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Anticancer and DNA binding studies of potential amino acids based quinazolinone analogs: Synthesis, SAR and molecular docking

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ABSTRACT

A novel series of amino acids conjugated quinazolinone-Schiff's bases were synthesized and screened for their *in vitro* anticancer activity and validated by molecular docking and DNA binding studies. In the present investigations, compounds **32, 33, 34, 41, 42** and **43** showed most potent anticancer activity against tested cancer cell lines and DNA binding study using methyl green comparing to doxorubicin and ethidium bromide as a positive control respectively. The structure-activity relationship (SAR) revealed that the tryptophan and phenylalanine derived electron donating groups (OH and OCH_3) favored DNA binding studies and anticancer activity whereas; electron withdrawing groups (Cl, NO_2, and F) showed least anticancer activity. The molecular docking study, binding interactions of the most active compounds **33**, **34**, **42** and **43** stacked with A–T rich regions of the DNA minor groove by surface binding interactions were confirmed.

1. Introduction

Cancer is a group of diseases characterized by uncontrolled cell growth having potential to spread other parts of the body [1–5] and it is second most leading cause covering 8.8 million deaths worldwide due to cancer [6]. The one of the most important reason for cancer is due to an abnormality in the DNA called as mutations by abnormalities in the repair mechanism due to unfavourable factors [7]. In recent decades, a significant research was progressed in the field of diagnosis, treatment, and preventing the targeted cancer types [8]. The number of chemotherapeutic agents is resistant to cancer types and obstacle for the treatment of cancer. The anthracycline and taxanes are not effective to breast cancer treatment due to the occurrence of resistance to drugs in tumour cells [9]. Due to this problem, the survival time found to be 8.3 months in brain metastases from breast cancer patients [10]. In specific, the conventional treatment for the cancer is successful also the re-emergence of a tumour is the greatest challenge in the field of cancer [11]. To date, the current treatment for the cancer is through chemotherapy, radiation, surgery, biological, and hormonal but, the main hurdle involved in the treatment of cancer is high cost and adverse side effects [12]. To step move forward in the field of cancer biology and toxicology, the current research area needs to focus on developing anticancer drugs as therapeutics by designing candidates to bind to the "central dogma" or DNA involved in the control of every action of the body.

The DNA consists of two similar complementary strands way in antiparallel containing sugar-phosphate polydeoxyribonucleotide backbone connected to a specific hydrogen bond between the nucleotide bases [13]. In this structure, the different chemical feature attributed to form grooves for molecular recognition of the small molecules and proteins. Numerous biological experiments have suggested that DNA is one of the primary cellular targets for many anticancer agents. Particularly, in cancer cells, DNA can be preferentially damaged, due to the interactions with anticancer agents, therefore inhibition/blockage of cell division causes cell death.

In development of drugs for the cancer, DNA is a one of the best target for development of specific drug candidates to cancer by overcoming drug resistance in cancer cell types. The DNA interacting molecules are usually bound to DNA non-covalently by three modes: intercalation, groove binding and static electronic interactions. Static electronic interactions refer to molecules that bind with the negatively charged DNA double helix externally through a non-specific interaction. In groove binding, the targeting molecules interact with DNA in the base edges of the major groove or minor groove, which had been discussed by many groups. The intercalation is another DNA binding mode that is closely related to the antitumor ability of many anticancer agents. Another thing need to understand while designing the drug in target specific by considering drug should not affect the normal physiological functions of the cells [14]. In this regard, the designing DNA binding drugs are leading approaches in the field of cancer therapy for the future.

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On the other hand, previous reports have shown that conjugation of different amino acids to various biologically active scaffolds has fetched outstanding results which are very hopeful and even enthusiastic [15–21]. Further, amino acid/peptide-based drugs have low toxicity, ample bioavailability and permeability, modest potency and good metabolic and pharmacokinetic properties [22]. Prompted by all these observations and with a further interest to develop more biologically active compounds, the present work encompasses the synthesis of novel amino acids conjugated quinazolinone-Schiff's base analogues as promising DNA binding and anticancer agents.

2. Results and discussion

2.1. Chemistry

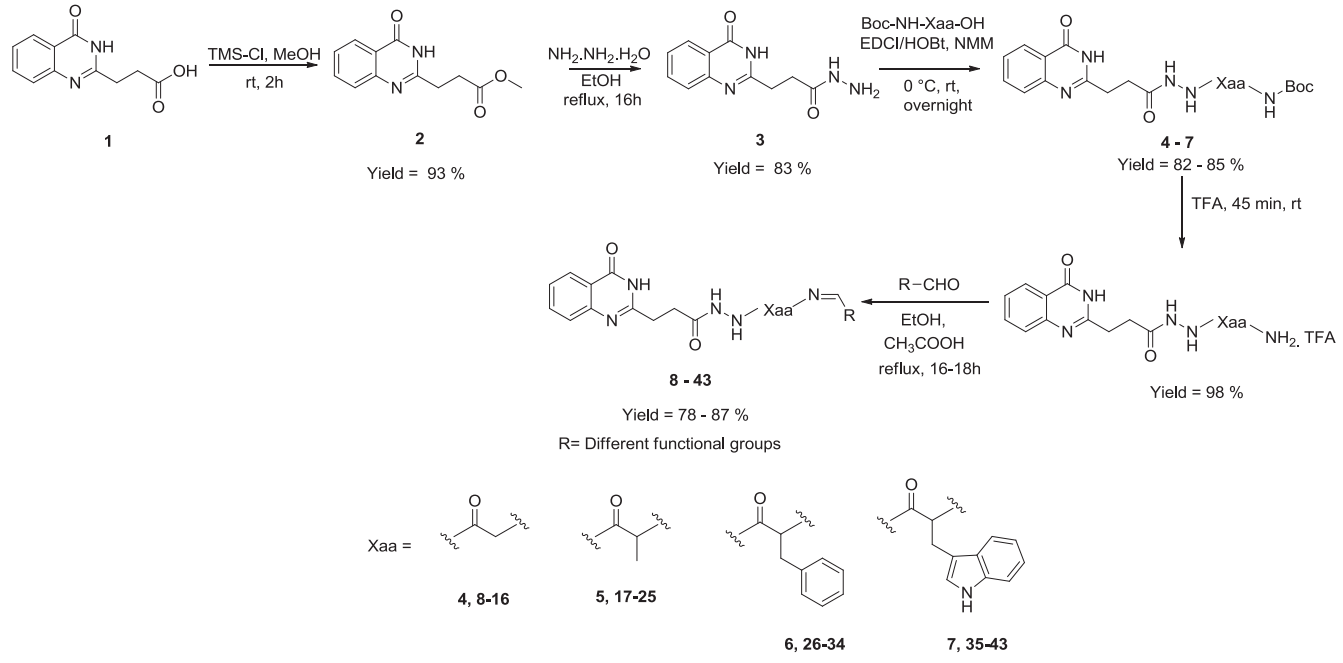
The heterocycle 3-(4-oxo-3,4-dihydroquinozolin-2-yl)propanoic acid (**1**) was synthesized following literature methods [23–25] which were esterified using trimethylsilylchloride (TMS-Cl)/methanol at room temperature, and treated with an excess of hydrazine hydrate to obtain corresponding quinazolinone hydrazides (**3**). These hydrazides were conjugated with Boc protected amino acids using EDCI (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide)/HOBt (hydroxybenzotriazole) as a coupling agent and NMM (*N*-methyl morpholine) as a base. Boc group of the conjugates was removed using TFA (trifluoroacetic acid) and a nucleophilic addition was carried out with variously substituted aldehydes in presence of catalytic amount of glacial acetic acid to obtain Schiff's base derivatives (**8–43,** Scheme 1). All the compounds were obtained in high yields. The formation of methyl esters (**2**) was confirmed by the appearance of a singlet at 3.68 δ for $-OCH_3$ and absence of COOH proton peak at 12.25 δ in ^{1}H NMR spectrum. In IR spectra, bands at 3310 and 3217 cm^{-1} for NH_2-NH-groups indicates the conversion of methyl esters into hydrazides. The stretching frequencies appeared at 1630–1644 cm^{-1} (−CO) and 3300 cm^{-1} (−NH) in IR spectra and the peak appeared at $\delta \sim 11.20$ as a singlet (−NH) confirms the conjugation. The formation of Schiff's bases was confirmed by the presence of absorption at 1612–1630 for imines i.e., −N=CH− in IR spectra. The presences of newly synthesized compounds including intermediates were confirmed by ^{1}H NMR, ^{13}C NMR and mass spectral analysis (spectra are provided in Supporting information).

2.2. Biology

2.2.1. Anticancer activity

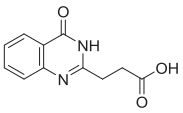
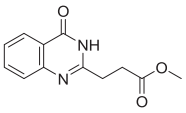
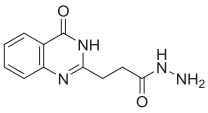
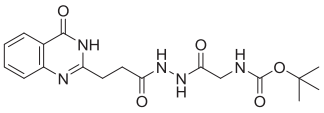
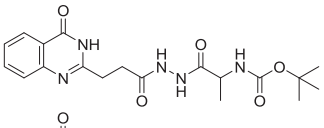
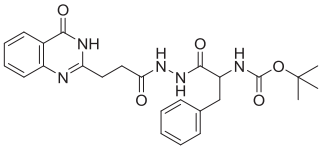
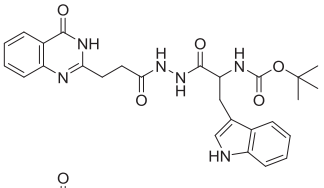
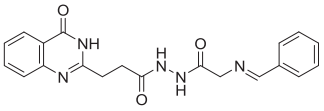
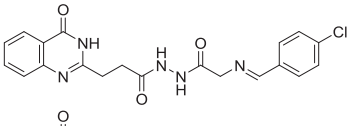
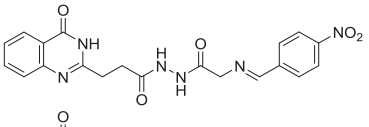
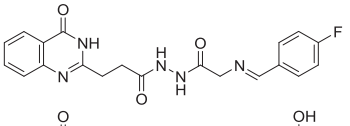
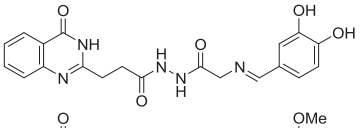
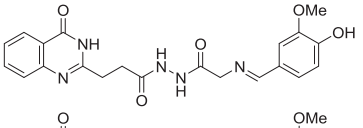
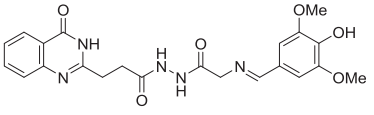
All the synthesized amino acids conjugated quinazolinone-Schiff's bases were evaluated as *in-vitro* anticancer activities against different human cancer cell lines such as MDA-MB-231, A546 and MCF7 and results were tabulated in the Table 1. The anticancer activities of our tested compounds were expressed as IC_{50} μg/mL values. Compounds **32, 33, 34, 41, 42** and **43** showed most potent anticancer activity against tested all cancer cell lines MCF7 (32.01 ± 1.17, 29.12 ± 1.33, 31.11 ± 1.19, 26.22 ± 0.69, 28.22 ± 1.30 and 30.34 ± 1.08 μg/mL respectively), MDA-MB-231 (31.11 ± 1.15, 32.19 ± 1.07, 30.11 ± 1.08, 30.69 ± 1.02, 24.36 ± 0.68 and 29.34 ± 0.98 μg/mL respectively), and A546 (32.07 ± 1.19, 32.08 ± 1.06, 29.11 ± 1.09, 28.09 ± 0.98, 26.55 ± 0.89 and 29.66 ± 0.67 μg/mL respectively), compared to standard drug doxorubicin 32.67 ± 1.09, 31.26 ± 1.06 and 32.81 ± 0.89 μg/mL respectively. Compounds **30**, **31**, **39** and **40** showed good anticancer activity against tested cancer cell lines with IC_{50} values slightly higher than that of standard drug doxorubicin. This fact may due to the presence of electron donating (OH and OCH_3) groups on the phenyl ring [26–28]. A compound containing electron withdrawing groups (**9–11**, **18–20**, **27–29** and **36–38**) showed least anticancer activity against tested cancer cell lines. Also, the anticancer activity of the designed compounds were satisfied by validating the cytotoxicity analysis against normal cell PBMC showed no significant toxicity compared to the standard doxorubicin (IC_{50} 2.23 μg/mL) in the present investigations. This suggests that, the designed compounds are promising candidates for the future anticancer small molecule discovery programs were expectable.

