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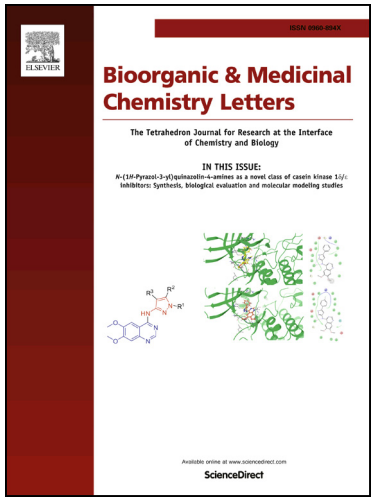

Design, synthesis and biological evaluation of 8-substituted-6-hydrazonoindolo [2, 1-*b*] quinazolin-12(6*H*)-one scaffolds as potential cytotoxic agents: IDO-1 targeting molecular docking studies

Ramu Guda, Rajashekar Korra, Siripireddy Balaji, Rambabu Palabindela, Rakesh Eerla, Harikiran Lingabathula, Narsimha Reddy Yellu, Girijesh Kumar, Mamatha Kasula

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Design, synthesis and biological evaluation of 8-substituted-6-hydrazonoindolo [2, 1-*b*] quinazolin-12(6*H*)-one scaffolds as potential cytotoxic agents: IDO-1 targeting molecular docking studies

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ABSTRACT

Herein, we have reported the synthesis of 18 novel 8-substituted tryptanthrin analogues based on our earlier work. All these tryptanthrin analogues were well characterized by ^{1}H & ^{13}C NMR, FT-IR, Mass Spectrometry and Elemental Analysis. All these 8-substituted analogues were screened for their anti-oxidant activity by DPPH radical scavenging assay. Out of all the tested compounds, **T^{11}**, **T^{12}**, **T^{17}** and **T^{18}** showed potent anti-oxidant activity. The anti-cancer activity have been performed by using MTT assay protocol and their results depicts that compounds having the 4-pyridyl or 4-carboxyphenyl substituents at the 8th position of the tryptanthrin framework are found to be the most promising cytotoxic agent against A549, MCF-7 and HeLa human cancer cell lines compared to others as well as with the standard drug cisplatin. Moreover, the comparative molecular docking studies against the three protein receptors IDO1, EGFR and HER2 strongly suggested that IDO1 is the best target protein, which exhibits lowest binding energies of –11.73 and –11.61 kcal mol^{-1} for **T^{11}** and **T^{12}** scaffolds, respectively towards the *in vitro* anti-cancer activity.

Keywords: tryptanthrin, cytotoxicity, anti-oxidant activity, MTT assay, DPPH, molecular docking, IDO1.

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Presently, cancer is one of the most challenging diseases found worldwide and also the most stimulating factor for the human morbidity and mortality. Therefore, the design and development of an anticancer drug is still one of the most challenging task for the people working in the domain of medicinal chemistry. As we know that the drug currently available in the market have several side effects on the normal cells and thus causing several abnormalities in the body despite of their enormous utilizations. The cost and availability are the another major concern of the present anti-cancer drugs.[1,2] Hence, developing the new therapeutic drugs having the advance proficiency and efficacy on the cancer cells is one of the foremost goal for the researchers.[3] In addition, to understand the molecular biology and pharmacology of cancer at a molecular level the target based drug-design and discovery is another emerging and challenging filed for the researchers.[4–6] In this regards, the highly developed technology in molecular biology advantages to identify many cancer targets in human body.[7] So, considerable attention and effort have been needed for the discovery and development of new and potential anti-cancer drugs.

In this regards, indoloquinazolines received considerable curiosity in the field of therapeutic and diagnostic medicines. The natural product tryptanthrin (Indolo [2,1-*b*]quinazoline-6,12-dione) is obtained from the Chinese medicinal plants *Polygonum tinctorium* and *Isatis tinctoria* (Chinese wood) and shows significant biological activities due to the presence of indoloquinazolines moiety (Fig. 1).[8] Furthermore, as tryptanthrin framework is derived from the isatin, which is well known inhibitors for anti-bacterial, anti-viral and anti-fungal agents and thereby also capable to influence their therapeutic effect of the concerned drugs.[9-15] These important classes of heterocycles have attracted remarkable attention[16] and possession of the researchers to investigate their synthesis, pharmacological and biological screening such as anti-leishmanial[17] anti-microbial[18,19] anti-tuberculosis[20] anti-inflammatory[21–23],

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anti-oxidant agents[24] and their cytotoxicity against many cancer cell lines in mammalian cells.[25] This compound can control the activity of COX-2[26,27] and inhibits the expression of nitric oxide synthase and prostaglandin E(2) in cells.[28–30] Even though tryptanthrin has wide range of applications it has some small drawback such as poor aqueous solubility as well. Inspiring with the aforementioned fascinating importance of the tryptanthrin scaffolds, we are interested to develop some new tryptanthrin based anti-cancer agents and IDO1 inhibitor.

In literature 8-floro substituted tryptanthrin derivatives were successfully demonstrated as potential anti-cancer agent and IDO1-inhibitor.[31] wherein, 8-bromo substituted hydrazono tryptanthrins were found to be an excellent cytotoxic agents as well as a good inhibitors for IDO-1 enzyme. The *in vitro* anti-cancer activity and molecular docking studies were also well exploited in our previous report.[32] Herein, we have disclosed the design and synthesis of a series of 18 novel 8-substituted (F, Cl, I, NO_2, 4-pyridyl or 4-carboxyphenyl) tryptanthrin analogues **T^1 – T^{18}** (Scheme 1 and Fig. 2) and investigated their anti-cancer evaluation by MTT assay using human cancer cell lines MCF-7, A549, HeLa and anti-oxidant activity screening by using DPPH radical scavenging method. The molecular docking studies were also performed using the three target proteins IDO1, EGFR and HER2. The tryptanthrin analogues **T^1 –T^4** were prepared by adapting the literature protocol and using the respective substituted (F, Cl, Br, I and NO_2) indoline-2,3-dione and 2H-benzo[d][1,3]oxazine-2,4(1*H*)-dione followed by the facile condensation using the hydrazine hydrate in presence of acid.[33] Compounds **T^1**–**T^4** afforded **T^7**–**T^{10}** and **T^{13}**–**T^{16}** after the reaction with benzoyl acetone and *o*-vanillin, respectively in presence of acetic acid and following the literature protocol.[34,35] Moreover, Suzuki coupling reaction of respective boronic acid and bromo substituted tryptanthrin analogue (see Scheme 1) gave **T^5** and **T^6**. However, **T^{11}** and **T^{12}** were prepared by refluxing **T^5** and **T^6** with benzoyl acetone. In similar

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manner, $\mathbf{T^5}$ and $\mathbf{T^6}$ also afforded $\mathbf{T^{17}}$ and $\mathbf{T^{18}}$ after the reaction with *o*-vanillin. The synthetic route adapted for the preparation of all the aforementioned 8-substituted-6-hydrazonoindolo [2,1-*b*]quinazolin-12-(6*H*)-one and its derivatives were illustrated in Scheme 1. Whereas, details of the synthesis and characterization of these tryptanthrin analogues $\mathbf{T^1}$ **–**$\mathbf{T^{18}}$ were depicted in Supporting Information (Fig. S1 – S14).

