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Design, synthesis and evaluation of novel 2-thiophen-5-yl-3*H*-quinazolin-4-one analogues as inhibitors of transcription factors NF-κB and AP-1 mediated transcriptional activation: Their possible utilization as anti-inflammatory and anti-cancer agents

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ABSTRACT

In an attempt to discover novel inhibitors of NF-κB and AP-1 mediated transcriptional activation utilizing the concept of chemical lead based medicinal chemistry and bioisosterism a series of 2-(2,3-disubstituted-thiophen-5-yl)-3*H*-quinazolin-4-one analogs was designed. A facile and simple route for the synthesis of the designed molecules was developed. Synthesized molecules were evaluated for their activity as inhibitors towards NF-κB and AP-1 mediated transcriptional activation in a cell line report-based assay. This series provides us with a substantial number of compounds inhibiting the activity of NF-κB and/or AP-1 mediated transcriptional activation. These compounds also exhibit anti-inflammatory and anti-cancer activity in in vivo models of inflammation and cancer. The 4-pyridyl group is found to be the most important pharmacophore on the third position of thiophene ring for inhibiting NF-κB and AP-1 mediated transcriptional activation. The relationships between the activities shown by these compounds in the in vivo and in vitro models have been established by using FVB transgenic mice model. These results suggest the suitability of the designed molecular framework as a potential scaffold for the design of molecules with inhibitory activity towards NF-κB and AP-1 mediated transcriptional activation, which may also exhibit anti-inflammatory and anti-cancer activity. This series of molecules warrants further study to explore their potential as therapies for use in chronic inflammatory conditions and cancer. Development of the synthetic protocol for the synthesis of this series of molecules, biological activities and a structure–activity relationship (SAR) have been discussed herein.

1. Introduction

The current treatment options for cancer and inflammatory diseases are by and large unsatisfactory due to poor overall efficacy and relatively significant side effect profiles.[1] A complex network of proteins involving multiple molecular pathways, for which a great deal of redundancy exists, act as mediators of inflammation and cancer.[2] In view of the polygenic nature of these diseases, with a number of pathways acting in parallel, it seems appropriate to look for therapies acting on more than one pathway.[3] The targeting of transcription factors with small molecules represents one such approach of modulating the activity of a number of proteins and pathways simultaneously.[4]

Transcription factors are regulatory proteins which bind to specific DNA sequences in the gene promoter or enhancer regions to activate or inhibit the transcription of genes.[5] The synthesis of new messenger RNA by the process of transcription is a major control point in the expression of many proteins. This is especially true for cytokines, cell adhesion molecules, erosive enzymes and other proteins that mediate inflammatory diseases and cancer. Nuclear factor-κB (NF-κB) and activating protein (AP-1) are key transcription factors that orchestrate expression of many genes involved in inflammation and cancer. NF-κB controls the expression of virtually all significant pro-inflammatory chemokines/cytokines, many angiogenic growth factors, TNF-α, inducible nitric oxides synthase, COX-2, ICAM-1, VCAM-1, E-selectin and pathways of cell proliferation and survival.[6,7] Similarly AP-1 regulates the activity of interleukin-2 (IL-2), IL-3 and granulocyte-macrophage colony stimulating factor (GM-CSF), metalloproteinases, collagenase and stromelysin.[8] Many other pro-inflammatory genes are also known to have binding sites for NF-κB and AP-1.

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The role of the immune system in cancer has also become better understood. The tumor microenvironment is highly influenced by inflammatory cells responsible for fostering tumor proliferation, survival and migration.[9] Thus, altering cytokine expression can have a profound affect on cancer progression as demonstrated by the success of Celgene's immunomodulatory drugs, thalidomide and revlimid. Therefore, the selective inhibition of a limited number of key transcription factors like NF-κB and AP-1 involved in the inflammatory process may control a plethora of mediators of inflammation and cancer. The dual targeting of these two transcription factors may also overcome some of the redundancy that is inherent to biological systems and possibly responsible for the failure of the more specific, 'magic bullet' targeted therapies.[10,11] The anti-inflammatory effect of steroids, retinoids and a variety of anti-rheumatic drugs act through a mechanism that converges on a limited number of transcription factors, most notably transcription factors NF-κB and AP-1.[11] Importantly, it is believed that the compounds that exert their anti-inflammatory effects by inhibiting the activation of NF-κB and AP-1 may cause much milder gastrointestinal mucosal damage and other side-effects associated with long term NSAID and glucocorticoid use.[12] Based on these observations, it appears that inhibition of NF-κB and/or AP-1 transcriptional activation may represent a potential and safe target in the development of novel agents to treat inflammatory diseases and cancer.[13,14]

Quinazoline scaffolds and their bioisosteres are well known as a privileged scaffold in drug discovery. During their search for small molecule inhibitors of NF-κB and AP-1, Palanki and his coworkers have reported quinazoline derivatives which have a thiophene ring attached at the second position of the quinozoline (**SPC-839**, Fig. 1).[15–17]

Further, tri-substituted thiophenes have been reported by us as inhibitors of the transcription factors, NF-κB and AP-1, and as anti-inflammatory agents.[18–20] The SAR study in our earlier work suggested that a morpholine at the second position, with aryl and heteroaryl substitutions at the third and fifth position of thiophene scaffold resulted in an advantageous pharmacophore for designing better inhibitors of NF-κB and AP-1.[18–20] In the process of engineering better inhibitors of transcriptional activation of NF-κB and AP-1 utilizing the concept of bioisosterism and chemical lead based medicinal chemistry, we delineated the gross framework for the design of several new compounds as 2-thiophen-5-yl-3*H*-quinazolin-4-one analogues **9** (Fig. 1). The monocyclic hetroaromatic ring system at the fifth position of thiophene ring was replaced with a bicyclic heteroaromatic ring system in the 3-aryl-3*H*-quinazolin-4-one scaffold, which can be considered as a bioisostere of the quinoline ring in SPC-839 and is a well known anti-inflammatory scaffold. Herein, we report the synthesis and evaluation of 2-thiophen-5-yl-3*H*-quinazolin-4-one analogues as inhibitors of transcription factors NF-κB and AP-1 mediated transcriptional activation. We also discuss their potential as anti-inflammatory and anti-cancer agents.

2. Chemistry

In designing the synthetic protocol for 2-thiophen-5-yl-3*H*-quinazolin-4-one analogues, we envisioned that 2-chloro methyl-3-aryl-3*H*-quinazoline-4-one **2** and 1,3-di-morpholin-4-yl-2-substituted-propenethione intermediate **5** may represent interesting starting materials for synthesis of designed compounds **9** (Scheme 1). A chemical interaction between **2** and **5** may result into the S-alkylation of the sulfur group of **5** by the active methylene group ($-CH_2-$) of **2**, yielding intermediate **1**. It can be observed from the structure of **1** that it is chemically not likely to be very stable, therefore an intramolecular cyclization reaction followed by stable aromatic ring formation would be the favored synthetic route (Schemes 1 and 2).

Mindful of this consideration we initiated to synthesize intermediates **2** and **5**. The synthesis of 2-chloromethyl-3-aryl-3*H*-quinazolin-4-one **2** was carried out using a modified Niementowski synthesis, beginning with the chloro-acetylation of anthranilic acid **4** to yield the corresponding 2-(2-chloro-acetyl amino) benzoic acid **3**. Acid **3** was then treated with trichlorophosphate (PCl_3) to convert into an acid chloride which was immediately treated with substituted aniline in situ to replace the chloro group of the acid chloride with substituted aniline. This was then refluxed in toluene to generate the cyclized product **2**.[21,22] 2-Aryl-1,3-dimorpholin-4-yl-propenethiones **5** were prepared using Willgerodt–Kindler reaction, where appropriate acetophenones **7** were reacted with elemental sulfur and morpholine **8** to yield the phenylthioacetic acid morpholide intermediates **6**.[23] Phenylthioacetic acid morpholide was reacted with morpholine and triethylorthoformate (as a one carbon donor), to yield 2-aryl-1,3-dimorpholin-4-yl-propenethiones **4**[24] (Scheme 2). In the final step, intermediate **2** was

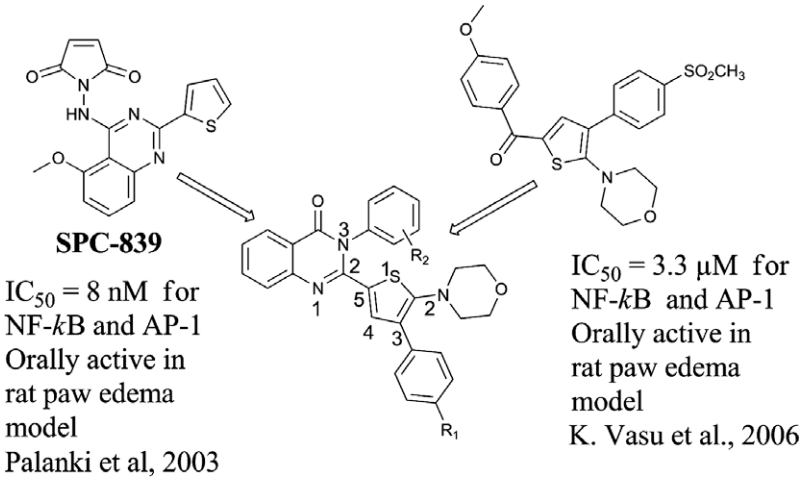
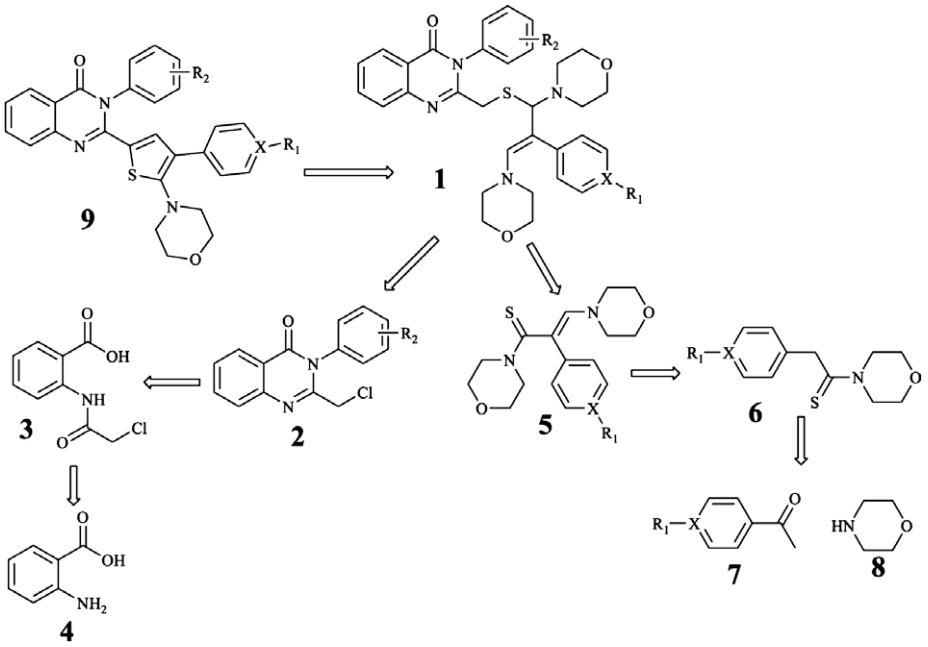
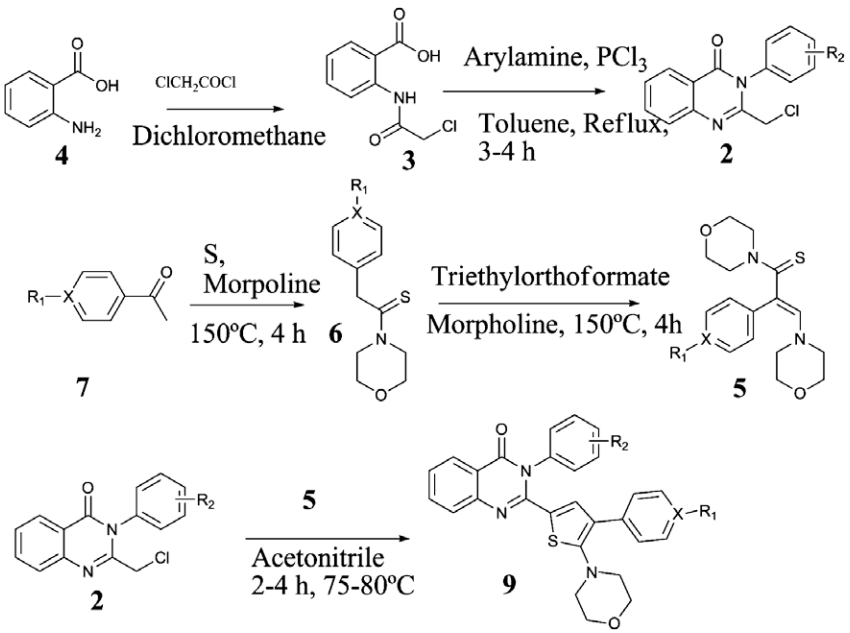


