like HeLa, MCF-7, A549 and HeLa. The compounds **17c**, **18a** and **18c** showed medium cytotoxicity on MCF-7, A549 and HeLa cancer cell lines. The compound **18b** showed no cytotoxicity among the four cancer cell lines. It was very crucial point that all the synthesized compounds do not showed any cytotoxic effect especially on HEK-293 cell lines (Human embryonic kidney cells). The anti-tumor activities of six compounds among the five cancer cell lines were shown in Table 1.

Molecular docking studies using Autodock 1.5.6 tools

Tubulin as attractive target has been extensively studied for bis indole class of compounds (Alvarez et al. 1991; Hwang et al. 2015). To correlate the in vitro anti-proliferative data for present analogs, colchicine binding site of tubulin was employed for the in silico predictive studies. The hydrophobic and hydrophilic active site of colchicine consists of a few noticeable crucial residues like Cys241, Val238, Ala316, Ala317, Leu242, Val318, Leu252, Thr314, Val315, Asn349 and Met259. The docking results of all the ligands were analyzed; indole ring with oxaacetyl chain of compound 17b exhibited a π–π interaction with Tyr312. This is due to the reason that the bulkier nature of bromine atom on indole nucleus might put its orientation towards Tyr312 and thus it might establish P–P interactions with Tyr312. The bromine atom might establish the salt bridges with amino acids and sometimes it may also able to interact with Tyr residue (Kortagere et al. 2008; Matter et al. 2009). The carbohydrazide NH of another bromo indole ring was found to be making H-bond with Ala317 of active site. The H-bond distance of NH with Ala317=2.117 Angstorm. Whereas, the binding pattern of compound 17a and 17c was not found to be similar. In case of compound 18a, 18b and 18c, the oxo group of triazinone ring was found to establishing H-bond interaction with Leu252. However, cyclization of bisindole derivatives (17a, 17b and 17c) might reduce their anti-proliferative activity. The unique π–π interaction of dibromo-substituted compound 17b might be responsible for its remarkable cytotoxicity. The docking score of **17** and **18** compounds is shown as below (see Figs. 3, 4).

The docking score of 17a compound = −9.30kcal/mol
The docking score of 17b compound = −9.40kcal/mol
The docking score of 17c compound = −8.76kcal/mol
The docking score of 18a compound = −9.12kcal/mol
The docking score of 18b compound = −9.27kcal/mol
The docking score of 18c compound = −9.14kcal/mol

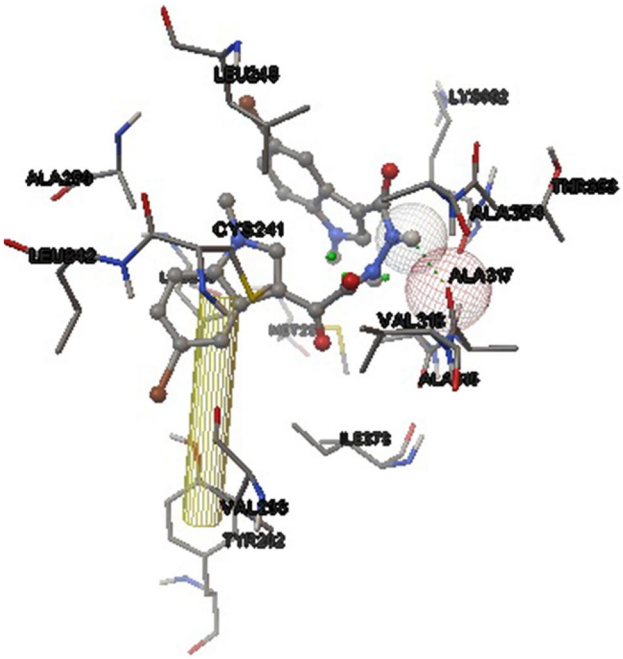


Fig. 3 Ball and stick residue indicated Compound 17b, yellow cylinder indicates π–π interaction, dotted line indicates H-bond and active site residues indicated by sticks