To study the structure-activity relationship (SAR) between the activity and synthesized analogs, compounds having electron donating groups (OH and OCH_3) presents on the phenyl ring (**32, 33, 34, 41, 42** and **43)** were found to be the most potent anticancer activity compared to electron withdrawing groups (Cl, NO_2, and F) presents on the phenyl ring. The biological activities also depends on the nature of amino acids presents on the heterocyclic moieties [29–31]. Based on these previous results, amino acids were playing a crucial role in increasing biological activities. Some of the amino acids/peptides were played a key factor for increasing the anticancer activity [32,33]. The tryptophan (**41, 42**



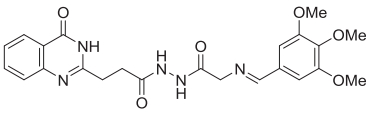
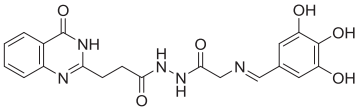
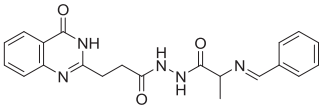
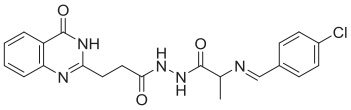
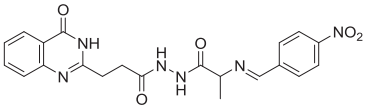
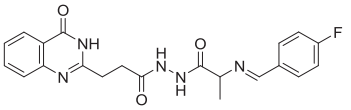
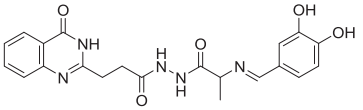
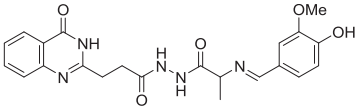
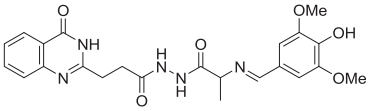
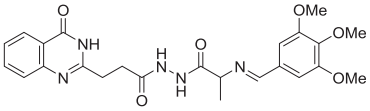
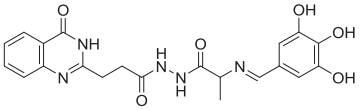
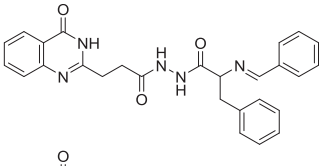
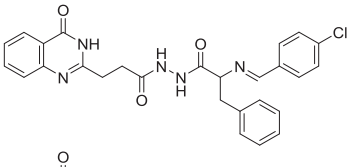
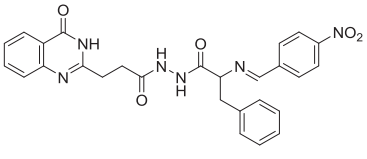
Scheme 1. Synthesis of the title compounds (**4–43**).

Table 1
Anticancer and DNA binding studies of synthesized compounds (**1–43**).

Sl.No	Structure	PBMC (IC_{50}, μg/mL)[a]	MCF7 (IC_{50}, μg/mL)[a]	MDA-MB-435 (IC_{50}, μg/mL)[a]	A549 (IC_{50}, μg/mL)[a]	DNA/methyl green (IC_{50}, μg/mL)[a]
1		–	–	–	–	–
2		–	–	–	–	–
3		–	–	–	–	–
4		–	–	95.17 ± 1.02	–	–
5		–	–	–	–	92.17 ± 1.28
6		64.16 ± 0.68	92.19 ± 1.27	90.15 ± 1.23	92.11 ± 1.24	88.13 ± 1.12
7		66.10 ± 0.15	95.18 ± 1.11	88.14 ± 1.69	90.32 ± 1.30	85.30 ± 1.27
8		30.16 ± 0.51	90.17 ± 1.07	82.19 ± 1.37	89.14 ± 1.57	80.17 ± 1.37
9		54.60 ± 0.51	78.27 ± 1.33	76.38 ± 1.57	72.66 ± 1.77	74.31 ± 1.01
10		65.06 ± 0.65	70.47 ± 1.41	72.47 ± 1.41	78.37 ± 1.90	84.17 ± 1.07
11		31.02 ± 0.31	72.37 ± 1.07	78.17 ± 1.56	74.18 ± 1.30	70.22 ± 1.37
12		61.10 ± 0.64	55.23 ± 1.37	58.45 ± 1.67	50.42 ± 1.33	60.31 ± 1.36
13		32.51 ± 0.02	46.31 ± 1.55	50.61 ± 1.72	58.63 ± 1.11	45.19 ± 1.58
14		32.05 ± 0.21	45.08 ± 1.11	48.23 ± 1.09	43.62 ± 1.27	55.12 ± 0.48

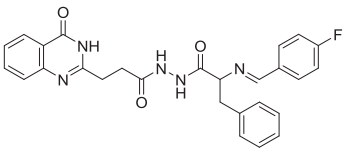
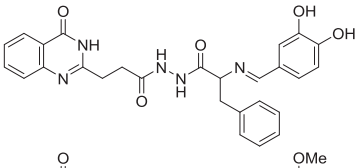
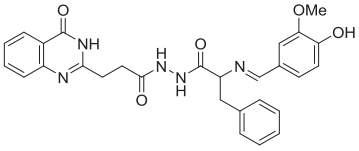
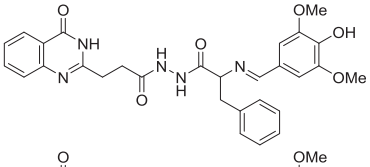
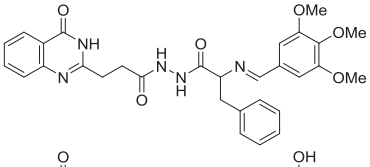
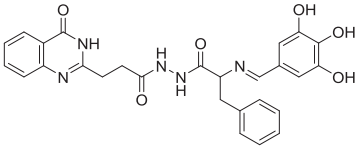
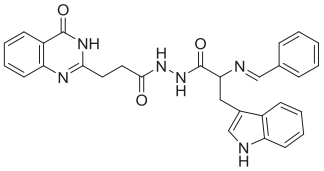
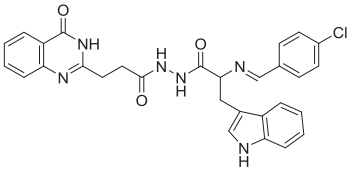
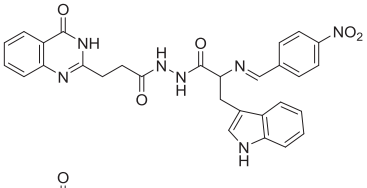
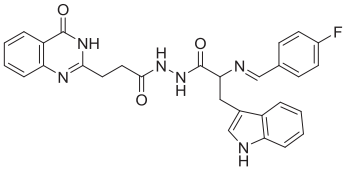
(*continued on next page*)

Table 1 (*continued*)

Sl.No	Structure	PBMC (IC_{50}, μg/mL)[a]	MCF7 (IC_{50}, μg/mL)[a]	MDA-MB-435 (IC_{50}, μg/mL)[a]	A549 (IC_{50}, μg/mL)[a]	DNA/methyl green (IC_{50}, μg/mL)[a]
15		21.06 ± 1.27	41.04 ± 1.34	46.31 ± 0.63	49.31 ± 1.22	50.30 ± 1.67
16		34.16 ± 0.02	55.02 ± 1.18	52.16 ± 1.49	50.37 ± 1.17	58.17 ± 1.27
17		34.60 ± 0.21	85.32 ± 1.33	84.11 ± 1.72	81.67 ± 1.73	76.18 ± 1.63
18		64.60 ± 0.20	76.31 ± 1.62	78.33 ± 1.07	72.33 ± 1.69	70.12 ± 1.30
19		79.16 ± 1.21	70.49 ± 1.04	68.14 ± 1.30	70.11 ± 1.06	69.12 ± 0.33
20		61.16 ± 0.26	75.33 ± 1.04	73.11 ± 1.02	68.33 ± 1.09	65.30 ± 1.08
21		64.61 ± 0.90	70.94 ± 1.65	72.49 ± 1.49	75.31 ± 1.47	70.36 ± 1.64
22		87.31 ± 1.32	72.33 ± 1.51	67.12 ± 1.62	74.64 ± 1.30	66.31 ± 1.09
23		34.03 ± 0.11	43.42 ± 1.34	45.44 ± 1.75	49.66 ± 1.74	70.45 ± 1.11
24		31.65 ± 0.11	44.12 ± 1.79	40.40 ± 1.33	45.19 ± 1.59	65.67 ± 1.28
25		35.50 ± 0.62	50.33 ± 1.52	45.33 ± 1.69	48.33 ± 1.69	60.56 ± 1.87
26		68.21 ± 0.15	78.11 ± 1.09	80.33 ± 1.02	76.31 ± 1.64	68.44 ± 1.49
27		62.16 ± 0.65	58.33 ± 1.64	60.63 ± 1.37	56.22 ± 1.07	54.33 ± 1.69
28		61.66 ± 0.35	54.69 ± 1.54	52.33 ± 1.07	55.12 ± 1.66	60.33 ± 1.69

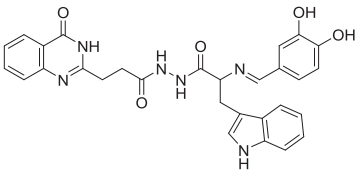
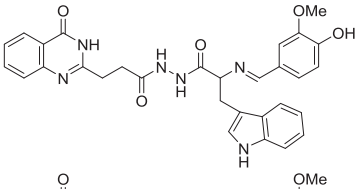
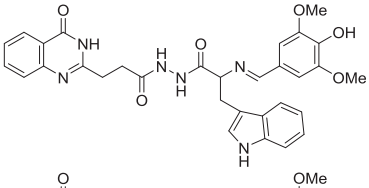
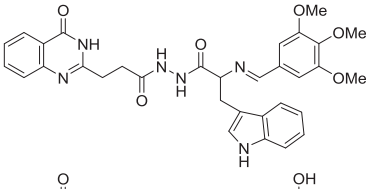
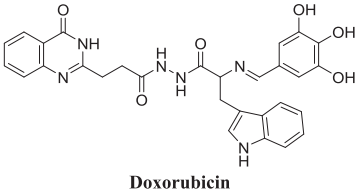
(*continued on next page*)

Table 1 (*continued*)

