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(RESEARCH ARTICLE)

Design and synthesis of novel 1-substituted -3-(4-oxo-2-phenylquinazolin-3(4H)-yl) urea and thiourea analogues targeting on TACE, as potent anticancer agents

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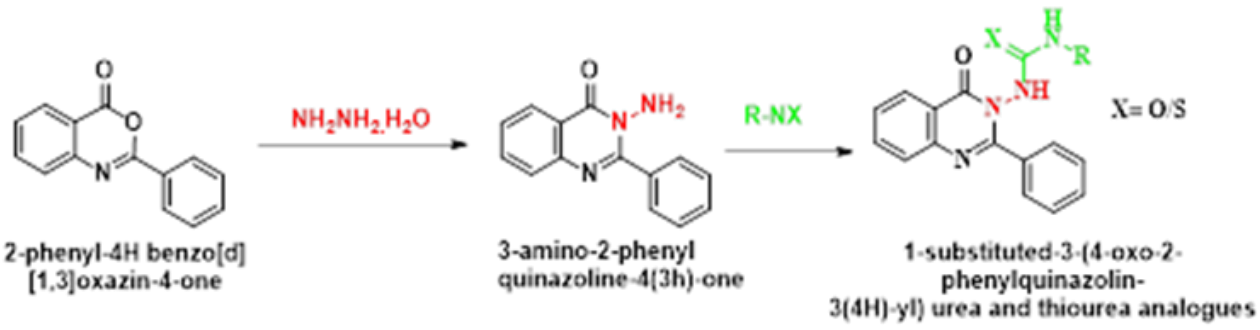
Abstract

1-substituted phenyl quinazolinones analogues were designed by performing molecular modelling studies against tumour necrosis factor alpha converting enzyme (TACE PDB Id: 2A8H) and *in-silico* Lipinski properties for drug likeness. From QSAR studies, it could be concluded that the urea and thiourea groups play a crucial role in enhancing cytotoxic effects of compounds. Substitution of halogens like trifluoromethyl, chloro and allylic functional group may enhance the cytotoxic effect of urea and thiourea analogues. Substitution of phenyl and benzoyl ring were not found effective against cancer. Also, the presence of substituted aromatic ring at position 3 and methyl or thiol group at position 2 are essential for antimicrobial activities of quinazolinone. The synthesized compounds were characterized by TLC, MP, IR, NMR and Mass spectral data and were screened for their anticancer activity. The *in vitro* anticancer studies were performed on six selected compounds using MTT assay against MDA MB-231 cell line using paclitaxel as a standard. The synthesized 1-substituted -3-(4-oxo-2-phenylquinazolin-3(4h)-yl) urea and thiourea derivatives exhibited significant anticancer activities.

Graphical Abstract



Keywords: Anticancer; MDA-MB 231; MTT assay; Quinazolinone analogues; TACE

1. Introduction

In medicinal chemistry research heterocyclic compounds particularly N-containing heterocyclic compounds are explored due to their wide scope of pharmacological activities [1]. The importance of heterocyclic compound in

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medicinal chemistry is well established, most of the drugs available today contain heterocyclic scaffolds. In medicinal chemistry quinazolinone scaffold is one of the most important and privileged pharmacophores. A quinazolinone attracts the research over 100 years due to its unique features of the pharmacophore [2]. Quinazolinones have wide spectrum of their antibacterial, anticonvulsant, antifungal, anticancer, anti-HIV, anti-inflammatory, antitubercular and analgesic activities [3]. Quinazoline being a central pharmacophore, holds different substitutions at multiple sites. Modifications based around the fused ring of quinazolinone subsequently evaluate its usefulness in treating various disease conditions [4].

We have mainly focused on anticancer activity, because cancer is severe non-communicable disease Worldwide, which accounts for about 18.1millions of new cases with 9.6 million of death each year, however India contributes about 1.2 million of cases every year. According to World Health Organization's recent reports, is second leading cause of death Worldwide [5]. There are over 100 types of cancer out of which lung, liver, colorectal, prostate, cervical, stomach and skin are more commonly observed. About 30% of patients die due to late detection of cancer in past few years [6]. The current treatment of cancer includes chemotherapy, hormonal and surgical treatment, but it cannot hold the nerve of the disease due to late detection of cancer and painful chemotherapy. Cancer treatment can also develop carcinogenicity in patients [7]. To overcome such problems there is urgent need to develop new anticancer agents. Quinazolinone possess potent anticancer activity against various enzymes like TACE (Tumor Necrosis Factor α Converting Enzyme), EGFR (Epidermal Growth Factor Receptor), Tyrosine kinase, MMP (Matrix metallopeptidases), Aurora B kinase [8-11]. etc.

