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Novel substituted hydrazono indolo[2,1-b]quinazoline-6,12-dione analogues as cytostatic agents: Synthesis, crystal structure, biological evaluation and molecular docking studies

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Abstract

A series of novel substituted hydrazono indolo[2,1-b]quinazoline-6,12-dione analogues have been synthesized and screened for their *in vitro* cytotoxic and antimicrobial activities. Among all the target compounds, **3c** exhibited the most potent inhibitory activity against three cancer cell lines MCF-7, A549, HeLa with IC_{50} values 07.14± 1.285 μM, 09.18± 0.968 μM and 10.57 ± 0.581 μM respectively, while maintaining low toxicity towards non-cancer originated cell line, HEK-293. The detailed studies about molecular interactions with probable target protein indoleamine 2, 3-dioxygenase (IDO1) were done by using docking simulations. The results from docking models are in consistent with the experimental *in vitro* cytotoxic activity conclusions i.e **3c** shows the highest binding energy -11.25Kcal/mol**.** Furthermore, antimicrobial studies revealed that the compound **3e** has shown excellent anti bacterial activity against four tested strains and the compounds **3b**, **3e** and **3f** have shown good anti fungal activity against two tested organisms as compared with their standard drugs.

Keywords: tryptanthrin, cytotoxicity, antibacterial activity, antifungal activity, molecular docking, IDO1.

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Globally nowadays greater number of population was affected by the cancer. In 2012, 14.2 million new cancer cases and 8.2 million deaths were registered, and up to 2030 it is expected to increase to about 19 million.[1] About 22% of cancer deaths are due to the usage of tobacco, 10% of cancer deaths due to lack of physical activities, junk diet, and devouring of alcohol.[2] Several times, conventional treatments like radiation and surgical are not suitable because of the improper location of tumor in the body. In most of the times these methods fail completely to remove tumors. So, for cell devastation without harming to other parts, chemotherapy is the systematic method and the best source for cancer treatment. But most of the drugs available in market for the treatment of cancer (chemotherapy) are having the undesirable side effects. Thus, it is a great challenge for the people working in the domain of medicinal chemistry to design and develop such an efficient, safe, suitable and potent anti cancer drug having the greater resistance without causing any adverse side effects and also which could be able to suppress the high cancer mortality rate in the world is the need of the contemporary research.[4–6]

On the other hand the most common infectious diseases in human beings are caused by the pathogens include bacteria and fungi. These infectious agents enter into the body produce a variety of manifestations and increases at an alarming rate causing deadly diseases. The treatment of infectious diseases remains as an important issue because of the rapid increase in multi-drug resistant microbial pathogens, encourages as in developing a new class of drugs.[7]

Polygonum tinctorium and *Isatis tincoria* (Chinese wood) are the natural products of Chinese medicinal plants, which are the main source for the indolo[2,1-b]quinazolin-6,12-dione (tryptanthrin).[8] The literature reveals that tryptanthrin shows anti microbial activity.[9,10] In 1998, Mitscher Baker reported that tryptanthrin and its analogues act as anti tubercular agents such as PA-505, PA-510[11] and exhibits potent anti inflammatory activities.[12,13] It also has structural similarity with batracyclin, which is a known cytotoxic agent.[14] These structural resemblances of tryptanthrin leads to develop their analogs as excellent therapeutic agents. Presently, studies on anti cancer activity of tryptanthrin are getting more and more popular.[15] Recent studies report that tryptanthrin induces apoptosis and differentiation of leukemia cells[16–18] and inhibits the drug resistant related gene in breast and colon cancer cells.[19,20] It also reduces the activity of cyclooxygenase-2 (COX-2) and reduces the expression of nitric oxide synthase and prostogladine (E2) in cells.[21–23] Some of the biologically active tryptanthrin analogues were shown in **Fig. 1**.

