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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and screening of 2-(2-(4-substituted piperazine-1-yl)-5-phenylthiazol-4-yl)-3-aryl quinazolinone derivatives as anticancer agents

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Abstract Synthesis of novel quinazolinone derivatives was performed from the reaction of *N*-benzoyl substituted piperazine-1-carbothioamide with 2-chloromethyl quinazolinone derivatives and screened for their in vitro cytotoxic activity by MTT assay. The cell lines used were NCI (human lung cancer cell), MCF 7 (Breast cancer cell), and HEK 293 (Normal epidermal kidney cell). Result of screening on cell line showed moderate to good anticancer activity for all the compounds. Compound **3d** ($IC_{50} = 1.1 \pm 0.03$ μM) was found to be the most active compared to standard methotrexate ($IC_{50} = 2.20 \pm 0.18$ μM) and 5-florouracil ($IC_{50} = 2.30 \pm 0.49$ μM). Structure activity relationship of synthesized analogs suggested that the presence of NH linker with aryl moiety at the third position of quinazolinone ring was important for potent anticancer activity. Electron donating group on phenyl ring at the third position of quinazolinone ring gave better anticancer activity then unsubstituted phenyl and electron withdrawing group. Activity by substituted piperazine at 2nd position of thiazole linked with quinazolinone scaffold gave better activity in the order of $H > CH_3 > CO–C_6H_5$. Our findings may impart new direction to medicinal chemists and biochemists for further investigations of quinazolinone-thiazole containing anticancer agents.

Keywords Anticancer · Quinazolinone · Thiazole · Piperazine · MTT assay

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Introduction

Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues. Number of cancer patients is increasing rapidly from day to day and good protection from cancer and with reduced adverse effects is the requirement of present scenario.

Quinazolinones are among the most useful heterocyclic compound from both synthetic and medicinal chemistry aspects. Most of the researchers focused on synthesis of quinazolinone and their anticancer property (Ahmed *et al*., 2010; Shang *et al.*, 2005). The structural design of quinazolinone have attracted a great deal of attention because of their ready accessibility, diverse chemical reactivity, and biological activities like anti-inflammatory (Ashok and Chatrasal, 2009), antimalarial (Shuren *et al*., 2010), anthelmintic (Rajiv and Anil, 2008), muscle relaxant (Buyuktimkin and Ekinici, 1992), antihyperlipidemic (Fawzia and Amry, 2005), antitubercular (Kunes *et al*., 2000), antimicrobial (Wasfy, 2003), and antihypertensive (Ashok and Chatrasal, 2009) activities. The synthetic flexibility of quinazolinone scaffold led to the synthesis of variety of its substituted analogs. Raltitrexed and thymitaq are now clinically used as anticancer drugs having quinazolinone moiety.

Thiazole, as a part of our designed molecule is a five member heterocyclic moiety having various pharmacological activities like anticancer, anti-inflammatory, and antibacterial. Thiazole is found in certain natural product like vitamin B_1 and penicillins. There are many reports available on the anticancer activity of 2-aminothiazole and benzothiazole derivatives.

In the design of new anticancer agent, the development of hybrid molecules through the combination of different pharmacophore i.e., quinazolinone and thiazole moiety in one frame may lead to compounds with interesting

anticancer profiles (Rajan *et al*., 2010a, b; Al Obaid *et al*., 2009). Quinazolinone and thiazole pharmacophore may serve as an important scaffold to develop new anticancer agents with improved activity.

We hypothesized that the designing of molecule with quinazolinone moiety as molecular scaffold by using strategies like linking with thiazole and by varying chain length using piperazine as linker and substituted phenyl group attach at position the third of quinazolinone ring in the target molecules for better anticancer activity.

Chemistry

This synthetic strategy began with the synthesis of quinazolinone by the reaction of 2-(2-chloroacetamido)-benzoic acid with aromatic amines in the presence of phosphorus oxychloride. In the IR spectrum of quinazolinone, the carbonyl frequency was observed at 1,729 cm^{-1} (Hui *et al.*, 2009; Shashikant *et al*., 2006). The synthesis of isothiocyanate oil by the reaction of benzoyl chloride with potassium thiocyanate in the presence of PEG 400 as phase separation catalyst was carried out and characterized by boiling point and TLC. The synthesis of *N*-(4-substituted piperazine-1-thioyl)-benzamide was carried out using a reported method (Rajan *et al*., 2010a, b). The IR spectrum of *N*-(4-substituted piperazine-1-thioyl)-benzamide showed C–N stretch, C–S stretch, and C=O stretch at 1,437, 1,555, and 1,695 cm^{-1}, respectively. Final product was synthesized by the reaction of different quinazolinones and *N*-(4-substituted piperazine-1-thioyl)-benzamides in the presence of triethylamine and acetonitrile as solvent. Reaction mixture was refluxed at temperature of 80 °C and monitored with TLC for completion. The yields of synthesized compound are given in Table 1. We reported the synthesis of novel quinazolinone derivatives and their cytotoxic potentials.