According to Mohan et. al. TACE is member of A disintegrin and metalloprotease (ADAM) family a key enzyme in cell cycle [12]. It belongs to multi-domain type I transmembrane protein that includes extracellular zinc dependent protease domain. Previously the enzyme was targeted for anti-inflammatory activity [13]. Gawad et. al. has synthesized some new substituted quinazolin- 4[3H]- ones and 3,4- dihydroquinazolin- 2[1H] analogues which exhibit potent anticancer activity against numerous cell lines that belong to various different tumor subpanels [14]. Ahemad et.al. (2013) has synthesized a three-novel series of 6,8- Dibromo-4 (3H) quinazolinone derivatives which have showed promising anticancer activity at very low concentrations against MCF-7 cell line using doxorubicin as control standard [15]. Noolvi et. al. has studied Qualitative structure activity realationship (QSAR) of synthesized substituted quinazoline and quinoxaline analogue. These novel analogues substituted with series of 2-Furano-4 (3H) quinazolinone were further screened for their anticancer potential and few analogues have shown good anticancer potential.

QSAR techniques enabled understanding the pharmacophoric requirement for quinazoline and quinoxaline derivatives. The overall outcome of the study revealed that the quinazoline ring is satisfactory backbone for antitumor activity [16]. Cao et.al. has synthesized a series of 4(3H)-quinazolinone derivatives with dithiocarbamate side chains which were synthesized and tested for their in vitro antitumor activity against human myelogenous leukemia K562 cells. Among them, (3,4-dihydro-2-methyl-4-oxoquinazolin-6-yl)- methyl 4-(4 fluorophenyl) piperazine-1-carbodithioate exhibited significant inhibitory activity against K562 cells with IC50 value of 0.51 M [17]. Xia et.al synthesized a series of 20, 30, 40, 6, 7-substituted 2-aryl quinazolinones and were screened for anticancer activity. Amongst them 17 compounds displayed significant growth inhibitory action against a panel of tumor cell lines. Dimethoxy substituted 2-aryl quinazolinone exhibited was found to be potent inhibitor of tubulin polymerization.

Compounds with flouro substitution displayed selective activity against P-gp-expressing epidermoid carcinoma of the nasopharynx [18]. Unnissa et. al. synthesized a series of disubstituted quinazolin 4(3H)-one derivatives which exhibited significant antitumour activity [19]. Saeedi. et.al. synthesized a novel series of quinazolinone-1,2,3-triazole hybrids which were evaluated for their in vitro α-glucosidase inhibitory activity leading to efficient anti-diabetic agents [20]. On the basis of literature and drug design tool, we designed 1-substituted-3-(4-oxo-2-phenylquinazolin- 3(4h)-yl) urea and thiourea analogues.

2. Material and methods

All chemicals were purchased from Sigma Aldrich Merck and were of synthetic grade. Reaction procedures were optimized on Radley's six station parallel combinatorial synthesizers and monitored on pre-coated aluminum plates (Merck silica gel 60F-254) using UV visualization technique and iodine vapors. All chemicals, reagents and solvents All the chemicals used were of synthetic grade. Pre-distilled solvents were used for reaction, characterization and purification. Melting points (uncorrected) were determined on programmable melting point and boiling point apparatus (VEEGO, India). IR spectra were recorded on JASCO V-530 FTIR 4100 .1H NMR was recorded on "Bruker Avance" Spectrometer at 100, 300, 400 MHz frequency in CDCl3 and DMSO in presence of TMS as internal standard (Chemical shift in ppm). Mass spectra were obtained from G6460A triple quadrupole/MS/MS system (Agilent technologies) equipped with electrospray ionization technique.

The In- silico drug properties calculation was performed using OSIRIS Data warrior (version 5.0.0.) which is based on Lipinski rule of five. In-vitro anticancer evaluation was carried out against MDA-MB 231 cell line at KLE University, Belgaum, Karnataka India.

2.1. Designing of target molecules (SS, SS-A, SS-B, SS-01, SS-02, SS-03, SS-04)

Designing of target molecules of quinazolinone was done by taking into consideration of two potent anticancer agents Gefitinib and Sorafenib which is given in (Figure. 1).