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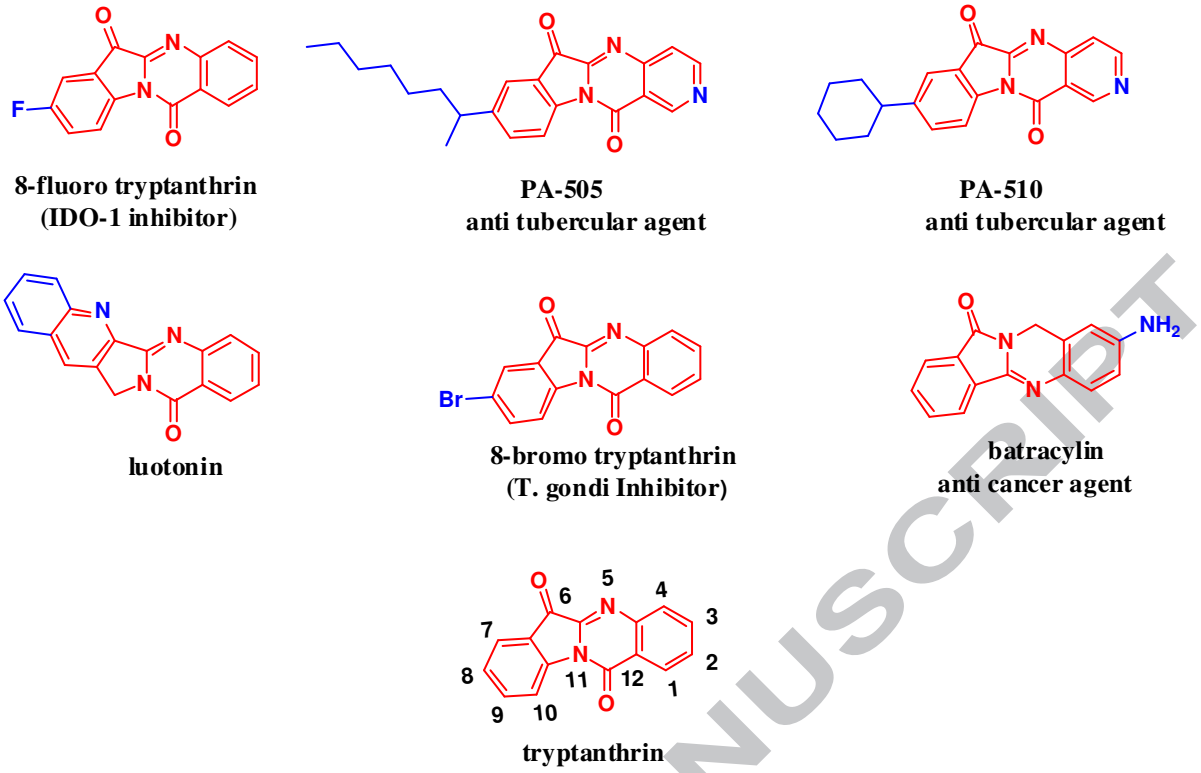

Figure 1. Some biologically potent tryptanthrin derivatives.

In the due course, to diagnose the various novel anti cancer agents, we were particularly interested on the parent molecule tryptanthrin, due to their distinct and wide range of biological properties.[24–26]

The targeted compounds **1a-f**, **2a-f**, **3a-f** and **4a** were synthesized by well established methods outlined in **scheme 1**. The key intermediates were synthesized from condensation of isatin and isotoic anhydride in toluene in presence of triethylamine as a base. Hydrazine derivatives of tryptanthrin were synthesized by condensation of intermediate **A** with hydrazine in THF solvent for 12 h by using reported synthetic protocol.[27] The titled compounds were synthesized by refluxing intermediate **B** with 1,3–diketones in acetic acid for 6–8 h with good yields. The newly synthesized compounds were characterized by ^{1}H and ^{13}C NMR, IR, Mass spectrometry, elemental analysis and single crystal X-ray analysis **(Fig. S1–S27, Supporting Information (SI) and Fig.2).** The IR spectrum of the targeted compound **3c** showed the prominent absorption bands at 3648 cm^{-1} (enolic-OH), 1678 cm^{-1} (C=O), 1544 cm^{-1} (C=N) and 1480 cm^{-1} (C=C). The synthesized molecules may exists in two forms i.e. *keto* (in crystalline state) and *enol* (in solution state) due to the probability of the shifting of enolic-OH hydrogen atom towards the adjacent nitrogen **(Scheme 1).** The ^{1}H NMR spectra of compounds **1a-f**, **2a-f**, **3a-f** and **4a**, shows singlet at the region δ15.89–13.24 ppm, which corresponds to the presence of enolic-OH; however, we did not observed any signals related to N–H proton. The appearance