Figure 1. Template based approach for the designing of novel 2-thiophene-5-yl-3*H*-quinazoline-4-one analogues as NF-κB and AP-1 inhibitors.



Scheme 1. Retro analysis of the designed molecule.



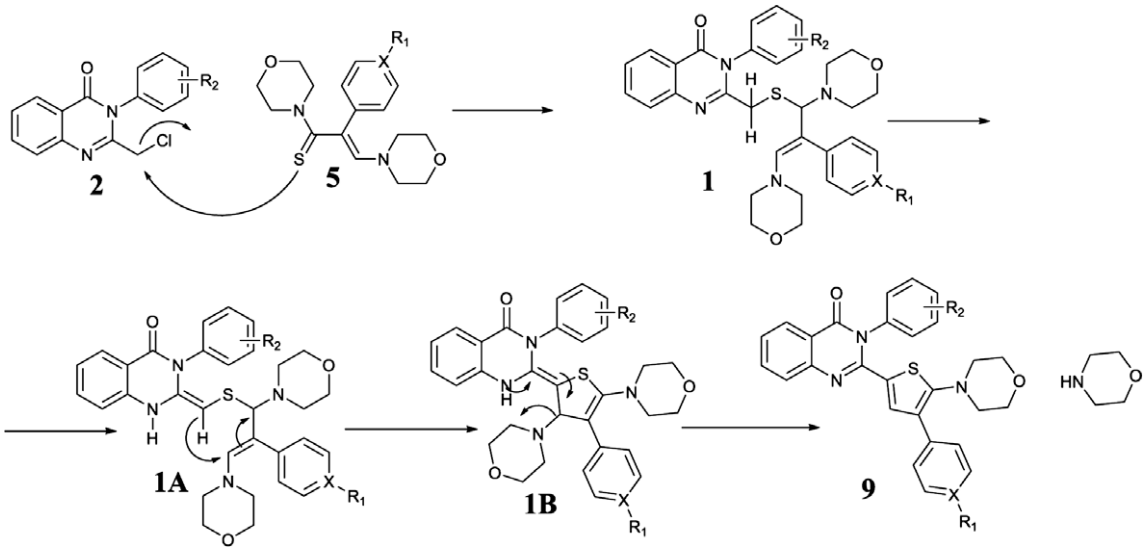
Scheme 2. Synthesis of designed compounds.

treated in acetonitrile with intermediate **5** in acetonitrile at 75–80 °C for 2–4 h to yield the desired 2-thiophen-5-yl-3*H*-quinazolin-4-one analogues.

In this synthesis, a functionalized 2-aryl-1,3-dimorpholin-4-yl-propenethiones **5** derivative provided three ring carbon (C-2, C-3, C-4) atoms and the heteroatom (S-1) of the resultant thiophene ring; the remaining carbon atom (C-5) was supplied by the methylene group of the 2-chlormethyl group at the second position of the quinazolinone ring **2**. The mechanism involves the S-alkylation of the –SH group of 2-aryl-1,3-dimorpholin-4-yl-propenethione **5** by $-CH_2-$ of chloromethyl at the second position of quinazolinone ring followed by the intramolecular cyclization through the nucleophilic attack of the active methylene group. Presumably this nucleophilic attack may take place due to the initial tautomerization of the quinazolinone double bond to generate an enamine like intermediate; the quinazolinone nitrogen then serves as an electron source for the nucleophilic attack. The driving force for the thiophene ring formation is the elimination of the morpholine **8** residue and the formation of the stable five membered aromatic heterocycle. The probable mechanism has been summarized in Scheme 3.

3. Results and discussion

The focus of our approach in this work was to optimize the substitutions on the phenyl ring at third (R_2) position of the quinazoli-



Scheme 3. Plausible mechanism for the synthesis of designed compounds.

none ring and on the third position of the thiophene ring (R_1) to generate maximum inhibition of NF-κB and/or AP-1 mediated transcriptional activation. The selection of substituents at both positions were mainly guided by lipophilicity and electronic considerations as defined by the σ–π Craig plot, chosen in such a way that they fall within the ±σ, ±π quadrant of the Craig plot.[25] One substituent was chosen for the R_1 position from every quadrant (i.e., $-CH_3$ from first, Cl from second, $-COCH_3$ from third and $-OCH_3$ from fourth quadrant) to generate a structure–activity profile of the compounds. Similarly, one substituent was chosen from every quadrant (i.e., $-SCH_3$ from first, –Cl from second, $-SO_2CH_3$ from third and $-NHCOCH_3$ from fourth quadrant) for the R_2 position.

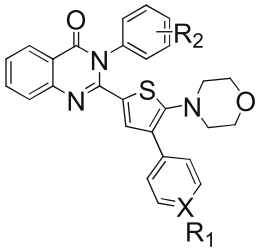
3.1. Inhibition of NF-κB and AP-1 mediated gene transcription

The ability of the synthesized 2-thiophen-5-yl-3*H*-quinazolin-4-one analogues to modulate NF-κB and AP-1 mediated transcriptional activation was evaluated using a luciferase reporter assay in human embryonic kidney (HEK-293) cells with a stably transfected NF-κB-luc and AP-1-luc gene. These cells maintain, through hygromycin selection, a chromosomal integration of a luciferase reporter construct regulated by multiple copies of the NF-κB and AP-1 response elements. For induction of transcription and to test the biological response of the promoters, the HEK/NF-κB cell line was stimulated with TNF-α (1 ng/μl) and the HEK/AP-1 cell line was stimulated with PMA (1 ng/μl) in the presence or absence of test compounds. All of the 37 compounds synthesized were evaluated for their activities. A reasonable number of compounds have been identified exhibiting moderate to excellent inhibitory activity towards NF-κB and/or AP-1 mediated transcriptional activation in this cell based in vitro assay at10 μM concentration (Table 1). Compounds showing more than 50% inhibition at 10 μM were taken forward for in vivo testing in carrageenan induced rat paw edema model of inflammation and were found to exhibit a significant protective activity in this model at 50 mg/kg, po in comparison to the existing anti-inflammatory drug ibuprofen (Table 2). The most promising compounds from the in vitro screening were tested in vitro for dose response from 10 nM to 10 μM. Compound **9bk** was found to be the most promising compound from the series with IC_{50} value of 10 μM and 5 μM for NF-κB and AP-1, respectively (Table 2). Compound **9bk** was also found to be the most active compound in the in vivo anti-inflammatory activity (Table 2).

3.1.1. Structure–activity relationship

Analysis of the data generated from this study suggests that: (1) the activity of the designed molecular framework is dependent upon the substituents at the R_1 and R_2 positions and/or their combination. (2) Compounds which have $-SO_2CH_3$ group at R_1 position were found to be more active for the biological activities discussed in this paper than compounds which have –H, –Cl, $-SCH_3$ and $-NHCOCH_3$ at R_1 position (Table 1). These results suggest that electron withdrawing hydrophilic substitutes (e.g., $-SO_2CH_3$) are more desirable at the R_1 position rather than electron withdrawing (e.g., –Cl), electron donating (e.g., $-SCH_3$) lipophilic substituents or electronically neutral hydrophilic substitutes (e.g., $-NHCOCH_3$) for achieving the desired activity. (3) The compounds substituted with –Cl on R_2 positions yielded the least active series of compounds in this study (except in combination with 4-pyridyl at the 3rd position of thiophene ring). The presence of –Cl at R_2 position is detrimental even where R_1 is $-SO_2CH_3$ (**9bf**, Table 1). Which suggests that electron withdrawing groups with lipophilic characteristics like –Cl may not be a ideal substitution on R_2 position to get the good activity from the designed molecular framework (**9bc–9bf** and **9bi**, Table 1) and presence of this kind of substitution may also eliminate potency of the $-SO_2CH_3$ group at R_1 position. (4) The compounds substituted with $-CH_3$ group at R_2 position were shown to exhibit mild to very weak activity in both the assays tested (**9ag, 9ah, 9aj, 9al–9ao, 9aq–9as, 9au, 9av,** Table 1). These results suggest that substituents with electron donating and lipophilic characteristics like $-CH_3$ may not be the ideal substituents on R_2 position for the desired activity but presence of this group does not eliminate the potency of the $-SO_2CH_3$ group at R_1 position (**9ai** and **9at**, Table 1). (5) The compounds substituted with electron donating and very low steric value like $-OCH_3$ at R_2 position were found to be more active in the designed series of compounds than compounds having electron donating and lipophilic characteristics like $-CH_3$ (**9ax, 9ay, 9ba** Table 1). (6) Compounds which have $-COCH_3$ group at R_2 position were found to be active for the biological activities discussed in this paper (**9bj** and **9bk**, Table 1). Combination of $-COCH_3$ group at R_2 position with –H and –Cl groups at R_1 proved to be detrimental for the activity of designed molecular framework. (7) Combination of $-SO_2CH_3$ group at R_1 position with $-COCH_3$ groups at R_2 position was not found to be beneficial as compared to the other combination (Table 1). (8) 4-Pyridyl group at the third position of the thiophene ring

Table 1
Evaluation of synthesized compounds in in vitro assays for their inhibitory activities towards NFkB and AP-1 mediated transcriptional activation