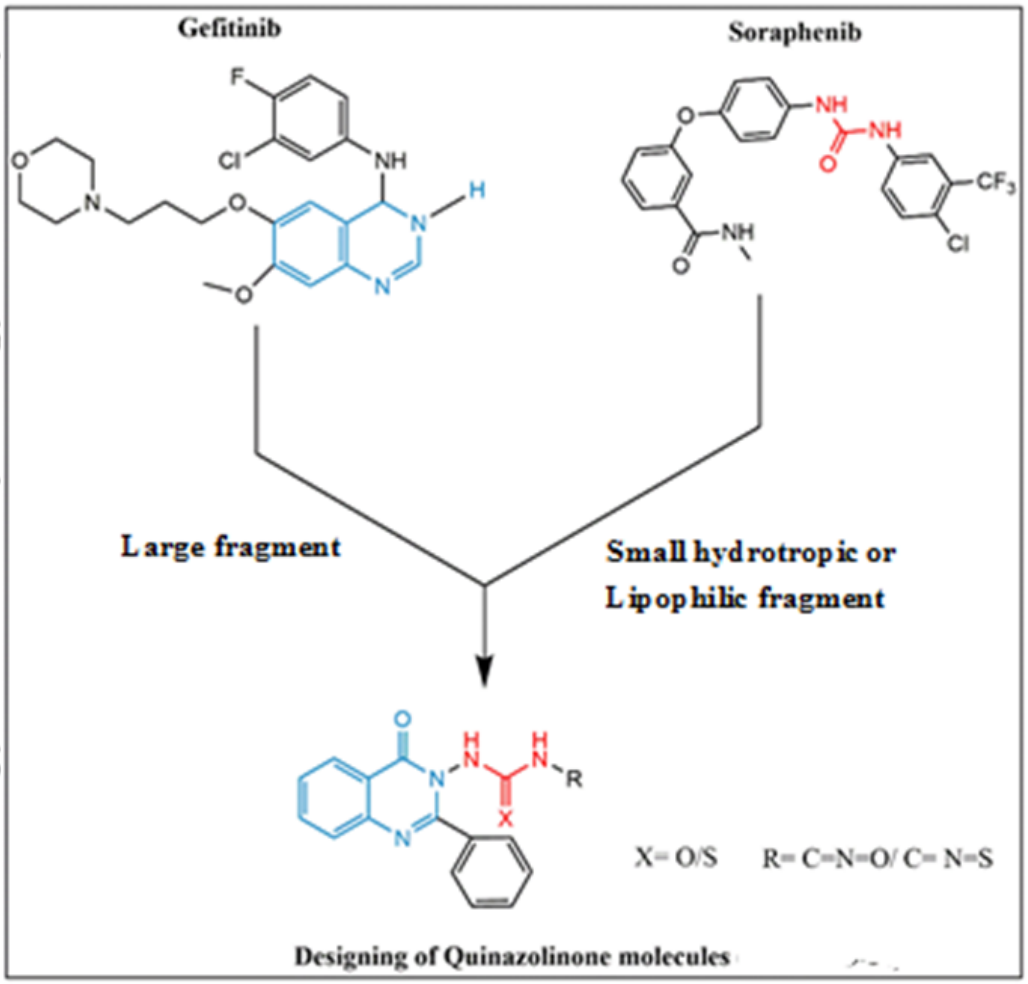


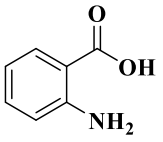
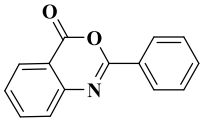
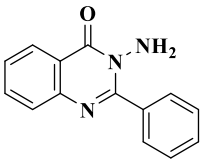
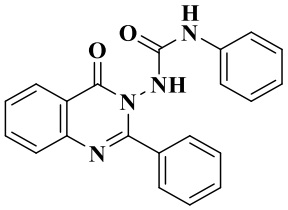
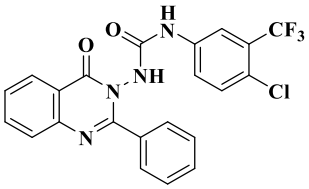
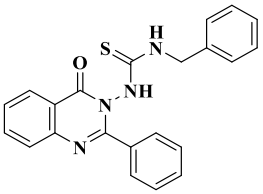
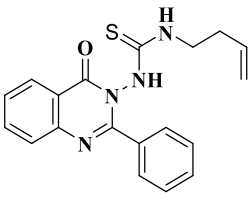
Figure 1 Designing of quinazolinone molecules

(In Figure 1. The quinazoline group from gefitinib which shown in blue color and the 4 Chloro-3- trifluoromethyl urea moiety shown in red color, were combined and modified to generate new molecules of quinazolinone)

2.2. Synthesis of analogues (SS, SS-A, SS-B, SS-01, SS-02, SS-03, SS-04)

On the basis of literature survey and structural activity relationship, six quinazolinone analogues were synthesized [21-24]. i. e. SS, SS-A, SS-B, SS-01, SS-02, SS-03, SS-04 as depicted in (Table 1).