Biology

All the synthesized compounds were screened for their cytotoxic activity on MCF-7 (human mammary gland adrenocarcinoma cell line), NCI (lung cancer cell line), and HEK-293 (human epidermal kidney cell line as normal cell line) by MTT assay. MCF-7, NCI, and HEK-293 cell cultures were procured from National Centre for Cell Sciences, Pune, India. The screening experiments were carried out at Department of Biotechnology, S.K. Patel College of Pharmaceutical Education and Research, Ganpat University, Gujarat, India. Cultures were observed using an inverted microscope to assess the degree of viability, and the absence of bacterial and fungal contaminants was confirmed. Cell monolayer was washed with PBS without Ca^{++}/Mg^{++} using a volume equivalent to half the volume of culture medium. Trypsin/EDTA was added on to the washed cell monolayer using 1 ml per 25 cm^2 of surface area. Flask was rotated to cover monolayer with trypsin and moved to the incubator and left for 2–4 min. The cells were examined using an inverted microscope to ensure that all the cells were detached and floated. The cells were resuspended in a small volume of fresh serum containing HEK-293 medium and 100–200 µl was removed to perform cell count. The required number of cells were transferred to a new labeled flask containing prewarmed HEK-293 medium and incubated as appropriate for the cell line (Rathi *et al*., 2009; Freshney, 2005). All the cytotoxicity experiments were carried out in 96-well plates. Methotrexate and 5-flourouracil was used as a reference standard for cytotoxic activity. All solutions of test compounds were prepared using DMSO. IC_{50} values were calculated, it is a drug concentration causing a 50 % inhibition of cell proliferation (Prakash *et al*., 2011).

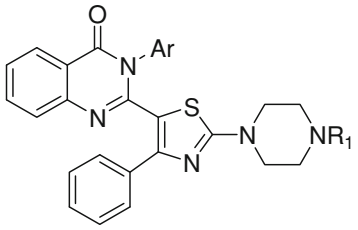
Results and discussion

The target molecules were designed by joining two different moieties i.e., quinazolinones and thiazoles. These novel quinazolinone-thiazole derivatives were synthesized with different aryl substitutions at the third position of quinazolinone ring and different substituted piperazines at 2nd position of thiazole ring. Synthesis of target molecule was carried out as per Scheme 1. Different piperazines were reacted with benzoyl isothiocyanate to give adducts (**1a–1c**) that were further subjected to reaction with different quinazolinones (**2a–2f**) to provide target molecules (**3a–3o**). The structure of all synthesized compound were confirmed by physical characterization i.e., melting point, R_f value, elemental analysis, and spectral characterization i.e., IR, MASS, and NMR spectroscopy.

In the target molecules, optimization was done at the third position of quinazolinone and the second position of thiazole ring. The third position of quinazolinone was substituted with different aryl groups (electron withdrawing, electron releasing group with and without NH as linker) and the second position of thiazole was substituted with different piperazines (H, CH_3, and COC_6H_5). All the synthesized compounds were subjected to in vitro cytotoxicity activity on MCF-7 (breast cancer), NCI (lung cancer), and HEK-293 (normal cell of epidermal kidney) cell lines by MTT assay for anticancer therapy. IC_{50} values were calculated for test and standard compounds.

Statistical significance of the data (expressed as mean ± SEM) was demonstrated by performing one-way ANOVA test followed by Dennett comparison of IC_{50} of the entire compounds against methotrexate and

Table1 In vitro cytotoxicity screening data of synthesized quinazolinone derivatives compared against the standard drugs