The ^{1}H NMR spectra of the compounds $\mathbf{T^1}$ **–** $\mathbf{T^6}$ showed two signals in the range of δ 11.02 – 11.35 ppm and δ 10.20 – 10.55 ppm, which is assigned as NH_2 protons. However, compounds $\mathbf{T^7}$ – $\mathbf{T^{12}}$ exhibits a signal in the region of δ 13.65 – 16.25 ppm, which corresponds to the enolic proton (-OH). The singlet appered in the region of δ 5.45 – 6.35 ppm attributed to the alkene (=CH-) proton. For compounds $\mathbf{T^{13}}$ – $\mathbf{T^{18}}$ singlet related to the =CH proton appread in the region δ 8.57 – 8.45 ppm. The ^{13}C NMR spectra showed the signals in the region of δ 198.2 – 187.8 and δ 167.8 –155.7 ppm related to the enolic carbon and carbonyl group of quinazoline ring and thus strengthens the aforementioned statement. The FT–IR spectrum of $\mathbf{T^{12}}$ also displays peaks in the region of 3332 – 3375 cm^{-1} and at 1738 cm^{-1}, which are assigned for the $\nu_{(OH)}$ and $\nu_{(C=O)}$, respectively. The LCMS spectrum of the compounds $\mathbf{T^{11}}$ and $\mathbf{T^{12}}$ shows peaks at m/z 485 and 425, which is related to the $[M+H]^+$ peak of the $\mathbf{T^{11}}$ and $\mathbf{T^{12}}$, respectively.

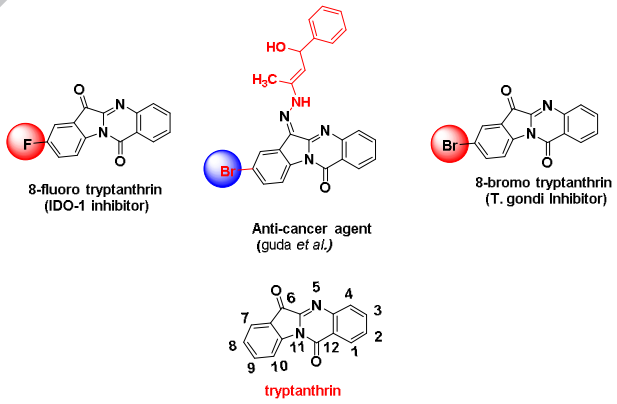

Fig. 1. Some biologically potent 8-substituted-6-hydrazonoindolo [2, 1-*b*] quinazolin-12-(6*H*)-one derivatives.

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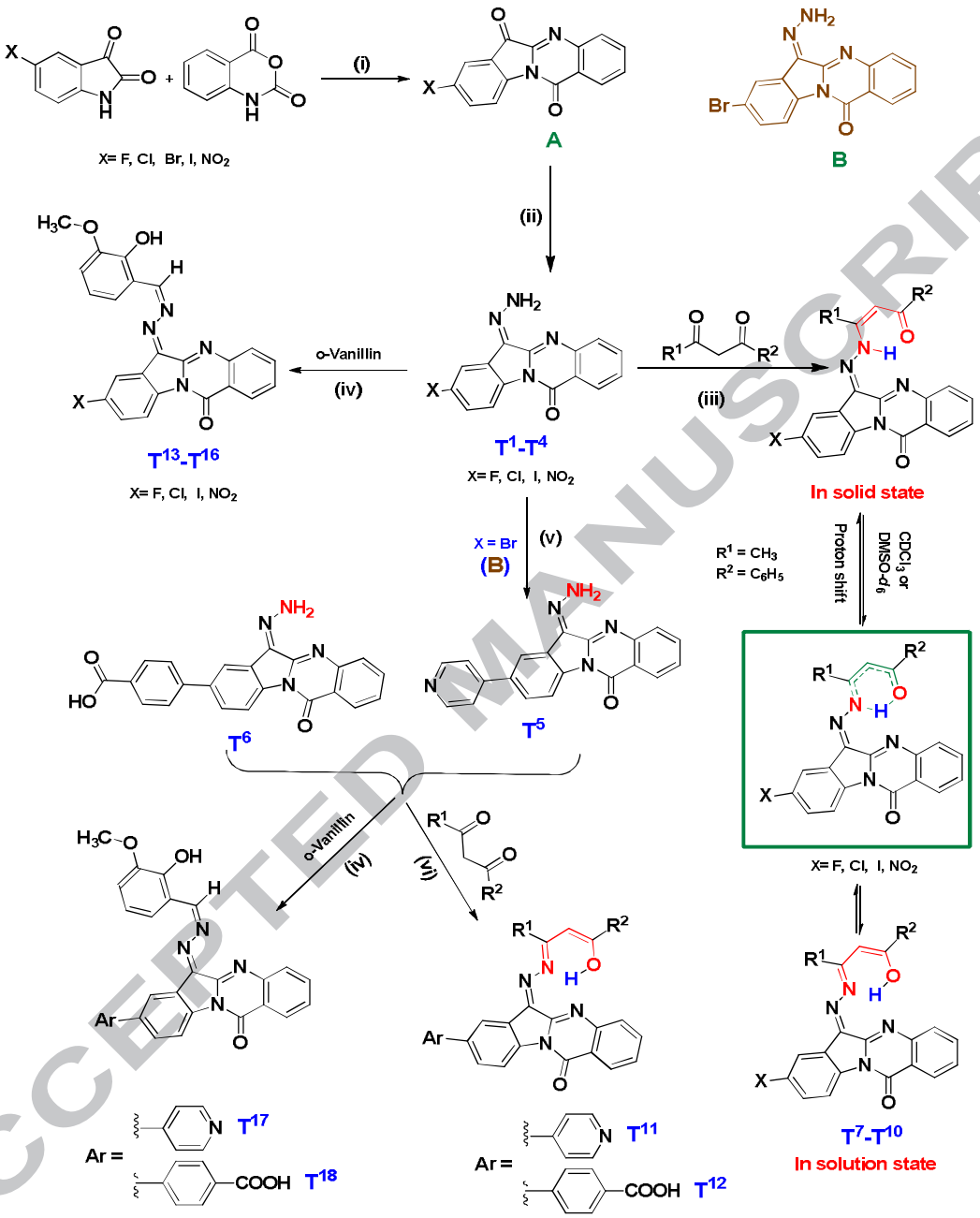

Scheme 1. (i) Et_3N, 2–4 h, toluene, reflux. (ii) NH_2-$NH_2.H_2O$, 12 h, THF, reflux. (iii) Acetic acid, benzoyl acetone, reflux. (iv) *o*-vanillin, CH_3OH, reflux 12 h. (v) Na_2CO_3 (20 mL, $PdCl_2(PPh_3)_2$, DMF, 9 h, 110 °C, 4-pyridineboronic acid or 4-carboxyphenyl boronic acid. (vi) Acetic acid, benzoyl acetone, reflux.

