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Non-classical antifolates. Part 2: Synthesis, biological evaluation, and molecular modeling study of some new 2,6-substituted-quinazolin-4-ones ☆

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ABSTRACT

A new series of 2,6-substituted-quinazolin-4-ones was designed, synthesized, and evaluated for their in vitro DHFR inhibition, antimicrobial, and antitumor activities. Compounds **22**, **33–37**, **39–43**, and **45** proved to be active DHFR inhibitors with IC_{50} range of 0.4–1.0 μM. Compound **18** showed broad-spectrum antimicrobial activity comparable to the known antibiotic gentamicin. Compounds **34** and **36** showed antitumor activity at GI_{50} (MG-MID) concentrations of 11.2, and 24.2 μM, respectively. Molecular modeling study including flexible alignment; electrostatic, hydrophobic mappings; and pharmacophore prediction were performed. A main featured pharmacophore model was developed which justifies the importance of the main pharmacophoric groups as well as of their relative distances. The substitution pattern and spatial considerations of the π-systems in regard to the quinazoline nucleus proved critical for biological activity.

1. Introduction

Dihydrofolate reductase (DHFR) catalyzes the reduction of folate or 7,8-dihydrofolate to tetrahydrofolate and intimately couples with thymidylate synthase (TS). TS is a crucial enzyme that catalyzes the reductive methylation of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) utilizing N^5,N^{10}-methylene-tetrahydrofolate as a cofactor, which functions as source of the methyl group as well as the reductant. This is the exclusive de novo sources of dTMP, hence inhibition of DHFR or TS activity in the absence of salvage, leads to 'thymineless death'.[1,2] Thus, DHFR inhibition has long been an attractive goal for the development of chemotherapeutic agents against bacterial and parasitic infections as well as cancer.[3] Upon reviewing the literature, it was noticed that in addition to the non-classical DHFR inhibitors: Trimethoprim (TMP, **1**),[4] trimetrexate (TMQ, **2**),[5,6] and piritrexim (PTX, **3**),[5,7] some other interesting inhibitors, most of them belong to the quinazoline heterocycle were also located; such as the anti-colorectal cancer thymitaq (**4**) with its 5-arylthio function,[8,9] the antimalarial drug **5** with its 6-arylthio moiety,[10] the antileukemic activity of the dithiocarbamate **6**, and the carbodithioate **7**,[11] then The 6-acrylamide analogue **8**,[12] (Chart 1).

In continuation of our previous efforts,[13–26] a new series of 2,6-substituted-quinazolin-4-one analogues of the general formulae **A**–**C** (Chart 2), was designed to possess 6-methyl, 6-nitro or 6-amino functions, representing electron donating and electron withdrawing substituents; a benzyl group at position 3-, based on what was concluded from a previous study, that DHFR inhibition activity could be obtained only, upon the existence of a benzyl group at N-3 of the quinazoline ring, rather than 3-methyl or 3-phenyl functions.[26] In addition, alkyl, allyl or cinnamyl thioether groups introduced at position 2- in resemblance to the lead compounds **4** and **5** (**A**). The 6-amino function of **A** was used to introduce an allyl and cinnamyl amines, or acrylamide and cinnamamide functions (**B**); these 6-amide compounds resemble the active 6-acrylamide analogue **8**. The secondary amines of **A** were converted to their corresponding tertiary amines (**C**) through their alkylation using methyl, allyl, or cinnamyl functions, to block the NH-function to produce methotrexate type analogues. Most of the functions designed to be accommodated on the quinazoline ring such as thioether, alkyl, aryl, arylalkyl and nitro groups are known to increase lipid solubility of polar compounds, a character very much needed for the non-classical DHFR

☆ For part 1 see Ref. 26.