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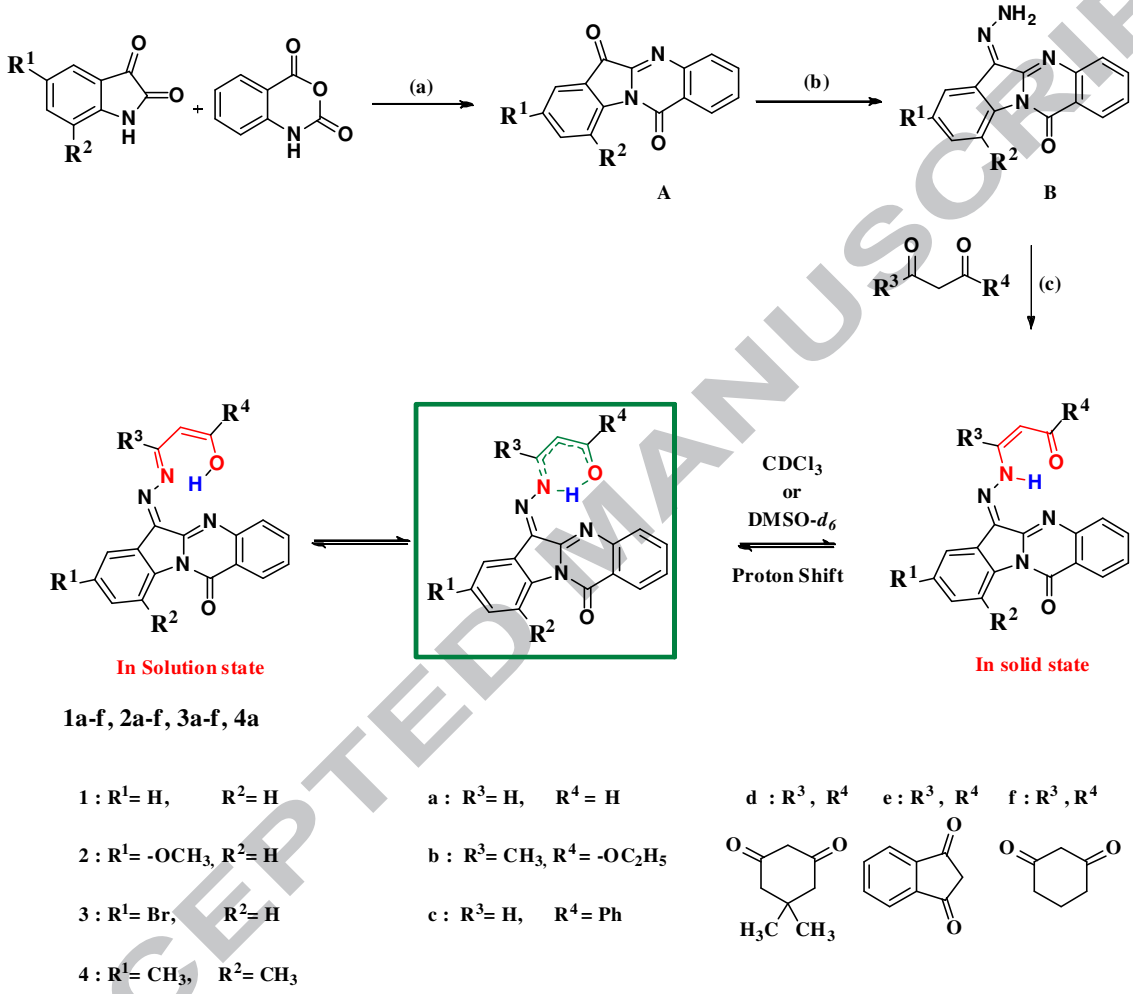
of methine proton of 1,3-diketone at the region 6.25 ppm to 5.72 ppm are evidence for the predominance of *enol* form over the *keto* form in solution state. However, signal at 197.6 ppm and 159.8 ppm in ^{13}C NMR, shows presence of enolic carbon and carbonyl group of quinazoline ring and further strengthens the above mentioned statement. The LCMS of **3c** showed [M+1] ion peak at m/z 437, whereas, elemental analysis data suggested the purity of the bulk sample of **3c.**



Scheme 1. Synthesis of titled compounds **1a-f**, **2a-f**, **3a-f, 4a**. (a) Et_3N, 2-4 h, toluene, reflux. (b) NH_2-$NH_2.H_2O$, 12 h, THF, reflux. (c) Acetic acid, reflux, 1,3-diketones (acetyl acetone, ethyl acetoacetate, benzoyl acetone, dimedone, indanedione,1,3-cyclohexanedione).

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The single crystal X-ray data confirms that the *keto* form predominates over *enol* form in the solid state with good *R*-factor 0.0476 and 0.0527 for **1a** and **3c**, respectively **(Fig. 2 and Table S1, SI).** In both, **1a** and **3c** the bond lengths of C17–O2 are found to be 1.236 Å and 1.243 Å, respectively **(Table S2, SI),** which are in between the standard bond lengths of C=O (1.21 Å) and C–O (1.43 Å). Similarly, the bond lengths of N14–C15 are found to be 1.375 Å and 1.370 Å for the compounds **1a** and **3c**, respectively **(Table S2, SI),** which is in between the standard bond lengths of C–N (1.47 Å) and C=N (1.25 Å). A deep observation concludes that delocalization of π–electrons and proton shifting takes place in between N and O atom as shown in the **Scheme 1**. Crystallographic data and structure refinement parameters are presented in **Table S1**. Both compounds **1a** and **3c** crystallize in monoclinic crystal system with the space group $P2_1/n$ (for **1a**) and $P2_1/c$ (for **3c**). The labeled ORTEP diagram (30% probability level) of **1a** and **3c** are depicted in **Fig. 2**. The selected bond distances of compounds **1a** and **3c** are listed in **Table S2**. Compound **1a** contains inter molecular interactions (C–H···O, C–H···π and C···N). Compound **3c** also includes the similar types of inter molecular interactions(C–H···O, C–H···π and C···N) and forms a one dimensional infinite chain, which is finally transformed into a 2D hydrogen bonded structure having the hexagonal channels. However, for clarity we have shown only C–H···π interactions **(Fig. S28 and S29; SI).** The CCDC numbers for the compounds **1a** and **3c** is **1474666** and **1474667**, respectively.