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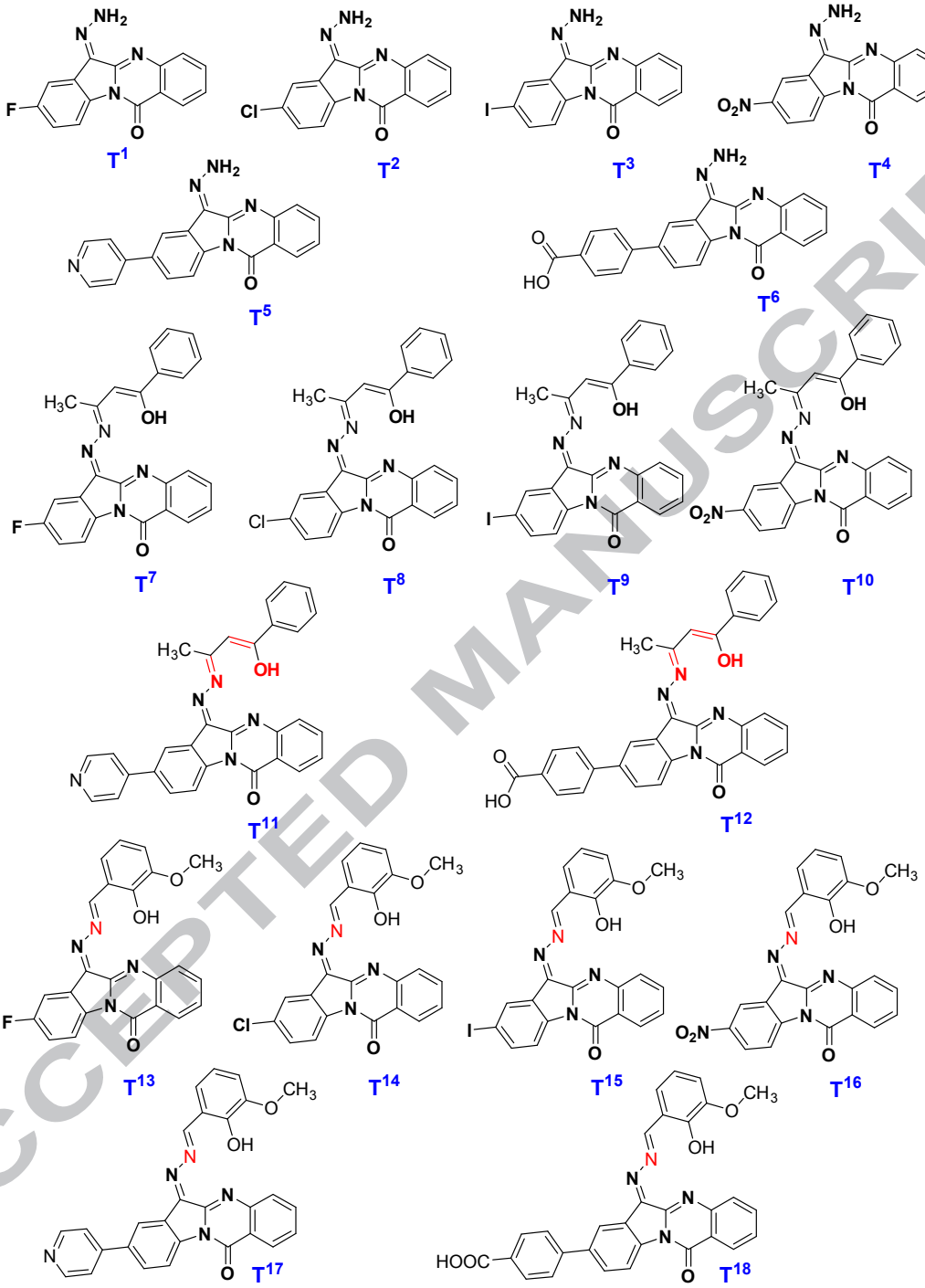

Fig. 2. Chemical structures of all the 8-substituted-6-hydrazonoindolo [2, 1-*b*] quinazolin-12-(6*H*)-one derivatives.

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Cytotoxic Activity. All these 8-substituted-6-hydrazonoindolo [2,1-*b*] quinazolin-12-(6*H*)-one derivatives **T[1]** – **T[18]** were evaluated for their *in vitro* anti-cancer activity against the human breast cancer MCF-7, Lung cancer A549 and HeLa cervical cancer cell lines according to procedure described in literature and taking cisplatin as the reference drug.[36,37] The structure of most potent tryptanthrin derivative is shown in Fig.3. The relationship between surviving fraction and drug concentration was plotted to obtain the survival curves of MCF-7, A549 and HeLa (Fig. 4). The response parameter calculated was the IC_{50} value, which corresponds to the concentration required for 50% inhibition of cell viability. Among all the 8-substituted-6-hydrazonoindolo [2, 1-*b*] quinazolin-12-(6*H*)-one derivatives used in this studies compounds **T[8]**, **T[9]**, **T[11]** and **T[12]** shows better potent behavior than others. In particular **T[11]** and **T[12]** are showing most potent cytotoxicity against three tumor cell lines with their IC_{50} values of 11.60 ± 1.821 µM, 9.42 ± 1.239 µM against MCF-7, 6.01 ± 1.116 µM, 7.19 ± 0.991µM against A549 and 12.20 ± 0.239 µM, 9.42 ± 1.594 µM against HeLa, respectively, compared to the standard drug cisplatin. Compounds **T[8]** and **T[9]** shown moderate IC_{50} values of 19.71 ± 0.937 µM, 19.89 ± 1.44 µM for MCF-7, 18.07 ± 0.375 µM, 20.36 ± 1.832 µM for A549 and 23.64 ± 1.629 µM, 18.82 ± 1.168 µM for HeLa, respectively. These results indicates that compounds **T[8]** – **T[12]** shows good cytotoxic activity towards the three cell lines MCF-7, A549 and HeLa. Under identical conditions the remaining compounds exhibits poor anti-cancer activity with the IC_{50} values ranging from 20.368± 0.190 µM to 67.68 ± 0.862 µM for the three cell lines. We have also screened cytotoxic activity of **T[8]** – **T[12]** against HEK-293 (Human Embryonic Kidney-293) using MTT-micro cultured tetrazolium assay and noticed IC_{50} values of 76.09±0.901µM, 64.12±1.482µM, 71.04±1.034µM and 82.54±0.694 µM for **T[11]**, **T[12]**, **T[8]** and **T[9]**, respectively and

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the detail results are presented in Table 1. Notably and importantly, the results summarized in Table 1 advocated that there are no adverse effects noticed on the normal cell lines.