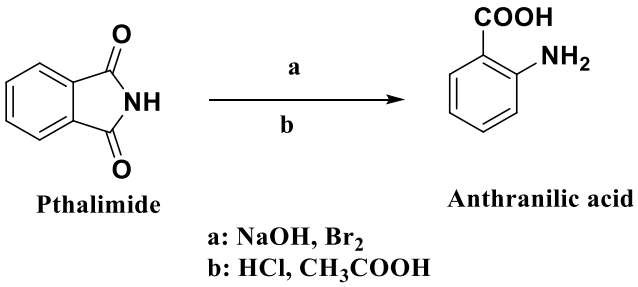
Table 1 Synthesized 1-substituted-3-(4-oxo-2-phenylquinazolin-3(4H)-yl) urea and thiourea analogues

Sr. No.	Compound Code	Structure	IUPAC Name
1	SS		2-amino benzoic acid
2	SS-A		2-phenyl-4H benzo[d][1,3]oxazin-4-one
3	SS-B		3-amino-2-phenyl quinazoline-4(3h)-one- 4-one
4	SS-01		1-(4-oxo-2-phenylquinazolin-3(4H)-yl)-3-phenylurea
5	SS-02		1-(4-chloro-3-(trifluoromethyl)phenyl)-3-(4-oxo-2-phenylquinazolin-3(4H)-yl)urea
6	SS-03		1-benzyl-3-(4-oxo-2-phenylquinazolin-3(4H)-yl) thiourea
7	SS-04		1-(but-3-en-1-yl)-3-(4-oxo-2-phenylquinazolin-3(4H)-yl) thiourea

2.2.1. Synthesis of 2 aminobenzene carboxylic acid (SS)

In a 350 ml conical flask a solution of 4.0 g (0.1 mol) of sodium hydroxide in 40 ml of water was prepared and cooled. To this flask 26.2 g (8.4 ml, 0.16 mol) of bromine was added by shaking vigorously till bromine gets reacted. The temperature was set at 0 °C. 2 g (0.05 mol) of sodium hydroxide was dissolved in 20 ml of water and 4 g (0.16 mol) finely powered phthalimide was added into one portion in to the cold solution of sodium hypobromite. Sodium

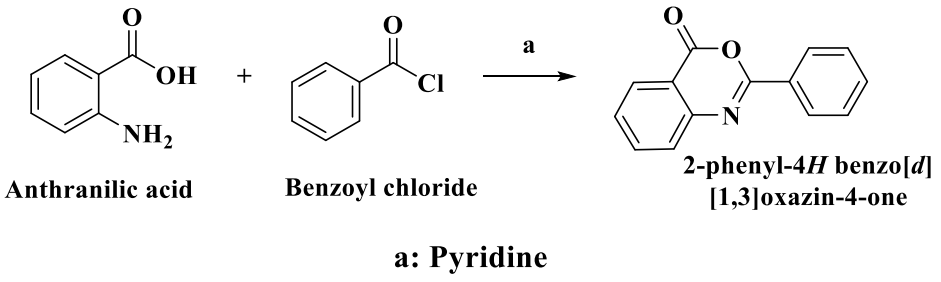
hydroxide solution was rapidly heated up to 80 °C for about 2 min, concentrated hydrochloric acid was slowly added to neutralize the solution. Anthranilic acid was precipitated completely by gradual addition of glacial acetic acid (20-25 ml). The precipitate was filtered at vacuum pump and was recrystallized from hot water with addition of little decolorizing carbon (Scheme 1).



Scheme 1 Scheme for synthesis of Anthranilic acid

2.2.2. Synthesis of 2-phenyl-4H benzo[d] [1,3] oxazin-4-one (SS-A)

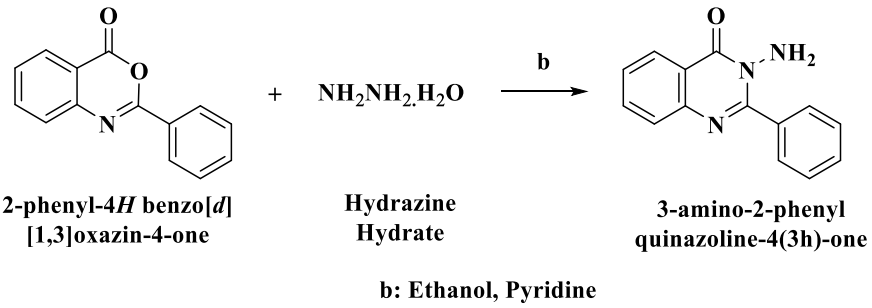
Anthranilic acid solution was prepared by adding 6.85 g (0.05 mol) of anthranilic acid to 60 ml of pyridine. To this mixture 5.67 ml (0.05 mol) of benzoyl chloride was added drop wise at 0-2º C for 2 hours. Reaction mixture was stirred for 2 hours. The reaction mixture was neutralized with saturated sodium bicarbonate solution and solid product was filtered and re-crystallized from ethanol (Scheme 2).



