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Short communication

Azide-alkyne cycloaddition *en route* to novel 1*H*-1,2,3-triazole tethered isatin conjugates with *in vitro* cytotoxic evaluation

Pardeep Singh [a], Pooja Sharma [b], Amit Anand [a], P.M.S. Bedi [b], Tandeep Kaur [c], A.K. Saxena [c], Vipan Kumar [a,*]

[a] *Department of Chemistry, Guru Nanak Dev University, Amritsar 143005, India*
[b] *Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar 143005, India*
[c] *Cancer Pharmacology Division, Indian Institute of Integrative Medicine, Jammu 180001, J & K, India*

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ABSTRACT

1*H*-1,2,3-triazole tethered isatin conjugates have been synthesized and evaluated for cytotoxicity on four human cancer cell lines. The results revealed **5a**, **5c**, **5e** and **5n** proved to be twice as potent as 5-fluorouracil on THP-1 cell line with **5a** and **5c** being most active exhibiting IC_{50} values of <1 against all cell lines except Caco-2. Activity profiles showed dependence on the substituents on isatin rings with a preference for hydrogen while a strong electron withdrawing fluoro as well as nitro substituents on either ring decreased the anticancer activity.

1. Introduction

The isatin (1*H*-indole-2,3-dione) moiety is a privileged scaffold with wide possibility for chemical modification and responsible for a broad spectrum of biological properties in many synthetically versatile molecules [1]. Among these properties, cytotoxic and antineoplastic activities of this moiety have been found to be interesting and mechanistically can be associated with its affinity to inhibit tyrosine kinase [2] and serine/threonine-specific protein kinases such as the cyclic-dependent kinases (CDKs) [3] and carbonic anhydrase isozymes (CAIs) [4]. Literature rationale reveals that in particular, halogenated isatin derivatives have been reported to exhibit enhanced anticancer activity compared to the parent molecule. 5-Bromo-3-*o*-nitrophenyl isatin hydrazone and a series of 5-bromo-(2-oxo-3-indolinyl) thiazolidine-2,4-diones substituted by various Mannich bases were found to exhibit anticancer activity against Walker carcinoma-256 and P388 lymphocytic leukemia in mice, respectively [5]. 6-Bromo-2-methylthio-3*H*-indol-3-one, tyrindoleninone, a brominated precursor to Tyrian purple, isolated from the egg masses of the Australian mollusk *Dicathais orbita* has been reported as a cytotoxic marine compound [6]. 6-bromo isatin, a major decomposition product formed through the oxidation of tyriverdin (precursor of Tyrian purple), has been shown to have a weaker anticancer activity against a human lymphoma cell line in comparison with 6-bromo-2-methylthio-3*H*-indol-3-one [7]. Moreover, *N*-benzylation of 5,7-dibromoisatin further increased the cytotoxicity against U937 (human monocyte-like histiocytic lymphoma) cells [8] targeting of microtubules and activity against a range of human cancer cell lines including a metastatic breast adenocarcinoma cell line (MDA-MB-231) [9]. Isatin-pyrazoline and isatin-4-thiazolidinone conjugates have been recently targeted as prospective anticancer agents with highly selective influence on the leukemia subpanel and moderate selectivity toward the CNS and renal cancer subpanels [10]. In the recently approved drugs by FDA a 5-fluoro-3-substituted-2-oxoindole, SU11248 (Sutent), is provided for the treatment of gastrointestinal stromal tumors and advanced renal-cell carcinoma [11,12].

Over the past few years, 1,2,3-triazoles have attracted the attention of organic and medicinal chemists because of their varied biological properties including anti-allergic, antibacterial, anti-fungal, anti-HIV, anticonvulsant, anti-inflammatory, antitubercular and anticancer activities [13]. The favorable properties of 1,2,3-triazole ring like moderate dipole character, hydrogen

* Corresponding author. Tel.: +91 183 2258802 09x3320; fax: +91 183 2258819 20.
E-mail address: vipan_org@yahoo.com (V. Kumar).

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bonding capability, rigidity and stability under *in vivo* conditions are evidently responsible for their enhanced biological activities [14].

Recent revelation from our lab has shown the synthesis and *in vitro* cytotoxic evaluation of a series of *N*-alkyl bromo and *N*-alkylphthalimido isatins against four human cancer cell lines [15]. As an extension to this approach and our interest in the synthesis of biologically imperative frameworks with medicinal potential [16], the present manuscript explicates the synthesis of novel 1*H*-1,2,3-triazole tethered isatin conjugates *via* azide-alkyne cycloaddition as elucidated in Fig. 1 along with their anticancer evaluation on four human cancer cell lines.

2. Synthetic chemistry and pharmacology

2.1. Synthetic chemistry

The procedure for the synthesis of *N*-alkyl azido isatin derivatives **3** was based on a combination of the literature methods [17] involving an initial base-assisted *N*-alkylation of substituted isatins with dibromoethane to yield the corresponding *N*-alkyl bromo isatins **2**, followed by subsequent reaction with sodium azide in DMF at 60 °C. The second precursor, substituted *N*-propargylated isatins **4** were prepared by base assisted propargylation as shown in Scheme 1. The target compounds **5** were synthesized by utilizing azide-alkyne cycloaddition reaction of appropriate precursors **3** and **4** in the presence of $CuSO_4 \cdot 5H_2O$/sodium ascorbate in ethanol:water mixture (Scheme 1). The purification of the reaction mixture, after usual work up, *via* column chromatography resulted in the isolation of desired scaffolds, the structures to which were assigned on the basis of spectral data and analytical evidences.

