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Original article

Synthesis and evaluation of quinazolinone derivatives as inhibitors of NF-κB, AP-1 mediated transcription and eIF-4E mediated translational activation: Inhibitors of multi-pathways involve in cancer

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ABSTRACT

In our effort to discover and develop small molecule multi-pathway inhibitors which may be useful as tools for treating cancerous conditions, we have synthesized a small library of 2-thiazole-5-yl-3*H*-quinazolin-4-one derivatives. Synthesized compounds were evaluated as inhibitors of NF-κB and AP-1 mediated transcriptional and eIF-4E mediated translational activation as these transcription and translation factors are known to play a pivotal role in initiation and progression of cancer. The results from the study suggest the utility of the 2-thiazole-5-yl-3*H*-quinazolin-4-one scaffold as a promising scaffold for the design of novel multi-pathway inhibitors, which can be explored as anti-cancer agents.

1. Introduction

Cancer is a complex disease that is the result of perturbations in multiple intracellular regulatory systems [1] leading to an overall increase in cell number and thus tumor formation. As cancer is a multifactor process involving the expression of a large number of proteins, a single molecule that can simultaneously modulate more than one pathway or target involved in cancer would be a significant advantage over the current combination therapy strategies. NF-κB and AP-1 are two important transcription factors for many cell types and are thought to be promising targets for the development of anti-cancer therapeutics. It has been well documented that the NF-κB and AP-1 pathways play an important role in promoting metastases [2–4], tumor progression [5], angiogenesis [6] and chemoresistance [7]. A variety of genes involved in cancer (IL-6, IL-8, MMP-9, COX-2, and MCP-1) are regulated by the combined action of NF-κB and AP-1. Furthermore, activated AP-1 and NF-κB are found in transformed keratinocytes, pancreatic cancers, and head and neck squamous cell carcinoma cell lines [8–10]. Similarly, the translation initiation factors, eIF-4E, eIF-4F, and eIF-4G, as well as mTOR have been shown to play a significant role in tumor progression [11–13]. The level of eIF-4E is found to be elevated in a majority of human breast, head and neck cancers. The over expression of eIF-4E is also correlated with elevated angiogenic growth factors and induced by hypoxia [14–16]. These studies provide a clear physiological role for these transcription (NF-κB and AP-1) and translation (eIF-4E) factors in cancer. These studies also suggest that the modulation of these transcription and translation factors represents a sound strategy for the treatment of cancer. Previously, we had developed an in vitro screening assay for the analysis of changes in transcriptional (AP-1 and NF-κB) and translational (eIF-4E) activity [17,18], which was used in the present study for screening of the newly synthesized molecules.

Quinazoline scaffolds and their bioisosteres are well known as a privileged scaffold in drug discovery. Quinazolinones and their analogs have shown to have utility in a number of therapeutic

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areas, including allergies [19,20], Parkinson's and Alzheimer's diseases, epilepsy and pain [21,22], cytotoxic agents [23], inflammation [24], malaria [25], tuberculosis [26], and cancer [27]. In our effort to discover and develop inhibitors of NF-κB and AP-1 mediated transcriptional activation as potential anti-inflammatory and anti-cancer agents, we have reported a series of 2-(2-alkylamino,4-substituted-thiazole-5-yl)-3-aryl-3*H*-quinazolin-4-one derivatives (Fig. 1) [28]. The results of the previous study encouraged us to synthesize additional analogues of 2-thiazole-5-yl-3*H*-quinazoline-4-one derivatives with more diverse substitutions and to analyze their activities against the transcription and translation factors (eIF-4E, NF-κB and AP-1) described above to identify multi-pathway inhibitors that may be useful in treating cancer. In the previous study, only alkyl amino and aryl amino substitutions were studied at the R_1 position, therefore carboxyethyl amino and benzoyl amino groups at this position were included in this study to expand the scope of the SAR. Similarly, a methyl substitution on the R_2 position was explored in the previous study, whereas in the current study phenyl substitutions at the R_2 position were explored. Para position in the phenyl ring at the 3rd position of quinazolinone was found to be the key for the activity of this scaffold [28] therefore, in present study effects of an electron withdrawing group (−Cl) and an electron donating group ($-OCH_3$) were investigated at the R_3 position in combination with the substitutions at the R_1 and R_2 positions. To get some insights into the potential mechanism of action of the compounds molecular docking study was performed and results of the same is also discussed herein with the results of in vitro experiments.