Sl.No	Structure	PBMC (IC_{50}, μg/mL)[a]	MCF7 (IC_{50}, μg/mL)[a]	MDA-MB-435 (IC_{50}, μg/mL)[a]	A549 (IC_{50}, μg/mL)[a]	DNA/methyl green (IC_{50}, μg/mL)[a]
29		32.10 ± 0.35	55.36 ± 1.94	60.64 ± 1.94	58.49 ± 1.30	55.61 ± 1.69
30		95.26 ± 1.64	64.44 ± 1.36	60.39 ± 1.08	68.59 ± 1.04	64.43 ± 1.42
31		65.26 ± 0.68	64.22 ± 1.44	60.22 ± 1.38	66.34 ± 1.68	58.69 ± 1.67
32		40.65 ± 0.60	32.01 ± 1.17	31.11 ± 1.15	32.07 ± 1.19	50.33 ± 1.09
33		34.26 ± 0.64	29.12 ± 1.33	32.19 ± 1.07	32.08 ± 1.06	52.33 ± 0.19
34		65.99 ± 1.51	31.11 ± 1.19	30.11 ± 1.08	29.11 ± 1.09	48.33 ± 0.18
35		94.16 ± 1.56	72.33 ± 1.39	75.31 ± 1.09	68.99 ± 1.75	66.98 ± 1.67
36		34.16 ± 0.66	66.66 ± 1.57	71.33 ± 1.69	78.32 ± 1.66	72.36 ± 1.69
37		65.19 ± 1.00	72.33 ± 1.69	60.34 ± 1.95	66.64 ± 1.67	70.66 ± 1.69
38		64.16 ± 0.15	68.87 ± 0.86	66.74 ± 0.92	68.85 ± 1.08	78.99 ± 1.06

(*continued on next page*)

Table 1 (*continued*)

Sl.No	Structure	PBMC (IC_{50}, μg/mL)[a]	MCF7 (IC_{50}, μg/mL)[a]	MDA-MB-435 (IC_{50}, μg/mL)[a]	A549 (IC_{50}, μg/mL)[a]	DNA/methyl green (IC_{50}, μg/mL)[a]
39		51.16 ± 0.87	40.59 ± 1.66	48.49 ± 1.34	35.94 ± 1.97	58.69 ± 1.67
40		43.65 ± 0.68	38.69 ± 1.66	35.69 ± 1.54	34.66 ± 1.54	50.66 ± 1.49
41		51.08 ± 0.84	26.22 ± 0.69	30.69 ± 1.02	28.09 ± 0.98	52.36 ± 0.99
42		66.03 ± 054	28.22 ± 1.30	24.36 ± 0.68	26.55 ± 0.89	50.66 ± 1.30
43		72.12 ± 0.06	30.34 ± 1.08	29.34 ± 0.98	29.66 ± 0.67	48.36 ± 1.39
Std	Doxorubicin	22.23 ± 0.19	32.67 ± 1.09	31.26 ± 1.06	32.81 ± 0.89	–

[a] Vaues are mean of three determinations, the ranges of which are < 5% of the mean in all cases. Doxorubicin used as standard. Note: PBMC- Peripheral blood mononuclear cells; MDA-MB-231-human triple negative breast cancer cells, A546-lung cancer, and MCF7-melanoma cell lines, and DNA/methyl green- calf thymus DNA/dye used as control to determine the efficacy of the synthesized compound respect to standard.

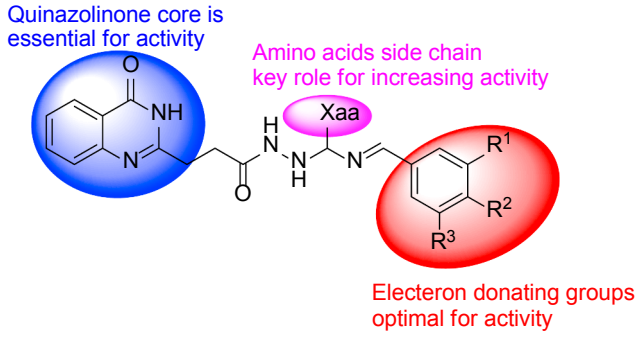


Fig. 1. SAR of the synthesized compounds against anticancer activity.

and **43**) and phenylalanine (**32, 33** and **34**) amino acids containing compounds showed more potent anticancer agents compared to the other two conjugated amino acids glycine (**14, 15** and **16**) and alanine (**23, 24** and **25**) derivatives. This fact may be explained on the basis of aromaticity and hydrophobicity of tryptophan and phenylalanine possesses good anticancer properties [34–37]. While the other two amino acid derivatives (glycine and alanine) were also moderate active, which may be due to their simple side chain functionalities and steric hindrance of aliphatic side chains The number of electron donating groups (**32**, **33**, **34**, **41, 42** and **43)** increases on the phenyl ring, the anticancer activity also increases compared to minimum number of electron donating groups resents on the phenyl ring of compounds (**30**, **31**, **39** and **40**). The compounds with electron withdrawing groups (**9–11**, **18–20**, **27–29** and **36–38**) substituents showed least anticancer activity (a schematic representation of SAR studies in Fig. 1).

2.2.2. DNA binding studies

The UV–Vis absorption spectroscopy recognized as one of the most common ways to investigate the affinity given compound to DNA based on the absorption of photons of monochromatic light [38]. The efficient DNA binding property was exhibited by the synthesized compounds (Table 1). The compounds **32, 33, 34, 41, 42** and **43** with IC_{50} values 50.33 ± 1.09, 52.33 ± 0.19, 48.33 ± 0.18, 52.36 ± 0.99, 50.66 ± 1.30 and 48.36 ± 1.39 μg/mL respectively, showed an excellent binding to DNA was investigated using methyl green comparing to ethidium bromide as positive control observing the complete loss of methyl green. This indicates the active nature of compounds involved in the binding to DNA was observed. The compounds **30, 31, 39** and **40** showed a moderate level of DNA binding was observed. It is well known that active small molecules can involve in interacting with double-stranded DNA through the intercalation with electrostatic interactions at minor groove binding site in B-DNA is becoming a site for developing new interesting drugs [39,40]. In the present investigation, the compounds **32, 33, 34, 41, 42** and **43** effectively bind to DNA minor grove by electrostatic interaction was highlightable and it is agreement with

Table 2
Molecular docking scores of all synthesized compounds against DNA dodecamerd as obtained through Glide docking.

Sl. No	Docking score	Glide gscore	Glide energy	XP HBond
4	−4.442	−6.047	−52.757	−1.803
5	−5.540	−5.583	−55.682	−1.569
6	−2.715	−2.758	−37.955	−1.396
7	−6.005	−7.609	−65.347	−1.598
8	−2.123	−2.192	−55.094	−0.499
9	−3.063	−4.411	−54.761	−1.761
10	−4.127	−4.191	−43.678	−0.916
11	−2.186	−2.250	−55.977	−0.613
12	−4.983	−6.331	−63.263	−2.161
13	−3.140	−3.204	−56.621	−1.666
14	−1.003	−1.067	−47.910	−1.391
15	−1.543	−3.537	−47.477	−1.502
16	−7.048	−8.406	−64.434	−3.609
17	−4.025	−5.377	−56.519	−1.044
18	−4.863	−6.211	−56.875	−0.959
19	−4.277	−5.625	−50.676	−0.962
20	−3.078	−4.426	−57.263	−0.584
21	−5.072	−5.136	−53.556	−2.847
22	−5.057	−5.121	−52.718	−1.254
23	−2.127	−2.191	−50.010	−1.070
24	−3.911	−3.996	−38.196	−1.260
25	−7.576	−7.651	−54.265	−3.568
26	−3.379	−3.444	−48.525	−1.987
27	−3.288	−3.352	−51.374	−1.062
28	−2.156	−2.220	−62.459	−0.969
29	−2.321	−2.385	−55.444	−1.018
30	−6.139	−6.203	−59.925	−2.496
31	−4.581	−4.646	−57.946	−1.780
32	−6.590	−6.654	−50.578	−2.360
33	−7.275	−7.346	−40.126	−1.646
34	−7.948	−7.022	−50.066	−2.191
35	−3.475	−3.539	−52.402	−1.265
36	−5.857	−5.921	−65.222	−0.634
37	−3.511	−3.554	−60.398	−1.163
38	−3.527	−3.591	−68.223	−2.026
39	−4.972	−5.036	−57.748	−3.056
40	−7.127	−7.191	−58.866	−2.021
41	−6.923	−6.987	−51.219	−1.299
42	−7.014	−7.083	−47.125	−0.842
43	−7.400	−7.474	−54.374	−2.812
Doxorubicin	−6.283	−6.299	−54.984	−1.769

in vitro anticancer activity and previous reports [41,42]. The presence of strong electron donating groups (OH and OCH_3) on the aromatic ring increases the hydrophobicity of the molecules and is responsible for the enhanced anticancer activity. This fact suggests the importance of the electron withdrawing groups for anticancer activity of this series.

2.2.3. Molecular docking studies

Molecular docking studies provided the correct information of biomolecules and chemical compounds interactions; henceforth we carried out to explore the binding of compounds (**4–43**) with DNA. Virtual predicted lowest negative Glide score (kcal/mol) along with parameters such as hydrophobic, hydrogen bonding and van der Waals interactions (Table 2) of compounds involve most excellent possible geometrical orientation of those compound, adjunction with the target DNA double helix, implies good binding affinity with DNA. The 3D structure of compounds **43** (Fig. 2**A**), **42** (Fig. 2**B**), **34** (Fig. 2**C**) and **33** (Fig. 2**D**) plays a significant role in deducing its structural consequences and conformational behavior as same as standard inhibitor Doxorubicin (Fig. 2**E**) during interactions with DNA [43]. Compounds **33**, **34**, **42**, and **43** stacked with A–T rich regions of the DNA minor groove by surface binding interactions (Fig. 2). Suggesting it might down regulate the expression of gene by binding towards the TATA box interfering minor-groove binding architectural proteins TBP, SRY, IHF, and HMG I in complex with their DNA targets. The compounds **33, 34, 42** and **43** intercalate with nucleotide and forming hydrogen bonds with hydroxyl groups present at DNA. Therefore, it suggests that there is a concordant explanation between *ex vivo* data and molecular docked model, which can be authenticated. The results could confer the anticancer activity of compounds **32–34** and **41–43** *ex vivo* to their abilities to bind to DNA minor groove as reported by Hassan et al. [42].

3. Conclusion

In conclusion, we designed and synthesized a novel series of amino acids conjugated quinazolinone-Schiff's base analogues were screened for their *in vitro* anticancer and DNA binding studies. The compounds 32, 33, 34, 41, 42 and 43 showed excellent anti-cancer activity against tested human MDA-MB-231, A546 and MCF7 cancer cell lines along with excellent DNA binding capability. To study the structure-activity relationship (SAR), hydrophobic and aromatic amino acids tryptophan and phenylalanine containing an electron donating groups most favour the anticancer activity. In additional, molecular docking studies of compounds 34 and 43 showed the highest docking G-scores for DNA binding studies. Our results presented here could be used as a preliminary stage for the development of powerful quinazolinone conjugated amino acids as anticancer therapies.

4. Chemistry

4.1. Experimental

4.1.1. General instrumentation and chemicals

All Boc-amino acids, EDCI, HOBt and TFA were purchased from Advanced Chem. Tech. (Louisville, Kentucky, USA). All the amino acids used except glycine were of *L*-configuration unless otherwise mentioned. NMM, DPPH, ABTS and DMPD were purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals and reagents were obtained from Aldrich (USA), Spectrochem Pvt. Ltd. (India) and Rankem Pvt. Ltd. (India) and were used without further purification. Progress of the reaction was monitored by TLC using silica gel coated on glass plates with solvent system comprising chloroform/methanol/acetic acid in the ratio 98:02:03 (R_f^a)/95:05:03 (R_f^b) and the compounds on TLC plates were detected by iodine vapors. Melting points were determined on a Superfit melting point apparatus (India) and are uncorrected. FT-IR was performed using a Jasco spectrometer (Japan) using nujol media. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Agilent Technologies (USA) using DMSO (d_6) as solvent and the chemical shifts are reported as parts per million (δ ppm) using TMS as an internal standard. High resolution mass spectroscopic analysis was performed on a Bruker MicroTOF QII mass spectrometer in positive mode.