2.2. Pharmacology

2.2.1. Anticancer evaluation

The human cancer cell lines were procured from National Cancer Institute, Frederick, PO Box B, Frederick, MD 21702-1201, U.S.A. Cells were grown in tissue culture flasks in complete growth medium (RPMI-1640 medium with 2 mM glutamine, pH 7.4, supplemented with 10% fetal calf serum, 100 μg/mL streptomycin and 100 units/mL penicillin) in a carbon dioxide incubator (37 °C, 5% CO_2, 90% RH). The cells at subconfluent stage were harvested from the flask by treatment with trypsin [0.05% in PBS (pH 7.4) containing 0.02% EDTA]. Cells with viability of more than 98% as determined by trypan blue exclusion, were used for determination of cytotoxicity. The cell suspension of 1×10^5 cells/mL was prepared in complete growth medium. Stock solutions (2×10^{-2} M) of compounds were prepared in DMSO. The stock solutions were serially diluted with complete growth medium containing 50 μg/mL of gentamycin to obtain working test solutions of required concentrations.

In vitro cytotoxicity against human cancer cell lines of different tissues was determined [18,19] using 96-well tissue culture plates. The 100 μl of cell suspension was added to each well of the 96-well tissue culture plate. The cells were allowed to grow in carbon dioxide incubator (37 °C, 5% CO_2, 90% RH) for 24 h. Test materials in complete growth medium (100 μl) were added after 24 h of incubation to the wells containing cell suspension. The plates were further incubated for 48 h in a carbon dioxide incubator. The cell growth was stopped by gently layering trichloroacetic acid (50%, 50 μl) on top of the medium in all the wells. The plates were incubated at 4 °C for 1 h to fix the cells attached to the bottom of the wells. The liquid of all the wells was gently pipetted out and discarded. The plates were washed five times with distilled water to remove trichloroacetic acid, growth medium low molecular weight metabolites, serum proteins etc and air-dried. The plates were stained with sulforhodamine B dye (0.4% in 1% acetic acid, 100 μl) for 30 min. The plates were washed five times with 1% acetic acid and then air-dried [19]. The adsorbed dye was dissolved in Tris–HCl Buffer (100 μl, 0.01 M, pH 10.4) and plates were gently stirred for 10 min on a mechanical stirrer. The optical density (OD) was recorded on ELISA reader at 540 nm. The cell growth was determined by subtracting mean OD value of respective blank from the mean OD value of experimental set. Percent growth in presence of test material was calculated considering the growth in absence of any test material as 100% and in turn percent growth inhibition in presence of test material was calculated.

3. Results and discussion

The synthesized 1,2,3-triazole tethered isatin conjugates were evaluated for their anticancer activity against four human cancer cell lines *viz*. A-549(lung), PC-3(prostate), THP-1(leukemia) and Caco-2(colon) using sulforhodamine B assay. The concentration dependent cytotoxicity of these scaffolds against human cancer cell lines is shown in Table 1. Paclitexal has been used as standard in case of lung (A-549), 5-fluorouracil in case of THP-1(leukemia) and Caco-2(colon) while mitomycin in case of PC-3(prostate). The analysis of Table 1 revealed interesting structure activity relationship (SAR) and substituent effect with regard to C-5 position of isatin rings A and B and considerable growth inhibition at low concentrations. As evident from Table 1, the test compound **5a** has shown almost complete growth inhibition on all cell lines except Caco-2 at 10 μM concentration and was 2–3 folds more active than 5-fluorouracil on

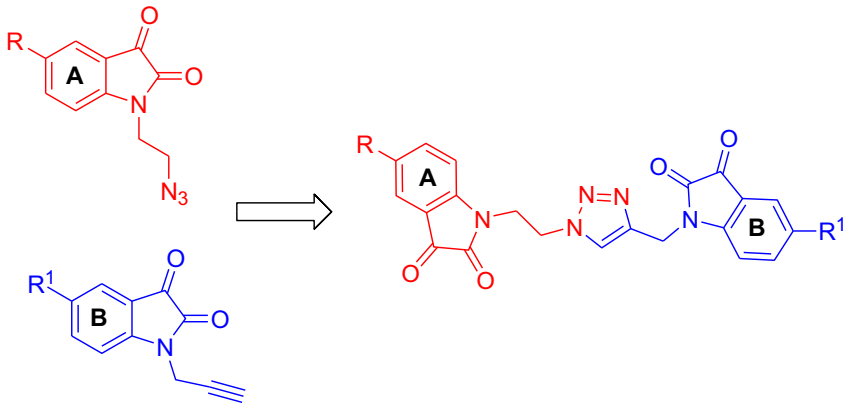
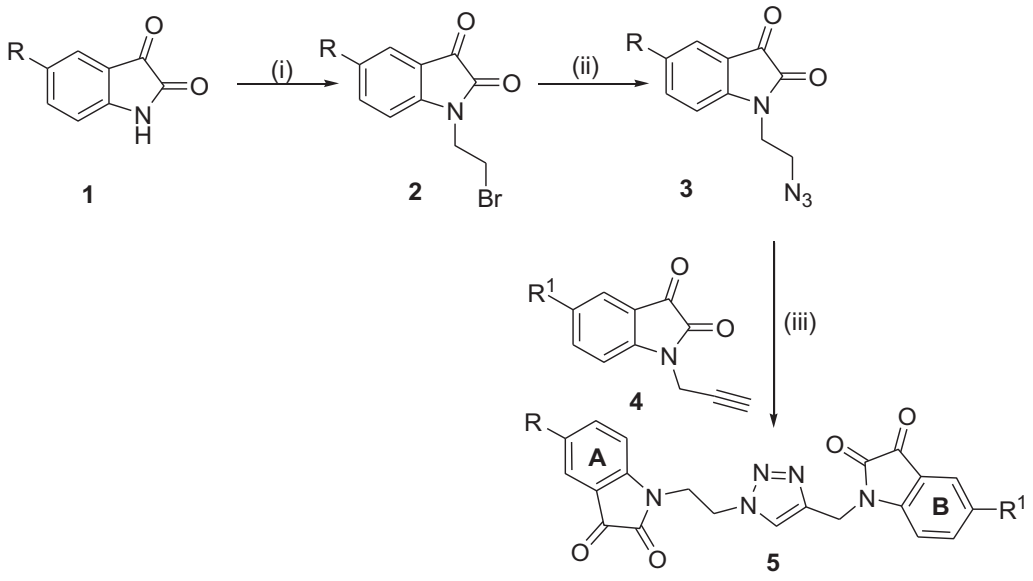


Fig. 1. General structure of lead compound and target hybrid compounds.