Table 1. Cytotoxic activities of newly synthesized 8-substituted-6-hydrazonoindolo [2,1-*b*] quinazolin-12-(6H)-one derivatives **T^1 – T^{18}** 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	T^1	54.55 ± 0.871	44.59± 0.974	67.68 ± 0.862	ND
2	T^2	67.51± 1.453	62.12 ± 1.190	55.67 ± 1.122	ND
3	T^3	44.94 ± 0.841	43.44 ± 1.454	51.05 ± 1.091	ND
4	T^4	53.56 ± 0.745	45.55 ± 0.896	50.29 ± 0.540	ND
5	T^5	50.38 ±0.977	47.76 ± 0.511	44.12 ± 0.801	ND
6	T^6	61.10 ±1.004	62.16 ± 0.540	54.26 ± 1.538	ND
7	T^7	20.368±0.190	32.00 ± 1.230	24.46 ± 0.836	ND
8	**T^8**	**19.71 ± 0.937**	**18.07 ± 0.375**	**23.64 ± 1.629**	**71.04±1.034**
9	**T^9**	**19.89 ± 1.44**	**20.36 ± 1.832**	**18.82 ± 1.168**	**82.54±0.694**
10	T^{10}	31.52 ± 1.234	28.39 ± 0.708	33.10 ± 0.300	ND
11	**T^{11}**	**11.60 ± 1.821**	**6.01 ± 1.116**	**12.20 ± 0.239**	**76.09±0.901**
12	**T^{12}**	**9.42 ± 1.239**	**7.19 ± 0.991**	**9.42 ± 1.594**	**64.12±1.482**
13	T^{13}	35.51 ± 1.11	31.20 ± 1.075	34.28 ± 0.886	ND
14	T^{14}	35.38 ± 0.982	40.84 ± 1.234	36.56 ± 1.547	ND
15	T^{15}	30.38± 1.021	32.29 ± 1.964	29.22± 1.237	ND
16	T^{16}	34.25± 0.237	40.06 ± 0.873	38.03 ± 0.966	ND
17	T^{17}	24.77 ± 1.568	32.87 ± 1.342	24.77 ± 1.452	ND
18	T^{18}	37.07± 1.471	39.25 ± 1.803	32.29 ± 1.053	ND
19	**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.

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A closer look of the structure-activity relationship demonstrates that usually the potency of heterocyclic compounds towards biological activity mostly depends on the position and type of substituents present on the core scaffold. Substituents present at C2 and C8 position of indole and quinazolone rings in tryptanthrin changes the cytotoxic activity against different cell lines.[31,32] However, substituents which can influence the core moiety either by donating electrons or by withdrawing the electron density of indole ring of the tryptanthrin also effects the cytotoxicity proficiency against the different human cancer cell lines. In this work we have noticed enhance cytotoxic activity in case of 4-pyridyl and 4-carbaxyphenyl substituted (at 8th position) tryptanthrin derivatives against three human cancer cell lines MCF-7, A549 and HeLa compared with non-substituted tryptanthrin derivatives and less cytotoxicity against HEK293 (normal human cancer cell line).

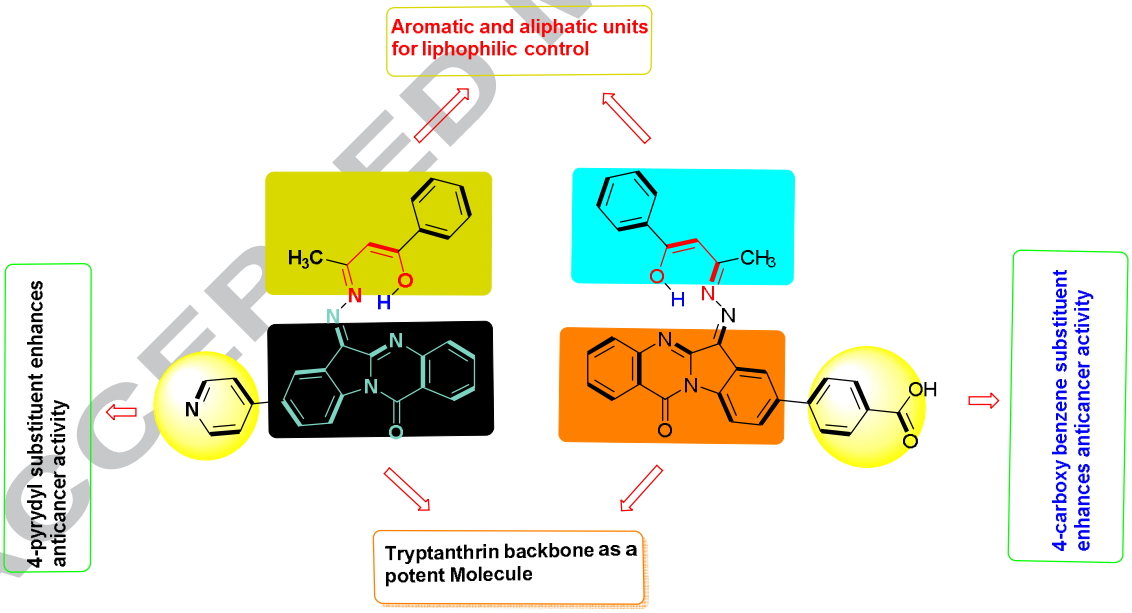

Fig. 3. Structure of most potent 8-substituted-6-hydrazonoindolo [2,1-*b*] quinazolin-12-(6*H*)-one analogues **T**[11] and **T**[12].