4.1.2. Synthesis of methyl 3-(4-oxo-3,4-dihydroquinazolin-2-yl) propanoate

To a solution of QZN (0.02 mol, 4.36 g) dissloved in methanol (40 mL), trimethylsilylchloride (0.02 mol, 3.80 mL) was added slowly. The reaction mixture was stirred for 4 h to complete the reaction (monitored by TLC). The solvent was removed under reduced pressure and the resultant precipitate was washed with ice cold water and filtered to yield the desired product **2.**

4.1.3. Synthesis of 3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanehydrazide

To a solution of **2** (0.015 mol, 4.02 g) dissloved in ethanol (40 mL), hydrazine hydrate (0.020 mol, 0.97 mL) was added. The reaction mixture was refluxed for 16 h for completion of the reaction (monitored by TLC). The solvent was removed under reduced pressure and cooled by adding ice cold water. The resulting precipitate was filtered, washed with cold water and recrystallized from ethanol to get the desired compound **3.**

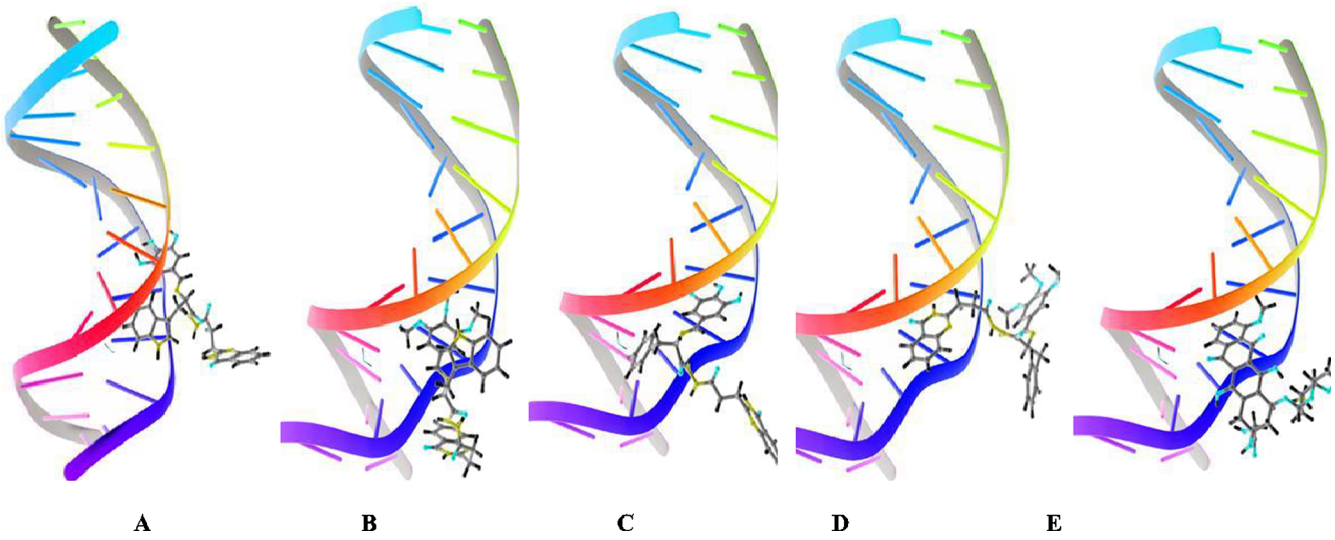


Fig. 2. Three dimensional binding mode of DNA with compound **43** (A), **42** (B), **34** (C), **33** (D) and Doxorubicin (E). DNA is represented in thin curved wire form wherein the nucleoside is colored as blue-thymine and dark orange as adenine. Compounds are represented red colored wired which shows binding at minor groove of DNA.

4.1.4. General procedure for the conjugation of Boc-amino acids to 3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoic acid (QZN)

Boc-amino acids (1 mmol) dissolved in dimethyl formamide (DMF) separately (10 mL/g of compound) and cooled to 0 °C was added NMM (1 mmol). EDCI (1 mmol) was added under stirring while maintaining the temperature at 0 °C and stirred for 15 min. HOBt (1 mmol) in DMF (2 mL) was added. The reaction mixture was stirred for an additional 10 min and a pre-cooled solution of 3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoic acid (QZN) (1 mmol) and NMM (1 mmol) in DMF was added slowly. After 20 min, pH of the solution was adjusted to 8 by the addition of NMM and the reaction mixture was stirred overnight at room temperature. DMF was removed under reduced pressure and the residue was poured into about 100 mL ice-cold 90% saturated $KHCO_3$ solution and stirred for 30 min. The precipitated product was taken into $CHCl_3$ and washed sequentially with 5% $NaHCO_3$ solution (2 × 20 mL), water (2 × 20 mL), 0.1 N cold HCl solution (2 × 20 mL) and finally brine (2 × 20 mL). The $CHCl_3$ layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The products so obtained were recrystallized from ether/petroleum ether to get desired products **4–7**.

4.1.5. Deblocking of Boc group

QZN-Xaa-Boc (1 mmol) was stirred with 2.0 mL of TFA for 45 min at room temperature. After completion of the reaction monitored by TLC, reaction mixture was concentrated at high vacuum to get QZN-Xaa-H.TFA which was then triturated with dry ether, filtered and dried.

*4.1.6. Procedure for the synthesis of Schiff's bases (**8–43**)*

QZN-Xaa-H.TFA (1 mmol) were dissolved in minimum quantity of DMF and then neutralized with NMM as a base, after the neutralization ethanol (10 mL/g of compound) was added and treated with appropriate aldehydes (1 mmol) in the presence of catalytic amount of glacial acetic acid. The reaction mixtures were refluxed for 16 h and the completion of reaction was monitored by TLC. After completion of the reaction, the solvent was removed under reduced pressure and cooled by adding ice cold water. The resulting precipitate was filtered, washed with water and recrystallized from ethanol to obtain the desired amino acids derived quinazolinone Schiff's bases **(8–43)**.

*4.1.7. 3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoic acid (**1**)*

Yield 86%, white solid, R_f^a = 0.40, R_f^b = 0.48, m.p. 206–207 °C, MS *m/z*: 219.1080, IR KBr (cm^{-1}): 1630, 1768, 2910, 3510, ^{1}H NMR (DMSO-d_6, 500 MHz) *δ*: 12.16 (2H, s, NH & COOH), 8.05–7.40 (4H, m, Ar-H), 2.83–2.78 (2H, t, CH_2), 2.72–2.69 (2H, t, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) *δ*: 173.9, 162.0, 156.7, 146.7, 134.6, 127.2, 126.4, 126.1, 121.3, 30.2, 29.5.

*4.1.8. Methyl 3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoate (**2**)*

Yield 93%, white solid, R_f^a = 0.66, R_f^b = 0.70, m.p. 184–185 °C, IR KBr (cm^{-1}): 1627, 1770, 2945, MS *m/z*: 219.1245, ^{1}H NMR (DMSO-d_6, 500 MHz) *δ*: 11.31 (1H, s, Het-NH), 8.15–7.59 (4H, m, Ar-H), 3.61 (3H, s, OCH_3), 3.11–3.08 (2H, t, CH_2), 3.00–2.97 (2H, t, CH_2); ^{13}C NMR (DMSO-d_6, 500 MHz) *δ*: 173.0, 168.0, 156.2, 146.7, 133.7, 129.2, 126.3, 126.1, 121.3, 51.8, 30.3, 29.1.

*4.1.9. 3-(4-Oxo-3,4-dihydroquinazolin-2-yl)propanehydrazide (**3**)*

Yield 83%, white solid, R_f^a = 0.32, R_f^b = 0.36, m.p. 220–221 °C, MS *m/z*: 247.1264, IR KBr (cm^{-1}): 1640, 1778, 1940, 3312, ^{1}H NMR (DMSO-d_6, 500 MHz) *δ*: 11.20 (1H, s, Het-NH), 8.99 (1H, s, NH), 8.02–7.38 (4H, m, ArH), 4.14 (2H, s, NH_2), 2.79–2.77 (2H, t, CH_2), 2.46–2.48 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 100 MHz) *δ*: 173.2, 167.0, 156.7, 146.1, 133.6, 128.2, 127.1, 126.8, 121.2, 30.1, 29.4.

*4.1.10. Tert-butyl (2-oxo-2-(2-(3-(4-oxo-3,4-dihydroquinazolin-2-yl) propanoyl)hydrazinyl) ethyl)carbamate (**4**)*

Yield 85%, white solid. R_f^a = 0.52, R_f^b = 0.59, m.p. 220–221 °C, MS *m/z*: (M + 1) 390.2564, IR KBr (cm^{-1}): 1633, 1780, 2947, 3214, ^{1}H NMR (DMSO-d_6, 500 MHz) *δ*: 11.10 (1H, s, Het-NH), 10.70 (1H, s, NH), 8.05–7.17 (4H, m, ArH), 6.73–6.72 (1H, d, NH), 3.90–3.89 (2H, s, $^{\alpha}CH_2$), 3.87 (1H, s, NH), 2.92–2.91 (2H, t, CH_2), 2.63–2.61 (2H, t, CH_2), 1.30 (9H, s, Boc-H), ^{13}C NMR (DMSO-d_6, 100 MHz) *δ*: 173.2, 171.1, 161.1, 156.7, 156.1, 148.1, 133.1, 127.4, 126.9, 126.1, 120.1, 78.8, 48.1, 30.1, 29.4, 24.3.

*4.1.11. Tert-butyl (1-oxo-1-(2-(3-(4-oxo-3,4-dihydroquinazolin-2-yl) propanoyl)hydrazinyl)propan-2-yl)carbamate (**5**)*

Yield 82%, white solid, R_f^a = 0.49, R_f^b = 0.58, m.p. 156–157 °C, MS *m/z*, (M + 1): 404.1548, IR KBr (cm^{-1}): 1630, 1748, 2917, 3312, ^{1}H NMR (DMSO-d_6, 500 MHz) *δ*: 11.10 (1H, s, Het-NH), 8.01–7.31 (4H, m, ArH), 6.91–6.89 (2H, d, 2NH), 3.98–3.94 (1H, m, $^{\alpha}CH$), 3.41 (1H, s, NH), 2.80–2.77 (2H, t, CH_2), 2.59–2.56 (2H, t, CH_2), 1.33 (9H, s, Boc-H), 1.22–1.20 (3H, d, CH_3), ^{13}C NMR (DMSO-d_6, 100 MHz) *δ*: 171.2, 170.0, 164.4, 159.6, 155.3, 149.7, 133.7, 126.7, 126.1, 125.4, 121.3, 78.4, 48.8, 31.3, 30.7, 28.6, 18.7.

*4.1.12. Tert-butyl (1-oxo-1-(2-(3-(4-oxo-3,4-dihydroquinazolin-2-yl) propanoyl)hydrazinyl)-3-phenylpropan-2-yl)carbamate **(6)***

Yield 82%, white solid, brown solid, R_f^a = 0.50, R_f^b = 0.63, m.p. 180–182 °C, MS *m/z*, (M + 1): 480.1659, IR KBr (cm^{-1}): 1640, 1785, 2917, 3216, ^{1}H NMR (DMSO-d_6, 500 MHz) *δ*: 10.71 (1H, s, Het-NH), 8.05–7.12 (9H, m, ArH), 6.90–6.92 (1H, d, NH), 4.22–4.17 (1H, m, $^{\alpha}CH$), 3.31 (3H, s, NH), 3.01–2.96 (d, 2H, d, $^{\beta}CH_2$), 2.88–2.84 (2H, m, CH_2), 2.67–2.66 (2H, t, CH_2), 1.28 (9H, s, Boc-H), ^{13}C NMR (DMSO-d_6, 100 MHz) *δ*: 171.4, 170.2, 164.4, 159.6, 156.7, 148.1, 137.1, 133.1, 128.7, 127.6, 127.1, 126.9, 126.1, 125.1, 121.1, 78.1, 58.4, 38.1, 30.1, 29.9, 25.7.