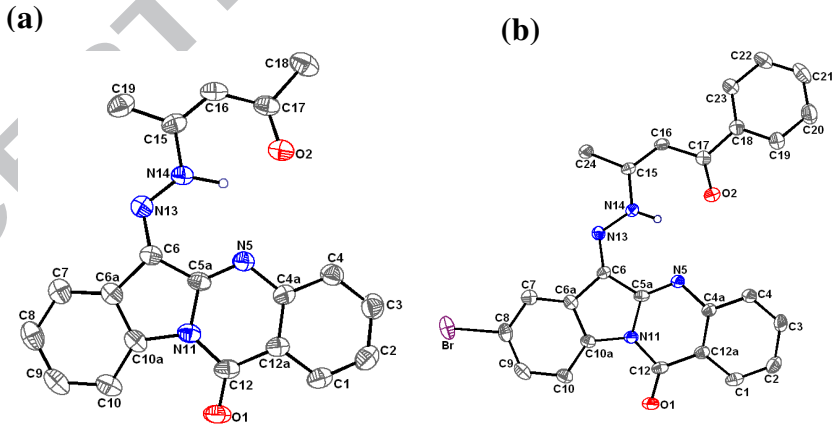

Figure 2. (a) ORTEP diagram of compound **1a**. (b) ORTEP diagram of compound **3c**. In both case the thermal ellipsoids are drawn at 30% probability level; ring hydrogen atoms have been omitted for clarity.

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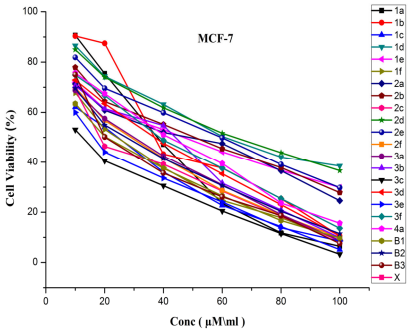
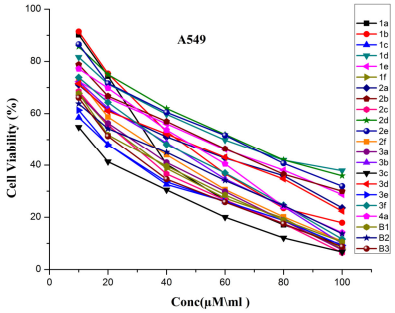
In vitro cytotoxic bioassay: The newly synthesized tryptanthrin derivatives were evaluated for their *in vitro* cytotoxic activities against three human cancer cell lines, according to the procedures described in literature.[28,29] The tumor cell line panel consists of MCF-7, A549 and HeLa cell lines. Cisplatin was used as the reference drug. The relationship between surviving fraction and drug concentration was plotted to obtain the survival curves of MCF-7, A549 and HeLa **(Fig. 3a, b, c).** The response parameter calculated was the IC_{50} value, which corresponds to the concentration required for 50% inhibition of cell viability **(Table 1).** The structure of most potent tryptanthrin derivative is shown in **Fig. 4**.

(a) Anticancer activity(MCF-7)



(b) Anticancer activity(A549)



(c) Anti cancer activity (HeLa)

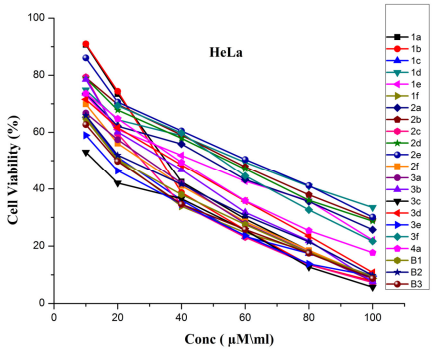


Figure 3. (a), (b), (c) Survival curves of MCF-7, A549, HeLa cell lines.

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As shown in **Table 1**, some of the tryptanthrin derivatives exhibited excellent activity against the three tumor cells. Remaining compounds showed moderate to good anti cancer activity.