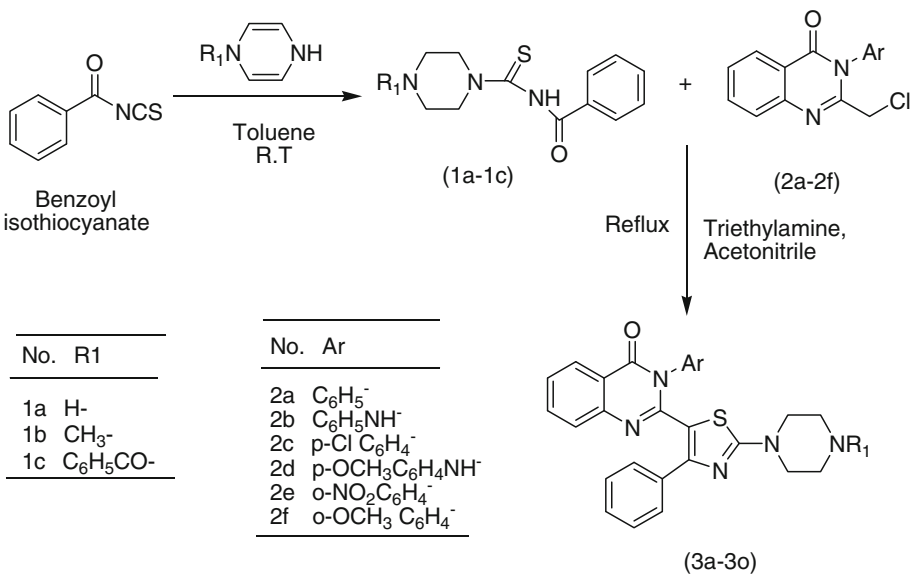
Compound No.	Ar	R_1	Mean $IC_{50}(\mu M) \pm SEM^*$		
			NCI	MCF-7	HEK-293
3a	C_6H_5	CH_3	$9.89 \pm 0.21^{aaa,bbb}$	$5.84 \pm 0.12^{aaa,bbb}$	$0.86 \pm 0.04^{NS,bbb}$
3b	C_6H_5	C_6H_5CO	$13.40 \pm 0.14^{aaa,bbb}$	$19.92 \pm 0.06^{aaa,bbb}$	$0.42 \pm 0.02^{aaa,bbb}$
3c	C_6H_5	H	$2.66 \pm 0.12^{NS,NS}$	$46.72 \pm 0.24^{aaa,bbb}$	$0.74 \pm 0.03^{aa,bbb}$
3d	C_6H_5 NH	CH_3	$1.11 \pm 0.03^{aa,bb}$	$0.16 \pm 0.16^{aaa,NS}$	$3.03 \pm 0.09^{aaa,bbb}$
3e	C_6H_5 NH	C_6H_5CO	$5.59 \pm 0.10^{aaa,bbb}$	$2.49 \pm 0.11^{NS,NS}$	$0.80 \pm 0.03^{NS,bbb}$
3f	p-$OCH_3C_6H_4NH$	H	$2.92 \pm 0.15^{NS,NS}$	$11.40 \pm 0.19^{aaa,bbb}$	$1.11 \pm 0.04^{NS,bbb}$
3g	p-Cl C_6H_4	C_6H_5CO	$15.57 \pm 0.16^{aaa,bbb}$	$>100^{aaa,bbb}$	$0.69 \pm 0.03^{aa,bbb}$
3h	p-Cl C_6H_4	CH_3	$>100^{aaa,bbb}$	$1.97 \pm 0.13^{NS,NS}$	$0.94 \pm 0.06^{NS,bbb}$
3i	p-Cl C_6H_4	H	$6.10 \pm 0.09^{aaa,bbb}$	$>100^{aaa,bbb}$	$14.36 \pm 0.73^{aaa,bbb}$
3j	o-$OCH_3C_6H_4$	CH_3	$9.43 \pm 0.21^{aaa,bbb}$	$1.50 \pm 0.09^{NS,NS}$	$0.67 \pm 0.03^{aa,bbb}$
3k	o-$OCH_3C_6H_4$	H	$4.33 \pm 0.98^{aaa,bbb}$	$3.70 \pm 0.08^{a,bbb}$	$0.55 \pm 0.04^{aaa,bbb}$
3l	o-$OCH_3C_6H_4$	C_6H_5CO	$5.93 \pm 0.29^{NS,b}$	$0.66 \pm 0.02^{NS,NS}$	$2.29 \pm 0.10^{aaa,NS}$
3m	o-$NO_2C_6H_5$	CH_3	$6.72 \pm 0.05^{aaa,bbb}$	$8.90 \pm 0.53^{aaa,bbb}$	$0.81 \pm 0.05^{NS,bbb}$
3n	o-$NO_2C_6H_5$	H	$8.39 \pm 0.07^{aaa,bbb}$	$5.86 \pm 0.31^{aaa,bbb}$	$1.07 \pm 0.04^{NS,bbb}$
3o	o-$NO_2C_6H_5$	C_6H_5CO	$11.03 \pm 0.16^{aaa,bbb}$	$6.49 \pm 0.27^{aaa,bbb}$	$0.73 \pm 0.02^{aa,bbb}$
Methotrexate	–	–	2.20 ± 0.18	2.26 ± 0.11	1.27 ± 0.06
5-Florouracil	–	–	2.30 ± 0.49	1.25 ± 0.07	2.15 ± 0.13

NS Not Significant

* $N = 3$; One-way ANOVA was performed using Dennett Test ^{aaa} <0.0001; ^{aa} <0.001

^{a} <0.05 (Methotraxate); ^{bbb} <0.0001; ^{bb} <0.001; ^{b} <0.05 (5-florouracil)

Scheme 1 Schematic representation for the synthesis of quinazolinone derivatives



5-flourouracil using Graph pad Prism (Version 5.0) software. Results of the biological screening and statistical analysis are shown in Table 1.