Table 1 Anti-tumor activity of the synthesized compounds

Test compound	IC_{50} (µM)				
	A549	MDA-MB-231	MCF-7	HeLa	HEK 293
17a	–	–	–	4.6 ± 0.5	–
17b	–	28.8 ± 0.55	16.8 ± 0.43	1.3 ± 0.11	–
17c	–	–	44.5 ± 0.76	–	–
18a	51.9 ± 0.99	–	–	–	–
18b	–	–	–	–	–
18c	–	–	–	35.3 ± 0.82	–
Doxorubicin	0.36 ± 0.14	0.47 ± 0.4	0.98 ± 0.14	0.89 ± 0.26	–

– Activity was not found at maximum concentrations, A549—Human alveolar adenocarcinoma cell line, HeLa—Human Cervical cancer cell line, MDA—MB-231—Human breast adenocarcinoma cell line, MCF-7—Human breast adenocarcinoma cell line, HEK 293—Human embryonic kidney cell line

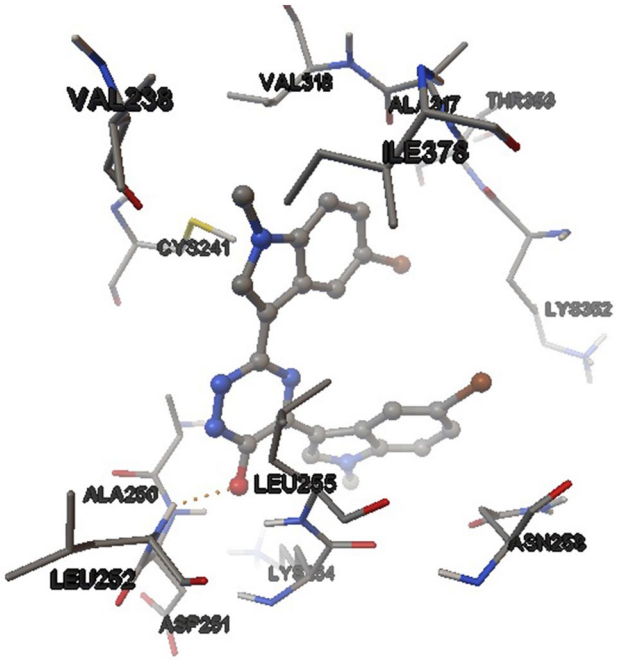


Fig. 4 Ball and stick residue indicated Compound 18b, dotted line indicates H-bond and active site residues indicated by sticks

The docking structures of **17b** and **18b** were shown in Figs. 3 and 4.

Experimental section

The salts, fine chemicals, reagents and catalysts used in this synthesis are purchased from AVRA laboratories PVT limited, Hyderabad, Telangana state, India and these items are used directly without any further purification process. Proton and carbon NMR spectra were recorded on Brucker 400 MHZ frequency instrument. Tetra Methyl Silane is taken as internal standard in recording the chemical shift value (δ). Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254, and visualization on TLC was achieved by UV light or iodine indicator. HRMS spectra were also recorded for accurate analysis of synthesized compounds.

General procedure for the preparation of-substituted indole-3-carboxylic acids (11a-b) 6 g of substituted indole-3-aldehydes (**10a–b**) was dissolved in 600 mL of acetone. To this $KMnO_4$ (9 g, 56 mmol) soluble in 180 mL water was added slowly for 30 min and the reaction mixture was allowed for stirring for 6 h at room temperature. After it was quenched by 6 mL of 30% H_2O_2, filtered and concentrated on rotavapor. Now it was solidified by conc.HCl, filtered, dried and recrystallized from methanol solvent which then afforded 5-substituted indole-3-carboxylic acids **11a–b**.

5-Bromo-1*H*-indole-3-carboxylic acid (11b) (Yoo et al. 2012) yellow color solid. yield: 65%. m.p:230–232 °C; IR (KBr): 3349, 2914, 2574, 1643 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.30 (1H, m, Ar–H), 7.435 (1H, d, J = 8.8 Hz, Ar–H), 8.05 (1H, d, J = 2.8 Hz, Ar–H), 8.12 (1H, d, J = 2 Hz, Ar–H), 12.01 (1H, bs, -NH), 12.13 (1H, s, -OH) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 107.06, 113.79, 114.28, 122.68, 124.70,127.79, 133.45, 135.16, 165.45 ppm; HRMS calculated for $C_9H_6NO_2BrNa$: 261.94741; found: 261.94711.