1 2 3 4 5

5a. R=H, R^1=H
5b. R=H, R^1=Br
5c. R=H, R^1=Cl
5d. R=H, R^1=F
5e. R=H, R^1=CH_3
5f. R=H, R^1=NO_2
5g. R=F, R^1=H
5h. R=F, R^1=Br
5i. R=F, R^1=Cl
5j. R=F, R^1=F
5k. R=F, R^1=CH_3
5l. R=F, R^1=NO_2
5m. R=Cl, R^1=Br
5n. R=Cl, R^1=CH_3
5o. R=Cl, R^1=NO_2

Scheme 1. Synthesis of isatin-conjugates: (i) 1,2-dibromoethane, NaH, DMF, 60 °C, 2 h; (ii) sodium azide, DMF, 60 °C, 3 h; (iii) sodium ascorbate, $CuSO_4 \cdot 5H_2O$, ethanol:H_2O, 8 h.

THP-1 cell line while compounds **5c**, **5e** and **5n** were twice as potent as 5-flouorouracil.The compound **5c** has also shown considerable growth inhibition on A-549 and PC-3 cell lines while **5b** and **5n** proved to be active against PC-3 and A-549 cell lines at low concentration. These preliminary results at low concentration indicated that the synthesized scaffold **5a** proved to be the most active of the series showing a marked preference for hydrogen substituent on both the rings. The activity profiles of **5b**, **5c** and **5e** also corroborates toward the preference of H-substituent on ring A while the poor activity exhibited by **5d** and **5f** might be attributed to the presence of strong electron withdrawing fluoro and nitro substituents on ring B. Careful scrutinizing of Table 1 proved the attribution of poor activity to electron withdrawing fluoro and nitro substituents on ring A as evident by the activity profiles of compounds **5g**–**l**. The reduction in cytotoxic profile with the introduction of electron withdrawing substituents has further authenticated by the poor activity profiles observed in case of **5j** (R = R^1 = F) and **5l** (R = F, R^1 = NO_2); both being least active in the series.

The evaluation studies at 50 μM further improved the cytotoxic profiles in case of all the test compounds, the effects being more pronounced in case **5b** (A-549, PC-3), **5d** (A-549, PC-3 and THP-1), **5e** (A-549 and PC-3), **5f** (A-549, PC-3, THP-1) and **5n** (A-549 and THP-1). The compound **5g** and **5k** showed a significant increase in cytotoxic activity against A-549 and THP-1 cell lines while **5f** showed the similar enhancements in case of A-549, PC-3 and THP-1 cell lines. The compound **5b** showed 96% and 99% growth inhibition on A-549 and PC-3 cell lines respectively at 50 μM while compounds **5h** and **5m** showed a significant increase in cytotoxic activity against A-549 and THP-1 cell lines. The series of compounds having fluoro substituent on ring A (**5g**–**l**) however does not show any marked improvement of anticancer profile at 50 μM except in case of **5h** (A-549 and THP-1) and **5k** (THP-1). The compound **5h** (R = R^1 = F) and **5l** (R = F, R^1 = NO_2) showed least improvement in cytotoxic profiles. A further increase in concentration to 100 μM showed complete growth inhibition in most of the compounds especially **5a**–**e** in all cancer cell lines tested except Caco-2 while **5h**, **5i**, **5k**, **5m** and **5n** showed almost complete growth inhibition (97–99%) on A-549 cell lines (Fig. 2). The IC_{50} values, which is the concentration required to inhibit 50% of the cell viability by the test compounds after exposure to cells, of the test compounds were calculated and the results are summarized in Table 2.

Table 1
In vitro cytotoxicity against human cancer cell lines.

Compound	Concentration	Cell lines				
		A-549	PC-3	THP-1	Caco-2	C log *P*[a] [20]
		% Age growth inhibition				
5a	10	96	99	100	9	0.148
	50	98	100	100	20	
	100	99	100	100	36	
5b	10	41	94	10	1	1.189
	50	96	99	53	10	
	100	97	100	88	25	
5c	10	94	99	80	6	1.039
	50	98	100	90	24	
	100	100	100	100	38	
5d	10	11	20	10	0	0.469
	50	97	98	96	1	
	100	99	100	100	22	
5e	10	35	63	89	0	0.647
	50	99	100	100	0	
	100	100	100	100	17	
5f	10	13	24	11	0	0.274
	50	96	97	97	4	
	100	98	99	100	18	
5g	10	6	0	8	0	0.469
	50	17	32	15	1	
	100	32	82	90	10	
5h	10	41	0	26	0	1.511
	50	79	2	85	7	
	100	98	51	94	13	
5i	10	64	0	20	2	1.361
	50	73	4	70	12	
	100	97	9	92	30	
5j	10	5	0	1	0	0.791
	50	23	10	13	0	
	100	62	30	92	10	
5k	10	8	0	14	0	0.968
	50	57	31	88	15	
	100	99	48	89	44	
5l	10	7	2	2	0	0.595
	50	22	11	14	0	
	100	64	33	90	0	
5m	10	10	29	28	36	2.081
	50	83	43	82	57	
	100	98	70	90	68	
5n	10	81	36	71	39	1.538
	50	95	55	91	52	
	100	98	70	92	56	
5o	10	11	30	29	17	1.165
	50	80	44	82	21	
	100	99	72	88	33	
Paclitaxel	1	69	ND	ND	ND	
5-Flourouracil	20	ND	ND	70	68	
Mitomycin-C	1	ND	60	ND	ND	

[a] Calculated using Chem Draw ultra 10.0; ND = Not Determined.