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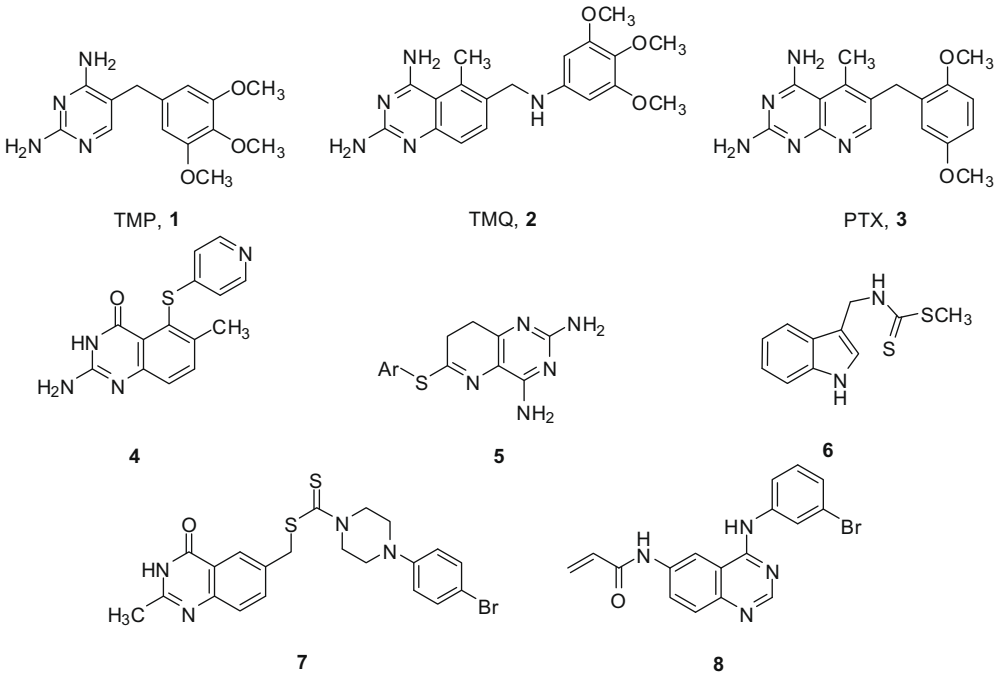


Chart 1.

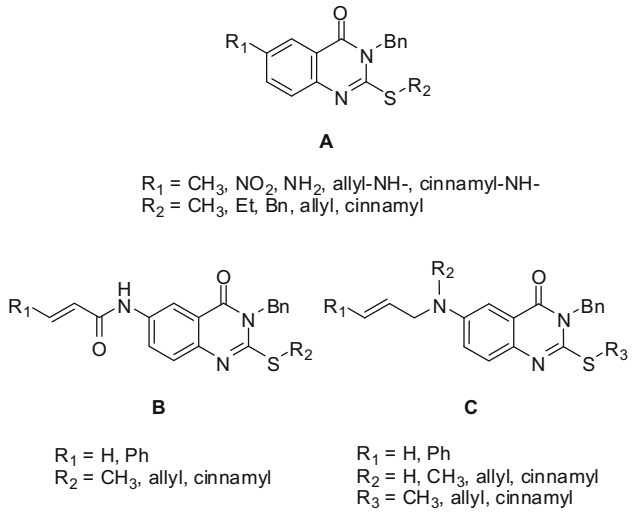


Chart 2.