General procedure for the preparation of 5-substituted-3-carbomethoxy Indoles (12a–b) 4 g of 5-substituted indole- 3- carboxylic acids was dissolved in 10 volumes (400 mL) of methanol and catalytic amount of conc.H_2SO_4 was added slowly. The total reaction mixture was refluxed over 5 h time period and the reaction mixture was cooled to room temperature slowly. Now ice cold water was added and the yellow color solid was filtered. This solid was recrystallized from ethanol yielded 5 -substituted -3- carbomethoxy indoles **12a–b**.

Methyl-5-bromo-1*H*-indole-3-carboxylate (12b) yellow color solid. yield 62%. m.p: 210–212 °C; IR (KBr): 3213, 3114, 2929, 1745 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.93 (3H, s, -OCH_3), 7.28 (1H, d, J = 8.8 Hz, Ar–H), 7.35 (1H, m, Ar–H), 7.91(1H, d, J = 2.8 Hz, Ar–H), 8.32 (1H, s, Ar–H), 8.67 (1H, s, -NH) ppm; ^{13}C NMR(400 MHz, $CDCl_3$ + DMSO-d_6): δ 49.93, 105.91, 112.88, 113.75, 122.40, 124.26, 126.65, 131.89, 134.33, 164.13 ppm; HRMS calculated for $C_{10}H_9NO_2Br$: 253.98112; found: 253.98102.

General procedure for the preparation of 5-substituted-1-methyl-3-Carbomethoxy indoles (13a–b) A mixture of 5-substituted-3-carbomethoxy indoles (3 g, 17 mmol), Potassium carbonate (1.5 g), *N*,*N*-dimethyl formamide(21 mL, 7 vol.) and dimethyl carbonate(4.286 mL, 50 mmol) was stirred and refluxed at 130 °C for 3.5 h. After, the reaction mass was allowed to 27 °C and ice was added. The yellow color solid was filtered and recrystallized from ethanol afforded 5-substituted-1-methyl-3-carbomethoxy indoles **13a–b.**

Methyl 5-bromo-1-methyl-1H-indole-3-carboxylate (13b) yellow color solid. yield: 61%. m.p: 65-67°c; IR (KBr): 3246, 3100, 2940, 1745 cm^{-1}; ^{1}H NMR (400 MHz,$CDCl_3$): δ 3.81 (3H, s, -NCH_3), 3.90 (3H, s, -OCH_3), 7.19 (1H, d, J = 8.8 Hz, Ar–H), 7.36 (1H, m, Ar–H), 7.75 (1H, s, Ar–H), 8.29 (1H, d, J = 1.6 Hz, Ar–H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 33.52, 51.03, 106.53, 111.17, 115.52, 124.13, 125.64, 127.97, 135.75, 135.86, 164.86 ppm; HRMS calculated for $C_{11}H_{11}NO_2Br$: 267.99677; found: 267.99707.

General procedure for the preparation of 5-substituted-1-methyl indole-3-Carbo hydrazides (14a-b) 5-substituted-1-methyl-3-carbomethoxy indoles(2 g, 10 mmol) were dissolved in ethanol(10 mL, 5vol) and hydrazine hydrate(1.55 mL, 32 mmol) was slowly added. The reaction

mixture was refluxed for 8 h and allowed to room temperature. Now required quantity of crushed ice was added to the reaction mixture, filtered and recrystallized from ethanol which yielded 5-substituted-1-methyl indole-3-carbohydrazides **14a–b.**

1-methyl-1*H*-indole-3-carbohydrazide(14a) (Alemany et al. 1975) yellow color solid. Yield: 61%. m.p: 150-152°c; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.80 (3H, s, -NCH_3), 4.14 (2H, s, -NH_2), 7.24-7.32 (3H, m, Ar–H), 7.34 (1H, d, J = 7.6 Hz, Ar–H), 7.66 (1H, s, -NH), 7.93 (1H, d, J = 7.6 Hz, Ar–H) ppm.