No.	Entry[a]	R_1	R_2	X	% Inhibition in vitro (10 μM)	
					NF-κB	AP-1
1	**9aa**	–H	–H	C	0	11
2	**9ab**	–Cl	–H	C	0	0
3	**9ac**	$-NHCOH_3$	–H	C	7	26
4	**9ad**	$-SO_2CH_3$	–H	C	9	28
5	**9ae**	$-SCH_3$	–H	C	0	0
6	**9af**	—	–H	N	30	38
7	**9ag**	–H	2-CH_3	C	23	27
8	**9ah**	–Cl	2-CH_3	C	1	32
9	**9ai**	$-SO_2CH_3$	2-CH_3	C	51	52
10	**9aj**	$-SCH_3$	2-CH_3	C	15	13
11	**9ak**	—	2-CH_3	N	53	0
12	**9al**	–Cl	3-CH_3	C	28	10
13	**9am**	$-NHCOCH_3$	3-CH_3	C	35	13
14	**9an**	$-SO_2CH_3$	3-CH_3	C	20	48
15	**9ao**	$-SCH_3$	3-CH_3	C	11	14
16	**9ap**	—	3-CH_3	N	42	35
17	**9aq**	–H	4-CH_3	C	0	17
18	**9ar**	–Cl	4-CH_3	C	15	27
19	**9as**	$-NHCOCH_3$	4-CH_3	C	4	35
20	**9at**	$-SO_2CH_3$	4-CH_3	C	55	51
21	**9au**	$-SCH_3$	4-CH_3	C	22	16
22	**9av**	—	4-CH_3	N	26	29
23	**9aw**	–H	2-OCH_3	C	0	0
24	**9ax**	$-SO_2CH_3$	2-OCH_3	C	55	33
25	**9ay**	—	2-OCH_3	N	55	33
26	**9az**	$-SO_2CH_3$	3-OCH_3	C	20	0
27	**9ba**	$-SO_2CH_3$	4-OCH_3	C	60	10
28	**9bb**	—	2-Cl	N	80	35
29	**9bc**	–H	4-Cl	C	0	23
30	**9bd**	–Cl	4-Cl	C	0	14
31	**9be**	$-NHCOCH_3$	4-Cl	C	0	17
32	**9bf**	$-SO_2CH_3$	4-Cl	C	0	7
33	**9bg**	—	4-Cl	N	68	32
34	**9bh**	–H	4-$COCH_3$	C	0	25
35	**9bi**	–Cl	4-$COCH_3$	C	0	7
36	**9bj**	$-SO_2CH_3$	4-$COCH_3$	C	46	31
37	**9bk**	—	4-$COCH_3$	N	66	84

[a] Synthesized compounds were characterized by their IR, NMR and LC–MS (Section 5). SPC-839, reduced luciferase activity by ~40% in the NF-κB and AP-1 cell lines at 1.0 μM.[20]

emerged as the most important pharmacophore for inhibiting NF-κB and AP-1 mediated transcriptional activation, as all the compounds (except **9av**, which was found to be least active in 4-pyridyl series) in this series were found to have highly significant activity in both the assays (Table 1). (9) To study the effect of $-COCH_3$ group at the R_2 position in combination with the 4-pyridyl group at the third position of the thiophene ring, compound **9bk** was synthesized and was found to be the most promising dual inhibitor of NF-κB and AP-1 mediated transcriptional activation.

These results suggest that the electron withdrawing hydrophilic substituents like $-COCH_3$ at R_2 position in combination with lipophilic cyclic ring containing weakly basic nitrogen may be the ideal feature for obtaining the dual inhibitory activity towards NF-κB and AP-1 mediated transcriptional activation from the designed molecular framework.

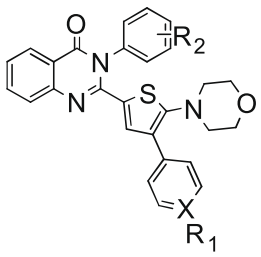
3.1.2. 9bk Decreased LPS-stimulated NF-κB activity by 43% in transgenic FVB mice

In our in vitro assay we have used human embryonic kidney cell line, (HEK293) stably expressed with a plasmid containing a minimal promoter and tandem copies of the NF-κB transcriptional element (5′-AGTTGAGGGGACTTTCCCAGGC-3′) or the AP-1 transcriptional element (TGACTAA), which regulates the expression of the luciferase reporter gene. The read out of our in vitro screening results suggest that our compounds are inhibiting the activation of NF-κB and AP-1 mediated transcriptional process. Therefore to see if our compounds inhibit the activation of NF-κB directly in vivo we tested the activity of **9bk** to inhibit the activation of NF-κB in vivo using transgenic FVB mice consisting of a minimal promoter and tandem NF-κB binding sites in all cells were used as in vivo 'reporters' of NFκB activity. Three transgenic mice which express an NF-κB-luciferase reporter gene in all tissues were taken per group. Mice injected with luciferin only (not stimulated with LPS) were shown to have very low endogenous levels of NF-κB activity limited to the thymus (Fig. 2A). All mice were confirmed to glow by injection of 60 μg LPS (Sigma) 3 h prior to injection of luciferin and imaging on the IVIS 100 system (Xenogen) (Fig. 2B). These results suggest that stimulation with LPS treatment is able to activate the NF-κB in vivo. The treatment group was treated for four days with 50 mg/kg of **9bk** daily once and on the fourth day all three mice were injected with 60 μg LPS and after 3 h all mice were injected with luciferin and were imaged on the IVIS 100 system (Xenogen) (Fig. 2C). Compound **9bk** treated mice (Fig. 2C) had a significantly decreased level of bioluminescence compared to the control (maximally) stimulated mice (Fig. 2B), indicating that **9bk** is inhibiting activation of NF-κB directly in vivo.

Inflammation and cancer have been shown to have a close association and hence anti-inflammatory drugs of steroidal as well as non-steroidal nature have been proven to decrease the risk of developing various malignancies including colon,[26] lung,[27] prostate and breast cancers.[28] Under this concept, the targets NF-κB and AP-1 have been widely described as very important links between the inflammatory mechanism and cancer and as a result this association has been studied in depth in various aspects of cancer.[28–30] Based on this understanding, the most active compound from this study **9bk** was tested for its ability to inhibit proliferation or cause apoptosis on various cancer cell lines, which are used as models for different types of cancers. Compound **9bk** was shown to inhibit the growth of LNCap and PC-3 (prostate cancer cell lines) and TCCSUP (bladder cancer cell line). Compound **9bk** was also found to inhibit tumor growth and tumor angiogenesis in a various human tumor xenograft models. In a cytokine profiling assay **9bk** was found to alter the expression of cytokines like TNF-α, MCP-3, MCP-4, BMP-4, BMP-6, RANTES, MCP-1, GCP-1 and various anti-apoptotic genes involved in several type of cancer progression.[31] These results suggest that **9bk** is potential inhibitor of NF-κB and AP-1 mediated transcriptional activation which may be utilized to treat inflammatory diseases and cancer.

A new molecular scaffold 2-(2,3-disubstituted-thiophen-5-yl)-3*H*-quinazolin-4-one, has been developed and can be considered as a potential molecular scaffold for designing of novel inhibitors of NF-κB and AP-1 mediated transcriptional activation, with potential for use as anti-inflammatory and anti-cancer therapies. This was developed by utilizing the molecular scaffold generated through a chemical lead based medicinal chemistry approach (Fig. 1) and incorporating the morpholine, aryl and heteroaryl groups on thiophene ring, which was an already established pharmacophore as an inhibitor of NF-κB and AP-1 mediated transcriptional activation and as an anti-inflammatory pharmacophore.

Table 2
Evaluation of most promising compounds in in vitro assays for their anti-inflammatory activity in carrageenin induced rat paw edema model of acute inflammation



Entry	R_1	R_2	X	IC_{50}[b] (μM)		% Protection[a] in in vivo (50 mg/kg)
				NF-kB	AP-1	
9ak	—	2-CH_3	N	7.1	na[c]	51
9ax	–SO_2CH_3	2-OCH_3	C	9.2	na[c]	63
9ay	—	2-OCH_3	N	12.2	na[c]	42
9ba	–SO_2CH_3	4-OCH_3	C	26	na[c]	53
9bb	—	2-Cl	N	10.3	na[c]	43
9bg	—	4-Cl	N	11.4	na[c]	52
9bk	—	4-$COCH_3$	N	10	5	81

[a] Oral administration for all test compounds for in vivo anti-inflammatory activity. For the standard drug ibuprofen, dose and % protection were 50 mg/kg, 52%, respectively.
[b] IC_{50} values for inhibition of luciferase production in pNF-κB-luc HEK and pNF-AP-1-luc HEK cells following TNF-α and PMA activation, respectively.
[c] na, not applicable (only compounds showing more than 50% inhibition in initial screening were subjected to six point dose response study. 9ai and 9at have shown promising activity in primary screening but failed to show good dose response).

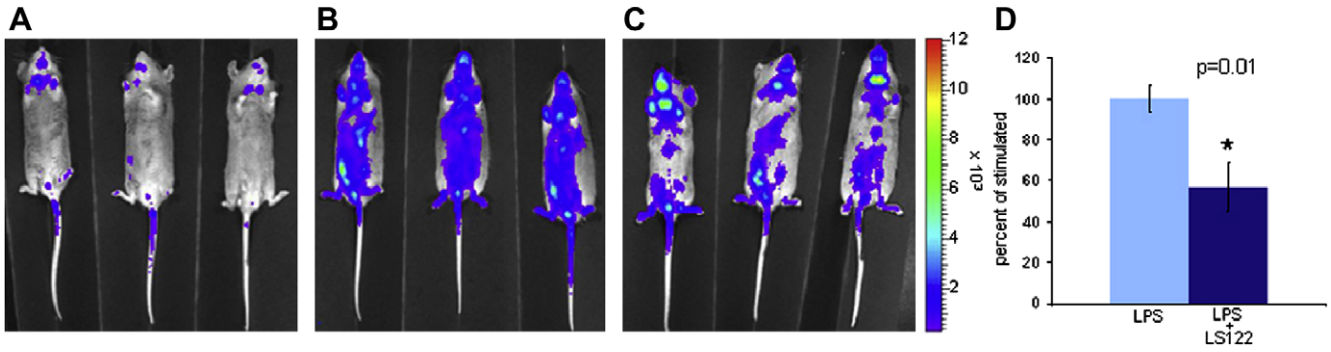


Figure 2. Compound **9bk** decreased LPS-stimulated NF-κB activity by 43% in luciferase reporter mice. Transgenic mice expressed an NFκB-luciferase reporter gene in all tissues. (A) Mice injected with luciferin only were shown to have very low endogenous levels of NF-κB activity limited to the thymus. (B) All mice were confirmed to glow by injection of 60 μg LPS (Sigma) 3 h prior to injection of luciferin and imaging on the IVIS 100 system (Xenogen). (C) Mice were given 50 mg/kg **9bk** by oral gavage for 4 consecutive days prior to LPS treatment, luciferin injection, and imaged as above. Representative images from similar exposures with the same color scale are shown. (D) Compound **9bk (LS-122)** treated mice had a significantly decreased level of bioluminescence compared to the control (maximally) stimulated mice, indicating that **9bk** is inhibiting NF-κB directly in vivo.