Biological screening data suggested that compounds **3c** ($IC_{50} = 2.66 \pm 0.12$ μM), **3d** ($IC_{50} = 1.11 \pm 0.03$ μM), and **3e** ($IC_{50} = 2.92 \pm 0.10$ μM) show high activity on NCI (lung cancer) cell line. Compound **3d** ($p < 0.001$, for both the standard drugs) was found to be more potent than other compounds and compared to standard drug methotrexate ($IC_{50} = 2.20 \pm 0.18$ μM) and 5-florouracil ($IC_{50} = 2.30 \pm 0.49$ μM) on this cell line activity. Compounds having NH as linker between the third position of quinazolinone and phenyl group gave high activity. Screening on MCF-7 (breast caner cell line), compound **3d** ($IC_{50} = 0.16 \pm 0.16$ μM), **3l** ($IC_{50} = 0.66 \pm 0.02$ μM), and **3j** ($IC_{50} = 1.50 \pm 0.09$ μM) showed high activity. The comparison of compounds (**3d, 3l**, and **3j**) also showed comparable activity than methotrexate ($IC_{50} = 2.26 \pm 0.11$ μM) and 5-florouracil ($IC_{50} = 1.25 \pm 0.07$ μM). The statistical analysis of compound **3l** with the standard drug suggested that the compound is nearly equally active as both the standard drugs for MCF-7 cell lines and inactive for NCI cell lines. Compound **3d** substituted with methyl piperazine on thiazole and phenyl amine on quinazolinone ring is attributed to the highest activity on MCF-7 cancer cell line. Assay on normal cell line HEK-293 suggested that compound, **3d** ($IC_{50} = 3.03 \pm 0.09$ μM), **3l** ($IC_{50} = 2.29 \pm 0.10$), and **3i** ($IC_{50} = 14.36 \pm 0.73$ μM) kill less to normal cells compared to methotrexate ($IC_{50} = 1.27 \pm 0.06$ μM) and 5-florouracil ($IC_{50} = 2.15 \pm 0.13$ μM). From the above results from the entire cell lines, one can conclude that compound **3d** is more potent among all the synthesized compounds and also as compared to the standard drug. Compounds **3g**, **3h**, and **3i** are almost inactive among all the synthesized compounds and more toxic to normal human cells also.

Structure activity relationship suggested that compound with NH linker on the third position of quinazolinone moiety was more active throughout the series. On the other hand, compound with *p*-Cl was found to be the least active in the series. It is also suggested that electron withdrawing group on phenyl ring at the third position of quinazolinone ring may be responsible for less activity and also more toxicity to normal human cell line. Compound having electron donating group on phenyl ring at the third position of quinazolinone ring i.e., **3j**, **3k**, and **3l** have moderate activity. Compounds **3a**, **3b**, and **3c** which has only unsubstituted phenyl ring at the third position of quinazolinone ring also gave moderate activity but their activity is less than compound with electron donating group. The substitution on piperazine of thiazole ring did not make much difference on the activity of the whole series. Even unsubstituted piperazines were more active than substituted piperazines on the compounds having thiazole ring.

Finally, the substitution C_6H_5NH at the third position of quinazolinone ring, methyl substituted piperazines at the second position of thiazole ring, and phenyl group at the fourth position of thiazole ring as in compound **3d** can be considered as a four-point pharmacophore for designing better anticancer agents.

Conclusion

A series of quinazolinone derivatives was synthesized and screened for their in vitro cytotoxic activity. Results of assay indicated that all the compounds were found to have good to moderate activity. Compound **3d** possesses higher activity than the standard drug (methotrexate and 5-flourouracil). The compound **3d** was particularly promising, since it was able to kill cancer cells more effectively than the non-cancerous cell which was observed from the result of HEK-293 cell line. Furthermore, it concluded that compound with NH linker between aryl moiety and the third position of quinazolinone ring has been recognized as potent anticancer agent. Therefore, this type of compound may further be optimized and evaluated with enzymatic assay and in vivo animal models in the line of the development and also can serve as a prototype molecule of new class of anticancer agents.