Scheme 2 Scheme for synthesis of 2-phenyl-4H benzo[d][1,3]oxazin-4s-one (SS-A)

2.2.3. Synthesis of 3-amino-2-phenyl quinazoline-4(3h)-one- 4-one (SS-B)

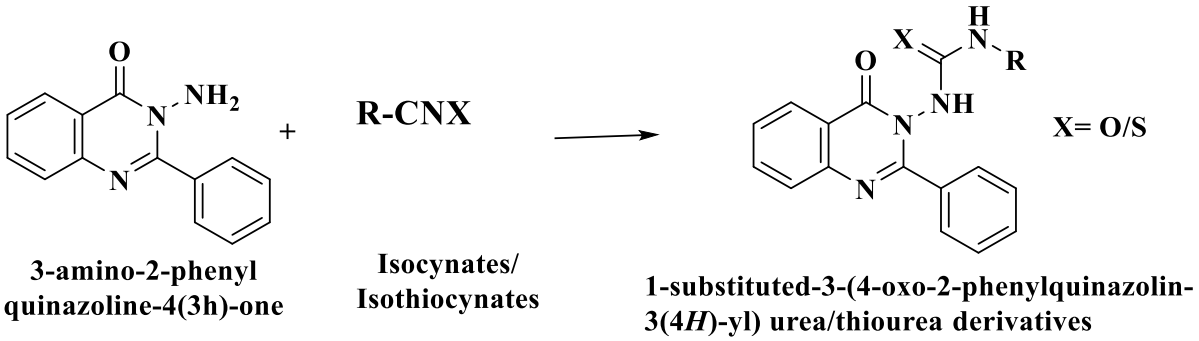
2.2 g (0.01 mol) of 4H-benzo[d] [1, 3] Oxazin-4-one was dissolved in ethanol and 0.5 ml (0.01mol) of hydrazine hydrate was added to it with catalytic amount of pyridine. Reaction mixture was refluxed for 1 hour and after cooling a crystalline product was filtered and re-crystallized from ethanol (Scheme 3).



Scheme 3 Synthesis of 3-amino-2-phenyl quinazoline-4(3h)-one- 4-one (SS-B)

2.2.4. Synthesis of 1-substituted-3-(4-oxo-2-phenylquinazolin-3(4H)-yl) urea and thiourea analogues

2 g (0.0090 mol) of 3-amino-4H-quinazolinone was dissolved in 10 ml di-chloro methane (DCM). After stirring the reaction mixture for 5 minutes at room temperature 1.99 g (0.00900 mol) of respective isocyanates were added in it and was stirred. After completion of reaction, product was filtered and re-crystallized from ethanol (Scheme 4).



Scheme 4 Scheme for synthesis of 1-substituted-3-(4-oxo-2-phenylquinazolin-3(4H)-yl) urea and thiourea analogues (SS-01 to SS-04)

In silico drug properties

The correlation of absorption, distribution, metabolism, excretion, toxicity and the prediction of physicochemical properties is necessary. For the prediction of physicochemical properties, OSIRIS Data warrior (version 5.0.0.) software was used which is based on Lipinski rule of five [25].

Characterization of Synthetic analogues

The synthesized analogues were characterized by thin layer chromatography, melting point, infrared spectroscopy, 1 H NMR and mass spectrometric analysis [26].

2.3. Biological Evaluation

2.3.1. Anticancer activity screening by Using MTT assay

The anticancer activity of quinazolinone analogues was calculated through MTT viability assay. The effect of synthesized analogues on the viability of MDA-MB was analyzed by using MTT dye. 5×10^3cells/well in a 96-well flat-bottom micro plate was maintained at 37^0C in 95% humidity and 5% CO_2 overnight. After 24 hours, the cells were incubated with fresh medium containing different concentrations (1000, 500, 250, 125, 62.5, 31.25 µg/ml) of derivatives and the plates were further incubated for 24 hrs. The MTT solution (5 mg/ml) was added to each well, followed by 4 hours incubation at 37uC in 5% CO_2 incubator. The intensity of colored formazan derivative was determined by measuring optical density (OD) with the ELISA microplate reader (Biorad, Hercules, CA) at 570 nm ($OD_{570-630\ nm}$). The cell viability was expressed in terms of percentage. Percent surviving and incubating cells were calculated by the fallowing formula [27].

$$\text{Surviving cells S (\%)} = \frac{\text{Mean OD of test compound}}{\text{Mean OD of negative control}} \text{ X } 100$$

Inhibiting cells (%) =100 - Surviving cells (S)