2. Chemistry

The synthesis of designed compounds is shown in Scheme 1. The thiourea derivatives **3** and 2-chloromethyl-3-aryl-3*H*-quinazolin-4-one derivatives **2** were prepared according to a previously reported procedure [28]. An appropriately substituted thiourea derivative **3** was S-alkylated by active methylene group of 2-chloromethyl-3-aryl-3*H*-quinazolin-4-one derivatives **2** to give intermediate **4** which in the same reaction follows the intramolecular cyclization followed by aromatic ring formation to give the desired 2-thiazole-5-yl-3*H*-quinazolin-4-one derivatives **5** (Scheme 1). The elaborated discussion of reaction mechanism for the synthesis of these compounds is previously discussed [28]. Table 1 shows the list of compounds that were prepared with modifications at R_1, R_2 and R_3.

3. Biology

Development of the screens for NF-κB, AP-1 and eIF-4E was previously discussed in detail [18]. In brief for the NF-κB and AP-1 screens, two HEK293 cell lines were purchased from Panomics, one was stably transfected with a plasmid in which luciferase expression is regulated by six copies of the NF-κB transcriptional element (5′AGTTGAGGGGACTTTCCCAGGC-3′) and the other in which luciferase expression is regulated by three copies of the AP-1 transcriptional element (TGACTAA). Activity of the compounds were

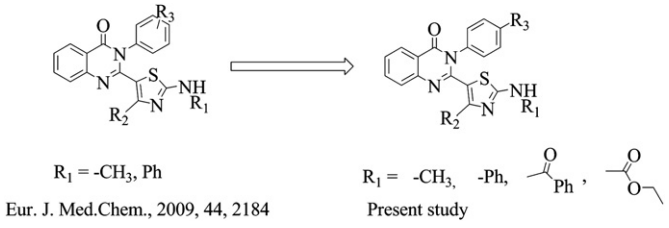
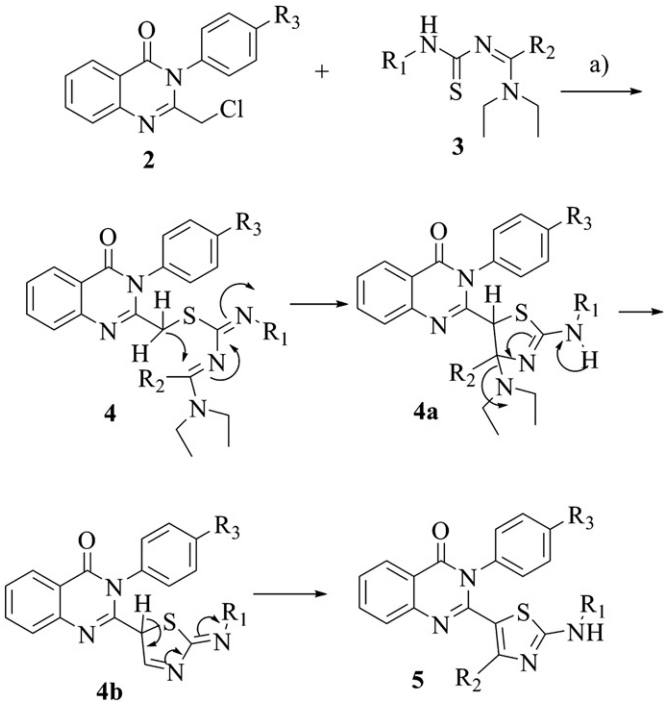
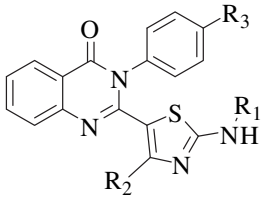


Fig. 1. General structure of compounds.



Scheme 1. Probable mechanism and the condition for the synthesis of desired compounds. (a) Acetonitrile, 75–80 °C, 4–5 h [28].