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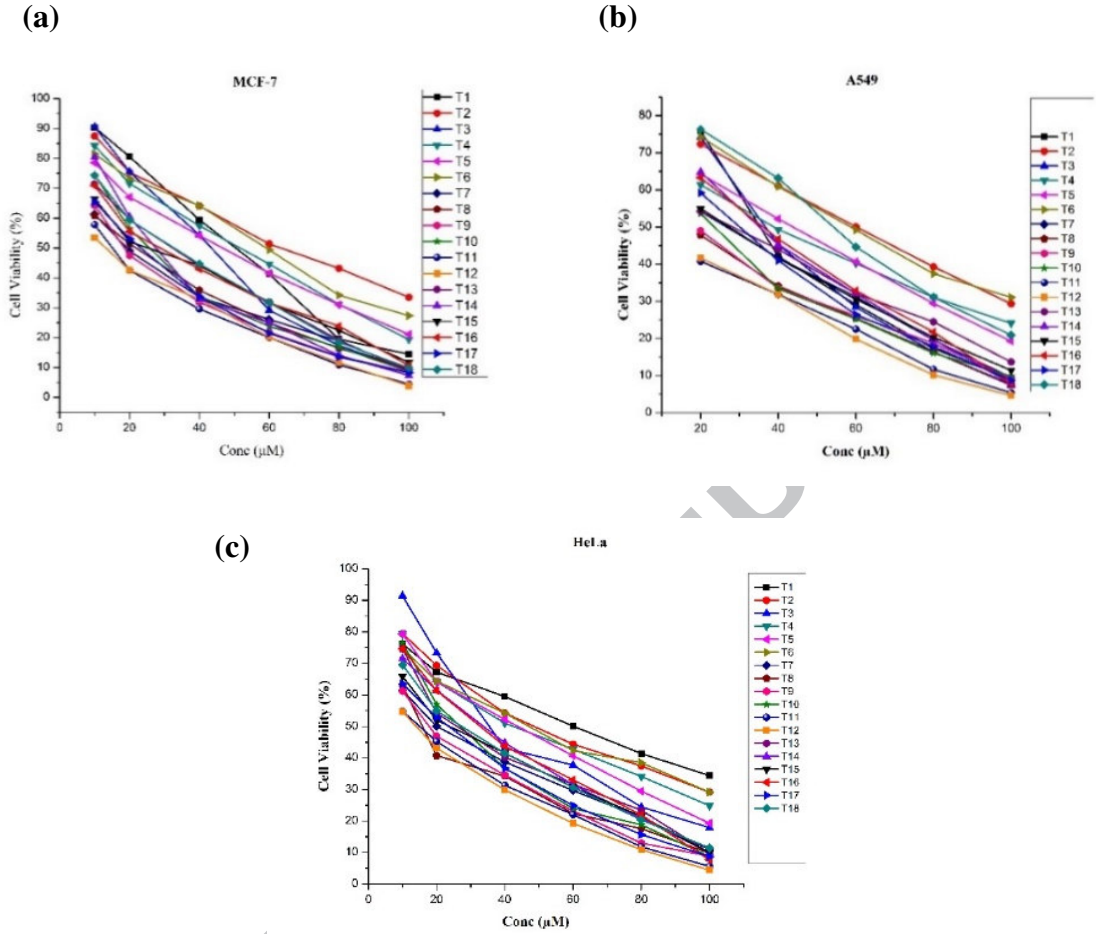

Fig. 4a–c. Survival curves of MCF-7, A549 and HeLa cell lines.

In Vitro Anti-Oxidant Assay. In order to investigate the potential application of $\mathbf{T^1 - T^{18}}$ towards the *in vitro* anti-oxidant activity, DPPH radical scavenging assay was performed. In this experiment, 10^{-6} molar methanolic solution of the concerned compounds was used followed by the seriel dilution. In small test tubes 1 mL (in each tube) mixture of scaffold (100μL) and DPPH radical (10^{-4} molar, 900μL) were taken and incubated at 37 °C under dark conditions for 30 min (French *et al*. 1994). The absorbance of the DPPH solution and mixture was recorded using the

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UV-visible spectrophotometer. The DPPH solution exhibits λ_{max} of 517 nm (typical range of DPPH radical, Blois *et al*. 1958; Lu and Foo, 2000; Zhu *et al.* 2002). Notably, the absorbance is decreases in presence of the concerned compounds with the colour change from the blue to yellow. In principle, DPPH has an odd electron and thus can accept an electron or hydrogen free radical and or also able to liberate this free radical, which can be scavenges by synthesized compounds. The shifting of absorbance value towards the lower wavelength side indicates the increasing radical scavenging activity of the test compounds (*Gulçin et al.* 2004*)*. The percentage of inhibition values were compared by using absorbance values of standard ascorbic acid under identical conditions. The anti-oxidant activity was considered in IC_{50} in µM (the effective concentration at which 50% of the radicals were scavenged) and shown in Table 2.[38,39] Moreover, all the samples were tested in triplicate to maintain the accuracy and to find out the standard deviation.[40] From Table 2, it is clear that these synthesized compounds showed remarkable anti-oxidant activity compared to the standard drug. Importantly, compounds **T**^{11}, **T**^{12}, **T**^{17} and **T**^{18} were found to be the most potent anti-oxidants with the least IC_{50} values of 6.23 µM (for **T**^{11}), 5.02 µM (for **T**^{12}), 4.98 µM (for **T**^{17}) and 5.31 µM (for **T**^{18}), respectively, among all the synthesized compounds.

We argued that, this better *in vitro* anti-oxidant activity of compounds **T**^{11}, **T**^{12}, **T**^{17} and **T**^{18} are might be due to the presence of electron-rich functionality such as 4-pyridyl or 4-carboxypheny in the tryptanthrin scaffolds. The proton exchange capability between carbonyl and –NH group of compounds **T**^{11} and **T**^{12} may possibly be another aspect for enhanced radical scavenging activity. Moreover, **T**^{5}, **T**^{8}, **T**^{9}, and **T**^{15} displaying moderate anti-oxidant activity compared with standard ascorbic acid having the IC_{50} values of 11.23 µM (for **T**^{5}), 12.45 µM (**T**^{8}), 11.60 µM (for **T**^{9}) and 10.90 µM (for **T**^{15}), respectively. The remaining compounds

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exhibits reasonable to poor anti-oxidant activity with IC_{50} value ranging from 16.45 µM to 29.71 µM. Notably, **T^{10}** and **T^{16}** shows poorest anti-oxidant activity among all the synthesized compounds with IC_{50} values of 29.71 µM and 28.68 µM, respectively (Table 2) attributed to the presence of electron withdrawing -NO_2 group at 8-position of the tryptanthrin framework.

Table 2. Anti-oxidant activity of 8-substituted-6-hydrazonoindolo [2, 1-*b*] quinazolin-12-(6*H*)-one derivatives (**T^1 – T^{18}**) by DPPH assay.