OD = Optical Density

3. Results and discussion

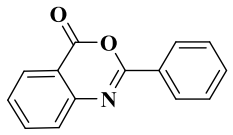
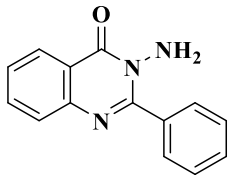
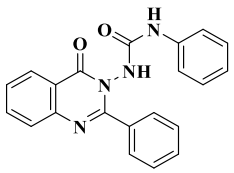
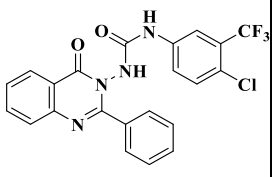
In this work, we have submitted the synthesized compounds against MDA- MB 231cell line for the analysis of Lipinski rule of five which indicates that whether a chemical compound could be orally active drug in humans. Our result showed that all the synthesized analogues fulfilled this rule. Less solubility of the synthesized compound**s** were predicted and was observed practically. The compounds were screened for anticancer activity on the basis of drug likeness score as shown in (Table 2).

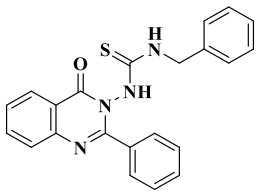
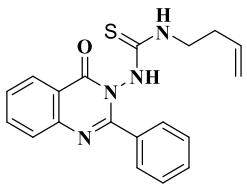
Table 2 OSIRIS calculation for Lipinski rule of five

Code	Mol. Weight	cLogP	cLogS	H-Acceptors	H-Donors	Drug likeness	Drug Score
SS-A	223.13	0.351	-3.341	3	0	4.81418	0.571266
SS-B	237.13	0.8997	-3.061	4	1	5.77379	0.598914
SS-01	356.13	2.5345	-4.389	5	1	0.2575	0.321117
SS-02	458.08	3.9888	-5.903	5	1	2.8979	0.716608
SS-03	386.13	2.5326	-4.354	5	1	0.35094	0.316608
SS-04	350.12	2.1636	-3.716	4	1	2.8868	0.716708

The anticancer activity of 1- substituted – 3-(4-oxo-2-phenylquinazolin-3(4H)-yl) urea and thiourea analogues can be enhanced by making minor substitution. On the basis of literature survey and drug designing tool molecules can be further screened for anti-inflammatory, analgesic and anti-oxidant activity [28-30]. The results obtained following the synthetic procedures for the preparation of 1- substituted – 3-(4-oxo-2-phenylquinazolin-3(4H)-yl) urea and thiourea analogues are presented in (Table 3). Which depicts melting point, percentage yield, molecular formula, R_f of the synthesized analogues.

Table 3 Physical Constants of Synthesized compounds

Code	Structure	Physical Constants			
		Melting Point (° C)	Yield (%)	Mol. Formula	R_f
SS-A		118.0-120.8	95	$C_{14}H_9NO_2$	0.85
SS-B		90.0-93.5	92	$C_{14}H_{11}N_3O$	0.60
SS-01		108.5-109.8	90	$C_{21}H_{16}N_4O_2$	0.65
SS-02		108.5-109.8	95	$C_{22}H_{17}N_4O_2$	0.65

SS-03		208.5-210.7	91	$C_{22}H_{18}N_4OS$	0.58
SS-04		101.5-103.8	95	$C_{22}H_{18}N_4OS$	0.47

3.1. *In silico* drug likeness

In this work, we have submitted the synthesized compounds against MDA- MB 231cell line for the analysis of Lipinski rule of five which indicates that whether a chemical compound could be orally active drug in humans. Our result showed that all the synthesized analogues fulfilled this rule. Less solubility of the synthesized compound**s** were predicted and was observed practically. The compounds were screened for anticancer activity on the basis of drug likeness score as shown in above table (Table 2).

The results obtained following the synthetic procedures for the preparation of 1- substituted – 3-(4-oxo-2-phenylquinazolin-3(4H)-yl) urea and thiourea analogues are presented in Table 3. Which depicts melting point, percentage yield, molecular formula, R_f of the synthesized analogues.

3.2. Spectroscopic characterization of synthesized analogues

(SS) 2-Aminobenzenecarboxylic acid: IR *vmax* (cm^{-1}) (KBr): 3324.17 (NH str, 2^0 amine), 3239.16 (=CH, str, aromatic), 2939.16 (-OH, Str, carboxylic acid),1719.06 (C=O, str carboxylic acid),1400.08 (C=C, ben aromatic); ^1H NMR (100 MH_Z in DMSO) δ 11.11 (s, -OH, 1H), 6.74 (d, $-NH_2$, 2H), 6.96 -7.84 (m, -Ar, 4H); Mass (ESI): m/z $[M+H]^+$ 137.16.

(SS-A) 2-phenyl-4H benzo[d][1,3]oxazin-4-one: IR *vmax* (cm^{-1}) (KBr): 3034.44 (C=C, str, aromatic), 1764.55 (C=N, str 2^0 amine), 1617.02 (=CH, str aromatic), 1256.4 (C-O -C, str); ^1H NMR (100 MH_Z in DMSO) δ 7.53-8.15 (m, -Ar, 9H); Mass (ESI): m/z $[M+H]^+$ 223.19.