4. Conclusion

In an attempt to discover novel inhibitors of NF-κB and AP-1 mediated transcriptional activation for use as potent anti-inflammatory and/or anti-cancer agents, a series of 2-(2,3-disubstituted-thiophen-5-yl)-3*H*-quinazolin-4-one analogs were designed, synthesized and evaluated for its biological activities. A facile and simple route for the synthesis of the designed compounds was developed. This series eventually turned out to be very promising such that a substantial number of compounds which inhibit the activation of NF-κB and AP-1 mediated transcriptional activation were generated. These inhibitors also demonstrated significant in vivo efficacy as anti-inflammatory agents. Compound **9bk**, the most active dual inhibitor of NF-κB and AP-1 mediated transcriptional activation from the entire series, was also found to demonstrate potential anti-cancer activity in vitro as well as in an in vivo model of cancer. The relationship between the activities shown by these compounds in the in vivo and in vitro models has been establish by using transgenic FVB mice model. Therefore, the activity of **9bk** in in vivo model may be atleast in part is due to its NF-κB inhibitory activity. These results suggest the suitability of the designed molecular framework for use in the development of anti-inflammatory and anti-cancer therapies.

5. Experimental

Melting points (mp) 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 analyses were done on a Perkin Elmer Applied Bio-Sciences API-165 in the analytical laboratory of B. V. Patel PERD Centre, Ahmedabad, India using electron spray ionization (EI) in positive mode. 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. Synthesis of 2-(2-chloro-acetyl amino) benzoic acid 3[21,22]

Anthranilic acid **4** (50.0 g, 0.37 mol) was taken into dichloromethane and equimolar amount of triethylamine was added, and then reaction was cooled to 0 °C. While maintaining this temperature chloro acetyl chloride (41.1 g, 0.37 mol) was added to it in 15 min. Reaction was stirred at room temperature for 4 h. White precipitates were filtered and washed with ample amount of water and dried. The crude compound was crystallized with hot water to get (55.0 g, 64.5%) of 2-(2-cholo-acetylamino) benzoic acid as a white solid. mp 183–185 °C, R_f: 0.40, LC–MS (*m/z*): 214 (M+1).

5.2. Synthesis of 2-chloromethyl quinazoline-4-one derivatives

5.2.1. Synthesize of 2-chloro methyl-3-(phenyl)-3*H*-quinazoline-4-one (2a)[22]

20.0 g (0.09 mol) of 2-(2-chloro-acetyl amino) benzoic acid **3** and equimolar amount of aniline (8.70 g, 0.09 mol) were taken in toluene and PCl_3 (18 mL) was added into it. Temperature was raised to reflux the reaction and maintained till TLC showed completion of reaction. Then the reaction was cooled to room temperature and solvent was evaporated in vacuo. To this residue water was added and reaction mixture was neutralized with the addition of sodium bicarbonate and extracted with chloroform (4 × 50 mL). The organic layers were combined and washed with water (2 × 30 mL). The organic layer was then dried over Na_2SO_4 evaporated in vacuo. Resulting crude was crystallized in methanol to get 2-chloromethyl-3-phenyl-3*H*-quinazoline-4-one (7.20 g, 28.0%) as a white solid. mp 152–153 °C, R_f: 0.48, LC–MS (*m/z*): 271 (M+1).

5.2.2. 2-Chloro methyl-3-(2-methyl-phenyl)-3*H*-quinazoline-4-one (2b)

2-(2-Chloro-acetyl amino) benzoic acid **3** (20.0 g, 0.09 mol) was reacted with 2-methyl aniline (9.80 g, 0.09 mol) using the same procedure described for **2a** to afford the title compound as an off white solid. Yield: 29.0%, mp 205–206 °C, R_f: 0.54, LC–MS (*m/z*): 285 (M+1).

5.2.3. 2-Chloro methyl-3-(3-methyl-phenyl)-3*H*-quinazoline-4-one (2c)

2-(2-Chloro-acetyl amino) benzoic acid **3** (20.0 g, 0.09 mol) was reacted with 3-methyl aniline (9.80 g, 0.09 mol) using the same procedure described for **2a** to afford the title compound as an off white solid. Yield: 32.5%, mp 183–185 °C, R_f: 0.53, LC–MS (*m/z*): 285 (M+1).

5.2.4. 2-Chloro methyl-3-(4-methyl-phenyl)-3*H*-quinazoline-4-one (2d)

2-(2-Chloro-acetyl amino) benzoic acid **3** (20.0 g, 0.09 mol) was reacted with 4-methyl aniline (9.80 g, 0.09 mol) using the same procedure described for **2a** to afford the title compound as an off white solid. Yield: 36.0%, mp 265–266 °C, R_f: 0.64, LC–MS (*m/z*): 285 (M+1).

5.2.5. 2-Chloro methyl-3-(2-methoxy-phenyl)-3*H*-quinazoline-4-one (2e)

2-(2-Chloro-acetyl amino) benzoic acid **3** (20.0 g, 0.09 mol) was reacted with 2-methoxy aniline (11.4 g, 0.09 mol) using the same procedure described for **2a** to afford the title compound as a light yellow solid. Yield: 31.4%, mp 193–194 °C, R_f: 0.62, LC–MS (*m/z*): 301 (M+1).

5.2.6. Chloro methyl-3-(3-methoxy-phenyl)-3*H*-quinazoline-4-one (2f)

2-(2-Chloro-acetyl amino) benzoic acid **3** (20 g, 0.094 mol) was reacted with 3-methoxy aniline (11.4 g, 0.094 mol) using the same procedure described for **2a** to afford the title compound as off light yellow solid. Yield: 32.2%, mp 180–182 °C, R_f: 0.62, LC–MS (*m/z*): 301 (M+1).

5.2.7. 2-Chloro methyl-3-(4-methoxy-phenyl)-3*H*-quinazoline-4-one (2g)

2-(2-Chloro-acetyl amino) benzoic acid **3** (20.0 g, 0.09 mol) was reacted with 4-methoxy aniline (11.4 g, 0.09 mol) using the same procedure described for **2a** to afford the title compound as a light yellow solid.Yield: 34.0%, mp 154–155 °C, R_f: 0.57, LC–MS (*m/z*): 301 (M+1).

5.2.8. 2-Chloro methyl-3-(2-chloro-phenyl)-3*H*-quinazoline-4-one (2h)

2-(2-Chloro-acetyl amino) benzoic acid **3** (20.0 g, 0.09 mol) was reacted with 2-choloro aniline (12.0 g, 0.09 mol) using the same procedure described for **2a** to afford the title compound as a light yellow solid. Yield: 26.4%, mp 145–146 °C, R_f: 0.61, LC–MS (*m/z*): 305 (M+1).

5.2.9. 2-Chloro methyl-3-(3-chloro-phenyl)-3*H*-quinazoline-4-one (2i)

2-(2-Chloro-acetyl amino) benzoic acid **3** (20.0 g, 0.09 mol) was reacted with 3-choloro aniline (12.0 g, 0.09 mol) using the same procedure described for **2a** to afford the title compound a light yellow solid. Yield: 27.4%, mp 202–205 °C, R_f: 0.59, LC–MS (*m/z*): 305 (M+1).

5.2.10. Chloro methyl-3-(4-chloro-phenyl)-3*H*-quinazoline-4-one (2j)

2-(2-Chloro-acetyl amino) benzoic acid **3** (20.0 g, 0.09 mol) was reacted with 3-choloro aniline (12.0 g, 0.09 mol) using the same procedure described for **2a** to afford the title compound a light yellow solid. Yield: 26.4%, mp 234–235 °C, R_f: 0.62, LC–MS (*m/z*): 305 (M+1).

5.2.11. 2-Chloro methyl-3-(p-acetyl phenyl)-3*H*-quinazoline-4-one (2k)

2-(2-Chloro-acetyl amino) benzoic acid **3** (20.0 g, 0.09 mol) was reacted with 4-amino acetophenone (12.7 g, 0.09 mol) using the same procedure described for **2a** to afford the title compound a light yellow solid. Yield: 36.6%, mp 180–181 °C, R_f: 0.54, LC–MS (*m/z*): 313 (M+1).

5.3. Synthesis of phenylthioacetic acid morpholide intermediates (6)[23]

5.3.1. 1-Morpholin-4-yl-2-phenyl-ethanethione (6a)

A 100 mL round-bottomed flask was charged with 24.0 g (0.20 mol) of acetophenone, 1.0 g of *p*-toluenesulfonic acid monohydrate, 36.0 g (0.41 mol) of morpholine, and 6.40 g (0.20 mol) of sulfur. The flask was equipped with a reflux condenser and was heated to reflux for 3 h. The resulting reddish brown solution was poured into 100 mL of stirred hot methanol. The resulting precipitates were filtered and washed twice with ice-cold methanol (0–5 °C, 50 mL). The crude was re-crystallized by methanol and water mixture to afford desire compounds (21.9 g) as a yellow solid. Yield: 50%, mp 78–79 °C, LC–MS (*m/z*): 222 (M+1).

5.3.2. 2-(4-Chloro-phenyl)-1-morpholin-4-yl-ethanethione (6b)

1-(4-Chloro-phenyl)-ethanone (10.0 g, 0.07 mol) was reacted with morpholine (0.13 mol) using the same procedure described for **6a** to afford the title compound (10.0 g) as a yellow solid. Yield: 60.15%, mp 175–177 °C, LC–MS (*m/z*): 256, 258 (M+1, M+2).

5.3.3. *N*-[4-(2-Morpholin-4-yl-2-thioxo-ethyl)-phenyl]-acetamide (6c)

N-(4-Acetyl-phenyl)-acetamide (10.0 g, 0.06 mol) was reacted with morpholine (0.13 mol) using the same procedure described for **6a** to afford the title compound (8.0 g) as a yellow solid. Yield: 50.72%, mp 210–212 °C, LC–MS (*m/z*): 279 (M+1).

5.3.4. 2-(4-Methanesulfonyl-phenyl)-1-morpholin-4-yl-ethanethione (6d)

1-(4-Methanesulfonyl-phenyl)-ethanone (10.0 g, 0.051 mol) was reacted with morpholine (0.13 mol) using the same procedure described for **6a** to afford the title compound (9.0 g) as a yellow solid. Yield: 60%, mp 198–199 °C, LC–MS (*m/z*): 300 (M+1).

5.3.5. 2-(4-Methylsulfanyl-phenyl)-1-morpholin-4-yl-ethanethione (6e)

1-(4-Methylsulfanyl-phenyl)-ethanone (10.0 g, 0.08 mol) was reacted with morpholine (0.13 mol) using the same procedure described for **6a** to afford the title compound (8.0 g) as a yellow solid. Yield: 48.39%, mp 158–160 °C, LC–MS (*m/z*): 268 (M+1).

5.3.6. 1-Morpholin-4-yl-2-pyridine-4-yl-ethanethione (6f)

1-Pyridine-4-yl-ethanone (10.0 g, 0.08 mol) was reacted with morpholine (0.13 mol) using the same procedure described for **6a** to afford the title compound (7.9 g) as a yellow solid. Yield: 47.4%, mp 162–163 °C, LC–MS (*m/z*): 223 (M+1).