Table 1
Evaluation of synthesized compounds for their inhibitory activity towards NF-κB, AP-1 and eIF-4e mediated transcriptional activation in vitro.



Entry	R_1	R_2	R_3	% Inhibition in vitro at 1 μM		
				NF-κB	AP-1	eIF–4E
5a	$-COOC_2H_5$	−Ph	−H	32	7	−63
5b	$-CH_3$	−Ph	−H	71	58	33
5c	−Ph	−Ph	−H	71	51	37
5d	−COPh	−Ph	−H	43	26	30
5e	4-Cl Phenyl	−Ph	−H	71	57	−66
5f	$-COOC_2H_5$	−Ph	$-OCH_3$	29	34	15
5g	−Ph	−Ph	$-OCH_3$	63	19	16
5h	−COPh	−Ph	$-OCH_3$	12	8	31
5i	4-Cl Phenyl	−Ph	$-OCH_3$	46	59	32
5j	$-COOC_2H_5$	$-CH_3$	−Cl	54	30	50
5k	$-CH_3$	$-CH_3$	−Cl	80	83	61
5l	−Ph	$-CH_3$	−Cl	66	29	67
5m	4-Cl Phenyl	$-CH_3$	−Cl	79	75	26
5n	$-COOC_2H_5$	−Ph	−Cl	24	20	18
5o	$-CH_3$	−Ph	−Cl	72	67	40
5p	−Ph	−Ph	−Cl	67	39	38
5q	−COPh	−Ph	−Cl	73	36	39
5r	4-Cl Phenyl	−Ph	−Cl	62	47	34

All the samples were analyzed in triplicate in each of two or three individual experiments and data presented here is the average of two to three individual experiments. Rapamycin a well-established inhibitor of eIF-4E was found to inhibit ~50% luciferase activity at 10 nM. A pyrimidine carboxymide compound that had previously been shown [29] to be an inhibitor of NF-κB and AP-1 mediated transcriptional activation, reduced luciferase activity by ~40% in the NF-κB and AP-1 cell lines @ 1 μM.

measured both following induction of transcription by a transcriptional stimulator and without induction. To test the biological activity following induction, the promoters the HEK/NF-κB cell line were stimulated with TNF-α (20 ng/ml) for 24 h and the HEK/AP-1 cell line with PMA (10 ng/ml) for 24 h. Both cell lines produced the desired response with NF-κB driven luciferase expression increasing by 100-fold and AP-1 driven expression by 30-fold following the appropriate stimulation. Screening for eIF-4E was carried out by cloning the highly structured 5′ UTR of FGF (which requires high levels of eIF-4E before protein synthesis can initiate) into the pMS110 plasmid (pGL3-control plasmid from Promega containing a neo selectable marker) and both the UTR and parental plasmids were stably transfected into a cell line that has high levels of eIF-4E (FaDu cell line), then analyzing luciferase activity in the presence and absence of compound [18].

4. Results and discussion

All the compounds synthesized were analyzed for activity in the three screens discussed above and the results are shown in Table 1. A number of the compounds inhibited luciferase expression under the control of the FGF 5′ UTR, while a few compounds actually increased luciferase expression. These former molecules may represent general inhibitors of translation, may reduce luciferase activity by reducing cell number through inhibition of growth or cell toxicity, or may actually act specifically to inhibit highly cap-dependent translation. All the compounds were then tested for their ability to modulate NF-κB and AP-1 activity. A number of these compounds exhibited mild to good inhibition in these assays (Table 1). Seven representative compounds (**5a**, **5i**, **5j**, **5k**, **5m**, **5o** and **5r**) were selected and retested in a six-point dose response beginning at 10 nM and increasing in ½ log units. Screening was carried out as described above monitoring the compound effects on basal NF-κB, AP-1 and FGF 5′ UTR dependent luciferase expression. Six of these compounds produced a very good dose dependent inhibition of NF-κB and AP-1 mediated transcriptional activity (Table 2). Conversely, none of the compounds tested for the eIF-4E elicited a good dose response inspite of the fact that during the primary screening for eIF-4E, a few of these compounds did demonstrate a good inhibition at 1 uM concentration. To analyze general effects of compounds on cell viability, the seven compounds were tested for their effects on cell number. An MTT assay was used to analyze this effect on FaDu cells since it would measure both inhibition of cell growth and cell toxicity. The compounds were tested in six point dose-response starting at 10 nM and increasing to 3.3 μM. Of these, only compound **5k** was found to be inhibiting cell growth at 3.3 μM by more than 20%.