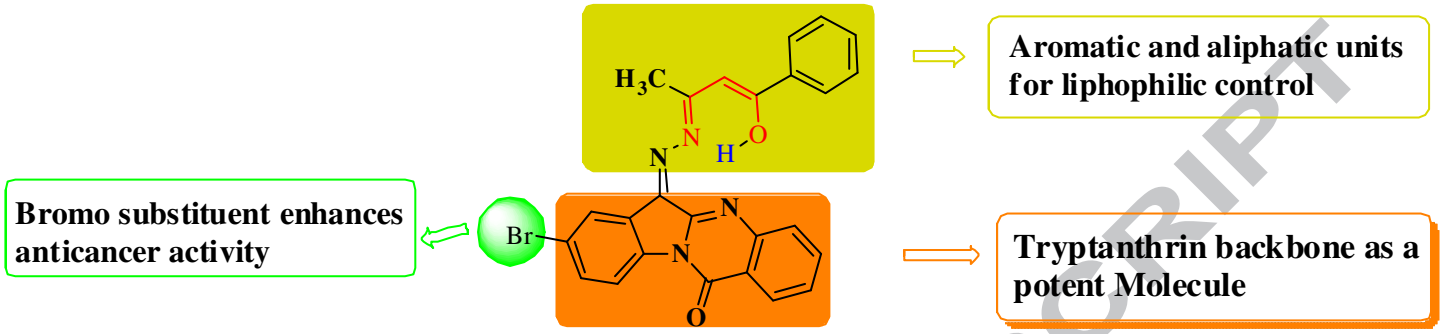

Figure 4. Structure of most potent tryptanthrin analogue **3c**.

Among all the compounds derived from tryptanthrin, **3c (Fig. 3)** has exhibited excellent activity against three cancer cell lines MCF-7, A549, HeLa with IC_{50} values 07.14± 1.285µM, 09.18± 0.968µM and 10.57 ± 0.581µM compared with the standard drug cisplatin. The compounds, **3e** has shown good activity against MCF-7, HeLa with IC_{50} values 15.88 ± 0.889 µM, 17.02 ± 1.201µM and **1c** shown against A549 cell line with IC_{50} value 17.59 ± 0.686 µM. Remaining compounds have exhibited promising activity with IC_{50} values ranging from 22.13 ± 1.028 to 68.75 ± 0.498 µM against MCF-7, A549 and HeLa cancer cell lines. We also screened for their cytotoxicity of active compounds against HEK-293 (Human Embryonic Kidney 293) using MTT-micro cultured tetrazolium assay, IC_{50} values of **1c, 3c, 3e** were 68.09±1.654µM, 46.10±1.453µM and 55.21±1.711µM. The results are presented in **Table 1**.

A deep observation into the structure activity relationship demonstrates that, the cytotoxic proficiency was highly dependent on the type and position of the pharmacophoric substituent on the indole and quinazoline ring of tryptanthrin. Various substituents at C2, C8 position of indole and quinazolone rings in tryptanthrin change the cytotoxic activity against different cell lines.[25] However, in particular the substituents like electron donating or electron withdrawing groups replace the hydrogen atom at C8 position of indole shows completely dissimilar results. Electron withdrawing group Br at C8 position enhances the cytotoxic activity appreciably and electron donating groups $-CH_3$, $-OCH_3$ groups reduces the cytotoxic activity against three cancer cell lines MCF-7, A549 and HeLa compared with non substituted tryptanthrin derivatives.

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Table 1. Cytotoxic activities of newly synthesized tryptanthrin derivatives on human cancer cell lines MCF-7, A549 and HeLa [*in vitro* (IC_{50} µM)][a].

S.No.	Compound	MCF-7	A549	HeLa	HEK293
1	B[1]	25.24 ± 0.791	28.74 ± 0.142	25.59 ± 0.537	ND
2	B[2]	32.29 ± 1.314	31.20 ± 0.219	29.22 ± 1.025	ND
3	B[3]	23.18 ± 0.339	23.09 ± 1.688	22.13 ± 1.028	ND
4	1a	42.08 ± 1.025	43.85 ± 0.393	44.76 ± 0.031	ND
5	1b	50.65 ± 0.977	51.05 ± 1.507	42.86 ± 1.796	ND
6	**1c**	**26.22 ± 0.556**	**17.59 ± 0.686**	**25.22 ± 1.686**	**68.09±1.654**
7	1d	68.75 ± 0.498	66.17 ± 1.283	59.29 ± 0.545	ND
8	1e	50.29 ± 1.545	56.36 ± 1.726	49.40 ± 0.573	ND
9	1f	27.92 ± 1.125	29.84 ± 1.251	23.89 ± 0.743	ND
10	2a	47.93 ± 0.750	46.67 ± 1.368	49.15 ± 0.851	ND
11	2b	54.53 ± 1.307	56.22 ± 0.280	58.23 ± 1.091	ND
12	2c	31.08 ± 1.024	29.33 ± 1.178	33.66 ± 1.389	ND
13	2d	68.69 ± 1.230	67.83 ± 0.388	56.93 ± 0.726	ND
14	2e	60.87 ± 0.431	63.49 ± 0.532	62.29 ± 0.779	ND
15	2f	32.68 ± 1.104	35.99 ± 0.700	32.10 ± 0.445	ND
16	3a	33.00 ± 0.562	31.11 ± 0.975	31.25 ± 1.567	ND
17	3b	36.56 ± 0.712	40.04 ± 0.573	36.68 ± 0.630	ND
18	**3c**	**07.14± 1.285**	**09.18± 0.968**	**10.57 ± 0.581**	**46.10±1.453**
19	3d	40.02 ± 0.980	46.33 ± 1.466	39.81 ± 0.845	ND
20	**3e**	**15.88 ± 0.889**	**20.36± 0.832**	**17.02 ± 1.201**	**55.21±1.711**
21	3f	43.87 ± 0.712	41.67 ± 1.412	35.81 ± 0.504	ND
22	4a	44.54 ± 1.193	46.56 ± 1.321	42.02 ± 1.267	ND
23	**Cisplatin**	**4.28± 0.355**	**5.14± 0.421**	**3.88± 0.354**	**ND**