*4.1.13. Tert-butyl (3-(1H-indol-3-yl)-1-oxo-1-(2-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)hydrazinyl)propan-2-yl)carbamate **(7)***

Yield 84%, reddish solid, R_f^a = 0.54, R_f^b = 0.63, m.p. 184–186 °C, MS *m/z*, (M + 1): 519.2365, IR KBr (cm^{-1}): 1640, 1772, 2942, 3220, ^{1}H NMR (DMSO-d_6, 500 MHz) *δ*: 11.23 (1H, s, Het-NH), 10.7 (1H, s, Indole-NH), 8.14–7.03 (8H, m, ArH), 6.72–6.71 (1H, d, NH), 4.95–4.94 (1H, m, $^{\alpha}CH$), 3.41–3.40 (2H, d, $^{\beta}CH_2$), 3.68 (2H, s, NH), 3.12–3.10 (2H, m, CH_2), 2.70–2.68 (2H, t, CH_2), 1.29 (9H, s, Boc-H), ^{13}C NMR (DMSO-d_6, 100 MHz) *δ*: 171.1, 170.7, 163.7, 159.1, 156.1, 148.7, 136.7, 133.4, 128.1, 127.1, 126.4, 126.0, 124.1, 122.1, 121.6, 119.2, 118.1, 114.1, 108.7, 79.0, 60.1, 31.1, 29.1, 28.40, 24.3.

*4.1.14. N-(2-(Benzylideamino)acetyl)-3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanehydrazide **(8)***

Yield 82%, white solid, R_f^a = 0.57, R_f^b = 0.68, m.p. 167–168 °C, MS *m/z*, (M + 1): 378.2315.

IR KBr (cm^{-1}): 1612, 1620, 1763, 2917, 3220, ^{1}H NMR (DMSO-d_6, 500 MHz) *δ*: 12.18 (1H, s, Het-NH), 11.46 (1H, s, NH), 8.19–8.18 (1H, d, NH), 8.05–7.37 (9H, m, ArH), 7.96 (1H, s, −N═CH), 4.22 (2H, s, $^{\alpha}CH_2$), 2.86–2.82 (2H, t, CH_2), 2.71–2.70 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 100 MHz) *δ*:171.9, 170.7, 162.0, 157.1, 147.0, 143.8, 134.6, 134.5, 130.2, 129.2, 128.7, 127.4, 126.3, 126.1, 121.3, 60.0, 31.7, 30.2.

*4.1.15. N-(2-((4-Chlorobenzylidene)amino)acetyl)-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanehydrazide **(9)***

Yield 88%, white solid, R_f^a = 0.45, R_f^b = 0.60, m.p. 188–191 °C, MS *m/z*, (M + 1): 412.2316, IR KBr (cm^{-1}): 1609, 1628, 1760, 2930, 3226, ^{1}H NMR (DMSO-d_6, 500 MHz) *δ*: 11.13 (1H, s, Het-NH), 10.80 (1H, s, NH), 8.14–7.16 (8H, m, ArH), 7.84 (1H, s, CH), 6.72–6.71 (1H, d, NH), 4.52–4.51 (2H, m, $^{\alpha}CH_2$), 8.87–2.85 (2H, t, CH_2), 2.66–2.64 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 100 MHz) *δ*: 171.4, 170.2, 163.1, 160.7, 156.1, 148.1, 137.1, 135.1, 133.7, 131.1, S129.4, 127.9, 127.0, 126.8, 120.1, 58.7, 30.9, 29.8.

*4.1.16. N4))-2)-'-Nitrobenzylidene)amino)acetyl)-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanehydrazide **(10)***

Yield 83%, yellow solid, R_f^a = 0.57, R_f^b = 0.63, m.p. 179–181 °C, MS *m/z* (M + 1): 423.6231, IR KBr (cm^{-1}): 1619, 1642, 1778, 2943, 3246, ^{1}H NMR (DMSO-d_6, 500 MHz) *δ*: 11.09 (s, 1H, Het-NH), 8.09–8.10 (m, 8H, Ar-H), 7.80 (s, 1H, – N = CH), 6.72–6.73 (d, 1H, NH), 4.52–4.53 (m, 2H, $^{\alpha}CH_2$), 3.70 (s, 2H, NH), 3.10–3.12 (m, 2H, CH_2), 2.66–2.68 (t, 2H, CH_2), ^{13}C NMR (DMSO-d_6, 100 MHz) *δ* ppm: 171.1, 170.3, 161.9, 160.0, 156.1, 149.4, 148.3, 143.0, 133.7, 130.2, 127.4, 126.7, 126.6, 125.4, 121.45, 60.9, 30.3, 29.2.

*4.1.17. N-(2-((4-Fluorobenzylidene)amino)acetyl)-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanehydrazide **(11)***

Yield 83%, brown solid, R_f^a = 0.50, R_f^b = 0.58, m.p. 166–167 °C, MS *m/z*, (M + 1): 396.2364, IR KBr (cm^{-1}): 1614, 1635, 1778, 2946, 3226, ^{1}H NMR (DMSO-d_6, 500 MHz) *δ*: 11.20 (1H, s, Het-NH), 10.27 (1H, s, NH), 8.07–7.26 (8H, m, ArH), 7.84 (s, 1H, CH), 6.71–6.72 (1H, d, NH), 4.52–4.50 (2H, m, $^{\alpha}CH_2$), 2.81–2.80 (2H, t, CH_2), 2.56–2.54 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 100 MHz) *δ*: 172.2, 171.1, 165.1, 161.7, 156.1, 148.1, 144.2, 135.1, 132.1, 129.2, 128.7, 127.6, 126.4, 120.9, 116.3, 60.2, 31.2, 29.7.

*4.1.18. N3,4))-2)-'-Dihydroxybenzylidene)amino)acetyl)-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanehydrazide **(12)***

Yield 77%, white solid, R_f^a = 0.37, R_f^b = 0.43, m.p. 186–188 °C, MS *m/z*, (M + 1): 410.2315, IR KBr (cm^{-1}): 1621, 1624, 1784, 2914, 3256, 3561, ^{1}H NMR (DMSO-d_6, 500 MHz) *δ*: 11.10 (1H, s, Het-NH), 10.88 (1H, s, NH), 9.85 (2H, s, OH), 8.03–7.10 (7H, m, Ar-H), 7.78 (s, 1H, −N═CH), 6.90–6.88 (1H, d, NH), 4.52–4.51 (2H, s, $^{\alpha}CH_2$), 2.90–2.88 (2H, t, CH_2), 2.66–2.64 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 500 MHz) *δ* ppm: 172.8, 170.8, 161.7, 156.6, 149.8, 148.5, 147.3, 143.9, 134.7, 133.8, 127.2, 126.7, 126.2, 123.9, 121.0, 117.8, 115.5, 59.2, 30.9, 29.2.

*4.1.19. N4))-2)-'-Hydroxy-3-methoxybenzylidene)amino)acetyl)-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanehydrazide **(13)***

Yield 76%, white solid, R_f^a = 0.54, R_f^b = 0.61, m.p. 155–157 °C, MS *m/z*, (M + 1): 424.2364, IR KBr (cm^{-1}): 1616, 1636, 1780, 2940, 3316, 3564, ^{1}H NMR (DMSO-d_6, 500 MHz) *δ*: 11.13 (1H, s, Het-NH), 10.22 (1H, s, NH), 9.12 (1H, s, OH), 8.10–7.03 (7H, m, Ar-H), 7.89 (s, 1H, −N═CH), 6.71–6.72 (1H, d, NH), 4.53–4.52 (2H, s, $^{\alpha}CH_2$), 3.72 (3H, s, $-OCH_3$) 2.89–2.87 (2H, t, CH_2), 2.60–2.58 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 100 MHz) *δ*: 173.2, 171.8, 161.4, 156.1, 153.6, 148.1, 146.5, 144.6, 135.7, 133.4, 128.2, 127.7, 126.2, 122.4, 120.8, 116.8, 112.5, 60.2, 52.7, 30.2, 29.8.

*4.1.20. N-(2-((4-Hydroxy-3,5-dimethoxybenzylidene)amino)acetyl)-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanehydrazide **(14)***

Yield 84%, white solid, R_f^a = 0.53, R_f^b = 0.70 m.p. 186–188 °C, MS *m/z*, (M + 1): 454.2364, IR KBr (cm^{-1}): 1626, 1640, 1783, 2954, 3217, 3567, ^{1}H NMR (DMSO-d_6, 500 MHz) *δ*: 11.30 (1H, s, Het-NH), 10.50 (1H, s, NH), 8.95 (1H, s, OH), 8.09–7.01 (6H, m, Ar-H), 7.98 (1H, s, −N═CH), 6.50–6.49 (1H, d, NH), 4.52–4.51 (2H, s, $^{\alpha}CH_2$), 3.71 (6H, s, $2OCH_3$), 2.80–2.78 (2H, t, CH_2), 2.62–2.60 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 100 MHz) *δ*: 174.2, 172.8, 161.5, 156.3, 148.7, 148.1, 143.7, 139.7, 135.5, 133.8, 127.7, 126.8, 126.4, 120.5, 104.9, 59.8, 52.8, 30.5, 29.2.

*4.1.21. 3-(4-Oxo-3,4-dihydroquinazolin-2-yl)-N-(2-((3,4,5-trimethoxybenzylidene)amino)acetyl)propanehydrazide **(15)***

Yield 82%, white solid, R_f^a = 0.56, R_f^b = 0.65, m.p. 190–192 °C, MS *m/z*, (M + 1): 468.2164, IR KBr (cm^{-1}): 1614, 1637, 1783, 2941, 3229, ^{1}H NMR (DMSO-d_6, 500 MHz) *δ*: 11.15 (s, 1H, Het-NH), 9.60 (1H, s, NH), 8.13–6.92 (6H, m, Ar-H), 7.79 (1H, s, −N═CH), 6.69–6.68 (1H, d, NH), 4.52–4.50 (2H, m, $^{\alpha}CH_2$), 3.81 (9H, s, $3OCH_3$), 2.90–2.88 (2H, m, CH_2), 2.63–2.61 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 100 MHz) *δ*: 171.19, 170.87, 163.10, 160.14, 156.20, 152.17, 148.70, 142.08, 138.16, 133.70, 128.14, 127.17, 126.90, 121.31, 104.60, 60.80, 59.80, 56.44, 30.14, 29.17.

*4.1.22. 3-(4-Oxo-3,4-dihydroquinazolin-2-yl)-N-(2-((3,4,5-trihydroxybenzylidene)amino)acetyl)propanehydrazide **(16)***

Yield 76%, white solid, R_f^a = 0.33, R_f^b = 0.41, m.p. 210–211 °C, MS *m/z*, (M + 1): 426.3216, IR KBr (cm^{-1}): 16139, 1620, 1758, 2940, 3245, 3641, ^{1}H NMR (DMSO-d_6, 500 MHz) *δ*: 11.25 (1H, s, Het-NH), 9.20 (1H, s, NH), 9.85 (2H, s, 2OH), 8.95 (IH, s, OH), 8.10–6.72 (6H, m, Ar-H), 7.79 (1H, s, −N═CH), 6.68–6.69 (1H, d, NH), 4.52–4.51 (2H, s, $^{\alpha}CH_2$), 2.78–2.76 (2H, t, CH_2), 2.60–2.58 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 100 MHz) *δ* ppm: 173.2, 171.7, 161.8, 156.9, 148.7, 146.5, 144.7, 139.5, 135.8, 133.8, 128.8, 127.1, 126.5, 120.8, 109.2, 59.8, 30.8, 29.6.

*4.1.23. 2-(Benzylideneamino)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl) propanoyl)propanehydrazide **(17)***

Yield 80%, white solid, R_f^a = 0.53, R_f^b = 0.60, m.p. 178–179 °C, MS

m/z, (M + 1): 392.1645, IR KBr (cm^{-1}): 1616, 1633, 1777, 2912, 3238, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.16 (1H, s, Het-NH), 9.90 (1H, s, NH), 8.03–7.36 (9H, m, Ar-H), 7.88 (1H, s, −N═CH), 6.71–6.70 (1H, d, NH), 4.21–4.20 (1H, m, $^{\alpha}$CH), 2.90–2.88 (2H, t, CH_2), 2.60–2.58 (2H, t, CH_2), 1.39–1.38 (3H, d, $^{\beta}CH_3$), ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 173.8, 171.6, 161.3, 156.7, 148.5, 143.9, 136.7, 133.5, 130.5, 129.5, 128.6, 127.1, 126.9, 126.2, 120.9, 69.1, 30.7, 29.8, 19.1.