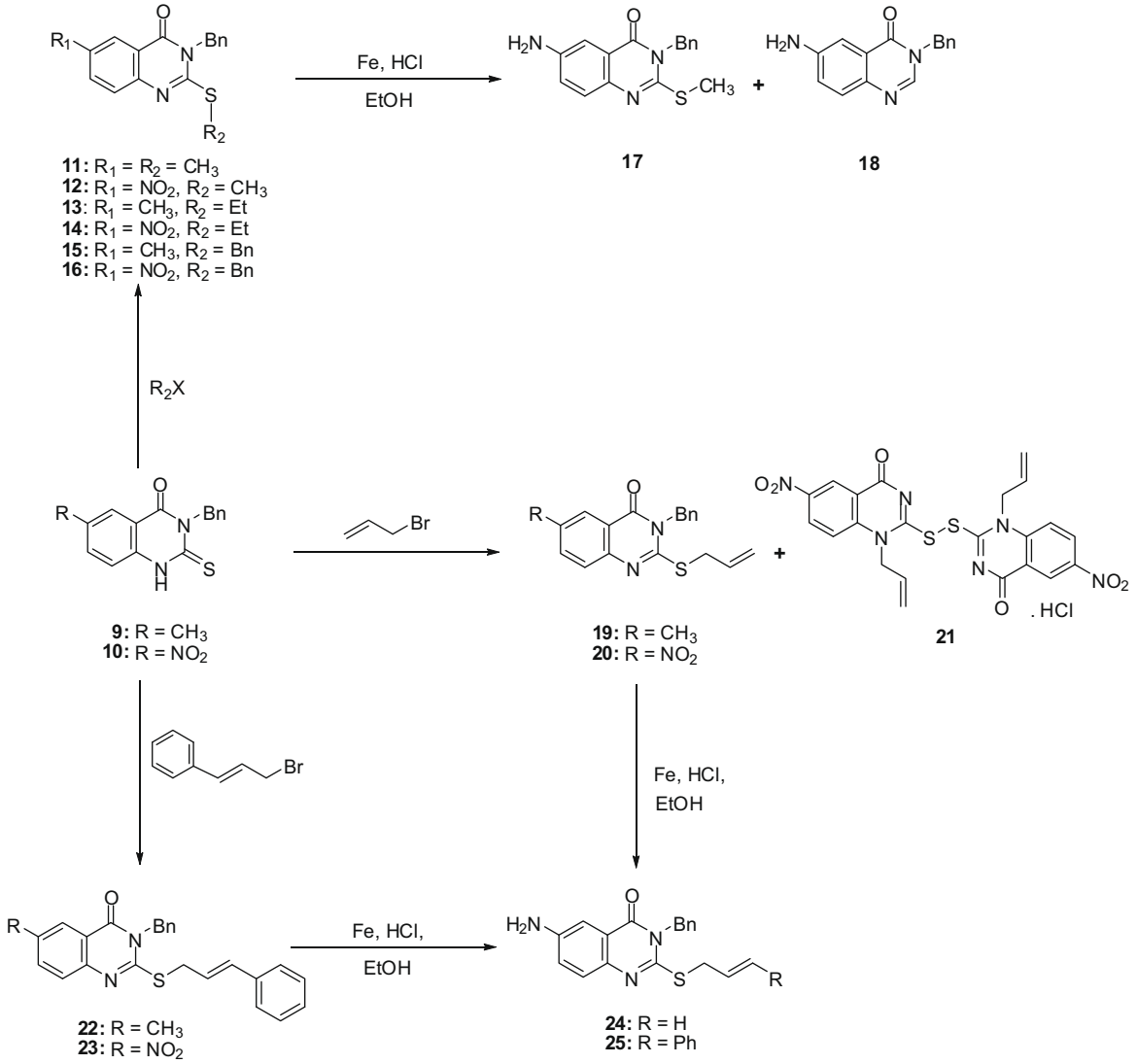
inhibition.[27,28] The aim of this study is to locate novel synthetic lead compound(s), and their in vitro testing as DHFR inhibitor(s). Compounds possessing such activity will be candidates for treating cancer, bacterial and parasitic infections.