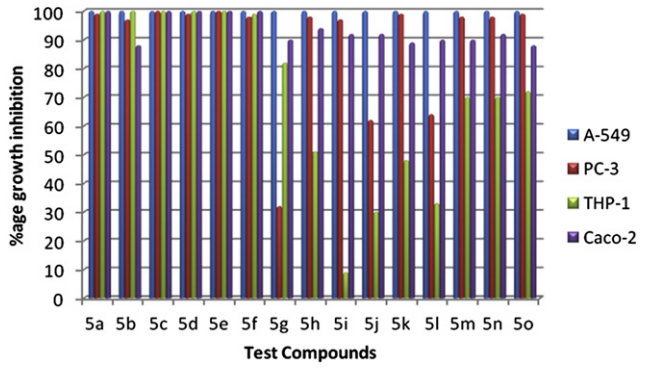


Fig. 2. Comparison of activity of test compounds on four cancer cell line at 100 μM.

Table 2
IC_{50} determination of cytotoxicity of compounds against human cancer cell lines.

Compound	Cell lines			
	Lung	Prostate	Leukemia	Colon
	A-549	PC-3	THP-1	Caco-2
	IC_{50} (μM)			
5a	<1	<1	<1	>100
5b	14.56	<1	51.86	>100
5c	<1	<1	<1	>100
5d	38.9	30.1	39.9	>100
5e	18.39	3.03	<1	>100
5f	39.2	30.7	42.8	>100
5g	>100	71.1	88.1	>100
5h	16.39	>100	27.6	>100
5i	2.65	>100	36.3	>100
5j	90.7	>100	83.3	>100
5k	49.9	85.2	38.75	>100
5l	93.2	95.2	86.2	>100
5m	42.2	51.4	26.9	32.2
5n	<1	32.6	<1	44.9
5o	39.3	53.2	26.0	>100

Among the four human cancer cell lines used, most of the compounds showed activity on all cancer cell lines except Caco-2 with the effect more pronounced against THP-1 followed by A-549 and PC-3 cell lines respectively. The tested compounds proved to be inactive against Caco-2 cell lines except **5m** and **5n** with IC_{50} values of 32.2 and 44.9 μM respectively. The most active analogs in the series proved to be **5a** and **5c** exhibiting IC_{50} values of <1 against A-549, PC-3 and THP-1 cell lines followed by **5n** with IC_{50} values of 0.03, 0.48, 32.6 and 44.9 μM against A-549, THP-1, PC-3 and Caco-2 cell lines respectively.

In conclusion, the present manuscript explicates the synthesis of novel 1*H*-1,2,3-triazole tethered isatin conjugates and their evaluation against a panel of four human cancer cell lines. The preliminary studies have shown that most of these compounds are active on all cancer cell lines except Caco-2 with activity showing dependence on C-5 substituent of the synthesized scaffolds. The compound **5a**, **5c**, **5e** and **5n** proved to be twice as potent as 5-fluorouracil on THP-1 cell line. These preliminary results indicate a marked dependence of cytotoxicity on the presence of substituent on the isatin ring with a distinct preference for hydrogen substituent. The presence of a strong electron withdrawing fluoro as well as nitro substituents markedly decreased the activity profiles with compound **5j** and **5l** being the least active of the test compounds. Based on these observations, the compound **5a** may be considered as a good hit in terms of %age growth inhibition, IC_{50} and partition coefficient C log *P* (0.148) values. Further studies in order to generalize substantiate and improve the activity profiles of the scaffolds are underway in the lab and will soon be communicated.

4. Experimental section

Melting points were determined by open capillary using Veego Precision Digital Melting Point apparatus (MP-D) and are uncorrected. IR spectra were recorded on a Shimadzu D-8001 spectrophotometer. ^{1}H NMR spectra were recorded in deuterochloroform and dimethylsulfoxide d_6 with Jeol 300 (300 MHz) and BRUKER AVANCE II (400 MHz) spectrometers using TMS as internal standard. Chemical shift values are expressed as parts per million downfield from TMS and *J* values are in hertz. Splitting patterns are indicated as s: singlet, d: doublet, t: triplet, m: multiplet, dd: double doublet, ddd: doublet of a doublet of a doublet, and br: broad peak. ^{13}C NMR spectra were recorded on Jeol 300 (75 MHz)

spectrometers in deuterochloroform using TMS as internal standard. Mass spectra were recorded on Shimadzu GCMS-QP-2000 mass spectrometer. Elemental analyses were performed on Heraus CHN-*O*-Rapid Elemental Analyzer. Column chromatography was performed on a silica gel (60–120 mesh).

4.1. Typical procedure for the synthesis of substituted N-alkyl bromo isatins (**2**)

To a stirred suspension of sodium hydride (1.5 mmol) in dry DMF (10 mL) was added isatin (1 mmol), resulting in the formation of purple colored anion. The solution was stirred at room temperature till the evolution of hydrogen ceases. To this reaction mixture was then added drop wise a solution of dibromoethane (1.1 mmol) in DMF. The reaction mixture was heated to 60 °C with constant stirring for about 2 h. After the completion of reaction, as evident by TLC, it was quenched by drop wise addition of water (20 mL) and subsequently extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification of the reaction mixture *via* column chromatography using hexane:ethyl acetate (8:2) mixture furnished the desired *N*-alkyl bromo derivatives in good yields.