4.1.24. 2-((4-Chlorobenzylidene)amino)-N–3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)propanehydrazide ***(18)***

Yield 81%, white solid, R_f^a = 0.54, R_f^b = 0.65, m.p. 197–198 °C, MS *m/z*, (M + 1): 426.3164, IR KBr (cm^{-1}): 1614, 1636, 1778, 2938, 3260, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.17 (1H, s, Het-NH), 9.90 (1H, s, NH), 8.10–6.88 (8H, m, Ar-H), 7.80 (1H, s, CH), 6.71–6.70 (d, 1H, NH), 4.77–4.75 (1H, m, $^{\alpha}$CH), 2.85–2.83 (2H, m, CH_2), 2.68–2.66 (2H, t, CH_2), 1.39–1.38 (3H, s, $^{\beta}CH_3$), ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.70, 170.11, 163.01, 160.87, 156.24, 148.14, 136.70, 134.17, 133.07, 129.14, 127.16, 127.01, 126.14, 121.19, 70.81, 30.17, 29.81, 18.67.

4.1.25. 2-((4-Nitrobenzylidene)amino)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)propanehydrazide ***(19)***

Yield 86%, yellow solid, R_f^a = 0.52, R_f^b = 0.58, m.p. 185–186 °C, MS *m/z*. (M + 1): 437.2364, IR KBr (cm^{-1}): 1627, 1629, 1785, 2964, 3306, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.14 (s, 1H, Het-NH), 8.50 (1H, s, NH), 8.12–6.86 (8H, m, Ar-H), 7.73 (1H, s, −N═CH), 6.63–6.61 (1H, d, NH), 4.71–4.70 (1H, m, $^{\alpha}$CH), 2.92–2.90 (2H, m, CH_2), 2.65–2.63 (2H, t, CH_2), 1.40–1.38 (3H, s, $^{\beta}CH_3$), ^{13}C NMR (DMSO-d_6, 500 MHz) δ: 171.16, 170.88, 163.71, 160.70, 156.23, 149.14, 148.28, 143.70, 133.70, 130.14, 128.70, 126.77, 126.01, 124.11, 121.10, 70.14, 30.16, 29.317, 18.70.

4.1.26. 2-((4-Fluorobenzylidene)amino)-N–3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)propanehydrazide ***(20)***

Yield 81%, brown solid, R_f^a = 0.47, R_f^b = 0.56, m.p. 147–149 °C, MS *m/z*, (M + 1): 410.3648, IR KBr (cm^{-1}): 1607, 1642, 1781, 2940, 3213, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.12 (1H, s, Het-NH), 9.56 (1H, s, NH), 8.03–7.24 (8H, m, Ar-H), 7.89 (1H, s, −N═CH), 6.68–6.67 (1H, d, NH), 4.22–4.21 (1H, m, $^{\alpha}$CH), 3.87–3.86 (2H, t, CH_2), 2.61–2.59 (2H, t, CH_2), 1.40–1.39 (3H, d, $^{\beta}CH_3$), ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.7, 170.5, 165.6, 161.4, 156.2, 148.9, 143.6, 134.7, 131.6, 130.5, 127.2, 127.1, 126.8, 120.5, 115.3, 68.7, 30.7, 29.5, 17.9.

4.1.27. 2-((3,4-Dihydroxybenzylidene)amino)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)propanehydrazide ***(21)***

Yield 85%, white solid, R_f^a = 0.46, R_f^b = 0.51, m.p. 170–171 °C, MS *m/z*, (M + 1): 424.2346, IR KBr (cm^{-1}): 1605, 1632, 1771, 2939, 3214, 3561, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.28 (1H, s, Het-NH), 9.50 (1H, s, NH), 8.59 (2H, s, 2OH), 8.11–6.68 (7H, m, Ar-H), 7.85 (1H, s, −N═CH), 6.63–6.62 (1H, d, NH), 4.26–4.25 (1H, m, $^{\alpha}$CH), 2.80–2.78 (2H, t, CH_2), 2.60–2.58 (2H, t, CH_2), 1.36–1.35 (3H, d, $^{\beta}CH_3$), ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 171.8, 170.6, 161.9, 156.2, 150.7, 148.7, 147.5, 144.2, 133.8, 133.1, 127.2, 126.8, 126.2, 124.2, 120.9, 118.2, 117.0, 68.1, 30.2, 29.3, 17.9.

4.1.28. 2-((4-Hydroxy-3-methoxybenzylidene)amino)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)propanehydrazide ***(22)***

Yield 85%, white solid, R_f^a = 0.53, R_f^b = 0.61, m.p. 175–176 °C, MS *m/z*, (M + 1): 438.4231, IR KBr (cm^{-1}): 1607, 1636, 1786, 2915, 3239, 3564, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.16 (1H, s, Het-NH), 9.89 (1H, s, NH), 8.59 (1H, s, OH), 8.09–6.88 (7H, m, Ar-H), 7.95 (1H, s, −N═CH), 6.55–6.54 (1H, d, NH), 4.28–4.27 (1H, m, $^{\alpha}$CH), 3.71 (3H, s, OCH_3), 2.84–2.83 (2H, t, CH_2), 2.79–2.77 (2H, t, CH_2), 1.37–1.36 (3H, d, $^{\beta}CH_3$), ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.3, 171.3, 161.8, 156.4, 152.6, 149.4, 147.1, 144.2, 134.5, 133.7, 128.1, 127.7, 126.2, 122.8, 120.7, 118.8, 111.7, 69.8, 52.6, 30.8, 29.7, 17.9.

4.1.29. 2-((4-Hydroxy-3,5-dimethoxybenzylidene)amino)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)propanehydrazide ***(23)***

Yield 79%, brown solid, R_f^a = 0.49, R_f^b = 0.57, m.p. 193–194 °C, MS *m/z*, (M + 1): 468.3264, IR KBr (cm^{-1}): 1612, 1636, 1784, 2905, 3236, 3564, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.32 (1H, s, Het-NH), 9.90 (1H, s, NH), 8.55 (1H, s, OH), 8.06–7.10 (6H, m, Ar-H), 7.88 (1H, s, −N═CH), 6.60–6.59 (1H, d, NH), 4.26–4.25 (1H, m, $^{\alpha}$CH), 3.82 (6H, s, $2OCH_3$), 2.86–2.84 (2H, t, CH_2), 2.59–2.57 (2H, t, CH_2), 1.38–1.37 (3H, d, $^{\beta}CH_3$), ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 172.7, 171.6, 162.0, 156.9, 148.8, 148.2, 143.9, 140.7, 135.7, 133.6, 127.8, 126.9, 126.1, 120.5, 104.7, 68.2, 52.7, 30.3, 29.8, 18.3.

4.1.30. 3-(4-Oxo-3,4-dihydroquinazolin-2-yl)-N-(2-((3,4,5-trimethoxybenzylidene)amino)propanoyl)propanehydrazide ***(24)***

Yield 78%, white solid, R_f^a = 0.59, R_f^b = 0.64, m.p. 210–212 °C, MS *m/z*, (M + 1): 482.3645, IR KBr (cm^{-1}): 1626, 1634, 1786, 2943, 3304, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.19 (s, 1H, Het-NH), 9.52 (1H, s, NH), 8.14–6.90 (6H, m, Ar-H), 7.71 (s, 1H, −N═CH), 6.61–6.59 (1H, d, NH), 4.73–4.72 (2H, m, $^{\alpha}$CH), 3.83 (9H. s, $3OCH_3$), 2.87–2.85 (2H, t, CH_2), 2.62–2.60 (2H, t, CH_2), 1.39–1.37 (3H, d, 3H, $^{\beta}CH_3$), ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.90, 170.27, 162.14, 160.88, 156.23, 153.27, 148.14, 142.14, 135.14, 133.19, 127.23, 126.92, 126.17, 121.14, 104.78, 70.07, 60.81, 56.44, 30.22, 29.18, 18.66.

4.1.31. 3-(4-Oxo-3,4-dihydroquinazolin-2-yl)-N-(2-((3,4,5-trihydroxybenzylidene)amino)propanoyl)propanehydrazide ***(25)***

Yield 85%, white solid, R_f^a = 0.34, R_f^b = 0.42, m.p. 169–170 °C, MS *m/z*, (M + 1): 440.1364, IR KBr (cm^{-1}): 1601, 1624, 1745, 2964, 3289, 3610, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.22 (1H, s, Het-NH), 10.60 (1H, s, NH), 9.10 (2H, s, 2OH), 8.89 (1H, s, OH), 8.08–6.72 (7H, m, Ar-H), 7.89 (1H, s, −N═CH), 6.56–6.54 (1H, d, NH), 4.23–4.22 (1H, m, $^{\alpha}$CH), 2.79–2.77 (2H, t, CH_2), 2.2.56–2.54 (2H, t, CH_2), 1.37–1.36 (3H, d, $^{\beta}CH_3$), ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 171.7, 170.6, 161.3, 156.8, 147.9, 146.5, 144.2, 139.7, 136.2, 133.5, 128.2, 127.1, 126.5, 120.4, 109.6, 67.9, 30.8, 29.4, 18.2.

4.1.32. 2-(Benzylideneamino)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl) propanoyl)-3-phenylpropanehydrazide ***(26)***

Yield 85%, white solid, R_f^a = 0.52, R_f^b = 0.60, m.p. 181–182 °C, MS *m/z*, (M + 1): 468.2368, IR KBr (cm^{-1}): 1613, 1633, 1786, 2951, 3260, ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 11.10 (1H, s, Het-NH), 10.23 (1H, s, NH), 8.07–7.26 (14H, m, Ar-H), 7.89 (1H, s, −N═CH), 6.71–6.70 (1H, d, NH), 4.46–4.48 (1H, m, $^{\alpha}$CH), 3.30–3.29 (2H, t, $^{\beta}CH_2$), 2.88–2.86 (2H, t, CH_2), 2.69–2.67 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 172.8, 171.0, 161.7, 156.4, 148.3, 143.7, 138.2, 136.5, 133.8, 130.4, 129.9, 128.7, 128.4, 127.9, 127.2, 126.9, 126.4, 125.3, 120.3, 74.6, 38.6, 30.7, 29.8.

4.1.33. 2-((4-Chlorobenzylidene)amino)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)-3-phenylpropanehydrazide ***(27)***

Yield 81%, white solid, R_f^a = 0.49, R_f^b = 0.60, m.p. 194–196 °C, MS *m/z*, (M + 1): 502.6521, IR KBr (cm^{-1}): 1611, 1626, 1776, 2938, 3229, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.23 (1H, s, Het-NH), 10.53 (1H, s, NH), 8.04–7.12 (13H, m, Ar-H), 7.83 (1H, s, −N═CH), 6.70–6.69 (1H, d, NH), 4.42–4.41 (1H, m, $^{\alpha}$CH), 3.31–3.29 (2H, t, $^{\beta}CH_2$), 2.79–2.77 (2H, t, CH_2), 2.68–2.67 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 172.8, 171.6, 161.9, 156.4, 148.7, 144.6, 137.8, 136.6, 134.9, 133.2, 130.7, 129.6, 128.4, 127.7, 127.2, 126.5, 126.2, 125.1, 120.7, 72.8, 38.6, 30.4, 29.6.