S. No	Compounds	IC_{50} (µm)
1	T^1	16.45
2	T^2	19.87
3	T^3	24.87
4	T^4	27.32
5	T^5	11.23
6	T^6	21.96
7	T^7	20.14
8	T^8	12.45
9	T^9	11.60
10	T^{10}	29.71
11	**T^{11}**	**6.23**
12	**T^{12}**	**5.02**
13	T^{13}	21.07
14	T^{14}	22.21
15	T^{15}	10.90
16	T^{16}	28.68
17	**T^{17}**	**4.98**
18	**T^{18}**	**5.31**
Standard	**Ascorbic acid**	**3.48**

Molecular docking studies. Docking investigation was performed to compounds **T^1 – T^{18}** with pharmacological target proteins, indoleamine 2,3-dioxygenase (IDO1), epidermal growth factor receptor (EFGR) and human epidermal growth factor receptor 2 (HER2) to compare their

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potential as drug candidates. IDO1, which is an attractive target in design of anti-cancer drugs as it catalyzes essential amino acid L-tryptophan to N-formylkynurenine.[41] In principle, IDO1 is the primary and rate-limiting enzyme of tryptophan catabolism and responsible for the depletion of tryptophan leading to halted growth of microbes as well as T-cells.[42] The EGFR is well known receptor for the members of epidermal growth factor family of extracellular protein.[43] It induces receptor dimerization and tyrosine autophoshorylation and leads to cell proliferation, differentiation, motility and cell servival [44–46] Over expression of EGFR is up regulated in colon cancers and most neoplasms. Whereas, mutations comprising EGFR lead to its constant activation and produces uncontrolled cell devision.[47,48] Moreover, HER2 is a member of human epidermal growth factor receptor (HER/EGFR/ERBB) family. The overexpression of oncogene play a vital role in the development and propagation of certain types of cancers such as breast, lung, stomach, ovarian, adenocarcinoma and uterine cancer (uterine serous epidermal carcinoma).[49–52] For the reason that we have chosen IDO1, EGFR and HER2 proteins as the target receptors for the docking studies and their comparative binding energy are compiled in Table 3 (readers are advised to refer the Supporting Information for the details of docking protocol). Whereas, hydrogen bonding profile for the compounds $\mathbf{T^1}$ – $\mathbf{T^{18}}$ are shown in Table 4 along with residues. The comparative molecular docking demonstrations of the organic scaffolds $\mathbf{T^1}$ – $\mathbf{T^{18}}$ with the target receptors IDO1, EGFR and HER2 (as shown in Table 3) clearly indicates that the affinity of these drug candidates with receptor IDO1 is better than that of receptors EGFR and HER2 and that's why we have chooses IDO1 as the target receptor for the details discussion. Out of these series of drug candidates exploited for the doc score, scaffolds $\mathbf{T^{12}}$, $\mathbf{T^{11}}$, $\mathbf{T^9}$ and $\mathbf{T^8}$ are found to be the best inhibitors and their probable reasons are discussed below.

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From Table 3 it is clear that **T^8** is a potential candidate for the anticancer drug with the binding energy of -11.20 kcal mol^{-1}. Notably, **T^8** exhibits strong hydrogen bonding interactions between amino acid His346 and N-atom of hydrazine tryptanthrin as well as between Ala264 and carbonyl group of benzoyl acetone with bond length of 2.7678 Å and 3.1517 Å, respectively (Fig. 5). All the other interactions are hydrophobic in nature and found between the amino acid of target receptor IDO1 and phenyl ring of tryptanthrin core with the bond distance of 3.6445 Å, which is off course larger than the drug – receptor hydrogen bonding interaction.

Table 3. Comparative binding energies of 8-substituted-6-hydrazonoindolo [2, 1-*b*] quinazolin-12-(6H)-one compounds **T^1 – T^{18}** against **IDO1**, **EGFR** and **HER2**.

S.No.	Compound	Binding Energy (kcal mol^{-1}) IDO1 (PDB id: 2dU0)	Binding Energy (kcal mol^{-1}) EGFR (PDB id: 4hjo)	Binding Energy (kcal mol^{-1}) HER2 (PDB id: 3ppo)
1	T^1	-8.22	-7.24	-7.80
2	T^2	-8.88	-7.69	-7.80
3	T^3	–9.47	-8.09	-7.68
4	T^4	-9.04	-7.86	-7.92
5	T^5	-9.92	-8.94	-9.07
6	T^6	-8.10	-9.08	-9.29
7	T^7	-10.79	-8.91	-10.79
8	**T^8**	**-11.20**	-10.43	-10.63
9	**T^9**	**-11.71**	-10.76	-9.59
10	T^{10}	-10.91	-11.00	-10.47
11	T^{11}	**-11.73**	-10.06	-10.20
12	**T^{12}**	**-11.61**	-10.49	-9.84
13	T^{13}	-10.52	-9.53	-9.84
14	T^{14}	-9.83	-9.05	-10.92
15	T^{15}	-10.31	-9.83	-10.88
16	T^{16}	-10.00	-9.59	-11.29
17	T^{17}	-10.80	-8.89	-10.84
18	T^{18}	-9.96	-9.41	-10.12

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In a similar fashion drug **T^{9}** (binding energy, -11.71 kcal mol^{-1}, Tables 3 and 4) also exhibit hydrogen bonding interaction with receptor IDO1 (Fig. 6). Which takes place between His346 and nitrogen atom of hydrazono group of the tryptanthrin core with bond distance of 2.6800 Å. Whereas, the hydrophobic interaction observed between the phenyl ring of quinazoline fused tryptanthrin core and Ser167 with bond distance 3.9972 Å. Furthermore, **T^{11}** also shows drug – receptor hydrogen bonding interaction (binding energy, -11.73 kcal mol^{-1}, Tables 3 and 4) analogous to **T^{9}** (-11.71 kcal mol^{-1}). In this case hydrogen bonding interaction took place in between His346 and nitrogen atom of hydrazono group appended to tryptanthrin moiety with bond distance of 2.7190 Å (Fig. 7). Drug **T^{11}** also features hydrophobic interactions between Ala264 and carbonyl group of benzoylactone attached with tryptanthrins core in addition to interactions between Ser167 and phenyl ring of quinazoline induced tryptanthrin framework having the distance of 3.2152 Å and 3.6072 Å, respectively. The structure of complex derived from the interaction of **T^{12}** and receptor IDO1 also displays hydrogen bonding interaction (binding energy, -11.61 kcal mol^{-1}, Tables 3 and 4) between His346 and nitrogen atom of hydrazono tryptanthrin core with bond distance of 3.0734 Å (Fig. 8). This drug-receptor complex also features hydrophobic interaction between Ala264 and carbonyl group of benzoylacetone group of tryptanthrin core with bond distance of 3.1209 Å. Notably, all these cases the synthesized organic scaffolds (**T^{12}**, **T^{11}**, **T^{9}** and **T^{8}**) are interacted with the target protein (receptor IDO1) through the hydrogen bonding between His346, Ala264 and/or Ser167 and different substituted functionality of the tryptanthrin core. Whereas, the affinity between the target receptor and drug candidates are enhanced *via* the hydrophobic interactions.[53] Notably, compounds other than **T^{12}**, **T^{11}**, **T^{9}** and **T^{8}** display moderate dock score and binding affinity against all the three protein receptors IDO1, EGFR and HER2 (see Table 3 for details).