(SS-B) 3-amino-2-phenylquinazolin-4(3H)-one: IR *vmax* (cm^{-1}) (KBr): 3355.53(N-NH_2, str 1^0 amine), 3306.36(C=NH, str 2^0 amine), 3034.44 (C=C, str, aromatic), 1617.02 (=CH, str aromatic); ^1H NMR (100 MH_Z in DMSO) δ 7.53-8.15 (m, -Ar, 9H, s, -NH, 2H); Mass (ESI): m/z $[M+H]^+$ 237.19.

(SS-01) 1-(4-oxo-2-phenylquinazolin-3(4H)-yl)-3-phenyl urea: IR *vmax* (cm^{-1}) (KBr): 3286.11, (-NH_2, str 1^0 amine), 3032.51 (C=0, str carboxylic acid), 1674.88 (C=O, str amide), 1572.66 (C=C, str aromatic), 1504.2 (=Ch, str aromatic); ^1H NMR (100 MH_Z in DMSO) δ 8.5380 (s, 1H, NH), 8.559 (s, 1H, NH), 6.9543-7.9363 (m 14H, -Ar); Mass (ESI): m/z $[M+H]^+$ 356.29.

(SS-02) 1-(4-chloro-3-(trifluoromethyl)phenyl)-3-(4-oxo-2-phenylquinazolin-3(4H)-yl) urea*:* IR *vmax* (cm^{-1}) (KBr): 3340 (-NH_2, str 1^0 amine), 1654(C=O, str amide), 1572.66 (C=C, str aromatic), 1504.2 (=Ch, str aromatic); ^1H NMR (100 MH_Z in DMSO) δ 11.86 (s, 1H), 9.39 (s, 1H), 7.522-8.670 (m, 12H); Mass (ESI): m/z $[M+H]^+$ 458.49.

(SS-03) 1-benzyl-3-(4-oxo-2-phenylquinazolin-3(4H)-yl) thiourea: IR *vmax* (cm^{-1}) (KBr): 3204.15 (-NH_2, str 1^0 amine), 2933.2 (C=O, str amide), 1660.14 (C=C, str aromatic), 1504.2 (=Ch, str aromatic); ^1H NMR (100 MH_Z in DMSO) δ 11.960 (s, 1H,-NH), 10.738 (s, 1H, -NH), 4.721(t, 2H, -CH_2-), 7.185-8.772 (m, 14H, -Ar); Mass (ESI): m/z $[M+H]^+$ 386.14.

(SS-04) 1-(but-3-en-1-yl)-3-(4-oxo-2-phenylquinazolin-3(4H)-yl) thiourea: IR *vmax* (cm^{-1}) (KBr): 3204.15 (-NH_2, str 1^0 amine), 2933.2 (C=O, str amide), 2260.44 (C)1660.14 (C=C, str aromatic), 1504.2 (C=C, str aromatic); ^1H NMR (100MH_Z in DMSO) δ 11.960 (s, 1H,-NH), 10.738 (s, 1H, -NH), 3.95 (t, 2H, -CH_2-), 7.185-8.772 (m, 9H, -Ar); Mass (ESI): m/z $[M+H]^+$ 350.44.

3.3. Anti-cancer activity

The anticancer activity of synthesized compounds was carried out by using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] cyto-toxicity assay on MDA MB 231 (breast cancer cell line). The anticancer activity was recorded against paclitaxel. The cytotoxic effects are summarized in (Table 4). The observed data did not follow a dose-response pattern, which implied no change in response to increasing doses as cell growth was not seen to be inhibited, due to which the IC_{50} value of the quinazolinone analogues was unable to determined. To compare the effects of individual analogues against basal, one -way (Analysis of Variance) ANOVA was used by using Graph Pad Prism v5 statistical analysis package (GraphPad Software Inc., La Jolla, CA). It was apparent that the effects of the analogues differ on cells showing that some drugs do inhibit the cell growth as shown in (Figure 2.) SS-02 showed a significant difference in cell viability at the lowest dose tested than basal and SS-04 showed significant difference at most of the tested concentrations as compared to basal.

Table 4 Anticancer activity of the synthesized compound on MDA-MB breast cancer cell line.