5-bromo-1-methyl-1*H*-indole-3-carbohydrazide(14b) yellow color solid. Yield: 74%. m.p: 250–252 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.81 (3H, s, -NCH_3), 4.11 (2H, s, -NH_2), 7.04 (1H, s, Ar–H), 7.21 (1H, d, J = 8.8 Hz, Ar–H), 7.38 (1H, m, Ar–H), 7.60 (1H, s, Ar–H), 8.13 (1H, s, -NH) ppm; HRMS calculated for $C_{10}H_{11}N_3OBr$: 268.00800; found:268.00775.

General procedure for the synthesis of triketo diazo compounds (17a–c)

A solution of oxalyl chloride (9.7 g, 7.6 mmol) was added drop wise to a 0 °C cold solution of N- methyl indoles (5 g, 38.11 mmol) soluble in 35 mL diethyl ether. The mixture was stirred at 0 °C over a period of 3 h under cooling condition and the precipitated solid was collected by suction filtration, washed with cold diethyl ether and dried under reduced pressure to give 2-oxo acetyl chloride indole derivatives **16a–b** as crude products. It was impossible to purify the tri keto diazo compounds in the column chromatography. The products **16a-b** are not purified because these products aredecomposed in silicagel during chromatography. Hence, we directly used these crude products for next reaction without further purification.

A solution of tri ethyl amine (9.02 mmol) in 5 volumes of THF was added dropwise to the above oxo acetyl chloride indole derivatives (9.02 mmol). The reaction mixture was refluxed over a period of 4 h and poured in crushed ice. This was neutralized with 10 mL HCl (0.01 N) to remove excess tri ethyl amine to get crude products and purified by column chromatography with 3:2 ratio ethyl acetate and hexane to give pure triazinone **17a–c** derivatives. The formation of these bis-acyl hydrazines was supported by literature of the synthesis of differently substituted hydrazine derivatives (Mielczarek et al. 2014).

5-bromo-1-methyl-*N*'-[(1-methyl-1*H*-indol-3-yl)(oxo)acetyl]-1*H*-indole-3-carbohydrazide (17a) yellow solid; Yield: 72%; m.p: > 300°c; IR (KBr): 3641, 3100, 2936, 1691 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.86 (3H, s, -NCH_3), 3.94 (3H, s, -NCH_3), 7.30-7.37 (3H, m, Ar–H), 7.50-7.53 (1H, d, J = 8.8 Hz, Ar–H), 7.60-7.62 (1H, d, J = 7.6 Hz, Ar–H), 8.08 (1H, s, Ar–H), 8.29-8.31 (1H, d, J = 7.2 Hz, Ar–H), 8.36 (1H, s, Ar–H), 8.85 (1H, s, Ar–H), 10.40 (2H, broad singlet, 2-NH) ppm; ^{13}C NMR: (400 MHz, DMSO–d_6): δ 32.5, 32.8, 110.1, 110.8, 111.6, 112.7, 120.4, 121.9, 123.9, 126.0, 127.0, 132.5, 134.4, 136.5, 140.8, 161.3, 161.5, 182.4 ppm. MS (ESI): m/z = 453 $(M + H)^+$; Anal Calcd for $C_{21}H_{17}N_4BrO_3$: C, 55.64; H, 3.78; N, 12.36. Found: C, 57.57; H, 3.69; N, 12.39.

5-bromo-*N*'-[(5-bromo-1-methyl-1*H*-indol-3-yl)(oxo)acetyl]-1-methyl-1*H*-indole-3-carbo hydrazide (17b) yellow solid; Yield: 79%; m.p: 150-152°c; IR (KBr): 3644, 3245, 3094, 2955, 1735 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.31 (3H, s, -NCH_3), 3.42 (3H, s, -NCH_3), 7.11-7.23 (2H, m, Ar–H), 7.62-7.74 (3H, m, Ar–H), 8.11 (1H, s, Ar–H), 8.20-8.23 (1H, m, Ar–H), 8.62 (1H, s, Ar–H), 11.30 (1H, broad singlet, NH); 12.25 (1H, broad singlet, NH) ppm; MS (ESI): m/z = 532 $(M + H)^+$; Anal Calcd for $C_{21}H_{16}N_5BrO$: C, 47.39; H, 3.03; N, 10.53. Found: C, 47.34; H, 2.98; N, 10.61.