4.1.34. 2-((4-Nitrobenzylidene)amino)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)-3-phenylpropanehydrazide ***(28)***

Yield 84%, yellow solid, R_f^a = 0.58, R_f^b = 0.64, m.p. 201–202 °C, MS *m/z*, (M + 1): 513.2364, IR KBr (cm^{-1}): 1620, 1630, 1745, 2962, 3231, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.32 (1H, s, Het-NH), 9.80 (1H, s, NH), 8.13–7.12 (13H, m, Ar-H), 7.85 (1H, s, −N═CH), 6.62–6.61

(1H, d, NH), 4.47–4.45 (1H, m, $^{\alpha}$CH), 3.27–3.26 (2H, t, $^{\beta}CH_2$), 2.80–2.78 (2H, t, CH_2), 2.62–2.61 (2H, t, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.7, 170.4, 161.3, 156.4, 151.7, 148.4, 144.7, 137.9, 136.8, 133.4, 130.6, 128.4, 127.9, 127.8, 126.6, 126.3, 125.5, 124.6, 120.7, 73.1, 38.4, 30.4, 29.8.

*4.1.35. 2-((4-Fluorobenzylidene)amino)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)-3-phenylpropanehydrazide **(29)***

Yield 82%, brown solid, R_f^a = 0.51, R_f^b = 0.59, m.p. 161–163 °C, MS *m/z*, (M + 1): 486.3246, IR KBr (cm^{-1}): 1614, 1632, 1775, 2952, 3214, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.28 (1H, s, Het-NH), 9.25 (1H, s, NH), 8.17–6.79 (14H, m, Ar-H & −N═CH), 5.98–5.91 (1H, d, NH), 4.04–3.99 (1H, m, $^{\alpha}$CH), 3.22–3.18 (2H, t, $^{\beta}CH_2$), 2.97–2.93 (2H, t, CH_2), 2.89–2.86 (2H, t, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.6, 171.3, 167.7, 161.7, 157.9, 148.2, 144.8, 138.3, 134.9, 132.7, 129.5, 129.2, 128.5 127.7, 126.7, 126.2, 120.1, 121.4, 116.0, 72.7, 37.4, 31.7, 29.7.

*4.1.36. 2-((3,4-Dihydroxybenzylidene)amino)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)-3-phenylpropanehydrazide **(30)***

Yield 78%, white solid, R_f^a = 0.42, R_f^b = 0.47, m.p. 175–177 °C, MS *m/z*, (M + 1): 500.3216, IR KBr (cm^{-1}): 1614, 1633, 1785, 2941, 3262, 3561, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 12.15 (1H, s, Het-NH), 11.33–11.15 (1H, d, NH), 9.20 (2H, s, 2OH), 8.42–6.73 (12H, m, Ar-H), 7.96 (1H, s, −N═CH), 6.75–6.73 (1H, d, NH), 4.54–4.49 (1H, m, $^{\alpha}$CH), 3.00–2.97 (2H, t, $^{\beta}CH_2$), 2.84–2.78 (2H, t, CH_2), 2.64–2.61 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.3, 171.4, 161.2, 156.5, 150.4, 148.7, 147.3, 144.3, 137.8, 134.4, 133.2, 129.7, 128.3, 127.1, 129.6, 126.5, 125.4, 123.4, 120.6, 117.3, 116.0, 73.4, 37.6, 30.7, 28.6.

*4.1.37. 2-((4-Hydroxy-3-methoxybenzylidene)amino)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)-3-phenylpropanehydrazide **(31)***

Yield 83%, white solid, R_f^a = 0.42, R_f^b = 0.51, m.p. 177–178 °C, MS *m/z*, (M + 1): 514.6521, IR KBr (cm^{-1}): 1606, 1632, 1745, 2956, 3269, 3561, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.18 (1H, s, Het-NH), 9.76 (1H, s, NH), 8.80 (1H, s, OH), 8.10–7.08 (12H, m, Ar-H), 7.89 (1H, s, −N═CH), 6.60–6.59 (1H, d, NH), 4.44–4.43 (1H, m, $^{\alpha}$CH), 3.78 (3H, s, OCH_3), 3.26–3.24 (2H, t, $^{\beta}CH_2$), 2.83–2.81 (2H, t, CH_2), 2.66–2.64 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 500 MHz) δ: 172.6, 171.4, 161.6, 156.3, 151.4, 150.3, 148.6, 144.3, 137.8, 135.5, 133.6, 129.7, 128.1, 127.2, 126.6, 126.1, 125.4, 123.3, 120.4, 117.7, 112.9, 73.2, 52.7, 37.6, 30.4, 28.9.

*4.1.38. 2-(4-Hydroxy-3,5-dimethoxybenzylidene)amino)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)-3-phenylpropanehydrazide **(32)***

Yield 78%, white solid, R_f^a = 0.47, R_f^b = 0.55, m.p. 160–161 °C, MS *m/z*, (M + 1): 544.3218, IR KBr (cm^{-1}): 1618, 1626, 1790, 2920, 3326, 3562, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.09 (1H, s, Het-NH), 9.89 (1H, s, NH), 8.80 (2H, s, 2OH), 8.11–7.05 (11*H*, m, Ar-H), 7.82 (1H, s, −N═CH), 6.62–6.61 (1H, d, NH), 4.43–4.42 (1H, m, $^{\alpha}$CH), 3.81 (6H, s, $2OCH_3$), 3.27–3.25 (2H, t, $^{\beta}CH_2$), 2.89–2.87 (2H, t, CH_2), 2.69–2.67 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 500 MHz) δ: 172.8, 170.7, 161.3, 156.4, 149.7, 148.7, 144.0, 139.5, 137.3, 135.2, 134.3, 128.8, 127.9, 127.8, 126.9, 126.3, 125.7, 120.4, 104.7, 73.0, 52.8, 37.4, 30.6, 28.7.

*4.1.39. 3-(4-Oxo-3,4-dihydroquinazolin-2-yl)propanoyl)-3-phenyl-2-((3,4,5-trimethoxybenzylidene)amino)propanehydrazide **(33)***

Yield 87%, brown solid, R_f^a = 0.56, R_f^b = 0.67, m.p. 204–206 °C, MS *m/z*, (M + 1): 558.6213, IR KBr (cm^{-1}): 1614, 1633, 1745, 2941, 3289, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.21 (1H, s, Het-NH), 9.90 (1H, s, NH), 8.08–7.15 (11*H*, m, Ar-H), 7.86 (1H, s, −N═CH), 6.60–6.58 (1H, d, NH), 4.40–4.38 (1H, m, $^{\alpha}$CH), 3.81 (9H, s, $3OCH_3$), 3.22–3.20 (2H, t, $^{\beta}CH_2$), 2.86–2.83 (2H, t, CH_2), 2.60–2.58 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.3, 170.4, 162.0, 156.7, 154.3, 148.6, 143.4, 142.1, 137.3, 134.6, 133.5, 128.6, 127.8, 127.7, 126.9, 126.4, 125.9, 120.7, 104.7, 73.6, 60.9, 52.7, 37.7, 30.4, 28.6.

*4.1.40. 3-(4-Oxo-3,4-dihydroquinazolin-2-yl)propanoyl)-3-phenyl-2-((3,4,5-trihydroxybenzylidene)amino)propanehydrazide **(34)***

Yield 83%, white solid, R_f^a = 0.32, R_f^b = 0.37, m.p. 210–211 °C, MS *m/z*, (M + 1): 516.2348, IR KBr (cm^{-1}): 1614, 1636, 1786, 2915, 3219, 3591, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.19 (1H, s, Het-NH), 9.92 (1H, s, NH), 9.30 (2H, s, 2OH), 8.47 (1H, s, OH), 8.08–6.60 (11*H*, m, Ar-H), 7.81 (1H, s, −N═CH), 6.67–6.66 (1H, d, NH), 4.40–4.39 (1H, m, $^{\alpha}$CH), 3.22–3.20 (2H, t, $^{\beta}CH_2$), 2.82–2.80 (2H, t, CH_2), 2.59–2.57 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 172.1, 171.6, 161.3, 156.7, 147.9, 146.4, 143.6, 139.2, 137.2, 135.4, 133.3, 129.1, 128.2, 127.4, 127.0, 126.4, 125.5, 120.4, 108.6, 73.5, 37.4, 30.6, 28.6.

*4.1.41. 2-(Benzylideneamino)-3-(1H-indol-3-yl)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)propanehydrazide **(35)***

Yield 80%, white solid, R_f^a = 0.57, R_f^b = 0.69, m.p. 192–193 °C, MS *m/z*, (M + 1): 507.2348, IR KBr (cm^{-1}): 1610, 1629, 1774, 2950, 3226, 3310, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.31 (1H, s, Het-NH), 10.24 (1H, s, Indole-NH), 9.92 (1H, s, NH), 8.08–6.62 (14H, m, Ar-H), 7.87(1H, s, −N═CH), 6.60–6.59 (1H, d, NH), 4.46–4.45 (1H, m, $^{\alpha}$CH), 3.18–3.17 (2H, t, CH_2), 3.14–3.12 (2H, t, $^{\beta}CH_2$), 2.80–2.79 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 173.3, 171.8, 162.1, 156.4, 147.9, 143.5, 138.8, 136.7, 133.5, 131.4, 129.8, 128.8, 127.4, 127.3, 126.6, 126.2, 123.4, 122.2, 121.7, 119.4, 115.6, 112.2, 111.7, 73.1, 34.4, 30.7, 28.6.

*4.1.42. 2-((4-Chlorobenzylidene)amino)-3-(1H-indol-3-yl)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)propanehydrazide **(36)***

Yield 79%, white solid, R_f^a = 0.56, R_f^b = 0.63, m.p. 190–191 °C, MS *m/z*, (M + 1): 541.2364, IR KBr (cm^{-1}): 1609, 1626, 1756, 2945, 3246, 3312, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.19 (1H, s, Het-NH), 10.20 (1H, s, Indole-NH), 9.98(1H, s, NH), 8.10–6.80 (13H, m, ArH), 7.80(1H, s, CH), 6.55–6.54 (1H, d, NH), 4.47–4.46 (1H, m, $^{\alpha}$CH), 3.14–3.13 (2H, t, CH_2), 3.10–3.08 (2H, t, $^{\beta}CH_2$), 2.82–2.80 (2H, t, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.7, 171.4, 162.7, 156.3, 148.2, 143.3, 137.1, 136.5, 134.3, 133.6, 130.4, 129.1, 128.7, 127.4, 127.1, 126.4, 123.5, 122.8, 121.6, 119.3, 118.7, 112.4, 111.3, 72.8, 35.7, 30.6, 28.4.

*4.1.43. 2-((4-Nitrobenzylidene)amino)-3-(1H-indol-3-yl)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)propanehydrazide **(37)***

Yield 81%, yellow solid, R_f^a = 0.55, R_f^b = 0.61, m.p. 184–186 °C, MS *m/z*, (M + 1): 552.6245, IR KBr (cm^{-1}): 1605, 1631, 1788, 2915, 3219, 3267, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.12 (1H, s, Het-NH), 10.26 (1H, s, Indole-NH), 9.56(1H, s, NH), 8.06–6.90 (13H, m, ArH), 7.88 (1H, s, CH), 6.62–6.61 (1H, d, NH), 4.43–4.42 (1H, m, $^{\alpha}$CH), 3.15–3.13 (2H, t, CH_2), 3.12–3.10 (2H, t, $^{\beta}CH_2$), 2.81–2.79 (2H, t, CH_2); ^{13}C NMR (DMSO-d_6, 500 MHz) δ: 172.1, 170.6, 161.3, 156.3, 151.7, 147.8, 144.3, 139.7, 137.6, 133.2, 130.1, 127.8, 127.4, 126.6, 126.2, 124.7, 123.6, 122.0, 121.8, 119.2, 118.4, 112.7, 111.4, 73.6, 34.8, 30.6, 28.1.