The above screening for transcriptional inhibition of luciferase expression was carried out in non-induced cell lines to determine the effects of compound on basal gene expression. However, it is also possible that in cancer these transcriptional factors are induced, similar to their activity in inflammation. To analyze the effects of the compounds on NF-κB induction of transcriptional activity, the stably transfected NF-κB cell line was plated in a 96-well plate then 24 h later, compounds **5i**, **5j** and **5r**, which demonstrated robust NF-κB inhibition in the un-induced cells, were added at six different concentrations for 2 h followed by addition of 20 ng/ml of TNF-α to induce NF-κB dependent gene transcription. Twenty-four hours later, the cells were lysed, luciferase activity recorded and the values normalized. Compounds **5i**, **5j** and **5r** strongly inhibited (>70%) TNF-α induced NF-κB gene expression at the 3.3 μM concentration. Similarly compounds **5k** and **5l** were also found to be giving IC_{50} of 3.3 and 5.5 μM respectively in this assay [28].

To further analyze the binding conformations and binding affinity of the active compounds from the above series, **5r** was chosen as a representative compound and analyzed for its potential binding interaction with NF-κB using the docking protocol Flexidock. The binding conformations and binding affinity of **5r** to the NF-κB's DNA binding site (the site at which the transcription factor binds to the DNA sequence) was obtained. The Sybyl 6.9 package was used to analyze if and how the active compound interacts with NF-κB. The thermodynamically stable conformations were first calculated and the automated docking procedure (FlexiDock) with manual adjustment was used to determine the most energetically favorable binding location and orientation of **5r**. The complex model with the lowest energy among several docking models was selected for binding observations. As an outcome of this study we have observed strong charged interactions of **5r** and NF-κB with a binding energy of −26.70 kcal/mol, where >C═O at 4th position of quinazoline ring presents two [H] bond acceptor sites for Ser 72 and Ser 78 residues, N-1 of quinazoline ring presents one [H] bond acceptor site for Gly66 and —NH— of p-Cl phenylamino group at second position of thiazole ring presents one [H] bond acceptor sites for Gly-52 residue of NF-κB (Fig. 2). These results suggest that a direct interaction of **5r** with NF-κB may lead to the reduced transcriptional activity. This potential interaction of the compound in the DNA binding pocket of NF-κB may inhibit its binding to DNA and thus inhibit transcriptional activation.

Table 2
IC_{50} values of the selected representative compounds.

Entry	IC_{50} (μM) in vitro	
	NF-κB	AP-1
5a	>10	5.7
5i	20	14
5j	16	10
5k	12	3.0
5m	15	1.2
5o	>250	>500
5r	3.3	4.3

IC_{50} values were calculated on the basis of dose response shown by the compounds in dose dependent screening by using graph pad software.