5.4. 2-Aryl-1,3-dimorpholin-4-yl-propenethiones derivatives (4)

5.4.1. 1, 3-Di-morpholin-4-yl-2-phenyl-propenethione (5a)

A mixture of 8.0 g of phenyl thioacetic acid morpholide **6a**, 8.0 g of morpholine and 20.0 g of triethylorthoformate was refluxed for 8 hours in an oil bath. Excess of triethylorthoformate was removed from reaction in vacuo. The residue was dissolved in mixture of warm chloroform: methanol (10 mL: 50 mL) and then cooled to 0 °C. The product separated as fine yellow crystals. The product was filtered, washed with methanol and dried to afford 6.1 g (56.0%). mp 115 °C, M.F.: $C_{17}H_{22}N_2O_2S$, LC–MS (*m/z*): 319 (M+1).

5.4.2. 2-(4-Chloro-phenyl)-1,3-di-morpholin-4-yl-propenethione (5b)

2-(4-Chloro-phenyl)-1-morpholin-4-yl-ethanethione **6b** (5.0 g, 0.02 mol) was reacted with morpholine and triethylorthoformate using the same procedure described for **5a** to afford title compound (5.40 g) as a yellow solid. Yield: 72%, mp 134–135 °C, M.F.: $C_{17}H_{21}N_2O_2SCl$, LC–MS (*m/z*): 353, 354 (M+1, M+2).

5.4.3. *N*-{4-[1-(Morpholine-4-carbothioyl)-2-morpholin-4-yl-vinyl]-phenyl}-acetamide (5c)

N-[4-(2-Morpholin-4-yl-2-thioxo-ethyl)-phenyl]-acetamide **6c** (5.0 g, 0.02 mol) was reacted with morpholine and triethylorthoformate using the same procedure described for **5a** to afford title compound (4.0 g) as a yellow solid. Yield: 59.26%, mp 145–147 °C, Molecular Formula $C_{19}H_{25}N_3O_3S$, LC–MS (*m/z*): 376 (M+1), ^{1}H NMR (300 MHz, DMSO-d_6), δ 2.02 (s, 3H), 3.19–3.38 (m, 8H), 4.19–4.50 (m, 8H), 4.67 (s, 1H), 6.36 (s, 1H), 7.20–7.34 (m, 2H), 7.70–7.80 (m, 2H).

5.4.4. 2-(4-Methanesulfonyl-phenyl)-1,3-di-morpholin-4-yl-propenethione (5d)

2-(4-Methanesulfonyl-phenyl)-1-morpholin-4-yl-ethanethione **6d** (5.0 g, 0.013 mol) was reacted with morpholine and triethylorthoformate using the same procedure described for **5a** to afford title compound (5.0 g) as a yellow solid. Yield: 75.5%, mp 167–169 °C, Molecular Formula $C_{18}H_{24}N_2O_4S_2$, LC–MS (*m/z*): 397 (M+1). ^{1}H NMR(300 MHz, DMSO-d_6), δ 3.05 (s, 3H), 3.29–3.62 (m, 8H), 4.15–4.44 (m, 8H), 6.5 (s, 1H), 7.27–7.49 (m, 2H), 7.65–7.76 (m, 2H).

5.4.5. 3-(4-Morpholino)-2-(4-methylmercapto-phenyl)-thioacrylic acid morpholide (5e)

2-(4-Methylsulfanyl-phenyl)-1-morpholin-4-yl-ethanethione **6e** (10.0 g, 0.04 mol) was reacted with morpholine and triethylorthoformate using the same procedure described for **5a** to afford title compound (5.8 g) as a yellow solid. Yield: 42%, mp 164–165 °C, M.F.: $C_{18}H_{24}N_2O_2S_2$, LC–MS (*m/z*): 365 (M+1).

5.4.6. 1,3-Di-morpholin-4-yl-2-pyridin-4-yl-propenthione (5f)

1-Morpholin-4-yl-2-pyridine-4-yl-ethanethione **6f** (5.0 g, 0.02 mol) was reacted with morpholine and triethylorthoformate using the same procedure described for **5a** to afford title compound (3.0 g) as a yellow solid. Yield: 39.68%, mp 122–125 °C, Molecular Formula $C_{16}H_{21}N_3O_2S$, LC–MS (*m/z*): 320 (M+1), ^{1}H NMR(300 MHz, DMSO-d_6), δ 3.19–3.59 (m, 8H), 3.7–4.2 (m, 8H), 6.09 (s, 1H), 7.01–7.32 (m, 4H).

5.5. Synthesis of 2-thiophen-5-yl-3*H*-quinazolin-4-one analogues

5.5.1. 2-(5-Morpholin-4-yl-4-phenyl-thiophene-2-yl)-3-phenyl-3*H*-quinazolin-4-one (9aa)

1,3-Di-morpholin-4-yl-2-phenyl-propenethione **5a** (1.0 g, 0.003 mol) was taken in 5 mL of acetonitrile and heated to 78 °C. At this temperature 2-chloro methyl-3-(phenyl)-3*H*-quinazoline-4-one **2a** (0.86 g, 0.003 mol) in 5 mL acetonitrile was added into it and this reaction mixture was heated at this temperature for 6–8 h. Then it was cooled to rt and solvent was removed in vacuo. The solid residue obtained was crystallized using methanol to afford title compound as yellow solid. Yield 58.0%, mp 218–220 °C, R_f: 0.53, IR (KBr, cm^{-1}); 3021, 1691,1539, 1308, 1281, 758, Molcular Formula $C_{28}H_{23}N_3O_2S$, LC–MS (*m/z*): 466 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.96–3.0 (m, 4H), 3.72–3.74 (m, 4H), 5.96 (s, 1H), 7.22–7.78 (m, 13H), 8.27–8.30 (m, 1H), Elemental Anal. Calcd: C, 72.23; H, 4.98; N, 9.03. Found: C, 72.22; H, 4.97; N, 8.45.

5.5.2. 2-[4-(4-Chloro-phenyl)-5-morpholin-4-yl-thiophen-2-yl]-3-phenyl-3*H*-quinazolin-4-one (9ab)

The title compound was prepared from 2-(4-chloro-phenyl)-1,3-di-morpholin-4-yl-propenethione **5b** (1.1 g, 0.003 mol) and 2-chloro methyl-3-(phenyl)-3*H*-quinazoline-4-one **2a** (0.81 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 51.0%, mp 229–230 °C, R_f: 0.52, IR (KBr, cm^{-1}); 3010, 1691, 1538, 1309, 1282, 758, Molcular Formula $C_{28}H_{22}N_3O_2SCl$, LC–MS (*m/z*): 500 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.93–2.96 (m, 4H), 3.70–3.73 (m, 4H), 5.91 (s, 1H), 7.20–7.79 (m, 12H), 8.25–8.28 (m, 1H).

5.5.3. *N*-(4-(2-Morpholino-5-(4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)thiophen-3-yl)phenyl)acetamide (9ac)

The title compound was prepared from *N*-{4-[1-(Morpholine-4-carbothioyl)-2-morpholin-4-yl-vinyl]-phenyl}-acetamide **5c** (1.0 gm, 0.003 mol) and 2-chloro methyl-3-(phenyl)-3*H*-quinazoline-4-one **2a** (0.70 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 57.0%, mp >275 °C, R_f: 0.34, IR (KBr, cm^{-1}): 3186, 3066, 1689, 1535, 1314, 1281, 758, Molcular Formula $C_{30}H_{26}N_4O_3S$, LC–MS (*m/z*): 523 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.18 (s, 3H), 2.94–2.97 (m, 4H), 3.70–3.73 (m, 4H), 5.94 (s, 1H), 7.22–7.78 (m, 13H), 8.27–8.3 (m, 1H).

5.5.4. 2-[4-(4-Methanesulfonyl-phenyl)-5-morpholin-4-yl-thiophen-2-yl]-3-phenyl-3*H*-quinazolin-4-one (9ad)

The title compound was prepared from 2-(4-methanesulfonyl-phenyl)-1, 3-di-morpholin-4-yl-propenethione **5d** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(phenyl)-3*H*-quinazoline-4-one **2a** (0.68 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 58.4%, mp >275 °C, R_f: 0.53, IR (KBr, cm^{-1}): 3212, 3036, 1681, 1608, 1448, 1326, 1275, 770, Molcular Formula $C_{29}H_{25}N_3O_4S_2$, LC–MS (*m/z*): 544 (M+1), ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.94–2.98 (m, 4H), 3.12 (s, 3H), 3.78–3.80 (m, 4H), 5.91 (s, 1H), 7.23–7.89 (m, 12H), 8.31–8.33 (m, 1H).

5.5.5. 2-[4-(4-Methylsulfanyl-phenyl)-5-morpholin-4-yl-thiphen-2-yl]-3-phenyl-3*H*-quinazolin-4-one (9ae)

The title compound was prepared from 3-(4-morpholino)-2-(4-methylmercapto-phenyl)-thioacrylic acid morpholide **5e** (0.98 g, 0.003 mol) and 2-chloro methyl-3-(phenyl)-3*H*-quinazoline-4-one **2a** (0.73 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 46%, mp 218–220 °C, R_f: 0.53, IR (KBr, cm^{-1}): 3042, 1688, 1438, 1281, 762, Molcular Formula $C_{29}H_{25}N_3O_2S_2$, LC–MS (*m/z*): 526 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.49 (s, 3H), 2.94–2.97 (m, 4H), 3.70–3.73 (m, 4H), 5.89 (s, 1H), 7.07–7.78 (m, 12H), 8.27–8.3 (m, 1H).

5.5.6. 2-(5-Morpholin-4-yl-4-pyridin-4-yl-thiophen-2-yl)-3-phenyl-3*H*-quinazolin-4-one (9af)

(*Z*)-1,3-Di-morpholin-4-yl-2-pyridin-4-yl-propenethione **5f**(1.0 g, 0.003 mol) was taken in dimethylformamide and 2-chloromethyl-3-phenyl-3*H*-quinazoline-4-one **2a** (0.84 g, 0.003 mol) in dimethylformamide was added into it at rt and reaction mass was stirred on room temperature for 24 h and then was poured into ice-cold water and stirred well for 10 min and then filtered to remove water. The residue was dried and recrystallised with methanol to get title compound as a yellow solid. Yield: 38.0%, mp 230–231 °C, R_f: 0.34, IR (KBr, cm^{-1}): 3053, 2908, 1689,1543, 1307,1281,758, 710, Molcular Formula $C_{27}H_{22}N_4O_2S_2$, LC–MS (*m/z*): 467 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.94–2.97 (m, 4H), 3.72–3.75 (m, 4H), 5.92 (s, 1H), 7.21–7.80 (m, 10H), 8.26–8.28 (m, 1H), 8.42–8.48 (m, 2H).

5.5.7. 2-(5-Morpholin-4-yl-4-phenyl-thiophene-2-yl)-3-*o*-tolyl-3*H*-quinazolin-4-one (9ag)

The title compound was prepared from 1,3-di-morpholin-4-yl-2-phenyl-propenethione **5a** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(2-methyl-phenyl)-3*H*-quinazoline-4-one **2b** (0.9 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 54.0%, mp 187–188 °C, R_f: 0.60, IR (KBr, cm^{-1}): 3045, 1687, 1445, 1282, 790, 750, Molecular Formula $C_{29}H_{25}N_3O_2S$, LC–MS (*m/z*): 480 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.15 (s, 3H), 2.95–2.98 (m, 4H), 3.70–3.73 (m, 4H), 5.97 (s, 1H), 7.17–7.76 (m, 12H), 8.26–8.28 (d, 1H).