[a]Values are expressed as mean ± SEM. Cytotoxicity as IC_{50} for each cell line, is the concentration of compound which is reduced by 50% the optical density of treated cell with respect to untreated cell using the MTT assay.

Anti bacterial activity: All the synthesized compounds were screened for their *in vitro* anti bacterial activity against two gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* and two gram-negative bacteria such as *Klebsiella pneumoniae* and *Escherichia coli* using broth dilution method.[30] The standard pathogenic microbial cultures were procured from the Microbial Type Culture Collection (MTCC), Chandigarh, India. Minimum inhibitory concentration (MIC) values for the tested compounds, as well as standards were measured in

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μg/mL. The results of in vitro anti bacterial screening reveals that, most of the compounds exhibit equipotent activity. The compound **3e** exhibited excellent anti bacterial activity against four tested strains *S. aureus* (1.562μg/mL), *B. subtilis* (1.562μg/mL), *K. pneumonia* (3.125 μg/mL) and *E. coli* (3.125 μg/mL) *subtilis* with respect to the standard drug streptomycin. Similarly the compounds **2e, 3b** exhibited good activity against *S. aureus* (1.562 μg/mL), *B. subtilis* (3.125μg/mL) and *E. coli* (6.25μg/mL). The compound **1e** also has shown good to moderate activity against *S. aureus* (3.125μg/mL), *B. subtilis* (6.25μg/mL), *K. pneumonia* (1.562 μg/mL) and *E. coli* (12.5 μg/mL). The Compounds **1f, 2f** and **3f** have shown good activity against *S. aureus, B. subtilis.* The enhanced activity of **3e** against all the strains may be due to the presence of indane ring. The minimum inhibitory concentrations (MIC) of the compounds are presented in **Table 2**.

Table 2. Anti bacterial activity of newly synthesized tryptanthrin derivatives (MIC).

	MIC (μg/mL)			
Compound	**S. *Aureus***	**B. *Subtilis***	**K.*pneumoniae***	**E. *Coli***
B[1]	50	>100	50	>100
B[2]	>100	>100	>100	>100
B[3]	>100	50	25	>100
1a	25	25	12.5	50
1b	6.25	6.25	12.5	12.5
1c	>100	>100	>100	>100
1d	50	50	12.5	50
1e	3.125	6.25	1.562	12.5
1f	6.25	3.125	12.5	6.25
2a	12.5	50	50	50
2b	6.25	6.25	3.125	12.5
2c	>100	>100	>100	>100
2d	>100	>100	>100	>100
2e	1.562	3.125	12.5	6.25
2f	3.125	6.25	6.25	12.5
3a	50	50	>100	50
3b	1.562	3.125	12.5	6.25
3c	>100	>100	>100	>100
3d	>100	>100	>100	>100
3e	1.562	1.562	3.125	3.125
3f	6.25	3.125	6.25	12.5
4a	12.5	12.5	6.25	25
Streptomycin	**6.25**	**3.125**	**1.562**	**6.25**

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Anti fungal activity: All the synthesized compounds were investigated for their *in vitro* anti fungal activity against the fungal strains *Aspergillus niger* and *Pencillium notatum* comparing with standard drug Ketoconazole with minimum inhibitory concentration (MIC) values. The results of the *in vitro* anti fungal activity in MIC of tested compounds (**Table 3**) revealed that the compounds **3b**, **3e** and **3f** exhibited excellent anti fungal activity against *Aspergillus niger and Pencillium notatum* with values for **3b** (6.25 µg/mL, 3.125 µg/mL), for **3e** (3.125 µg/mL, 6.25 µg/mL) and for **3f** (3.125 µg/mL, 6.25 µg/mL). Compounds **1b**, **1e** and **1f** exhibited moderate to good activity against *Aspergillus niger* strains with values 12.5 µg/mL, 6.5 µg/mL, and 12.5 µg/mL. Furthermore, compound **2f** registered moderate activity against *Pencillium notatum* organism with 12.5 µg/mL. Remaining compounds are moderate to poor in anti fungal activity against the tested fungal strains.