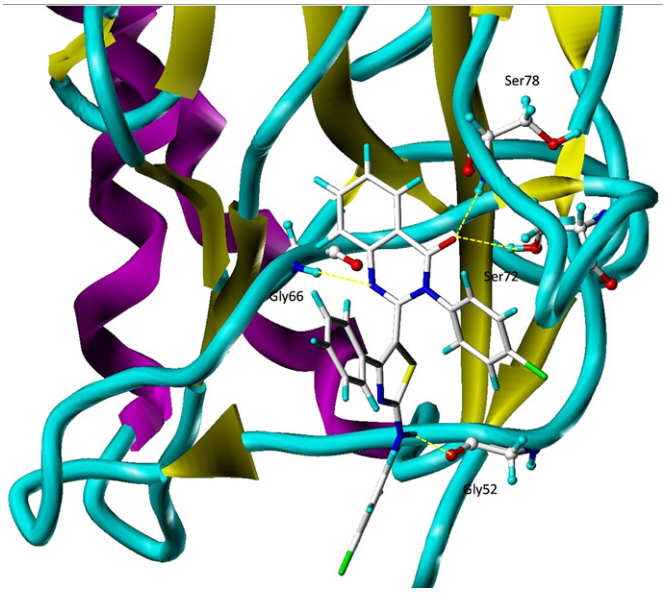


Fig. 2. Compound **5r** bound to NF-κB's DNA binding site in ribbon tube representation (The protein model of NF-κB (p50 homodimer) was taken from the Protein Data Bank entry 1NFK [31]). Where α-helix is purple colored, β-stand is yellow and loops are in blue. Selected protein residues are rendered in ball stick representation. The ligand (**5r**) is rendered I capped stick representation. Color code for atoms: Carbon (white), Hydrogen (cyan), Nitrogen (blue), Oxygen (red), Sulfur (yellow), Chlorine (green). H-bonds are depicted by yellow dotted lines.

Though a more detailed study is necessary before concluding this is the primary mechanism of action of the compound, these docking results partially explain a probable mechanism for transcriptional inhibition elicited by these compounds.

As discussed in our previous communication [28] 2-(2,4-disubstituted-thiazole-5-yl)-3-aryl-3*H*-quinazolin-4-one has again proven to be a reasonable molecular scaffold to develop as inhibitor of NF-κB and AP-1 mediated transcriptional activation. Although not as robust, this scaffold may also show some promise in modulating eIF-4E mediated translational activity. Although the limited number of compounds and mixed biological response makes it very difficult to develop a good SAR from this study the data does suggest a more elaborated study is warranted to explore the full potential of this scaffold. As the transcription factors, NF-κB and AP-1, and the translation factor, eIF-4E, have been demonstrated to play a pivotal role in cancer, these inhibitors may represent novel multi targeted anti-cancer agents. Additional characterization of these compounds and related analogs will be reported in due course.

In conclusion we have synthesized and evaluated 2-thiazole-5-yl-3*H*-quinazolin-4-one derivatives as inhibitors of NF-κB and AP-1 mediated transcriptional and eIF-4E mediated translational activation. The outcome of this study suggest that the 2-thiazole-5-yl-3*H*-quinazolin-4-one derivative is a promising scaffold for developing multi-pathway inhibitors and can be explored to its full potential as an anti-cancer scaffold.

5. Experimental

Unless mentioned otherwise all the starting materials and solvents were purchased from commercially available sources. Melting points were determined on a Toshniwal melting point apparatus using open glass capillary tubes and represent the uncorrected values. Proton NMR spectra were measured on a Bruker spectrometer using the specified solvents at RSIC Chandigarh. LC-MS analysis was done on a Perkin Elmer Applied Bio-Sciences API-165 in the analytical laboratory of B. V. Patel PERD Centre, Ahmedabad. Elemental analysis was carried out at RSIC, IIT Powai on Perkin Elmer 2400 CHN elemental analyzer. All the reactions were monitored using thin layer chromatography (TLC) using glass plate coated with silica gel G or GF_{254}. TLC plates were developed in iodine and toluene: acetonitrile (7:3) was taken as mobile phase, unless mentioned otherwise.

5.1. General synthesis of 2-(2,4-disubstituted-thiazole-5-yl)-3-aryl-3H-quinazolin-4-one derivatives

The general procedure for synthesis of compounds with general structure **5** is as follows. 0.01 mol of thiourea derivatives **3** in 5 ml of acetonitrile was added to a stirred solution of 0.01 mol of the respective 2-chloromethyl-3-aryl-3H-quinazolin-4-one **2** in 5 ml of acetonitrile at 75–80 °C. This reaction mixture was stirred at this temperature for 2–4 h and then cooled to room temperature. The compound that precipitated was filtered and recrystallized in methanol to obtain solid crystals.