5.5.8. 2-[4-(4-Chloro-phenyl)-5-morpholin-4-yl-thiophen-2-yl]-3-*o*-tolyl-3*H*-quinazolin-4-one (9ah)

The title compound was prepared from 2-(4-chloro-phenyl)-1,3-di-morpholin-4-yl-propenethione **5b** (1.1 g, 0.003 mol) and 2-chloro methyl-3-(2-methyl-phenyl)-3*H*-quinazoline-4-one **2b** (0.9 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 53.0%, mp 228–230 °C, R_f: 0.62, IR (KBr, cm^{-1}): 3044, 1670, 1440, 1275, 769, Molcular Formula $C_{29}H_{24}N_3O_2SCl$, LC–MS (*m/z*): 514 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm] = 2.42 (s, 3H), 2.93–2.96 (m, 4H), 3.71–3.74 (m, 4H), 5.91 (s, 1H), 7.16–7.87 (m, 11H), 8.26–8.28 (d, 1H).

5.5.9. 2-[4-(4-Methanesulfonyl-phenyl)-5-morpholin-4-yl-thiophen-2-yl]-3-*o*-tolyl-3*H*-quinazolin-4-one (9ai)

The title compound was prepared from 2-(4-methanesulfonyl-phenyl)-1,3-di-morpholin-4-yl-propenethione **5d** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(2-methyl-phenyl)-*3H*-quinazoline-4-one (**2b**) (0.74 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 56.0%, mp 255–258 °C, R_f: 0.42, IR (KBr, cm^{-1}): 3218, 3047, 1692, 1610, 1451, 1320, 1279, 771, Molcular Formula $C_{30}H_{27}N_3O_4S_2$, LC–MS (*m/z*): 558 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.1–2.2 (s, 3H), 2.97–3.0 (m, 4H), 3.1 (s, 3H) , 3.75–3.78 (m, 4H), 5.93 (s, 1H), 7.28–7.88 (m, 11H), 8.29–8.32 (m, 1H).

5.5.10. 2-[4-(4-Methylsulfanyl-phenyl)-5-morpholin-4-yl-thiophen-2-yl]-3-*o*-tolyl-3*H*-quinazolin-4-one (9aj)

The title compound was prepared from 3-(4-morpholino)-2-(4-methylmercapto-phenyl)-thioacrylic acid morpholide **5e** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(2-methyl-phenyl)-*3H*-quinazoline-4-one **2b** (0.78 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid.

Yield 56.0%, mp 255–258 °C, R_f: 0.42, IR (KBr, cm^{-1}): 3042, 1688, 1441, 1281, 761, Molcular Formula $C_{30}H_{27}N_3O_4S_2$, LC–MS (*m/z*): 526 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 2.12 (s, 3H), 2.32 (s ,3H), 2.95–2.98 (m, 4H), 3.71–3.74 (m, 4H), 5.91 (s, 1H), 7.08–7.76 (m, 11H), 8.26–8.29 (m, 1H).

5.5.11. 2-(5-Morpholin-4-yl-4-pyridin-4-yl-thiophen-2-yl)-3-*o*-tolyl-3*H*-quinazolin-4-one (9ak)

The title compound was prepared from 1,3-di-morpholin-4-yl-2-pyridin-4-yl-propenthione **5f** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(2-methyl-phenyl)-*3H*-quinazoline-4-one **2b** (1.0 g, 0.003 mol) using the same procedure described for **9af** to afford title compound as a yellow solid. Yield: 39.0%, mp 208–210 °C, R_f: 0.38, IR (KBr, cm^{-1}): 3021, 2911, 1686,1538, 1305,1240,750, Molcular Formula $C_{28}H_{24}N_4O_2S$, LC–MS (*m/z*): 481(M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.14 (s,3H), 2.95–2.98 (m, 4H), 3.73–3.76 (m, 4H), 5.9 (s, 1H), 7.2–7.8 (m, 9H), 8.27–8.30 (dd, 1H, J = 8.7, 1.2 Hz), 8.30–8.44 (m, 2H).

5.5.12. 2-[4-(4-Chloro-phenyl)-5-morpholin-4-yl-thiophen-2-yl]-3-*m*-tolyl-3*H*-quinazolin-4-one (9al)

The title compound was prepared from 2-(4-chloro-phenyl)-1,3-di-morpholin-4-yl-propenethione **5b** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(3-methyl-phenyl)-*3H*-quinazoline-4-one (**2c**) (0.78 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 56.0%, mp 228–230 °C, R_f: 0.57, IR (KBr, cm^{-1}): 3040, 1680, 1445, 1285, 759, Molcular Formula $C_{29}H_{24}N_3O_2SCl$, LC–MS (*m/z*): 514.5 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.42 (s, 3H), 2.93–2.96 (m, 4H), 3.71–3.74 (m, 4H), 5.91 (s, 1H), 7.16–7.87 (m, 11H), 8.26–8.28 (d, 1H).

5.5.13. *N*-{4-[2-Morpholin-4-yl-5-(4-oxo-3-*m*-tolyl-3–4-dihidro-quinazolin-2-yl)-thiophe-ne-3-yl]-phenyl}-acetamide (9am)

The title compound was prepared from *N*-{4-[1-(morpholine-4-carbothioyl)-2-morpholin-4-yl-vinyl]-phenyl}-acetamide **5c** (0.98 g, 0.003 mol) and 2-Chloro methyl-3-(3-methyl-phenyl)-*3H*-quinazoline-4-one **2c** (0.74 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as yellow solid. Yield 52%, mp >275 °C, R_f: 0.61, IR (KBr, cm^{-1}): 3216, 1682, 1455, 1275, 763, Molcular Formula $C_{31}H_{28}N_4O_3S$, LC–MS (*m/z*): 537.5 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.18 (s, 3H), 2.42 (s, 3H), 2.94–2.97 (m, 4H), 3.71–3.74 (m, 4H), 5.94 (s, 1H), 7.12–7.84 (m, 12H), 8.24–8.27 (d, 1H).

5.5.14. 2-[4-(4-Methanesulfonyl-phenyl)-5-morpholin-4-yl-thiophen-2-yl]-3-*m*-tolyl-3H-quinazolin-4-one (9an)

The title compound was prepared from 2-(4-methanesulfonyl-phenyl)-1,3-di-morpholin-4-yl-propenethione **5d** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(3-methyl-phenyl)-*3H*-quinazoline-4-one **2c** (0.71 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 54.5%, mp 208–210 °C, R_f: 0.62, IR (KBr, cm^{-1}): 3066, 1687, 1456, 1302, 1280, 760, Molcular Formula $C_{30}H_{27}N_3O_4S_2$, LC–MS (*m/z*): 558 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.18 (s, 3H), 2.93–2.99 (m, 4H), 3.71–3.75 (m, 4H), 5.99 (s, 1H), 7.10–7.74 (m, 12H), 8.26–8.28 (d, 1H).

5.5.15. 2-[4-(4-Methylsulfanyl-phenyl)-5-morpholin-4-yl-thiphen-2-yl]-3-*m*-tolyl-3*H*-quinazolin-4-one (9ao)

The title compound was prepared from 3-(4-morpholino)-2-(4-methylmercapto-phenyl)-thioacrylic acid morpholide **5e** (1.1 g, 0.003 mol) and 2-chloro methyl-3-(3-methyl-phenyl)-*3H*-quinazoline-4-one **2c** (0.88 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 43%, mp 220–221 °C, R_f: 0.59, IR (KBr, cm^{-1}): 3010, 1685, 1439, 1276, 762, Molcular Formula $C_{30}H_{27}N_3O_2S_2$, LC–MS (*m/z*): 526.5 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.33 (s, 3H), 2.43 (s, 3H), 2.95–2.98 (m, 4H), 3.71–3.74 (m, 4H), 5.97 (s, 1H), 7.08–7.78 (m, 12H), 8.25–8.27 (d, 1H).

5.5.16. 2-(5-Morpholin-4-yl-4-pyridin-4-yl-thiophen-2-yl)-3-*m*-tolyl-3*H*-quinazolin-4-one (9ap)

The title compound was prepared from 1,3-di-morpholin-4-yl-2-pyridin-4-yl-propenthione **5f** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(3-methyl-phenyl)-*3H*-quinazoline-4-one **2c** (0.88 g, 0.003 mol) using the same procedure described for **9af** to afford title compound as a yellow solid. Yield: 36%, mp 230–232 °C, R_f: 0.57, IR (KBr, cm^{-1}): 3047, 2929, 1691,1534, 1324,1252,710, Molcular Formula $C_{28}H_{24}N_4O_2S$, LC–MS (*m/z*): 481 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.43 (s, 3H), 2.95–2.98 (m, 4H), 3.73–3.76 (m, 4H), 5.96 (s, 1H), 7.17–7.77 (m, 9H), 8.26–8.29 (dd, 1H, J = 8.1, 1.2 Hz), 8.43–8.48 (m, 2H).

5.5.17. 2-(5-Morpholin-4-yl-4-phenyl-thiophene-2-yl)-3-*p*-tolyl-*3H*-quinazolin-4-one (9aq)

The title compound was prepared from 1,3-di-morpholin-4-yl-2-phenyl-propenethione **5a** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(4-methyl-phenyl)-*3H*-quinazoline-4-one **2d** (0.9 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 54.0%, mp 207–209 °C, R_f: 0.63, IR (KBr, cm^{-1}): 3054, 1682, 1441, 1284, 770, Molcular Formula $C_{29}H_{25}N_3O_2S$, LC–MS (*m/z*): 481 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.48 (s, 3H), 2.95–2.98 (m, 4H), 3.71–3.74 (m, 4H), 5.99 (s, 1H), 7.15–7.74 (m, 12H), 8.25–8.27 (d, 1H).

5.5.18. 2-[4-(4-Chloro-phenyl)-5-morpholin-4-yl-thiophen-2-yl]-3-*p*-tolyl-3*H*-quinazolin-4-one (9ar)

The title compound was prepared from 2-(4-chloro-phenyl)-1,3-di-morpholin-4-yl-propenethione **5b** (1.1 g, 0.003 mol) and 2-chloro methyl-3-(4-methyl-phenyl)-*3H*-quinazoline-4-one **2d** (0.9 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 51.0%, mp 228–230 °C, R_f: 0.63, IR (KBr, cm^{-1}): 3035, 2965, 1685, 1435, 1275, 786, Molcular Formula $C_{29}H_{24}N_3O_2SCl$, LC–MS (*m/z*): 514.5 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.14 (s, 3H), 2.93–2.96 (m, 4H), 3.71–3.75 (m, 4H), 5.98 (s, 1H), 7.17–7.76 (m, 11H), 8.26–8.28 (d, 1H).

5.5.19. *N*-{4-[2-Morpholin-4-yl-5-(4-oxo-3-*p*-tolyl-3-,4-dihidro-quinazolin-2-yl-thiophene-3-yl]-phenyl}-acetamide (9as)

The title compound was prepared from *N*-{4-[1-(morpholine-4-carbothioyl)-2-morpholin-4-yl-vinyl]-phenyl}-acetamide **5c** (0.97 g, 0.003 mol) and 2-chloro methyl-3-(4-methyl-phenyl)-*3H*-quinazoline-4-one **2d** (0.73 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid.