Table 3. Anti fungal activity of newly synthesized tryptanthrin derivatives (MIC).

Minimum Inhibitory Concentration MIC (µg/mL)		
Compound	*Asperegillus niger*	*Pencillium notatum*
B[1]	50	>100
B[2]	>100	50
B[3]	50	50
1a	>100	>100
1b	12.5	25
1c	25	25
1d	100	100
1e	6.25	12.5
1f	12.5	25
2a	>100	100
2b	50	50
2c	25	50
2d	100	>100
2e	50	50
2f	100	12.5
3a	>100	>100
3b	6.25	3.125
3c	25	25
3d	100	>100
3e	3.125	6.25
3f	6.25	6.25
4a	100	100
Ketoconazole	**3.125**	**3.125**

Standard = *Ketoconazole* ; Strains: *Asperegillus niger, pencillium notatum*

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Molecular docking studies: To illustrate the interaction mechanism of most active tryptanthrin derivatives with enzyme and to correlate with its docking score with the experimental cytotoxic activity results, we have chosen a computer aided experimental method that is molecular docking. The 2D structures of synthesized target compounds were drawn in Chem Draw Ultra 12.0 and structures have been fully optimized with small **hf/3-21g*** [31] basic set by using Guassian 09.[32]

Table 4. Binding energies of tryptanthrin inhibitors against IDO1 receptor.

S.No	Compound	Binding Energy (K cal/mol)
1	B^1	-8.57
2	B^2	-8.88
3	B^3	-8.27
4	1a	-9.60
5	1b	-9.70
6	1c	-10.99
7	1d	-10.64
8	1e	-10.71
9	1f	-10.18
10	2a	-10.30
11	2b	-9.22
12	2c	-10.91
13	2d	-10.81
14	2e	-11.03
15	2f	-10.56
16	3a	-10.02
17	3b	-9.78
18	**3c**	**-11.25**
19	3d	-10.77
20	3e	-11.04
21	3f	-10.13
22	4a	-9.17

The crystallographic 3D structure of IDO1 enzyme was extracted from RSC PDB *(an information portal to biological macromolecular structure)* PDB ID: 2d0U. It is an attractive target in the cancer disease and it catalyzes essential amino acid L-tryptophan to N-formylkynurenine.[33] Indoleamine 2,3-dioxygenase is the first and rate-limiting enzyme of tryptophan catabolism through kynurenine pathway, thus causing depletion of tryptophan which can cause halted growth of microbes as well as T cells.[34] Protein structure was cleaned by

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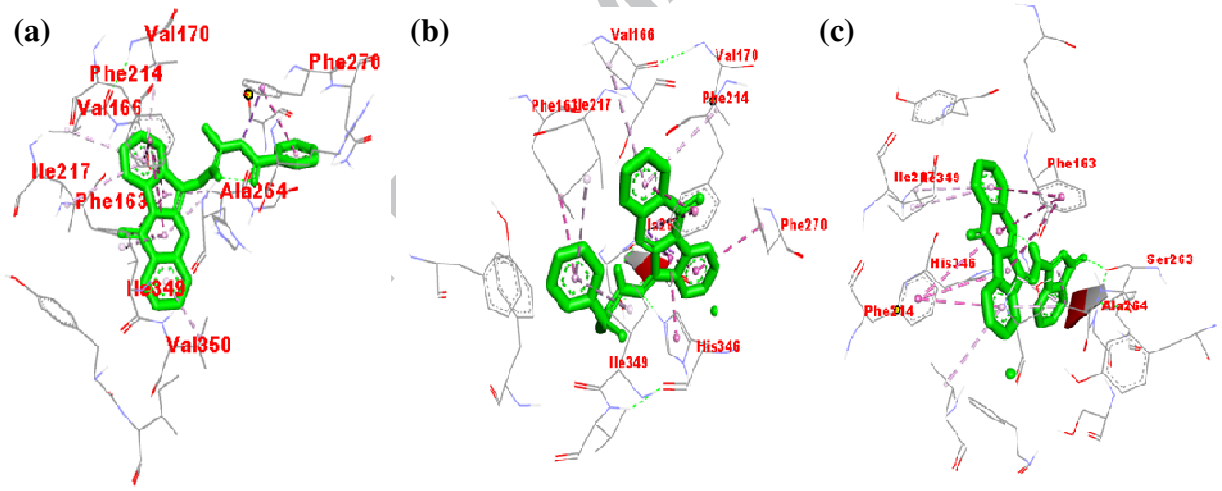
removing water molecules, ligands using UCSF chimera 1.10.1 software. Such energy minimized compounds and receptors are used for docking.