Experimental protocol

Chemistry

Melting points of all synthesized compounds were determined in open capillaries using Veego melting point apparatus, Model VMP-D (Veego India ltd., Mumbai, India) and were uncorrected. Infrared spectra were recorded using KBr pellets on SHIMADZU-FT-IR 8400S instrument. Mass spectra were recorded on PerkinElmer LC–MS PE Sciex API/65 Spectrophotometer. The ^1H NMR spectra were recorded on Brucker Avance-300 (300 MHz) model spectrophotometer in $CDCl_3$ using DMSO as solvent and TMSi as internal standard with ^1H resonant frequency of 300 MHz. The TLC was performed on precoated alumina silica gel 60 F_{254} (Merck). The mobile phase was benzene:methanol (9:1) and detection was made using UV light. The resulting compounds were purified by recrystallization using suitable solvent. The elemental analyses were done on elementar Vario EL 3 Carlo erba 1108 and were well in accordance with the structures assigned to the compound. The entire compound gave C, H, and N analysis within ±0.4 % of the theoretical

values. Synthetic grade chemicals procured from SD fine chemicals, Baroda, India were used for the synthesis of the target compounds. All the compounds of step I and 2 (**1a**–**1c** and **2a**–**2e**) were prepared according to the literature procedures with some minor modifications (Hui *et al.*, 2009; Rajan *et al.*, 2010a, b). General synthetic procedures used for the preparation of the target compound are as follows:

Synthesis of *N*-benzoyl 4-substituted piperazine-1-carbothiomide (**1a–1c**)

Equimolar quantity of benzoyl isothiocyanate was added from the dropping funnel to *N*-substituted piperazines in toluene. Stirring was continued for 2 h at room temperature. Crude solid product of *N*-benzoyl substituted piperazine-1-carbothioamide (**1a–1c**) was precipitated out. The collected precipitate was repeatedly washed with small portions of toluene and recrystallized with ethyl acetate to yield product.

Synthesis of 2-chloro methyl quinazolinone derivatives (**2a–2f**)

Addition of 2-(2-chloroacetamido)-benzoic acid to the equal mole of different substituted aromatic amines in acetonitrile in the presence of phosphorus oxychloride was carried out at room temperature and reaction was allowed to reflux. Completion of reaction was monitored with TLC. Then, sodium bicarbonate was added to neutralize reaction mixture. Aqueous layer was extracted with chloroform. The chloroform was evaporated under reduced pressure and product (**2a–2f**) was collected. Crude compound was recrystallized using toluene.

Synthesis of 2-(2-(4-substituted piperazine-1-yl)-5-phenylthiazol-4-yl)-3-aryl quinazolinone (**3a–3o**)

Equimolar quantity of *N*-benzoyl substituted piperazine-1-carbothioamide (**1a–1c**) and 2-chloromethyl quinazolinone derivatives (**2a–2e**) in acetonitrile were reacted in the presence of triethylamine. The reaction mixture was allowed to reflux and completion of reaction was checked by TLC. Precipitate was obtained after pouring the reaction mixture to crushed ice and finally compounds were recrystallized using methanol.

*2-(2-(4-Methylpiperazin-1-yl)-5-phenylthiazol-4-yl)-3-phenylquinazolin-4(3H)-one (**3a**)*

Light brown product; R_f value 0.60, Yield 66 %; M.p. 140–142 °C; Elem. analysis Calcd. for $C_{28}H_{25}N_5OS$: C, 70.12; H, 5.25; N, 14.60 %. Found: C, 70.08; H, 5.27; N, 14.57. IR (KBr, υ_{max}, cm^{-1}): 1,680 (C=O), 1,579 (C–N Stretch), 3,149 (C–H Stretch); ^{1}H NMR (300 MHz, δ ppm, DMSO): 7.92–6.88 (m, 14H, Ar–H), 4.34 (s, 3H, CH_3), 3.30–2.49 (m, 8H, CH–piperazine) ppm; and MS: m/z 480.6 (M + 1).

*2-(2-(4-Benzoylpiperazin-1-yl)-5-phenylthiazol-4-yl)-3-phenylquinazolin-4(3H)-one (**3b**)*

Brown product; R_f value 0.54, Yield 64 %; M.p. 105–109 °C; Elem. analysis Calcd. for $C_{34}H_{27}N_5O_2S$: C, 71.68; H, 4.78; N, 12.29 %. Found: C, 71.63; H, 4.74; N, 12.25; IR (KBr, υ_{max}, cm^{-1}): 1,643 (C=O), 1,571 (C–N Stretch), 3,117 (C–H Stretch), 1,582 (NH); ^{1}H NMR (300 MHz, δ ppm, DMSO): 7.95–6.97 (m, 19H, Ar–H), 3.62–3.45 (m, 8H, CH–piperazine) ppm; and MS: m/z 579.9 (M + 1).