5.2. Characterization of synthesized compounds

5.2.1. [5-(4-Oxo-3-phenyl-3,4-dihydro-quinazolin-2-yl)-4-phenyl-thiazol-2-yl]-carbamic acid ethyl ester (**5a**)

Yield: 79%, mp 245-7 °C, R*f*: 0.41, M.F.: $C_{26}H_{20}N_4O_3S$, LC-MS (m/e): 469,491 (M+1, M+23), ^{1}H NMR (300 MHz, DMSO-d^6) δ 1.90 (t, 3H), 4.24 (q, 2H), 6.81–7.92 (m, 13H), 8.1 (d, 1H), 10.5 (s, 1H).

5.2.2. 2-(2-Methylamino-4-phenyl-thiazol-5-yl)-3-phenyl-3H-quinazolin-4-one (**5b**)

Yield: 48%, mp 210-2 °C, R*f*: 0.48, M.F.: $C_{24}H_{18}N_4OS$, LC-MS (m/e): 411 (M+1), ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.85 (s, 3H), 5.92 (d, 1H), 6.42–7.79 (m, 12H), 8.27 (d, 1H), 9.61 (s,1H)

5.2.3. 3-Phenyl-2-(4-phenyl-2-phenylamino-thiazol-5-yl)-3H-quinazolin-4-one (**5c**)

Yield: 78%, mp 210-2 °C, R*f*: 0.70, M.F.: $C_{29}H_{20}N_4OS$, LC-MS (m/e): 473 (M+1), ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.47–6.49 (m, 2H), 6.89–7.12 (m, 5H), 7.24–7.83 (m, 11H), 8.29 (d, 1H), 9.5 (s, 1H).

5.2.4. N-[5-(4-Oxo-3-phenyl-3,4-dihydro-quinazolin-2-yl)-4-phenyl-thiazol-2-yl]-benzamide (**5d**)

Yield: 75%, mp >275 °C, R*f*: 0.67, M.F.: $C_{30}H_{20}N_4O_2S$, LC-MS (m/e): 501 (M+1), ^{1}H NMR (300 MHz, DMSO-d^6) δ 6.65–7.12 (m, 5H), 7.18–7.77 (m, 9H), 7.84 (d, 1H), 7.91 (dd, 1H), 8.08–8.18 (m, 3H), 11.91 (s, 1H)

5.2.5. 2-[2-(4-Chloro-phenylamino)-4-phenyl-thiazol-5-yl]-3-phenyl-3H-quinazolin-4-one (**5e**)

Yield: 71%, mp >275 °C, R*f*: 0.68, M.F.: $C_{29}H_{19}N_4OSCl$, LC-MS (m/e): 507 (M+1), ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.60 (dd, 2H), 7.07–7.84 (m, 15H), 8.3 (dd, 1H), 9.38 (s, 1H)

5.2.6. {5-[3-(4-Methoxy-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-4-phenyl-thiazol-2-yl}-carbamic acid ethyl ester (**5f**)

Yield: 46%, mp 256-7 °C, M.F.: $C_{27}H_{22}N_4O_4S$, LC-MS (m/e): 499,521 (M+1, M+23), ^{1}H NMR (300 MHz, DMSO-d^6) δ 1.23 (t, 3H), 2.47 (s, 3H), 4.20 (q, 2H), 6.85–7.95 (m, 12H), 8.16 (d, 1H), 11.9 (s, 1H).

5.2.7. 3-(4-Methoxy-phenyl)-2-(4-phenyl-2-phenylamino-thiazol-5-yl)-3H-quinazolin-4-one (**5g**)

Yield: 49%, mp 135-7 °C, R*f*: 0.58, M.F.: $C_{30}H_{22}N_4O_2S$, LC-MS (m/e): 503 (M+1), ^{1}H NMR (300 MHz, DMSO-d^6) δ 3.43 (s, 3H), 6.76–7,75 (m, 17H), 8.2 (d, 1H), 10.42 (s, 1H).

5.2.8. N-{5-[3-(4-Methoxy-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-4-phenyl-thiazol-2-yl}-benzamide (**5h**)

Yield: 57%, mp >275 °C, R*f*: 0.61, M.F.: $C_{31}H_{22}N_4O_3S$, LC-MS (m/e): 531, 553, 569 (M+1, M+23, M+39), ^{1}H NMR (300 MHz, DMSO-d^6) δ 3.64 (s, 3H,), 6.62–7.65 (m, 13H), 7.81 (d, 1H), 7.90 (dd, 1H), 8.08–8.18 (m, 3H), 12.88 (s, 1H).