2. Chemistry

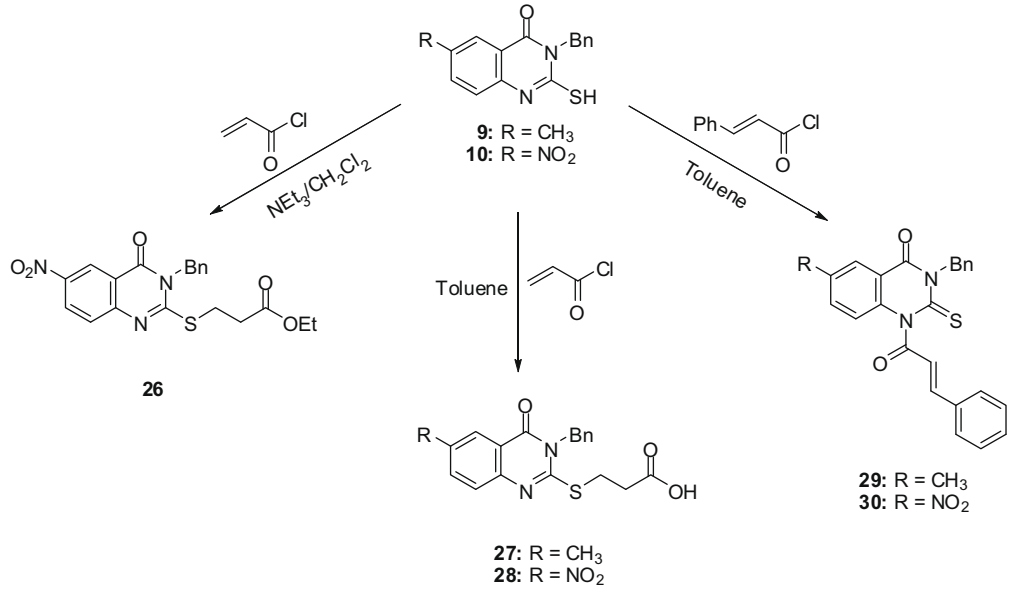
The synthetic strategy to obtain the targets **A–C** (Chart 2) is depicted in Schemes 1–3. The starting materials 3-benzyl-2-mercapto-6-methyl-quinazolin-4(3*H*)-one (**9**) and 3-benzyl-2-mercapto-6-nitro-quinazolin-4(3*H*)-one (**10**) were prepared adopting reported procedures.[24–26] The 2-thioxo- function of **9** and **10** was then alkylated using variety of alkyl halides to give the thioether derivatives **11–16**. A close examination of the NMR spectrum of 3-benzyl-2-ethylthio-6-nitro-quinazolin-4(3*H*)-one revealed its existence in two inseparable positional isomers; (*S*-Et:*N*-Et) in ratio of 3:1. The 6-nitro function of **12** was subjected to metal reduction using Fe/HCl. Chromatographic separation of the obtained products allowed the identification of the reported 6-amino analogue **17**[26] along with 6-amino-3-benzyl-quinazolin-4(3*H*)-one (**18**) resulted from the reductive elimination of 2-CH_3S- function. The 2-thioxo function of **9** and **10** was then alkylated using allyl or cinnamyl bromide to give the thioether derivatives **19**, **20**, **22**, and **23**. Chromatographic purification of the crude products obtained upon treating **10** with allyl bromide, allowed the separation of the target compound **20**, along with the disulfide analogue **21** resulted from the oxidation of the 2-thiol function. Oxidation of thiol into disulfide is well documented.[29] The 6-nitro function of both **20** and **23** was subjected to metal reduction using Fe/HCl to produce the corresponding 6-amino analogues **24** and **25**, respectively (Scheme 1).

Variety of amines and mercaptanes undergo Michael-type additions to α,β-unsaturated functions to produce the β-amino or β-mercapto derivatives.[30–34] Upon treating **10** with acryloyl chloride in NEt_3/CH_2Cl_2, a material was isolated and identified to be the β-mercapto analogue, ethyl 3-(3-benzyl-6-nitro-4-oxo-3,4-dihydroquinazolin-2-ylthio)-propanoate (**26**). The only possible source of ethyl group to generate the ester **26** is the NEt_3 exist in the reaction medium. This was proven by repeating the reaction without using such base, where the free acids 3-(3-benzyl-6-methyl or nitro-4-oxo-3,4-dihydroquinazolin-2-ylthio)-propanoic acid (**27** and **28**), were produced instead. The same procedure was adopted to prepare 3-benzyl-1-cinnamoyl-6-(methyl or nitro)-2-thioxo-2,3-dihydro-quinazolin-4(1*H*)-one (**29** and **30**). Spectral analysis of **29** and **30** revealed that acylation with cinnamoyl chloride occurred at N-1 of the quinazoline ring instead of the Michael addition of –SH group to the α,β-unsaturated function, as evidenced by the existence of the C=S absorptions at δ 173.5, 174.5 ppm, respectively. This might be attributed to the steric hindrance caused by the phenyl ring of the cinnamoyl moiety (Scheme 2).