*3-Phenyl-2-(5-phenyl-2-(piperazin-1-yl)thiazol-4-yl)quinazolin-4(3H)-one (**3c**)*

Brown product; R_f value 0.50, Yield 72 %; M.p. 144–146 °C; Elem. analysis Calcd. for $C_{27}H_{23}N_5OS$: C, 69.65; H, 4.98; N, 15.04 %. Found: C, 69.62; H, 4.99; N, 14.99; IR (KBr, υ_{max}, cm^{-1}): 1,645 (C=O), 1,556 (C–N Stretch), 3,086 (C–H Stretch); ^{1}H NMR (300 MHz, δ ppm, DMSO): 7.73–6.90 (m, 14H, Ar–H), 3.24–3.16 (m, 8H, CH–piperazine), 2.17 (s, 1H, NH) ppm; and MS: m/z 466.8 (M + 1).

*3-(4-Methoxy phenylamino)-2-(5-phenyl-2-(piperazin-1-yl)-thiazol-4-yl)quinazolinone (**3d**)*

Dark brown product; R_f value 0.56, Yield 64 %; M.p. 105–109 °C; Elem. analysis Calcd. for $C_{28}H_{26}N_6OS$: C, 67.99; H, 5.30; N, 16.99 %. Found: C, 67.80; H, 5.27; N, 16.97; IR (KBr, υ_{max}, cm^{-1}): 1,662 (C=O), 1,520 (C–N Stretch), 3,187 (C–H Stretch), 1,590 (NH); ^{1}H NMR (300 MHz, δ ppm, DMSO):, 7.55–6.89 (m, 14H, Ar–H), 4.19 (s, 1H, PhNH), 3.36–3.11 (m, 8H, CH–piperazine), 2.38 (s, 3H, CH_3) ppm; and MS: m/z 495.2 (M + 1).

*2-(2-(4-Benzoylpiperazin-1-yl)-5-phenylthiazol-4-yl)-3-(phenylamino)quinazolin-4(3H)-one (**3e**)*

Yellowish green product; R_f value 0.52, Yield 66 %; M.p. 130–132 °C; Elem. analysis Calcd. for $C_{34}H_{28}N_6O_2S$: C, 69.84; H, 4.83; N, 14.37 %. Found: C, 69.80; H, 4.80; N, 14.36, IR (KBr, υ_{max}, cm^{-1}): 1,680 (C=O), 1,527 (C–N Stretch), 3,110 (C–H Stretch), 1,418 (N=O); ^{1}H NMR (300 MHz, δ ppm, DMSO): 7.96–6.31 (m, 19H, Ar–H), 4.09 (s, 1H, PhNH), 3.46–2.86 (m, 8H, CH–piperazine) ppm; and MS: m/z 585.73 (M + 1).

*3-(4-Methoxyphenylamino)-2-(5-phenyl-2-(piperazin-1-yl)thiazol-4-yl)quinazolin-4(3H)-one (**3f**)*

Green product; R_f value 0.60, Yield 70 %; M.p. 129–133 °C; Elem. analysis Calcd. for $C_{28}H_{28}N_6O_2S$: C, 65.86; H, 5.13; N, 16.46 %. Found: C, 65.84; H, 5.10; N, 16.49, IR (KBr, υ_{max}, cm^{-1}): 1,749 (C=O), 1,556 (C–N Stretch), 3,023 (C–H Stretch); ^{1}H NMR (300 MHz, δ ppm, DMSO): 8.13–6.9 (m, 18H, Ar–H), 4.07 (s, 1H, PhNH), 3.79 (s, 3H, OCH_3), 3.19–2.77 (m, 8H, CH–piperazine) 2.19 (s, 1H, NH) ppm; and MS: m/z 510.61 (M)

*2-(2-(4-Benzoylpiperazin-1-yl)-5-phenylthiazol-4-yl)-3-(4-chlorophenyl)quinazolin-4(3H)-one (**3g**)*

Yellowish green product; R_f value 0.60, Yield 72 %; M.p. 130–132 °C; Elem. analysis Calcd. for $C_{34}H_{26}ClN_5O_2S$: C, 67.60; H, 4.34; N, 11.59 %. Found: C, 67.55; H, 4.34; N, 11.61, IR (KBr, υ_{max}, cm^{-1}): 1,713 (C=O), 1,570 (C–N Stretch), 3,043 (C–H Stretch), 1,566 (NH); ^{1}H NMR (300 MHz, δ ppm, DMSO): 8.33–6.62 (m, 18H, Ar–H), 2.72–2.07 (m, 8H, piperazine) ppm; and MS: m/z 603.97 (M + 1).