The inhibitors **1c, 2e, 3c** and **3e** bind strongly to receptor as inferred by their minimum binding energies -10.99, -11.03, -11.25 and -11.04 K cal/mol respectively. The docking results were in good coincidence with experimental IC_{50} values **(Table 4). The Fig. 5,** shows the best conformations of **1c, 3c** and **3e** forming a cluster into the binding pocket of receptor. Molecular docking results reveal that in IDO1 enzyme complex, all the interactions of **1c** are hydrophobic interactions with amino acids and there were no hydrogen bonding interactions. In **Ic,** the phenyl ring of 1,3-diketone interacts with amino acid Phen270 through π- π stacking, in tryptanthrin skeleton indole-6-membered aromatic ring with amino acids Phen214, Ile217, Val166, Val170 and indole 5-membered ring with amino acids Phe163, Phe214 through π–π stacking. Quinazoline 6-membered ring in tryptanthrin interacts with amino acids Ala264, Phen163, Phen214, Ile349 and aromatic ring with amino acid Val350 through hydrophobic interactions.



Figures 5. (**a**), (**b**), (**c**) shows the binding poses and interactions of tryptanthrin analogues **1c**, **3c** and **3e** to the binding sites of IDO1 enzyme.

3c has only one hydrogen bonding interaction with His346 by N-atom, hydrophobic interactions with the phenyl ring of di-ketone with amino acids Phen163, Ile217 and Ile349 through π–π stacking. Indole-6-membered ring with amino acids Phe270, Ala264 and indole 5-membered aromatic ring with Ala264, His346 interacts through π–π stacking. Quinazolone 6-membered

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ring in tryptanthrin with amino acids Ala264, Phen214 and aromatic ring with amino acids Phen214, Val166 and Val170 through hydrophobic interactions. Compound **3e** has two hydrogen bonding interactions with His346 by N-atom, and another by keto group of indane ring with Ser263 amino acid. The remaining all are hydrophobic interactions through π–π stacking. Indole 6-membered aromatic ring with amino acids Phen214, Ala264, Val170 and indole 5-membered ring with amino acids Phen214, Ala263 through π–π stacking interaction. Quinazolone 6-membered ring in tryptanthrin with following amino acids Phen163, phen214 and aromatic ring with amino acids Phen163, Ile217 and Ile349 through hydrophobic interactions.

In summary, we have synthesized novel 1,3-diketone substituted hydrazono tryptanthrin derivatives. These compounds were evaluated for their *in vitro* anti cancer activity against three cancer cell lines MCF-7, A549 and HeLa. The molecular docking studies of these inhibitors against target IDO 1gave the highest binding energies -11.25kcal/mol,-11.04 kcal/mol for **3c** and **3e**. The binding energies and H-bonding interactions to the amino acids of active sites of the target enzyme well supported the experimental *in vitro* cytotoxicity (IC_{50}) results. Furthermore, **3e** has exhibited the excellent anti bacterial activity against two gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, two gram-negative bacteria such as *Escherichia coli* and *Klebsiella pneumonia.* The compounds **3b**, **3e** and **3f** have shown most promising anti fungal activity against *Pencillium notatum* and *Aspergillus niger* strains. These encouraging results are useful in designing and developing new drugs from these derivatives.

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Supporting information

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org.....

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Graphical Abstract

Novel substituted hydrazono indolo[2,1-b]quinazoline-6,12-dione analogues as cytostatic agents: Synthesis, crystal structure, biological evaluation and molecular docking studies

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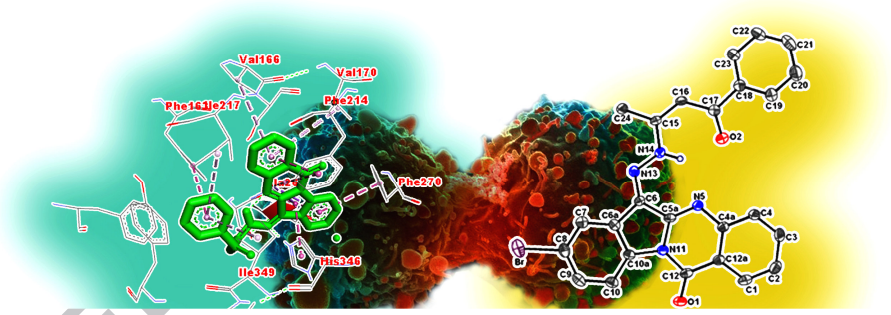
New tryptanthrin derivatives have shown moderate to excellent anti cancer activity against three human cancer cell lines MCF-7, A549 and HeLa.



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Research Highlights

- *Keto* form predominates in the solid state of hydrazono-tryptanthrin analogues.
- Compound with highest binding energy **-11.25**Kcal/mol shown excellent cytotoxicity.
- Anti-bacterial activity for indane substituted hydrazono-tryptanthrin was outstanding.
- Three 8-bromotryptanthrin analogues shown promising anti-fungal activity.
- IDO1 protein: a potential target for tryptanthrin analogues.

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