*4.1.44. 2-((4-Fluorobenzylidene)amino)-3-(1H-indol-3-yl)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)propanehydrazide **(38)***

Yield 77%, brown solid, R_f^a = 0.46, R_f^b = 0.54, m.p. 157–158 °C, MS *m/z*, (M + 1): 525.1648, IR KBr (cm^{-1}): 1610, 1636, 1788, 2945, 3266, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.21 (1H, s, Het-NH), 10.35 (1H, s, Indole-NH), 9.59(1H, s, NH), 8.10–6.96 (13H, m, ArH), 7.89 (1H, s, CH), 6.60–6.59 (1H, d, NH), 4.42–4.40 (1H, m, $^{\alpha}$CH), 3.14–3.12 (2H, t, CH_2), 3.08–3.07 (2H, t, $^{\beta}CH_2$), 2.86–2.84 (2H, t, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 171.7, 170.6, 166.6, 162.3, 156.3, 147.9, 144.5, 137.7, 133.5, 132.4, 130.6, 128.7, 127.1, 126.8, 126.4, 123.6, 122.2, 120.4, 119.6, 118.4, 115.6, 112.8, 111.3, 73.6, 34.5, 30.8, 28.4.

*4.1.45. 2-((3,4-Dihydroxybenzylidene)amino)-3-(1H-indol-3-yl)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)propanehydrazide **(39)***

Yield 83%, white solid, R_f^a = 0.42, R_f^b = 0.49, m.p. 182–184 °C, MS *m/z*, (M + 1): 539.6231, IR KBr (cm^{-1}): 1616, 1639, 1785, 2962, 3226,

3542, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.28 (1H, s, Het-NH), 10.10 (1H, s, Indole-NH), 9.80 (1H, s, NH), 8.40 (2H, s, 2OH), 8.10–6.70 (12H, m, ArH), 7.76 (1H, s, CH), 6.52–6.50 (1H, d, NH), 4.40–4.38 (1H, m, $^{\alpha}$CH), 3.19–3.18 (2H, t, CH_2), 3.10–3.08 (2H, t, $^{\beta}CH_2$), 2.85–2.83 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 500 MHz) δ: 172.8, 171.7, 161.4, 156.3, 150.7, 149.5, 148.7, 143.3, 136.4, 135.4, 133.2, 128.7, 127.4, 126.5, 126.1, 124.1, 123.3, 122.6, 120.5, 119.4, 118.7, 117.4, 116.3, 112.1, 111.4, 73.2, 34.5, 30.7, 28.6;

4.1.46. 2-((4-Hydroxy-3-methoxybenzylidene)amino)-3-(1H-indol-3-yl)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)propanehydrazide ***(40)***

Yield 81%, white solid, R_f^a = 0.43, R_f^b = 0.50, m.p. 179–181 °C, MS *m/z*, (M + 1): 553.4268, IR KBr (cm^{-1}): 1609, 1616, 1784, 2940, 3246, 3564, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.24 (1H, s, Het-NH), 10.28 (1H, s, Indole-NH), 9.91 (1H, s, NH), 8.2 (1H, s, OH), 8.06–6.86 (12H, m, ArH), 7.80 (1H, s, CH), 6.70–6.68 (1H, d, NH), 4.45–4.44 (1H, m, $^{\alpha}$CH), 3.77 (3H, s, OCH3), 3.20–3.19 (2H, t, CH_2), 3.07–3.05 (2H, t, $^{\beta}CH_2$), 2.68–2.65 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 500 MHz) δ: 172.7, 171.4, 161.6, 156.3, 149.6, 148.3, 144.6, 137.7, 134.5, 133.3, 128.7, 127.4, 126.9, 126.8, 123.5, 123.0, 122.4, 121.8, 119.7, 118.4, 117.1, 112.6, 111.4, 111.2, 73.6, 52.4, 33.8, 30.5, 28.6.

4.1.47. 2-((4-Hydroxy-3,5-dimethoxybenzylidene)amino)-3-(1H-indol-3-yl)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoyl)propanehydrazide ***(41)***

Yield 86%, white solid, R_f^a = 0.43, R_f^b = 0.51, m.p. 193–195 °C, MS *m/z*, (M + 1): 583.1264, IR KBr (cm^{-1}): 1609, 1639, 1785, 2936, 3226, 3312, 3549, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.22 (1H, s, Het-NH), 10.33 (1H, s, Indole-NH), 9.82 (1H, s, NH), 8.20 (1H, s, OH), 8.06–6.80 (11*H*, m, ArH), 7.82 (1H, s, CH), 6.68–6.67 (1H, d, NH), 4.44–4.43 (1H, m, $^{\alpha}$CH), 3.77 (6H, s, 2OCH_3), 3.16–3.14 (2H, t, CH_2), 3.05–3.03 (2H, t, $^{\beta}CH_2$), 2.70–2.68 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.7, 171.7, 161.8, 156.3, 149.5, 148.6, 144.5, 140.7, 137.8, 135.6, 133.5, 128.6, 127.1, 126.9, 126.5, 123.9, 122.8, 121.7, 119.5, 118.7, 112.1, 111.7, 104.8, 73.6, 52.8, 35.1, 30.8, 28.7.

4.1.48. 3-(1H-indol-3-yl)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl) propanoyl)-2-((3,4,5-trimethoxybenzylidene)amino)propanehydrazide ***(42)***

Yield 83%, R_f^a = 0.58, R_f^b = 0.64, m.p. 176–177 °C, IR KBr (cm^{-1}): MS *m/z*: 597.1258, IR KBr (cm^{-1}): 1614, 1628, 1783, 2942, 3226, 3312, ^{1}H NMR (DMSO-d_6) δ ppm: 11.20 (1H, s, Het-NH), 10.18 (1H, s, Indole-NH), 9.90 (1H, s, NH), 8.10–6.96 (11*H*, m, ArH), 7.88 (1H, s, CH), 6.60–6.58 (1H, d, NH), 4.40–4.38 (1H, m, $^{\alpha}$CH), 3.81 (9H, s, 3OCH_3), 3.19–3.17 (2H, t, CH_2), 3.10–3.09 (2H, t, $^{\beta}CH_2$), 2.71–2.69 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 173.6, 170.5, 161.4, 156.7, 152.4, 148.4, 143.3, 142.7, 136.4, 135.1, 133.4, 128.2, 127.5, 126.8, 126.4, 123.4, 121.3, 120.9, 119.4, 118.0, 112.4, 111.7, 104.4, 75.3, 60.8, 56.7, 37.2, 30.6, 28.6.

4.1.49. 3-(1H-indol-3-yl)-N-(3-(4-oxo-3,4-dihydroquinazolin-2-yl) propanoyl)-2-((3,4,5-trihydroxybenzylidene)amino)propanehydrazide ***(43)***

Yield 80%, brown solid, R_f^a = 0.35, R_f^b = 0.39, m.p. 177–179 °C, MS *m/z*, (M + 1): 555.3648, IR KBr (cm^{-1}): 1606, 1636, 1781, 2943, 3214, 3526, ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 11.25 (1H, s, Het-NH), 10.18 (1H, s, Indole-NH), 9.90 (1H, s, NH), 8.59 (2H, s, 2OH), 8.40 (1H, s, OH), 8.11–6.90 (11*H*, m, ArH), 7.86 (1H, s, CH), 6.61–6.59 (1H, d, NH), 4.46–4.45 (1H, m, $^{\alpha}$CH), 3.17–3.16 (2H, t, CH_2), 3.11–3.10 (2H, t, $^{\beta}CH_2$), 2.70–2.69 (2H, t, CH_2), ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 172.5, 170.6, 161.8, 156.4, 148.7, 146.3, 144.3, 138.4, 135.3, 134.7, 133.5, 128.3, 127.4, 126.9, 126.1, 123.4, 121.4, 120.5, 119.7, 118.5, 111.7, 110.9, 109.8, 75.6, 37.6, 30.4, 28.6.

5. Biological assays

5.1. In vitro anticancer assay

5.1.1. Cell culture

The Peripheral blood mononuclear cells (PBMC), human triple negative breast cancer cells (MDA-MB- 231), lung cancer (A546), and melanoma cell (MCF7) lines were purchased from the National Center for Cell Sciences (NCCS), Pune, India. The cancer cells were maintained in Dulbecco's modified eagles medium (DMEM) supplemented with 2 mM l-glutamine and balanced salt solution (BSS) adjusted to contain 1.5 g/L Na2CO3, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM l-glutamine, 1.5 g/L glucose, 10 mM (4-(2-hydroxyethyl)-1- piperazineethane sulfonic acid) (HEPES) and 10% fetal bovine serum (GIBCO, USA). Penicillin and streptomycin (100 IU/100 µg) were adjusted to 1 mL/L. The cells were maintained at 37 °C with 5% CO2 in a humidified CO2 incubator.

5.1.2. Evaluation of cytotoxicity

The inhibitory concentration (IC_{50}) value was evaluated using an MTT [3-(4,5-dimethylthiazol-2- yl)-2,5- diphenyltetrazolium bromide] assay [43]. Cancer cells were grown (1×10^4 cells/well) in a 96-well plate for 48 h in to 75% confluence. The medium was replaced with fresh medium containing serially diluted synthesized compounds, and the cells were further incubated for 48 h. The culture medium was removed, and 100 µL of the MTT [3-(4,5- dimethylthiozol-2-yl)-3,5- diphenyl tetrazolium bromide] (Hi-Media) solution was added to each well and incubated at 37 °C for 4 h. After removal of the supernatant, 50 µL of DMSO was added to each of the wells and incubated for 10 min to solubilize the formazan crystals. The optical density was measured at 620 nm in an ELISA multiwell plate reader (Thermo Multiskan EX, USA). The OD value was used to calculate the percentage of viability using the following formula.

$$\%\ \text{of vaibility} = \frac{\text{OD value of experimental sample}}{\text{OD value of experimental control}} \times 100$$

DNA binding affinity study: a colorimetric investigation

5.1.3. DNA binding assay

The DNA binding study was conducted as per the protocol of Burres et al. [44] with slight modifications. Briefly, the DNA methyl green (20 mg) was suspended in 100 mL of 0.05 M Tris-HCl buffer (pH 7.5) containing 7.5 mM MgSO4, then the mixture was stirred at 37 °C with a magnetic stirrer for 24 h. Different concentrations of test samples (10–100 µg/mL) were dissolved in ethanol in Ependoff tubes, solvent was removed under vacuum, and 200 µL of the DNA/methyl green solution were added to each tube. Samples were incubated in the dark at ambient temperature. After 24 h, the final absorbance of the samples was determined at 645 nm. Readings were corrected for initial absorbance and normalized as the percentage of the untreated standard using Ethidium bromide as positive control.

5.2. Methodology

5.2.1. Molecular docking studies

The structural drawing and geometry cleaning of the amino acids conjugated quinazolinone derivatives **4–43** were performed in Maestro 10.1 of Schrödinger suite 2015-1 platform and then subjected to other parameters viz energy minimization by using OPLS 2005 force field, addition of hydrogen atoms, neutralization of charged groups, generation of ionization states and set pH 7.5 using Epik. Generation of tautomers and stereoisomers of 32 per ligand and low-energy ring conformations and optimize the geometries followed by generating low energy ring conformation per ligand were computed, optimized by LigPrep and used for molecular docking.

5.2.2. Protein preparation for docking

Molecular docking studies were carried, which was reported previously by Savithri et al. [45] Briefly, The X-ray crystallographic structure of the DNA dodecamerd (CGCAAATTTGCG) with a bifurcated hydrogen-bonded conformation of the AT base pairs and its complex with distamycin A was selected from the Protein Data Bank (PDB code: 2DND) for the docking study. Crystal structure was imported and refined by a multistep process in Maestro 10.1, which includes energy minimization using OPLS-2005 force field, correct bond orders were assigned, hydrogen atoms were added and the water molecules where removed beyond 5 Å from hetero atom were optimized. Using PROPKA, pH was fixed and optimized to 7.5. Non-hydrogen atoms were minimized by restrained minimization to default RMSD to 0.3 Å. Using Extra-precision (XP) docking and scoring each compound were docking into the receptor grid of radii 20 Å × 20 Å × 20 Å and the docking calculation were judge based on the Glide score.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.03.038.

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