Yield: 40.0%, mp >275 °C, R_f: 0.61, IR (KBr, cm^{-1}): 3265, 3052, 2895, 1685, 1600, 1456, 1275, 760, Molcular Formula $C_{31}H_{28}N_4O_3S$, LC–MS (*m/z*): 537.52 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.18 (s, 3H), 2.48 (s, 3H), 2.94–2.97 (m, 4H), 3.71–3.74 (m, 4H), 5.89 (s, 1H), 7.16–7.78 (m, 12H), 8.25–8.27 (d, 1H).

5.5.20. 2-[4-(4-Methanesulfonyl-phenyl)-5-morpholin-4-yl-thiophen-2-yl]-3-*p*-tolyl-*3H*-quinazolin-4-one (9at)

The title compound was prepared from 2-(4-methanesulfonyl-phenyl)-1, 3-di-morpholin-4-yl-propenethione **5d** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(4-methyl-phenyl)-*3H*-quinazoline-4-one **2d** (0.71 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 57.0%, mp 265–266 °C, R_f: 0.57, IR (KBr, cm^{-1}): 3066, 1680, 1610, 1438, 1309, 1281, 765, Molcular Formula $C_{30}H_{27}N_3O_4S_2$, LC–MS (*m/z*): 558 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.51 (s, 3H), 2.96–2.99 (m, 4H), 3.08 (s, 3H) , 3.74–3.77 (m, 4H), 5.91 (s, 1H), 7.26–7.86 (m, 11H), 8.27–8.29 (m, 1H).

5.5.21. 2-[4-(4-Methylsulfanyl-phenyl)-5-morpholin-4-yl-thiphen-2-yl]-3-*p*-tolyl-3*H*-quinazolin-4-one (9au)

The title compound was prepared from 3-(4-morpholino)-2-(4-methylmercapto-phenyl)-thioacrylic acid morpholide **5e** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(4-methyl-phenyl)-*3H*-quinazoline-4-one **2d** (0.71 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 42.0%, mp 238–240 °C, R_f 0.59, IR (KBr, cm^{-1}): 3025, 2897, 1684, 1598, 1444, 1287, 790, Molcular Formula $C_{30}H_{27}N_3O_2S_2$, LC–MS (*m/z*): 527 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.13 (s, 3H), 2.30 (s, 3H), 2.93–2.987(m, 4H), 3.71–3.75 (m, 4H), 5.98 (s, 1H), 7.18–7.76 (m, 11H), 8.26–8.28 (m, 1H).

5.5.22. 2-(5-Morpholin-4-yl-4-pyridin-4-yl-thiophen-2-yl)-3-*p*-tolyl-*3H*-quinazolin-4-one (9av)

The title compound was prepared from 1,3-di-morpholin-4-yl-2-pyridin-4-yl-propenthione **5f** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(4-methyl-phenyl)-*3H*-quinazoline-4-one **2d** (0.88 g, 0.003 mol) using the same procedure described for **9af** to afford title compound as a yellow solid. Yield: 39.0%, mp 220–221 °C, R_f: 0.52, IR (KBr, cm^{-1}): 3011, 2897, 1679, 1540, 1321, 1270, 750, Molcular Formula $C_{28}H_{24}N_4O_2S$, LC–MS (*m/z*): 481 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.25 (s, 3H), 2.95–2.97 (m, 4H), 3.73–3.76 (m, 4H), 5.91 (s, 1H), 7.25–7.76 (m, 9H), 8.26–8.28 (m, 1H), 8.44–8.51 (m, 2H).

5.5.23. 3-(2-Methoxy-phenyl)-2-(5-morpholin-4-yl-4-phenyl-thiophen-2-yl)-3*H*-quinazolin-4-one (9aw)

The title compound was prepared from 1,3-di-morpholin-4-yl-2-phenyl-propenethione **5a** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(2-methoxy-phenyl)-*3H*-quinazoline-4-one **2e** (0.96 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 43.0%, mp 152–154 °C, R_f: 0.56, IR (KBr, cm^{-1}): 3016, 2952, 1680, 1453, 1278, 761, Molcular Formula $C_{29}H_{25}N_3O_3S$, LC–MS (*m/z*): 496.5 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.93–2.94 (m, 4H), 3.70–3.72 (m, 7H), 6.21 (s, 1H), 7.04–7.77 (m, 12H), 8.23–8.28 (d, 1H).

5.5.24. 2-[4-(4-Methanesulphonyl-phenyl)-5-morpholin-4-yl-thiophen-2-yl]-3-(2-methoxy-phenyl)-3*H*-quinazolin-4-one (9ax)

The title compound was prepared from 2-(4-methanesulfonyl-phenyl)-1,3-di-morpholin-4-yl-propenethione **5d** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(2-methoxy-phenyl)-3*H*-quinazoline-4-one **2e** (0.75 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 55.0%, mp 222–223 °C, R_f: 0.62, IR (KBr, cm^{-1}): 3056, 1682, 1611, 1434, 1314, 1278, 761, Molcular Formula $C_{30}H_{27}N_3O_5S_2$, LC–MS (*m/z*): 574 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.89–2.97 (m, 4H), 3.2 (s, 3H) , 3.72–3.78 (m, 4H), 3.91 (s, 3H), 5.93 (s, 1H), 7.27–7.89 (m, 11H), 8.28–8.30 (m, 1H).

5.5.25. 3-(2-Methoxy-phenyl)-2-(5-morpholin-4-yl-4-pyridin-4-yl-thiophen-2-yl)-3*H*-quinazolin-4-one (9ay)

The title compound was prepared from 1,3-di-morpholin-4-yl-2-pyridin-4-yl-propenthione **5f** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(2-methoxy-phenyl)-3*H*-quinazoline-4-one **2e** (0.93 g, 0.003 mol) using the same procedure described for **9af** to afford title compound as a yellow solid. Yield: 41.0%, mp 204–205 °C, R_f: 0.58, Molcular Formula $C_{28}H_{24}N_4O_3S$, LC–MS (*m/z*): 497 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.94–2.98 (m, 4H), 3.71–3.77 (m, 4H), 3.92 (s, 3H), 5.91 (s, 1H), 7.26–7.79 (m, 9H), 8.27–8.29 (m, 1H), 8.42–8.48 (m, 2H).

5.5.26. 2-[4-(4-Methanesulphonyl-phenyl)-5-morpholin-4-yl-thiophen-2-yl]-3-(3-methoxy-phenyl)-3*H*-quinazolin-4-one (9az)

The title compound was prepared from 2-(4-methanesulfonyl-phenyl)-1,3-di-morpholin-4-yl-propenethione **5d** (1.0 g, 0.003 mol) 2-chloro methyl-3-(3-methoxy-phenyl)-3*H*-quinazoline-4-one **2f** (0.75 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 57.0%, mp 203–205 °C, R_f: 0.62, IR (KBr, cm^{-1}): 3059, 1680, 1606, 1438, 1313, 1279, 763, Molcular Formula $C_{30}H_{27}N_3O_5S_2$, LC–MS (*m/z*): 574 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.90–2.98 (m, 4H), 3.25 (s, 3H) , 3.74–3.79 (m, 4H), 3.93 (s, 3H), 5.91 (s, 1H), 7.26–7.90 (m, 11H), 8.27–8.31 (m, 1H).

5.5.27. 2-[4-(4-Methanesulphonyl-phenyl)-5-morpholin-4-yl-thiophen-2-yl]-3-(4-methoxy-phenyl)-3*H*-quinazolin-4-one (9ba)

The title compound was prepared from 2-(4-methanesulfonyl-phenyl)-1,3-di-morpholin-4-yl-propenethione **5d** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(4-methoxy-phenyl)-3*H*-quinazoline-4-one **2g** (0.75 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield: 56.0%, mp 240–243 °C, R_f: 0.49, IR (KBr, cm^{-1}): 3053, 2935, 1680, 1452, 1320, 1275, 765, Molcular Formula $C_{30}H_{27}N_3O_5S$, LC–MS (*m/z*): 574 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.97–3.0 (m, 4H), 3.1 (s, 3H), 3.76–3.79 (m, 4H), 3.93 (s, 3H), 6.1(s, 1H), 7.1–7.8 (m, 11H), 8.27–8.30 (dd, 1H, *J* = 9.0, 1.0 Hz).

5.5.28. 3-(2-Chloro-phenyl)-2-(5-morpholin-4-yl-4-pyridin-4-yl-thiophen-2-yl)-3H-quinazolin-4-one (9bb)

The title compound was prepared from 1,3-di-morpholin-4-yl-2-pyridin-4-yl-propenthione **5f** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(2-chloro-phenyl)-3*H*-quinazoline-4-one **2h** (0.94 g, 0.003 mol) using the same procedure described for **9af** to afford title compound as a yellow solid. Yield: 39%, mp 179–180 °C, R_f: 0.58, Molecular Formula $C_{27}H_{21}ClN_4O_2S$, LC–MS (*m/z*): 501 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.92–2.99 (m, 4H), 3.71–3.75 (m, 4H), 6.11 (s, 1H), 7.27–7.80 (m, 9H), 8.24–8.29 (m, 1H), 8.43–8.46 (m, 2H).

5.5.29. 3-(4-Chloro-phenyl)-2-(5-morpholin-4-yl-4-phenyl-thiophen-2-yl)-3*H*-quinazolin-4-one (9bc)

The title compound was prepared from 1,3-di-morpholin-4-yl-2-phenyl-propenethione **5a** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(4-chloro-phenyl)-3*H*-quinazoline-4-one **2j** (0.97 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 54.0%, mp 223–224 °C, R_f: 0.68, IR (KBr, cm^{-1}): 3054, 1682, 1600, 1441, 1285, 772, Molcular Formula $C_{28}H_{22}ClN_3O_2S$, LC–MS (*m/z*): 500 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.93–2.98 (m, 4H), 3.71–3.75 (m, 4H), 5.96 (s, 1H), 7.12–7.72 (m, 12H), 8.22–8.25 (d, 1H).

5.5.30. 3-(4-Chloro-phenyl)-2-[4-(4-chloro-phenyl)-5-morpholin-4-yl-thiophen-2-yl]-3*H*-quinazolin-4-one (9bd)

The title compound was prepared from 2-(4-chloro-phenyl)-1,3-di-morpholin-4-yl-propenethione **5b** (1.1 g, 0.003 mol) and 2-chloro methyl-3-(4-chloro-phenyl)-3*H*-quinazoline-4-one **2j** (0.97 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 55.0%, mp 240–243 °C, R_f: 0.68, IR (KBr, cm^{-1}): 3032, 2967, 1682, 1432, 1276, 790, Molcular Formula $C_{28}H_{21}Cl_2N_3O_2S$, LC–MS (*m/z*): 514.5 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.91–2.95 (m, 4H), 3.70–3.74 (m, 4H), 5.99 (s, 1H), 7.21–7.77 (m, 11H), 8.29–8.31 (d, 1H).