Conc. (µg/ml)	% Cell Viability (expressed as % of basal)					
	SS-A	SS-B	SS-01	SS-02	SS-03	SS-04
0	100	100	100	100	100	100
31.25	92.3847	86.6859	83.6956	62.1376	75.8152	58.9673
62.05	87.8711	74.6359	92.9347	68.2065	81.1594	67.4818
125.00	100.0957	81.5194	86.5942	70.8333	74.3659	59.8731
250.00	70.7101	67.8418	93.0253	79.5289	100.9057	70.4710
500.00	79.2899	75.6852	87.6811	72.4637	73.7318	64.8550
1000	73.2246	70.5310	86.8659	68.2065	73.8224	64.1304

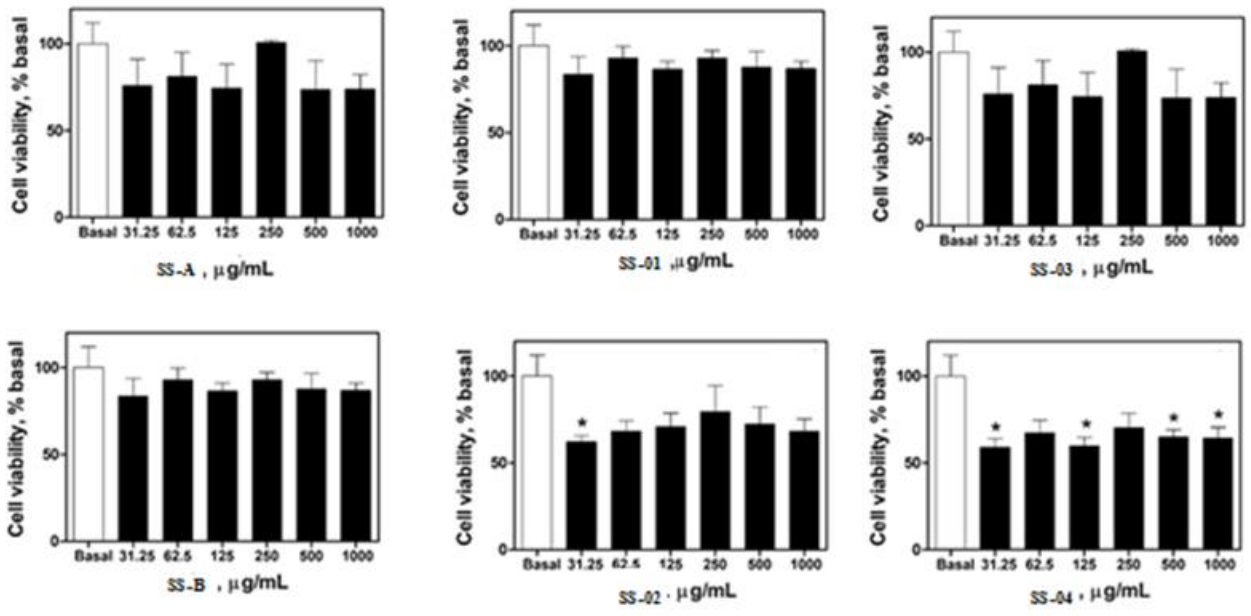


Figure 2 Basal graph of individual analogues

4. Discussion

The 1- substituted – 3-(4-oxo-2-phenylquinazolin-3(4H)-yl) urea and thiourea was obtained in the range of 90-95 %. It indicates that the synthetic pathway used for synthesis is suitable for quinazolinone urea and thiourea analogues of this kind. The Lipinski's rule of five for prediction of drug likeness of new chemical entities. The existing quinazolinone

compounds possesses exhibit greater bioavailability, solubility and potent anticancer activity. The anticancer activity of 1- substituted – 3-(4-oxo-2-phenylquinazolin-3(4H)-yl) urea and thiourea analogues can be enhanced by making minor substitution. The observed data did not follow a dose-response pattern, which implied no change in response to increasing doses as cell growth was not seen to be inhibited, due to which the IC_{50} value of the quinazolinone analogues was unable to determined. To compare the effects of individual analogues against basal, one -way ANOVA was used by using Graph Pad Prism v5 statistical analysis package (GraphPad Software Inc., La Jolla, CA). It was apparent that the effects of the analogues differ on cells showing that some drugs do inhibit the cell growth as shown in (Figure 2.) SS-02 showed a significant difference in cell viability at the lowest dose tested than basal and SS-04 showed significant difference at most of the tested concentrations as compared to basal. On the basis of literature survey and drug designing tool molecules can be further screened for anti-inflammatory, analgesic and anti-oxidant activity [28-29].

5. Conclusion

The current investigation of anticancer activity of synthesised 1-(4-oxo-2-phenylquinazolin-3(4H)-yl) urea and thiourea against MDA-MB 231 cancer cell line describes about the basic scaffold design required for anticancer analogues. The molecular modelling studies indicates the TACE can be more useful target for anticancer drug development. The results show that the current research work develops an efficient protocol for the synthesis of phenyl quinazolinone analogues, and the molecules can be further considered for development of potent anticancer agents.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this research article.

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