5.2.9. 2-[2-(4-Chloro-phenylamino)-4-phenyl-thiazol-5-yl]-3-(4-methoxy-phenyl)-3H quinazolin-4-one (**5i**)

Yield: 52%, mp 220-21 °C, R*f*: 0.62. M.F.: $C_{30}H_{21}N_4O_2SCl$, LC-MS (m/e): 537 (M+1), ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.73 (s, 3H), 6.50–7.82 (m, 16H), 8.32 (dd, 1H), 9.63 (s, 1H).

5.2.10. {5-[3-(4-Chloro-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-4-methyl-thiazol-2-yl}-carbamic acid ethyl ester (**5j**)

Yield: 46%, mp >275 °C, R*f*: 0.46, M.F.: $C_{21}H_{17}N_4O_3SCl$, LC-MS (m/e): 441, 463 (M+1, M+23), ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (t, 3H), 2.3 (s, 3H), 4.24 (q, 2H), 7.18–7.84 (m, 7H), 8.28 (dd, 1H), 9.85 (s, 1H).

5.2.11. 3-(4-Chloro-phenyl)-2-(4-methyl-2-methylamino-thiazol-5-yl)-3H-quinazolin-4-one (**5k**)

Yield: 41%, mp 195-7 °C, R*f*: 0.61, M.F.: $C_{19}H_{15}N_4OSCl$, LC-MS (m/e): 384 (M+1), ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.33 (s, 3H), 2.82 (s, 3H), 7.17–7.82 (m, 7H), 8.28–8.30 (m, 1H), 9.37 (s, 1H).

*5.2.12. 3-(4-Chloro-phenyl)-2-(4-methyl-2-phenylamino-thiazol-5-yl)-3H-quinazolin-4-one (**5l**)*

Yield: 51%, M.P.: 277–80 °C, R*f*: 0.84, M.F.: $C_{24}H_{17}N_4O_2SCl$, LC-MS (m/e): 446 (M+1), ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.46 (s, 3H), 6.94–7.85 (m, 12H), 8.23–8.25 (dd, 1H), 9.91 (s, 1H).

*5.2.13. 3-(4-Chloro-phenyl)-2-[2-(4-chloro-phenylamino)-4-methyl-thiazol-5-yl]-3H-quinazolin-4-one(**5m**)*

M.F.: $C_{24}H_{16}N_4OSCl_2$, LC-MS (m/e): 479, 480, 502 (M+, M+2, M+23), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.29 (s, 3H), 7.29 (d, 2H), 7.44–7.58 (m, 7H), 7.72 (d, 1H), 7.86 (dd, 1H), 8.14 (d, 1H), 10.33 (s, 1H), Elemental analysis results were: calculated for $C_{24}H_{16}N_4OSCl_2$; C 60.25, H 4.18, N 7.27, obtained; C 60.26, H 4.09, N 7.26.

*5.2.14. {5-[3-(4-Chloro-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-4-phenyl-thiazol- 2-yl}-carbamic acid ethyl ester (**5n**)*

Yield: 50%, mp >275 °C, R*f*: 0.62, M.F.: $C_{26}H_{19}N_4O_3SCl$, LC-MS (m/e): 502, 505, 525 (M+, M+3, M+23), ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.67 (t, 3H), 4.22–4.30 (q, 2H), 6.47–7.88 (m, 12H), 8.25–8.32 (m, 1H), 11.40 (s, 1H), Elemental analysis results were calculated for $C_{26}H_{19}N_4O_3SCl$; C 62.09, H 3.81, N 11.14, obtained; C 62.12, H 3.77, N 11.15.