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Table 4. Binding energies, number of hydrogen bonds and residues involved in hydrogen bonding of compounds 8-substituted-6-hydrazonoindolo [2, 1-*b*] quinazolin-12-(6H)-one **T^1– T^{18}** against **IDO1**.

S.No.	Compound	Binding Energy (kcal mol^{-1})	Number of hydrogen bonds	Residues
1	T^1	-8.22	2	Ser167
2	T^2	-8.88	2	Ser267, Glu171
3	T^3	–9.47	2	Ser267, Glu171
4	T^4	-9.04	2	Ser167
5	T^5	-9.92	2	Glu171
6	T^6	-8.10	2	Arg343
7	T^7	-10.79	1	His346
8	**T^8**	**-11.20**	**2**	**His346, Ala264**
9	**T^9**	**-11.71**	**1**	**His346**
10	T^{10}	-10.91	2	His346, Ser263
11	**T^{11}**	**-11.73**	**1**	**His346**
12	**T^{12}**	**-11.61**	**1**	**His346**
13	T^{13}	-10.52	3	Ser267, Ser167, His346
14	T^{14}	-9.83	2	His346
15	T^{15}	-10.31	2	His346
16	T^{16}	-10.00	2	Ser267, His346
17	T^{17}	-10.80	1	Ala234
18	T^{18}	-9.96	1	Arg343

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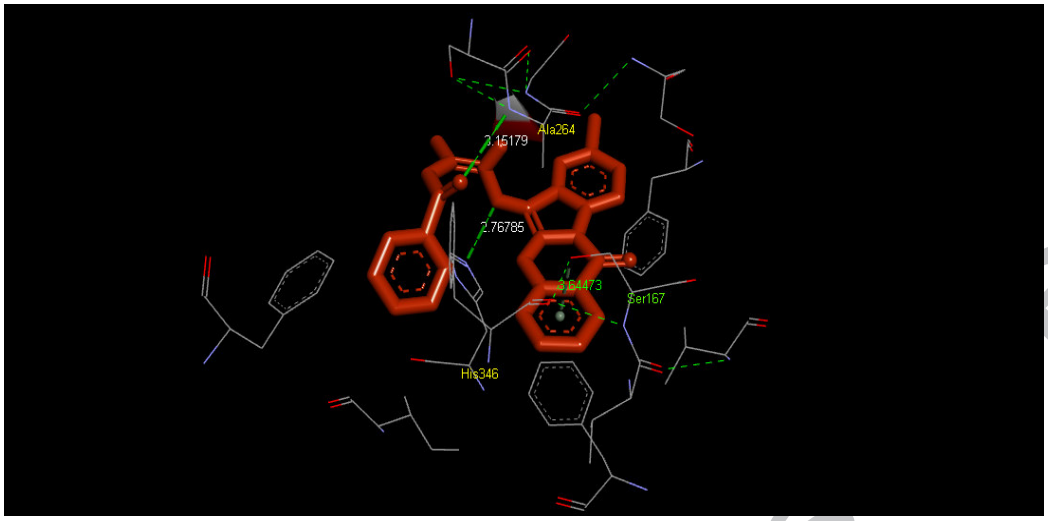

Fig. 5. Shows the binding poses and interactions of 8-substituted-6-hydrazonoindolo [*2,1-b*] quinazolin-12-(*6H*)-one analogue **T**[8] to the binding sites of target protein **IDO1** (PDB ID: 2d0U).

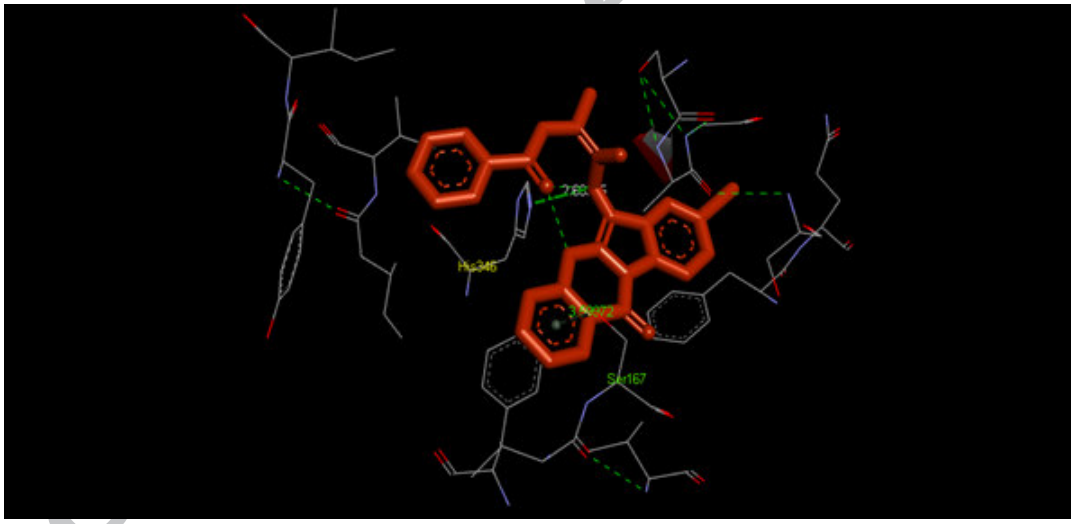

Fig. 6. Shows the binding poses and interactions of 8-substituted-6-hydrazonoindolo [*2,1-b*] Quinazolin-12-(*6H*)-one analogue **T**[9] to the binding sites of target protein **IDO1** (PDB ID: 2d0U).

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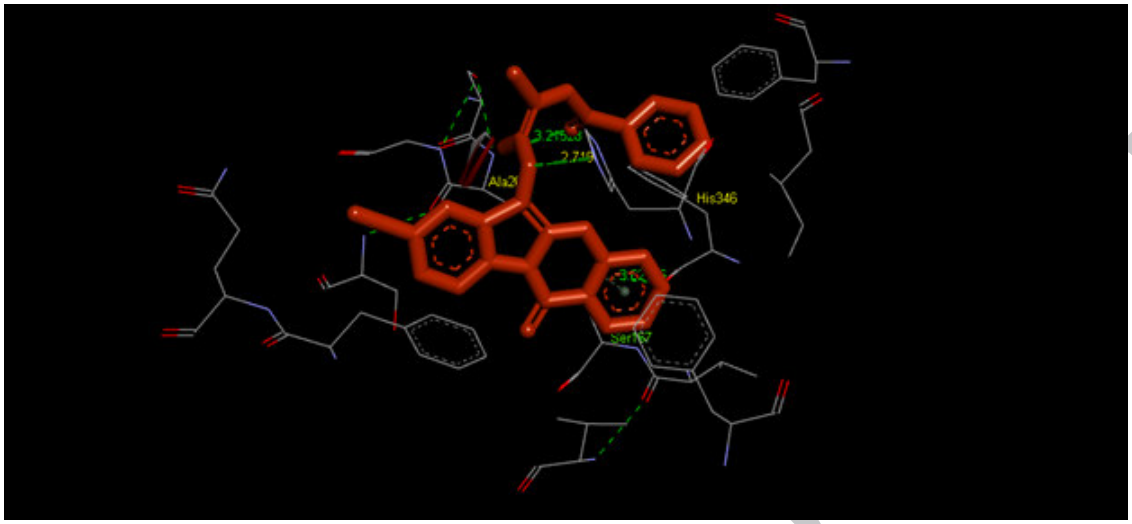

Fig. 7. Shows the binding poses and interactions of 8-substituted-6-hydrazonoindolo [*2,1-b*] quinazolin-12-(*6H*)-one analogue **T**[11] to the binding sites of target protein **IDO1** (PDB ID: 2d0U).