*3-(4-Chlorophenyl)-2-(2-(4-methylpiperazin-1-yl)-5-phenylthiazol-4-yl)quinazolin-4(3H)-one (**3h**)*

Green product; R_f value 0.52, Yield 55 %; M.p. 190–192 °C; Elem. analysis Calcd. for $C_{28}H_{24}ClN_5OS$: C, 65.42; H, 4.71; N, 13.62 %. Found: C, 65.41; H, 4.69; N, 13.63, IR (KBr, υ_{max}, cm^{-1}): 1,664 (C=O), 1,511 (C–N Stretch), 3,080 (C–H Stretch), 795 (C–Cl); ^{1}H NMR (300 MHz, δ ppm, DMSO): 7.34–6.53 (m, 13H, Ar–H), 3.84 (s, 3H, CH_3), 3.76–2.84 (m, 8H, CH–piperazine) ppm; and MS: m/z 513.9 (M)

*3-(4-Chlorophenyl)-2-(5-phenyl-2-(piperazin-1-yl) thiazol-4-yl)quinazolin-4(3H)-one (**3i**)*

Green product; R_f value 0.56, Yield 64 %; M.p. 122–126 °C; Elem. analysis Calcd. for $C_{27}H_{22}ClN_5OS$: C, 64.86; H, 4.43; N, 14.01 %. Found: C, 64.83; H, 4.41; N, 13.98, IR (KBr, υ_{max}, cm^{-1}): 1,692 (C=O), 1,542 (C–N Stretch), 3,072 (C–H Stretch), 814 (C–Cl), 1,577 (NH); ^{1}H NMR (300 MHz, δ ppm, DMSO): 7.41–6.46 (m, 13H, Ar–H), 3.84–2.71 (m, 8H, piperazine), 2.22 (s, 1H, NH) ppm; and MS: m/z 500.1 (M)

*3-(2-Methoxyphenyl)-2-(2-(4-methylpiperazin-1-yl)-5-phenylthiazol-4-yl)quinazolin-4(3H)-one (**3j**)*

Yellow product; R_f value 0.59, Yield 66 %; M.p. 125–127 °C; Elem. analysis Calcd. for $C_{29}H_{27}N_5O_2S$: C, 68.35; H, 5.35; N, 13.74 %. Found: C, 68.36; H, 5.32; N, 13.72, IR (KBr, υ_{max}, cm^{-1}): 1,723 (C=O), 1,577 (C–N Stretch), 3,022 (C–H Stretch); ^{1}H NMR (300 MHz, δ ppm, DMSO): 7.86–6.84 (m, 13H, Ar–H), 3.79 (s, OCH_3), 3.14–2.88 (m, 8H, CH–piperazine), 2.62 (s, CH_3) ppm; and MS: m/z 510.2 (M + 1).

*3-(2-Methoxyphenyl)-2-(5-phenyl-2-(piperazin-1-yl)thiazol-4-yl)quinazolin-4(3H)-one (**3k**)*

Brown product; R_f value 0.63, Yield 59 %; M.p. 101–103 °C; Elem. analysis Calcd. for $C_{28}H_{25}N_5O_2S$: C, 67.86; H, 5.08; N, 11.68 %. Found: C, 67.81; H, 5.10; N, 14.10, IR (KBr, υ_{max}, cm^{-1}): 1,666 (C=O), 1,543 (C–N Stretch), 3,126 (C–H Stretch), 1,570 (NH); ^{1}H NMR (300 MHz, δ ppm, DMSO 8.10–6.74 (m, 13H, Ar–H), 2.61–2.19 (m, 8H, CH–piperazine) 2.26 (s, 1H, NH) ppm; and MS: m/z 496.3 (M + 1).

*2-(2-(4-Benzoylpiperazin-1-yl)-5-phenylthiazol-4-yl)-3-(2-methoxyphenyl)quinazolin-4(3H)-one (**3l**)*

Brown product; R_f value 0.55, Yield 62 %; M.p. 125–128 °C; Elem. analysis Calcd. for $C_{35}H_{29}N_5O_3S$: C, 70.10; H, 4.87; N, 11.68 %. Found: C, 70.07; H, 4.83; N, 11.67, IR (KBr, υ_{max}, cm^{-1}): 1,670 (C=O), 1,556 (C–N Stretch), 3,090 (C–H Stretch), 810 (C–Cl); ^{1}H NMR (300 MHz, δ ppm, DMSO): 8.35–7.36 (m, 18H, Ar–H), 3.68–2.39 (m, 8H, CH–piperazine) ppm; and MS: m/z 601.2 (M + 1).