5.5.31. *N*-(4-{5-[3-(4-Chloro-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl]-2-morpholin-4-yl-thiophene-3-yl}-acetamide (9be)

The title compound was prepared from *N*-{4-[1-(morpholine-4-carbothioyl)-2-morpholin-4-yl-vinyl]-phenyl}-acetamide **5c** (1.2 g, 0.003 mol) and 2-chloro methyl-3-(4-chloro-phenyl)-3*H*-quinazoline-4-one **2j** (0.97 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 54.0%, mp >275 °C, R_f: 0.29, IR (KBr, cm^{-1}): 3245, 3042, 1690, 1610, 1445, 1280, 761, Molcular Formula $C_{30}H_{25}ClN_4O_3S$, LC–MS (*m/z*): 557 (M+1),^{1}H NMR (300 MHz, $CDCl_3$) δ 2.18 (s, 3H), 2.94–2.98 (m, 4H), 3.71–3.74 (m, 4H), 5.99 (s, 1H), 7.25–8.26 (m, 13H).

5.5.32. 2-[4-(4-Methanesulphonyl-phenyl)-5-morpholin-4-yl-thiophen-2-yl]-3-(4-chloro-phenyl)-3*H* quinazolin-4-one (9bf)

The title compound was prepared from 2-(4-methanesulfonyl-phenyl)-1,3-di-morpholin-4-yl-propenethione **5d** (1.3 g, 0.003 mol) and 2-chloro methyl-3-(4-chloro-phenyl)-3*H*-quinazoline-4-one **2j** (0.97 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 57.0%, mp >275 °C, R_f: 0.51, , IR (KBr, cm^{-1}): 3021, 1684, 1435, 1311, 1281, 760, Molcular Formula $C_{29}H_{24}N_3O_4SCl$, LC–MS (*m/z*): 578 (M+1), ^{1}H NMR (300 MHz, DMSO-d_6), δ 2.98–3.04 (m, 4H), 3.10 (s, 3H), 3.76–3.79 (m, 4H), 6.04 (s, 1H), 7.28–7.92 (m, 11H), 8.26–8.29 (dd,1H, *J* = 9.3, 1.2 Hz), Elemental Anal. Calcd: C, 60.25; H, 4.18; N, 7.27. Found: C, 60.26; H, 4.09; N, 7.26.

5.5.33. 3-(4-Chloro-phenyl)-2-(5-morpholin-4-yl-4-pyridin-4-yl-thiophen-2-yl)-3*H*-quinazolin-4-one (9bg)

The title compound was prepared from 1,3-di-morpholin-4-yl-2-pyridin-4-yl-propenthione **5f** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(4-chloro-phenyl)-3*H*-quinazoline-4-one **2j** (0.94 g, 0.003 mol) using the same procedure described for **9af** to afford title compound as a yellow solid. Yield: 39.0%, mp 195–197 °C , R_f: 0.57, Molcular Formula $C_{27}H_{21}ClN_4O_2S$, LC–MS (*m/z*): 501 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.94–2.98 (m, 4H), 3.73–3.76 (m, 4H), 6.12 (s, 1H), 7.26–7.78 (m, 9H), 8.25–8.28 (m, 1H), 8.45–8.46 (m, 2H).

5.5.34. 3-(4-Acetyl-phenyl)-2-(5-morpholin-4-yl-4-phenyl-thiophen-2-yl)-*3H*-quinazolin-4-one (9bh)

The title compound was prepared from 1,3-di-morpholin-4-yl-2-phenyl-propenethione **5a** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(*p*-acetyl phenyl)-*3H*-quinazoline-4-one **2k** (1.0 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 52.0%, mp >275 °C, R_f: 0.47, IR (KBr, cm^{-1}): 3015, 2894, 1685, 1452, 1284, 761, Molcular Formula $C_{30}H_{25}N_3O_3S$, LC–MS (*m/z*): 558 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.67 (s, 3H), 2.94–2.97(m, 4H), 3.70–3.73 (m, 4H), 5.98 (s, 1H), 7.17–8.17 (m, 12H), 8.2–8.3 (m, 1H).

5.5.35. 3-(4-Acetyl-phenyl)-2-[4-(4-chloro-phenyl)-5-morpholin-4-yl-thiophen-2-yl]-*3H*-quinazolin-4-one (9bi)

The title compound was prepared from 2-(4-chloro-phenyl)-1,3-di-morpholin-4-yl-propenethione **5b** (1.1 g, 0.003 mol) and 2-chloro methyl-3-(*p*-acetyl phenyl)-*3H*-quinazoline-4-one **2k** (0.96 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound a yellow solid. Yield: 50.0%, mp 222–4 °C, R_f: 0.45, M.F.:$C_{30}H_{24}N_3O_3SCl$, LC–MS (*m/z*): 542 (M+1), ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.68 (s, 3H), 2.87–2.93 (m, 4H), 3.65–3.71 (m, 4H), 5.89 (s, 1H), 7.20–7.80 (m, 11H), 8.21–8.24 (m, 1H).

5.5.36. 3-(4-Acetyl-phenyl)-2-[4-(4-methanesulfonyl-phenyl)-5-morpholin-4-yl-thiophen-2-yl]-*3H*-quinazolin-4-one (9bj)

The title compound was prepared from 2-(4-methanesulfonyl-phenyl)-1,3-di-morpholin-4-yl-propenethione **5d** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(*p*-acetyl phenyl)-*3H*-quinazoline-4-one **2k** (0.80 g, 0.003 mol) using the same procedure described for **9aa** to afford title compound as a yellow solid. Yield 54.0%, mp 239–240 °C, R_f: 0.51, IR (KBr, cm^{-1}): 3024, 2913, 1680, 1440, 1308, 1276, 664, Molcular Formula $C_{31}H_{27}N_3O_5S_2$, LC–MS (*m/z*): 586 (M+1), ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.71 (s, 3H), 2.95–2.98 (m, 4H), 3.08 (m, 3H), 3.75–3.77 (m, 4H), 6.11 (s, 1H), 7.28–8.2 (m, 11H), 8.27–8.30 (dd, 1H, *J* = 9.0, 1.2 Hz).

5.5.37. 3-(4-Acetyl-phenyl)-2-(5-morpholin-4-yl-4-pyridin-4-yl-thiophen-2-yl)-*3H*-quinazolin-4-one (9bk)

The title compound was prepared from 1,3-di-morpholin-4-yl-2-pyridin-4-yl-propenthione **5f** (1.0 g, 0.003 mol) and 2-chloro methyl-3-(*p*-acetyl phenyl)-*3H*-quinazoline-4-one **2k** (0.97 g, 0.003 mol) using the same procedure described for **9af** to afford title compound as a yellow solid. Yield: 42.0%, mp 241–242 °C, R_f: 0.53, Molcular Formula $C_{29}H_{24}N_4O_3S$, LC–MS (*m/z*): 509 (M+1), ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.69 (s, 3H), 2.92–2.95 (m, 4H), 2.71–2.75(m, 4H), 6.12 (s, 1H), 7.19–8.16 (m, 9H), 8.28–8.48 (m, 1H), 8.43–8.45 (m, 2H), Elemental Anal. Calcd: C, 68.49; H, 4.76; N, 11.02. Found: C, 68.44; H, 4.64; N, 11.54.

6. Biology

6.1. In vivo acute model of inflammation

The carrageenan induced rat paw edema test was used for the determination of in vivo anti-inflammatory activity.[32] Sprague-Dawley rats (bred in the animal colony at the B. V. Patel PERD, Centre, Ahmedabad) of either sex, in the weight range of 150–250 g were used for the study. Animals were fasted for 18 h prior to the experiment. Each group designating a single compound was tested against a control group receiving only the vehicle treatment (*n* = 4/group). All compounds were administered orally (50 mg/kg body weight) in 0.2% agar suspension prepared fresh prior to use. One hour post dosing, 0.1 ml of 1% carrageenan solution in normal saline (ripened for 7 days) was injected in the subplantar region of the right hind paw of each rat. After (3 h) the carrageenan injection, the reduction in the paw volume compared to vehicle control was measured plethysmometrically. All experimental protocols were reviewed and accepted by the Institutional Animal House Ethics Committee (IAEC) prior to the initiation of the experiment.

All the final compounds were tested simultaneously with the vehicle control to evaluate their anti-inflammatory activities. The percentage protection at 50 mg/kg dose was calculated according to the following formula:

$$\% \text{ Protection} = [(\text{Control} - \text{Test})/\text{Control}] \times 100$$

6.2. In vitro cell line based assays for NF-κB and AP-1

The human embryonic kidney cell line, (HEK293), from Panomics, Inc, Freemont, CA, was used for the in vitro cell based screening for inhibition of NF-κB and AP-1 activity. These cells stably expressed a plasmid containing a minimal promoter and tandem copies of the NF-κB transcriptional element (5′-AGTTGAGGGGACTTTCCCAGGC-3′) or the AP-1 transcriptional element (TGACTAA), which regulates the expression of the luciferase reporter gene. Cells were plated at 5×10^4 cells /well in 96 well plates at 37 °C in 5% CO_2 for 24 h. Test compounds were dissolved in 0.1% DMSO, added at desired concentrations (10 μM–1 nM) to replicate wells, and incubated at 37 °C in 5% CO_2 for an additional 24 h. For induction of transcription and to test the biological response of the promoters, the HEK/NF-κB cell line was stimulated with TNF-α (1 ng/μl) and the HEK/AP-1 cell line was stimulated with PMA (1 ng/μl) and the cells were incubated at 37 °C in 5% CO_2 for 20–24 h. After incubation, cell lysis buffer containing the luciferase substrate (Bright-Glo Luciferase Assay System, Promega) was added to each well. Luminescence was immediately measured using the Rosys Anthos Lucy II or Victor III (Perkin Elmer) luminometer. The luminescence of each test compound was reported as relative light units (RLU) by taking the mean of the replicate wells and normalizing them to the maximal control values. In separate assays, viability of the cells incubated with similar concentrations of compound was analyzed to confirm that changes in RLU were not due to cytotoxicity (data not shown).

6.3. In vivo regulation of transgenic model for NF-κB

Transgenic FVB mice carrying a transgene consisting of a minimal promoter and tandem NFκB binding sites in all cells were used as in vivo 'reporters' of NFκB activity. Three groups of 8–10 week old mice (*n* = 3) were used including (1) untreated control, (2) LPS treated, or (3) mice treated with a daily dose (50 mg/kg) of **9bk** for 3 days before LPS stimulation. On the 4th day, mice in groups 2 and 3 were treated with an intraperitoneal (60 μg/mouse) dose of bacterial lipopolysaccharide (from *E. coli* 0111:B4) in 100 μl sterile saline. The mice were transferred to the LSUHSC-S Small Animal in vivo Imaging Core for bioluminescence imaging (IVIS 100; Xenogen, Inc.). Approximately 4 h after LPS inoculation, mice were anesthetized with 150 mg/kg ketamine and 5 mg/kg xylazine in 100 μl sterile saline and injected with 100 μl ip 15 mg/kg luciferin in sterile saline, and imaged approximately 5–10 min later using the IVIS imager. Representative images are included showing endogenous NF-κB activity limited to the thymus in unstimulated control mice, maximally stimulated NF-κB activity after LPS inoculation, and mice treated with **9bk**. The percentage change in bioluminescence was determined by comparing the mean luminescence minus the background activity in the thymus between maximal LPS stimulation and **9bk** + LPS treatment ($p = 0.03$).

6.4. Statistical analysis

All biological experiments in in vitro assays were performed in triplicates. Representative results are depicted in this report. IC_{50} data were analyzed and presented using the GraphPad software. The correlation study was performed using a linear fitting model in GraphPad. Significance was set at $P < 0.05$.

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