4.2. Typical procedure for the synthesis of substituted N-alkyl azido isatins (**3**)

To the stirred suspension of *N*-alkyl bromo isatin in dry DMF was added NaN_3 (1.5 mmol) and the reaction mixture was heated at 60 °C for about 3 h (monitored by TLC). Upon completion, the reaction mixture was extracted with ethyl acetate and the organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield *N*-alkyl azido isatin derivatives in good yields.

4.3. Typical procedure for the synthesis of substituted N-propargy lated isatins (**4**)

To a stirred suspension of sodium hydride (1.5 mmol) in dry DMF (10 mL) was added isatin (1 mmol), resulting in the formation of purple colored anion. Solution was stirred at room temperature till the evolution of hydrogen ceases followed by the drop wise addition of a solution of propargyl bromide (1.5 mmol) in DMF. The reaction mixture was stirred at room temperature for about 2 h and on completion, as evident by TLC, was quenched by drop wise addition of water (20 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification of the reaction mixture *via* column chromatography using hexane:ethyl acetate (8:2) mixture furnished the desired *N*-propargylated isatin derivatives in good yields.

4.4. Typical procedure for the synthesis of triazole tethered functionalized isatin-conjugates (**5**)

To the stirred solution of *N*-propargylated isatin (1 mmol) and *N*-alkyl azido isatin (1 mmol) in ethanol:water (10:1) was added in succession copper sulfate (0.055 mmol) and sodium ascorbate (0.143 mmol) at room temperature. On completion of the reaction, as monitored by TLC, water (15 mL) was added to the reaction mixture and extracted with chloroform (2 × 50 mL). Combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduce pressure to result in a crude product which was recrystallized using chloroform:methanol (10:2) mixture.

4.4.1. 1-{1-[2-(2,3-Dioxo-2,3-dihydro-indol-1-yl)-ethyl]-1H-[1,2,3] triazol-4-ylmethyl}-1H-indole-2,3-dione (**5a**)

Orange solid; yield: 71%; m.p: 124–126 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm = 4.45–4.48 (t, J = 5.4 Hz, 2H, CH_2), 4.67–4.71 (t, J = 5.4 Hz, 2H, CH_2), 4.92 (s, 2H, CH_2), 7.10–7.15 (m, 3H, ArH), 7.28–7.59 (m, 5H, ArH), δ 8.22 (s, 1H, H-triazole ring). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm = 35.4, 41.7, 48.1, 112.9, 115.6, 116.3, 118.5, 121.1, 123.6, 123.8, 125.2, 127.6, 137.7, 139.5, 139.9, 141.2, 144.1, 156.5, 158.1, 181.4, 182.0. IR (cm^{-1}): 1749, 1732, 1648, 1627, 1512. MS (EI) m/z: 401 (M^+). Calcd for $C_{21}H_{15}N_5O_4$: C, 62.84; H, 3.77; N, 17.45. Found C, 63.04; H, 3.99; N, 17.23.

4.4.2. 5-Bromo-1-{1-[2-(2,3-dioxo-2,3-dihydro-indol-1-yl)-ethyl]-1H-[1,2,3]triazol-4-ylmethyl}-1H-indole-2,3-dione (**5b**)

Yellow solid; yield: 78.6%; m.p: 112–114 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm = 4.55–4.57 (t, J = 5.5 Hz, 2H, CH_2), δ 4.71–4.73 (t, J = 5.5 Hz, 2H, CH_2), 4.98 (s, 2H, CH_2), 7.17–7.49 (m, 4H, ArH), 7.59–7.61 (dd, 1H, J = 3.6, 8.4 Hz, ArH), 7.72–7.74 (d, 1H, J = 8.4 Hz, ArH), 7.77–7.78 (d, 1H, J = 3.6 Hz, ArH), 7.83 (s, 1H, H-triazole ring). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm = 35.5, 42.1, 48.6, 113.9, 115.7, 117.4, 119.5, 122.1, 123.6, 124.8, 127.0, 128.7, 138.2, 140.4, 141.9, 144.7, 149.6, 157.5, 158.9, 181.6, 182.4. IR (cm^{-1}): 1757, 1738, 1633, 1607, 1517. MS (EI) m/z: 480 (M^+). Calcd for $C_{21}H_{14}BrN_5O_4$: C, 52.52; H, 2.94; N, 14.58. Found C, 52.71; H, 3.05; N, 14.53.

4.4.3. 5-Chloro-1-{1-[2-(2,3-dioxo-2,3-dihydro-indol-1-yl)-ethyl]-1H-[1,2,3]triazol-4-ylmethyl}-1H-indole-2,3-dione (**5c**)

Orange solid; yield: 68.7%; m.p: 138–140 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm = 4.55–4.57 (t, J = 5.7 Hz, 2H, CH_2), 4.71–4.73 (t, J = 5.7 Hz, 2H, CH_2), δ 4.98 (s, 2H, CH_2), 7.20–7.44 (m, 4H, ArH), 7.53–7.56 (dd, 1H, J = 3.6, 8.0 Hz, ArH), 7.56–7.58 (m, 2H, ArH), 7.72 (s, 1H, H-triazole ring). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm = 36.1, 42.9, 49.1, 114.3, 115.9, 117.7, 120.7, 122.8, 124.3, 125.1, 127.7, 129.4, 138.9, 141.2, 141.7, 142.4, 145.6, 156.8, 157.8, 181.1, 183.5. IR (cm^{-1}):1763, 1744, 1654, 1613, 1515. MS (EI) m/z: 436 (M^+). Calcd for $C_{21}H_{14}ClN_5O_4$: C, 57.87; H, 3.24; N, 16.07. Found C, 58.02; H, 3.41; N, 15.87.