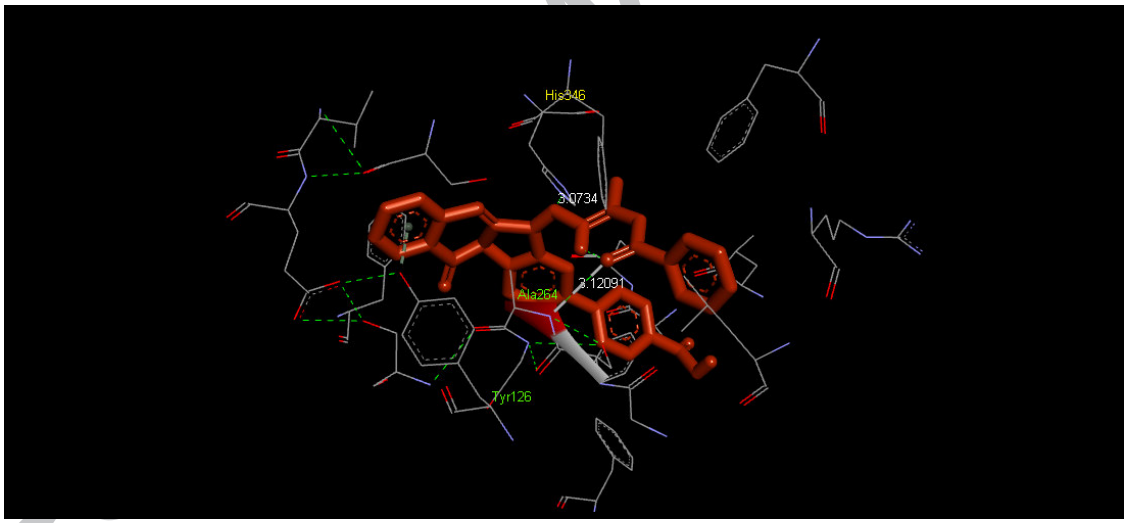

Fig. 8. Shows the binding poses and interactions of 8-substituted-6-hydrazonoindolo [*2,1-b*] quinazolin-12-(*6H*)-one analogue **T**[12] to the binding sites of target protein **IDO1** (PDB ID: 2d0U).

In summary, 18 novel, 8-substituted-6-hydrazonoindolo [2,1-*b*] quinazolin-12-(6*H*)-ones **T**[1] – **T**[18] were synthesized by well-established synthetic protocol followed by the Suzuki coupling

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reaction and screened for their anti-oxidant, anti-cancer and molecular docking studies. The compounds **T^{12}**, **T^{11}**, **T^{17}** and **T^{18}** were found to be the most potent anti-oxidant agents by DPPH radical scavenging assay. Whereas, **T^{12}**, **T^{11}**, **T^{8}** and **T^{9}** are showing a promising anti-cancer activity against the MCF-7, A549, HeLa human cancer cell lines using the MTT assay protocol and using cisplatin as a reference drug. We reasoned that the presence of 4-pyridyl or 4-carboxyphenyl substituents at 8th position of tryptanthrin framework might be a cause for their potent anti-cancer and anti-oxidant activity. The comparative molecular docking studies have been also performed against the three protein receptors IDO1, EGFR and HER2 and found IDO1 is the best one with binding energies of -11.73 kcal mol^{-1}, -11.61 kcal mol^{-1}, -11.20 kcal mol^{-1} and -11.20 kcal mol^{-1} for compounds **T^{12}**, **T^{11}**, **T^{8}**, and **T^{9}**, respectively. Importantly, molecules having the lowest binding energies are also displaying the highest cytotoxicity against the tested MCF-7, A549, HeLa human cancer cell lines. In a similar fashion, compounds with the better binding energies also showing excellent anti-oxidant. Moreover, this results will provide advantage to the medicinal chemist in the design and development of new tryptanthrin based anti-cancer drugs in near future.

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Research Highlights

- Designed and synthesized 18-novel tryptanthrin analogues as potential cytotoxic agents
- Compounds T^{11} and T^{12} were found to be best cytotoxic agents with lowest binding energies in docking
- Compounds T^{11}, T^{12}, $T^{17}$and T^{18} were found to be promising anti-oxidants
- IDO-1 protein is the best target for 8-substitued tryptanthrin analogues.

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Graphical Abstract

Design, synthesis and biological evaluation of 8-substituted-6-hydrazonoindolo [2,1-*b*]quinazolin-12(6*H*)-one scaffolds as potential cytotoxic agents: IDO-1 targeting molecular docking studies

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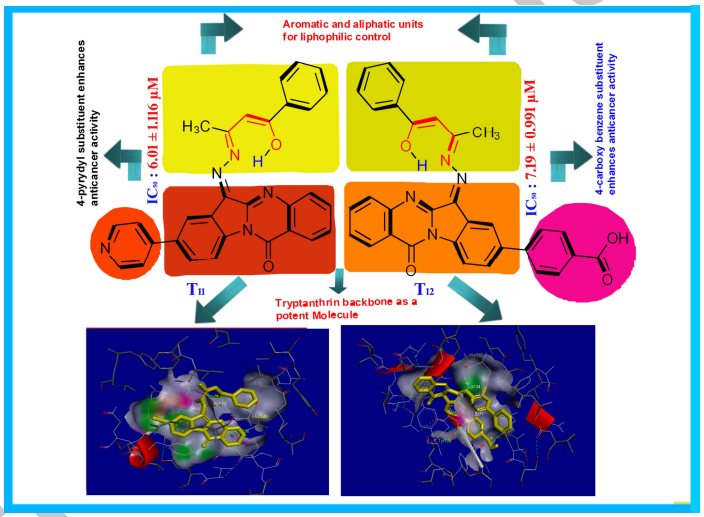
Novel tryptanthrin derivatives have exhibited excellent anti-cancer activity against three human cancer cell lines MCF-7, A549 and HeLa.



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