*2-(2-(4-Methylpiperazin-1-yl)-5-phenylthiazol-4-yl)-3-(2-nitrophenyl)quinazolin-4(3H)-one (**3m**)*

Yellowish green product; R_f value 0.58, Yield 62 %; M.p. 175–177 °C; Elem. analysis Calcd. for $C_{28}H_{24}N_6O_3S$: C, 64.11; H, 4.61; N, 16.02 %. Found: C, 64.09; H, 4.58; N, 15.98, IR (KBr, υ_{max}, cm^{-1}): 1,716 (C=O), 1,507 (C–N Stretch), 2,978 (C–H Stretch), 1,580 (NH); ^{1}H NMR (300 MHz, δ ppm, DMSO): 8.16 (d, H, NO_2Ar–H), 7.95–7.33 (m, 12H, Ar–H), 3.36–2.61 (m, 8H, piperazine), 2.31 (s, 3H, CH_3) and MS: m/z 524.29 (M).

*3-(2-Nitrophenyl)-2-(5-phenyl-2-(piperazin-1-yl)thiazol-4-yl)quinazolin-4(3H)-one (**3n**)*

Pale yellow product; R_f value 0.54, Yield 64 %; M.p. 180–182 °C; Elem. analysis Calcd. for $C_{27}H_{22}N_6O_3S$: C, 63.52; H, 4.34; N, 16.46 %. Found: C, 63.81; H, 4.51; N, 16.18, IR (KBr, υ_{max}, cm^{-1}): 1,726 (C=O), 1,510

(C–N Stretch), 2,928 (C–H Stretch), 1,575 (NH); ^{1}H NMR (300 MHz, δ ppm, DMSO): 8.21 (d, H, NO_2Ar–H), 7.98–7.24 (m, 12H, Ar–H), 3.44–2.32 (m, 8H, CH–piperazine) 2.15 (s, 1H, NH) ppm; and MS: m/z 511.71 (M + 1).

*2-(2-(4-Benzoylpiperazin-1-yl)-5-phenylthiazol-4-yl)-3-(2-nitrophenyl)quinazolin-4(3H)-one (**3o**)*

Yellowish product; R_f value 0.51, Yield 67 %; M.p. 175–178 °C; Elem. analysis Calcd. for $C_{34}H_{26}N_6O_4S$: C, 66.44; H, 4.26; N, 13.67 %. Found: C, 66.47; H, 4.31; N, 13.61, IR (KBr, υ_{max}, cm^{-1}): 1,723 (C=O), 1,570 (C–N Stretch), 3,013 (C–H Stretch), 1,546 (NH); ^{1}H NMR (300 MHz, δ ppm, DMSO): 8.21 (d, H, NO_2Ar–H), 7.92–7.31 (m, 17H, Ar–H), 3.36–3.44 (m, 8H, piperazine) ppm; and MS: m/z 615.57 (M + 1).

Cytotoxic assay

The cells were preincubated at a concentration of 1×10^6 cells/ml in culture medium for 3 h at 37 °C and 6.5 % CO_2. Then, the cells were seeded at a concentration of 5×10^4 cells/well in 100 µl culture medium and at various concentrations (0.005–100 µM/ml) of standard methotrexate and synthesized compounds [dissolved in 2 % DMSO (dimethylsulfoxide) solution] into microplates (tissue culture grade, 96 wells, flat bottom) and incubated for 24 h at 37 °C and 6.5 % CO_2. The cell proliferation is based on the ability of the mitochondrial succinate-terazolium reductase system to convert 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a blue-colored formazan. The test denotes the survival cells after toxic exposure. Then, 10 µl MTT labeling mixture was added and incubated for 4 h at 37 °C and 6.5 % CO_2. Each experiment was done in triplicate. Then, 100 µl of solubilization solution was added into each well and incubated overnight. The spectrophotometric absorbance of the samples was measured using a microplate (ELISA) reader. The wavelength to measure absorbance of the formazan product is between 550 and 600 nm according to the filters available for the ELISA reader was used. The reference wavelength should be more than 650 nm. IC_{50} was then calculated as the drug concentration causing a 50 % inhibition of cell proliferation. HEK 293 cell line was used to find out cytotoxic effects of synthesized compound on non-cancerous cells (Prakash *et al.*, 2011; Rathi *et al.*, 2009).

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