4.4.4. 1-{1-[2-(2,3-Dioxo-2,3-dihydro-indol-1-yl)-ethyl]-1H-[1,2,3] triazol-4-ylmethyl}-5-fluoro-1H-indole-2,3-dione (**5d**)

Orange solid; yield: 70.56%; m.p: 107–109 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm = 4.53–4.56 (t, J = 5.7 Hz, 2H, CH_2), 4.75–4.77 (t, J = 5.7 Hz, 2H, CH_2), 4.94 (s, 2H, CH_2), 7.19–7.48 (m, 5H, Ar–H), 7.52–7.53 (d, 1H, J = 3.3 Hz, ArH), 7.71–7.73 (d, 1H, J = 8.1 Hz, ArH), 7.78 (s, 1H, H-triazole ring). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm = 37.9, 43.9, 49.8, 116.1, 117.4, 119.3, 121.5, 123.9, 125.9, 127.1, 129.3, 131.4, 140.6, 142.8, 143.3, 145.3, 147.7, 160.1, 163.6, 182.9, 184.4. IR (cm^{-1}): 1788, 1759, 1658, 1622, 1512. MS (EI) m/z: 420 (M^+). Calcd for $C_{21}H_{14}FN_5O_4$: C, 60.14; H, 3.36; N, 16.70. Found C, 60.01; H, 3.49; N, 16.58.

4.4.5. 1-{1-[2-(2,3-Dioxo-2,3-dihydro-indol-1-yl)-ethyl]-1H-[1,2,3] triazol-4-ylmethyl}-5-methyl-1H-indole-2,3-dione (**5e**)

Orange solid; yield: 71.3%; m.p: 128–130 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm = 2.35 (s, 3H, CH_3), 4.51–4.54 (t, J = 5.7 Hz, 2H, CH_2), 4.70–4.73 (t, J = 5.7 Hz, 2H, CH_2), 4.91 (s, 2H, CH_2), 7.03–7.06 (d, 1H, J = 7.8 Hz, ArH), 7.19–7.45 (m, 5H, ArH), 7.65 (s, 1H, ArH), 8.15 (s, 1H, H-triazole ring); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm = 24.5, 40.7, 47.5, 50.1, 110.9, 114.8, 115.7, 117.5, 120.5, 123.1, 123.6, 124.6, 127.0, 136.6, 138.2, 139.5, 140.2, 143.4, 155.3, 157.8, 181.3, 182.0. IR (cm^{-1}):1766, 1728, 1639, 1617, 1515. MS (EI) m/z: 416 (M^+). Calcd for $C_{22}H_{17}N_5O_4$: C, 63.61; H, 4.12; N, 16.86. Found C, 63.87; H, 4.21; N, 16.73.

*4.4.6. 1-{1-[2-(2,3-Dioxo-2,3-dihydro-indol-1-yl)-ethyl]-1H-[1,2,3] triazol-4-ylmethyl}-5-nitro-1H-indole-2,3-dione (**5f**)*

Orange solid; yield: 71%; m.p: 126–128 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm = 4.19–4.21 (t, J = 5.4 Hz, 2H, CH_2), 4.69–4.72 (t, J = 5.7 Hz, 2H, CH_2), δ 4.91 (s, 2H, CH_2), 7.19–7.71 (m, 4H, ArH), 7.77 (s, 1H, H-triazole ring); 8.10–8.51 (m, 3H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm = 37.9, 43.7, 49.9, 117.9, 118.7, 119.5, 121.9, 123.7, 126.5, 128.8, 129.5, 131.7, 140.7, 142.9, 143.8, 145.9, 147.5, 158.3, 160.7, 183.7, 185.3. IR (cm^{-1}):1767, 1738, 1643, 1627, 1517. MS (EI) m/z: 446 (M^+). Calcd for $C_{21}H_{14}N_6O_6$: C, 56.51; H, 3.16; N, 18.83; Found C, 56.65; H, 3.11; N, 18.91.

*4.4.7. 1-{2-[4-(2,3-Dioxo-2,3-dihydro-indol-1-ylmethyl)-[1,2,3] triazol-1-yl]-ethyl}-5-fluoro-1H-indole-2,3-dione (**5g**)*

Orange solid; yield: 76%; m.p: 108–110 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm = 4.16–4.19 (t, J = 5.4 Hz, 2H, CH_2), 4.64–4.67 (t, J = 5.7 Hz, 2H, CH_2), δ 4.90 (s, 2H, CH_2), 6.33–6.37 (dd, 1H, J = 3.6, 8.4 Hz, ArH), 7.09–7.58 (m, 6H, Ar–H), 7.70 (s, 1H, H-triazole ring); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm = 37.6, 42.9, 49.0, 116.5, 118.1, 119.6, 121.7, 123.2, 126.4, 128.6, 129.3, 131.4, 140.6, 142.8, 143.3, 145.3, 147.7, 158.4, 160.3, 183.2, 184.9. IR (cm^{-1}):1767, 1738, 1643, 1627, 1517. MS (EI) m/z: 420 (M^+). Calcd for $C_{21}H_{14}FN_5O_4$: C, 60.14; H, 3.36; N, 16.70. Found C, 60.27; H, 3.31; N, 16.62.