***N*'-[(5-bromo-1-methyl-1*H*-indol-3-yl)(oxo)acetyl]-1-methyl-1*H*-indole-3-carbohydrazide (17c)** yellow solid; Yield: 66%; m.p: 163-164°c; IR (KBr): 3345, 3064, 2932, 1678 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.84 (3H, s, -NCH_3), 3.90 (3H, s, -NCH_3), 7.18-7.42 (7H, m, Ar–H), 8.02 (1H, s, Ar–H), 8.20 (1H, d, J = 7.55 Hz, Ar–H); 8.74 (1H, broad singlet, -NH), 8.89 (1H, broad singlet, -NH) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 24.75, 34.92, 108.7, 112.3, 113.3, 124.2, 124.6, 129.3, 136.2, 137.3, 160.4, 189.04 ppm; MS (ESI): m/z = 453 $(M + H)^+$; Anal Calcd for $C_{21}H_{17}N_4BrO_3$: C, 55.64; H, 3.78; N, 12.36. Found: C, 57.59; H, 3.72; N, 12.41.

General procedure for the synthesis of triazinones (18a–c) The synthesis of triazinones was achieved through cyclo condensation between amidrazone and ketoester functions (Garg and Stoltz 2005). But we proposed different methodology for this triazinone synthesis. The appropriate tri keto diazo compounds **17a–c** (2.207 mmol) were dissolved in 7 volumes of acetic acid and methanol mixture (1:1 ratio). To this mixture, ten times ammonium acetate was added and allowed the reaction mixture for reflux over 10 h. Crushed ice was added to the reaction mixture and neutralized with aqueous sodium carbonate to yield the crude products. The pure products are obtained from crude products by column chromatography with 3:2 ratio ethyl acetate and hexane to give pure triazinone **18a-c** derivatives.

3-(5-bromo-1-methyl-1*H*-indol-3-yl)-5-(1-methyl-1*H*-indol-3-yl)-1,2,4-triazin-6(1H)-one (18a) yellow solid; Yield: 60%; m.p: 165-167°c; IR (KBr): 3348, 3015, 2986, 1694 cm^{-1}; ^{1}H NMR (400 MHz, DMSO – d_6): δ 3.92 (6H, s, 2 –NCH_3), 7.21–7.39 (4H, m, Ar–H), 7.50 (2H, d, J = 7.36HZ, Ar–H), 7.55 (1H, broad, NH), 7.85 (1H, broad, N=C–OH), 8.31 (1H, d, J = 7.55 HZ, Ar–H), 8.811 (2H, s, Ar–H) ppm; ^{13}C NMR (400 MHz, DMSO – d_6): δ 31.6, 32.3, 98.7, 108.6, 114.6, 117.3, 122.2, 122.8, 125.8, 127.2,

128.9, 130.8, 131.2, 134.9, 136.3, 140.4, 146.3, 148.2, 151.6, 162.4, 168.3 ppm; MS (ESI): $m/z = 435$ $(M + H)^+$; Anal Calcd for $C_{21}H_{16}N_5BrO$: C, 58.08; H, 3.71; N, 16.13. Found: C, 57.97; H, 3.66; N, 16.20.

3,5-bis(5-bromo-1-methyl-1*H*-indol-3-yl)-1,2,4-triazin-6(1H)-one (18b) yellow solid; Yield: 65%; m.p: 173–175 °C; IR (KBr): 3356, 3067, 2943, 1663 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 3.71 (3H, s, -NCH_3), 3.90 (3H, s, -NCH_3), 7.20-7.72 (6H, m, Ar–H), 8.22-8.39 (2H, m, Ar–H), 9.87 (1H, broad, NH) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 31.0, 32.8, 97.7, 108.6, 111.2, 113.3, 115.5, 116.3, 120.2, 122.4, 123.2, 124.1, 126.3, 127.6, 128.6, 130.3, 139.3, 140.1, 146.2, 158.3, 166.9 ppm; MS (ESI): $m/z = 512$ $(M + H)^+$; Anal Calcd for $C_{21}H_{15}N_5Br_2O$: C, 49.15; H, 2.95; N, 13.65. Found: C, 49.07; H, 2.86; N, 13.71.