*5.2.15. 3-(4-Chloro-phenyl)-2-(2-methylamino-4-phenyl-thiazol-5-yl)-3H-quinazolin-4-one (**5o**)*

Yield: 45%, mp >275 °C, R*f*: 0.61, M.F.: $C_{24}H_{17}N_4OSCl$, LC-MS (m/e): 446 (M+1), ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.87 (s, 3H), 5.96 (d, 1H), 6.46–7.84 (m, 11H), 8.29 (d, 1H), 9.47 (s, 1H), Elemental analysis results were: calculated for $C_{24}H_{17}N_4OSCl$; C 64.79, H 3.85, N 12.59, obtained; C 64.78, H 3.84, N 12.58.

*5.2.16. 3-(4-Chloro-phenyl)-2-(4-phenyl-2-phenylamino-thiazol-5-yl)-3H-quinazolin-4-one (**5p**)*

Yield: 53%, mp 218-20 °C, R*f*: 0.64, M.F.: $C_{29}H_{19}N_4OSCl$, LC-MS (m/e): 508 (M+1), ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.47–6.49 (m, 2H), 7.01–7.83 (m, 15H), 8.3 (dd, 1H), 8.5 (s, 1H).

*5.2.17. N-{5-[3-(4-Chloro-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-4-phenyl-thiazol-2-yl}-benzamide (**5q**)*

Yield: 53%, mp >275 °C, R*f*: 0.54, M.F.: $C_{30}H_{19}N_4O_2SCl$, LC-MS (m/e): 535 (M+1), ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.50–8.18 (m, 17H), 8.27–8.29 (dd, 1H), 12.2 (s, 1H)

*5.2.18. 3-(4-Chloro-phenyl)-2-[2-(4-chloro-phenylamino)-4-phenyl-thiazol-5-yl]-3H-quinazolin-4-one (**5r**)*

Yield: 39%, mp 271-2 °C, R*f*: 0.64, M.F.: $C_{29}H_{18}N_4OSCl_2$, LC-MS (m/e): 542 (M+1), ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–8.21 (m, 16H), 8.22–8.23 (m, 1H), 10.62 (s, 1H), Elemental analysis results were: calculated for $C_{29}H_{18}N_4OSCl_2$; C 64.33, H 3.35, N 10.35, obtained; C 64.25, H 3.45, N 10.35.

6. Biological activities

Synthesised compounds were characterised for the reported biological activities as per previously reported procedure [18,28]

7. Molecular modeling protocol

All ligand structures were constructed using the Sketch Molecule of SYBYL 6.9 [30]. Conformational search of **5r** was performed by grid search, rotating three rotatable bonds in 60 °C increments and the amide bond at 0 °C or 180 °C. A random search was also performed, using the following options for all rotatable bonds; 3000 iterations, 3-kcal energy cutoffs and no chirality checking. In all cases, MMFF force field and charge were applied with the use of distance-dependent dielectric constants and the conjugate gradient method until the gradient reached 0.001 kcal mol^{-1} Å^{-1}.

Representative conformers selected from clusters of low-energy conformers obtained in the conformational search were re-optimized by semi empirical molecular orbital calculations with the PM3 method in the MOPAC 6.0 package. The protein model of NF-κB (p50 homodimer) was taken from the Protein Data Bank entry 1NFK [31]. DNA-binding region, amino acid residues 59–71 (59: Arg, Tyr, Val, Cys, Glu, Gly, Pro, Ser, His, Gly, Gly, Leu, Pro: 71) of NFkB were identified the as active site. This site was used for the docking of ligand [32,33].

Flexible docking was facilitated through the FlexiDock utility in the Biopolymer module of SYBYL 6.9. During flexible docking, the ligand and the side chains of hydrophilic amino acids in the putative binding site were defined as rotatable bonds. After the hydrogen atoms were added to the receptor, atomic charges were recalculated by using Kollman All-atom for the protein and Gasteiger–Huckel for the ligand. H-bonding sites were marked for all residues in the active site and ligands with H-bond donor or acceptor. Ligands were pre-positioned in the putative binding cavity guided by several superimposition results. Default FlexiDock parameters were set at 3000-generations for genetic algorithms. To increase the binding interaction, the torsion angles of the side chains within 5 Å of the ligands were manually adjusted from the results of FlexiDock. Finally, the complexes were minimized by using the powell method with a fixed dielectric constant (4.0), until the conjugate gradient reached 0.001 kcal mol^{-1} Å^{-1}.

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