*4.4.8. 1-{2-[4-(2,3-Dioxo-2,3-dihydro-5-bromo-indol-1-ylmethyl)-[1,2,3]triazol-1-yl]-ethyl}-5-fluoro-1H-indole-2,3-dione (**5h**)*

Yellow-orange solid; yield: 77%; m.p: 105–107 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm = 4.07–4.09 (t, J = 5.2 Hz, 2H, CH_2), 4.60–4.63 (t, J = 5.2 Hz, 2H, CH_2), δ 4.87 (s, 2H, CH_2), 6.74–6.77 (dd, 1H, J = 2.8, 8.8 Hz, ArH), 7.02–7.04 (d, 1H, J = 8.4 Hz, ArH), 7.20–7.25 (dd, 1H, J = 2.8, 8.8 Hz, ArH), 7.35–7.37 (d, 1H, J = 8.8 Hz, ArH), 7.70–7.71 (d, 1H, J = 2.8 Hz, ArH), 7.96–7.97 (d, 1H, J = 2.8 Hz, ArH), 8.19 (s, 1H, H-triazole ring). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm = 46.1, 49.5, 49.7, 111.1, 111.4, 113.2, 115.1, 118.0, 119.0, 123.6, 123.9, 124.6, 126.6, 139.8, 141.2, 146.3, 148.8, 157.1, 158.0, 181.7, 182.1. IR (cm^{-1}):1754, 1728, 1720, 1648, 1518. MS (EI) m/z: 498 (M^+). Calcd for $C_{21}H_{13}BrFN_5O_4$: C, 50.62; H, 2.63; N, 14.06. Found C, 50.81; H, 2.76; N, 13.93.

*4.4.9. 1-{2-[4-(2,3-Dioxo-2,3-dihydro-5-chloro-indol-1-ylmethyl)-[1,2,3]triazol-1-yl]-ethyl}-5-fluoro-1H-indole-2,3-dione (**5i**)*

Orange solid; yield: 72%; m.p: 120–122 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm = 4.14–4.17 (t, J = 5.4 Hz, 2H, CH_2), 4.66–4.69 (t, J = 5.4 Hz, 2H, CH_2), δ 4.88 (s, 2H, CH_2), 6.80–6.83 (dd, 1H, J = 2.4, 8.4 Hz, ArH), 7.11–7.13 (d, 1H, J = 8.4 Hz, ArH), 7.23–7.27 (dd, 1H, J = 2.8, 8.4 Hz, ArH), 7.42–7.44 (d, 1H, J = 8.4 Hz, ArH), 7.80–7.84 (m, 2H, ArH), 8.21 (s, 1H, H-triazole ring); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm = 46.9, 49.8, 50.7, 110.9, 112.8, 113.9, 119.8, 121.2, 123.9, 125.2, 131.9, 137.4, 138.2, 143.7, 146.1, 147.9, 148.4, 159.2, 160.9, 181.9, 182.2. IR (cm^{-1}): 1748, 1730, 1640, 1630, 1515. MS (EI) m/z: 454 (M^+). Calcd for $C_{21}H_{13}ClFN_5O_4$: C, 55.58; H, 2.89; N, 15.43. Found C, 55.71; H, 3.01; N, 15.22.

*4.4.10. 1-{2-[4-(2,3-Dioxo-2,3-dihydro-5-flouro-indol-1-ylmethyl)-[1,2,3]triazol-1-yl]-ethyl}-5-fluoro-1H-indole-2,3-dione (**5j**)*

Orange solid; yield: 65%; m.p: 110–112 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm = 4.01–4.03 (t, J = 5.1 Hz, 2H, CH_2), 4.46–4.49 (t, J = 5.1 Hz, 2H, CH_2), δ 4.83 (s, 2H, CH_2), 7.23–7.27 (dd, 1H, J = 2.8, 8.1 Hz, ArH), 7.32–7.36 (dd, 1H, J = 2.8, 8.1 Hz, ArH), 7.50–7.82 (m, 4H, ArH), 8.00 (s, 1H, H-triazole ring). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm = 45.5, 47.6, 49.7, 109.4, 110.8, 111.4, 117.5, 119.8, 121.7, 123.4, 129.2, 135.6, 137.9, 141.3, 145.1, 146.9, 149.8, 158.5, 159.4, 180.6, 180.9. IR (cm^{-1}): 1737, 1724, 1635, 1621, 1513. MS (EI) m/z: 438 (M^+). Calcd for $C_{21}H_{13}F_2N_5O_4$: C, 57.67; H, 3.00; N, 16.01. Found C, 57.85; H, 3.07; N, 15.92.

*4.4.11. 1-{2-[4-(2,3-Dioxo-2,3-dihydro-5-methyl-indol-1-ylmethyl)-[1,2,3]triazol-1-yl]-ethyl}-5-fluoro-1H-indole-2,3-dione (**5k**)*

Orange solid; yield: 69%; m.p: 119–121 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm = 2.31 (s, 3H, CH_3), 4.44–4.46 (t, J = 5.4 Hz, 2H, CH_2), 4.57–4.59 (t, J = 5.4 Hz, 2H, CH_2), δ 4.78 (s, 2H, CH_2), 7.21–7.25 (dd, 1H, J = 2.8, 8.1 Hz, ArH), 7.32–7.35 (dd, 1H, J = 2.8, 8.4 Hz, ArH), 7.50–7.53 (d, 1H, J = 2.8 Hz, ArH), 7.59–7.81 (m, 3H, ArH), 8.03 (s, 1H, H-triazole ring). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm = 22.3, 46.0, 49.7, 52.1, 108.7, 112.6, 113.4, 115.8, 118.7, 121.1, 122.6, 123.3, 125.0, 134.9, 137.1, 137.2, 139.0, 142.8, 153.3, 155.2, 181.1, 182.8. IR (cm^{-1}):1744, 1731, 1638, 1627, 1516. MS (EI) m/z: 434 (M^+). Calcd for $C_{22}H_{16}FN_5O_4$: C, 60.97; H, 3.72; N, 16.16. Found C, 61.13; H, 3.85; N, 15.98.