5-(5-bromo-1-methyl-1H-indol-3-yl)-3-(1-methyl-1H-indol-3-yl)-1,2,4-triazin-6(1H)-one (18c) yellow solid; Yield: 62%; m.p: > 250°c; IR (KBr): 3309, 3108, 2937, 1718 cm^{-1}; ^1H NMR (400 MHz, DMSO – d_6): δ 3.85 (6H, s, 2 –NCH_3); 7.21-7.58 (9H, m, Ar–H), 8.50 (1H, broad, NH); 8.90 (1H, broad, N = C–OH) ppm; ^{13}C NMR (400 MHz, DMSO – d_6): δ 31.0, 31.9, 98.1, 110.1, 110.2, 112.9, 113.0, 116.2, 116.3, 118.2, 123.2, 123.8, 124.1, 125.7, 125.8, 128.3, 128.4, 135.8, 142.8, 142.9, 165.3 ppm; MS (ESI): $m/z = 435$ $(M + H)^+$; Anal Calcd for $C_{21}H_{16}N_5BrO$: C, 58.08; H, 3.71; N, 16.13. Found: C, 57.99; H, 3.64; N, 16.22.

In vitro cytotoxicity assay

MTT assay method (Mosmann 1983) is used for screening the cytotoxicity of synthesized compounds. The four different human cancer cell lines MDA-MB-231, A549, MCF-7 and HeLa used in this assay are derived from ATCC No. HTB-26, ATCC No. CCL-185, ATCC No. HTB-22 and ATCC No. CCL-2. The human kidney cell lines were also derived from ATCC No. CRL-1573. All these cell lines were brought from United States of America. These cancer cell lines were grown in a modified DMEM medium along with 0.2% sodium hydrogen carbonate, 1% sodium pyruvate, 1% non-essential amino acids except L-glutamine, 1% antibiotic mixture (10 mg of streptomycin per mL and 10000 units penicillin) and 10% fetal bovine serum. These cancer cell lines were cleaned and re-suspended in the above DMEM medium. Now, this 100 mL suspension was added into a 96-well bottom plate. These cancer cell lines are kept in 2406 model Shellab CO_2 incubator at 37 °C under the 5% CO_2 atmosphere. This suspension was left over 24-h incubation. The ten synthesized compounds were soluble in DMSO solution. The cancer cell lines were treated with the ten synthesized compounds over 2 days with varying concentrations of 0.1–100 μM and these were assayed at the completion of second day. After the completion of incubation over 48 h, these cancer cell lines were subjected to MTT cell proliferation assay (5 mg m L^{-1}). The effects of our synthesized compounds towards the viability of the cancer cell lines were recorded with the help of 540 nm Infinite® M200Pro multimode reader. Doxorubicin was taken as standard positive control for the comparison of their IC_{50} values towards the synthesized compounds, while 1% DMSO behaves as vehicle control. To get the accurate values then subtract the values obtained from test compounds to the values obtained for DMSO control. Dose–response curves were plotted and calculated the IC_{50} values by these curves. GraphPad Software, Inc, La Jolla, CA, USA was used for statistical analysis. IC_{50} values (in μM) were recorded in four experiments of mean ± SD independently. Student's *t* test was used for all experimental data.

Conclusion

In conclusion, a series of novel bis indolyl tri keto diazo compounds and 3,5-bis(3′-indolyl) triazinones were designed and synthesized. Among them, **17a and 17b** compounds showed potent cytotoxicity with IC_{50} values of 4.6 μM and 1.3 μM especially on Human Cervical cancer cell line. Further, the compound **17b** acts as potent drug lead with IC_{50} value of 1.3 μM towards HeLa than the positive control doxorubicin with IC_{50} value of 0.89 μM.

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