*4.4.12. 1-{2-[4-(2,3-Dioxo-2,3-dihydro-5-nitro-indol-1-ylmethyl)-[1,2,3]triazol-1-yl]-ethyl}-5-fluoro-1H-indole-2,3-dione (**5l**)*

Orange solid; yield: 64%; m.p: 132–134 °C. δ ppm = 4.00–4.02 (t, J = 5.1 Hz, 2H, CH_2), 4.49–4.52 (t, J = 5.1 Hz, 2H, CH_2), δ 4.85 (s, 2H, CH_2), 7.25–7.29 (dd, 1H, J = 2.8, 8.1 Hz, ArH), 7.50–7.82 (m, 2H, ArH), 8.00 (s, 1H, H-triazole ring); 8.10–8.45 (m, 3H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm = 45.9, 47.0, 49.9, 109.6, 110.9, 111.1, 117.9, 119.6, 121.5, 123.8, 129.3, 135.7, 137.0, 141.4, 145.5, 146.8, 149.9, 158.0, 159.7, 180.3, 181.2. IR (cm^{-1}): 1737, 1724, 1635, 1621, 1513. MS (EI) m/z: 464 (M^+). Calcd for $C_{21}H_{13}FN_6O_6$: C, 54.32; H, 2.82; N, 18.10; Found C, 54.23; H, 3.01; N, 18.22.

*4.4.13. 1-{2-[4-(2,3-Dioxo-2,3-dihydro-5-bromo-indol-1-ylmethyl)-[1,2,3]triazol-1-yl]-ethyl}-5-chloro-1H-indole-2,3-dione (**5m**)*

Orange solid; yield: 74.7%; m.p: 122–124 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm = 4.41–4.44 (t, J = 5.7 Hz, 2H, CH_2), 4.47–4.50 (t, J = 5.7 Hz, 2H, CH_2), 4.61 (s, 2H, CH_2), 7.51–7.54 (dd, 1H, J = 3.6, 8.4 Hz, ArH), 7.72–7.80 (m, 4H, ArH), 7.96 (d, 1H, J = 3.6 Hz, ArH), 8.13 (s, 1H, H-triazole ring); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm = 46.3, 49.2, 52.0, 108.9, 111.7, 112.6, 116.2, 119.4, 120.7, 123.5, 124.3, 125.6, 132.9, 136.8, 137.9, 140.4, 141.6, 153.8, 156.2, 181.6, 182.0. IR (cm^{-1}):1756, 1744, 1624, 1622, 1513. MS (EI) m/z: 514 (M^+). Calcd for $C_{21}H_{13}BrClN_5O_4$: C, 49.00; H, 2.55; N, 13.61. Found C, 49.13; H, 2.71; N, 13.49.

*4.4.14. 1-{2-[4-(2,3-Dioxo-2,3-dihydro-5-methyl-indol-1-ylmethyl)-[1,2,3]triazol-1-yl]-ethyl}-5-chloro-1H-indole-2,3-dione (**5n**)*

Orange solid; yield: 70.5%; m.p: 124–126 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm = 2.11 (s, 3H, CH_3), 4.23–4.27 (t, J = 5.7 Hz, 2H, CH_2), 4.46–4.49 (t, J = 5.7 Hz, 2H, CH_2), 4.58 (s, 2H, CH_2), 7.38–7.41 (dd, 1H, J = 3.6, 8.4 Hz, ArH), 7.53–7.56 (dd, 1H, J = 3.6, 8.4 Hz, ArH), 7.71–7.80 (m, 4H, ArH), 7.99 (s, 1H, H-triazole ring); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm = 21.3, 45.9, 49.2, 52.3, 109.2, 112.3, 112.8, 116.5, 118.9, 121.7, 123.2, 123.8, 125.5, 135.4, 137.8, 138.4, 139.6, 143.1, 153.9, 155.7, 181.9, 182.3. IR (cm^{-1}): 1738, 1732, 1616, 1609, 1510. MS (EI) m/z: 450 (M^+). Calcd for $C_{22}H_{16}ClN_5O_4$: C, 58.74; H, 3.59; N, 15.57. Found C, 58.63; H, 3.71; N, 15.49.

*4.4.15. 1-{2-[4-(2,3-Dioxo-2,3-dihydro-5-nitro-indol-1-ylmethyl)-[1,2,3]triazol-1-yl]-ethyl}-5-chloro-1H-indole-2,3-dione (**5o**)*

Orange solid; yield: 63.2%; m.p: 137–139 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm = 4.40–4.44 (t, J = 5.7 Hz, 2H, CH_2), 4.48–4.51 (t, J = 5.7 Hz, 2H, CH_2), 4.66 (s, 2H, CH_2), 7.53–7.80 (m, 3H, ArH), 8.13 (s, 1H, H-triazole ring); 8.23–8.71 (m, 3H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm = 46.2, 49.1, 52.7, 108.8, 111.9, 112.5, 116.9, 119.5, 120.8, 123.6, 124.3, 125.1, 133.3, 136.2, 137.9, 140.0, 141.8, 153.5, 156.1, 181.9, 182.4. IR (cm^{-1}):1756, 1744, 1624, 1622, 1513. MS (EI) m/z: 480 (M^+). Calcd for $C_{21}H_{13}ClN_6O_6$: C, 52.46; H, 2.73; N, 17.48; Found C, 52.43; H, 2.82; N, 17.58.

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