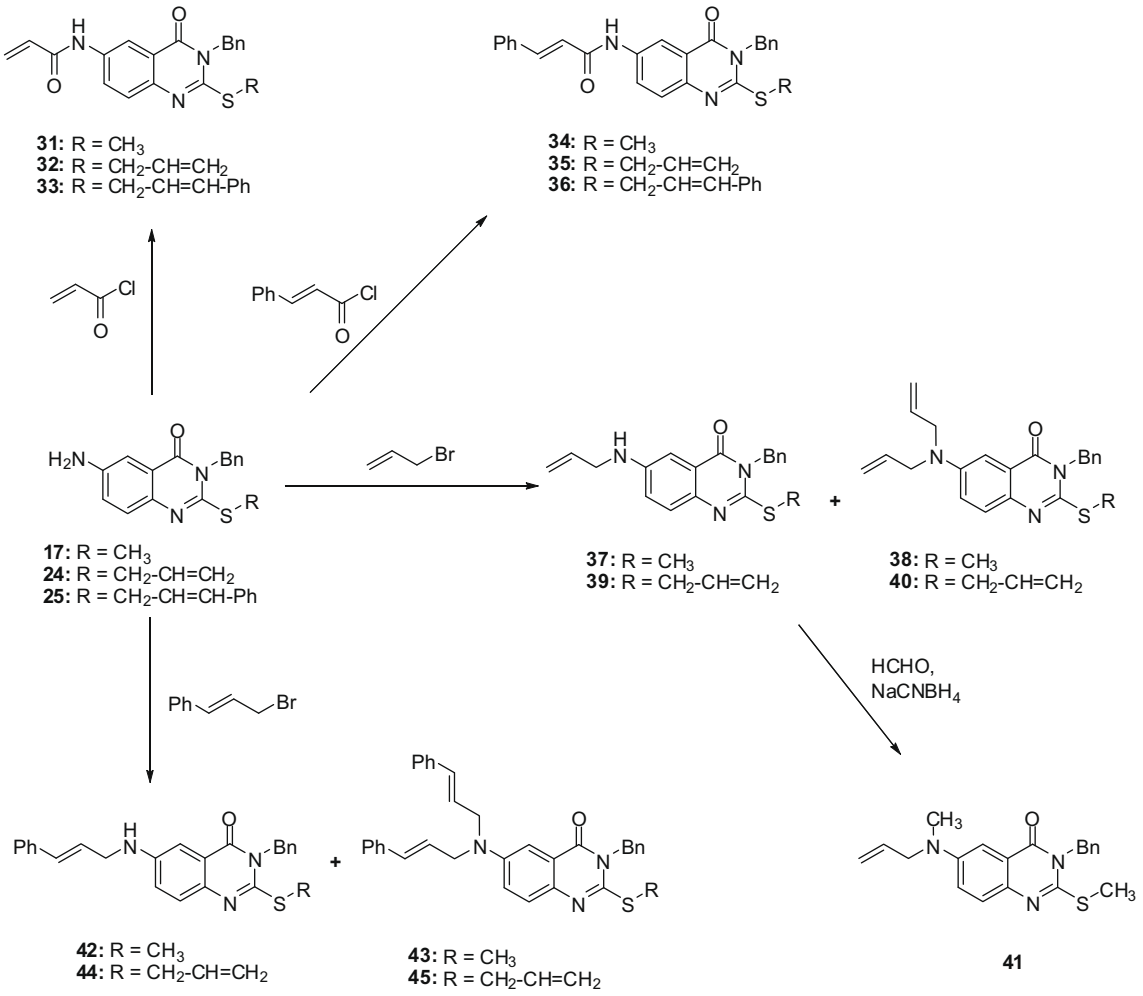
The synthesized 6-amino-quinazolines **17**, **24**, and **25** were then acylated using acryloyl chloride or cinnamoyl chloride to afford the target acrylamides (**31–33**), and cinnamamides (**34–36**), respectively, in reasonable yields. Meanwhile, the 6-amino function of **17** and **24** were alkylated using allyl bromide. Chromatographic



Scheme 1.



Scheme 2.



Scheme 3.

purification of the obtained products allowed the separation of 6-allylamino-3-benzyl-2-methylthio-quinazolin-4(3*H*)-one (**37**) along with the 6-diallyl amino **38**, also 6-allylamino-2-allylthio-3-benzylquinazolin-4(3*H*)-one (**39**) in addition to the 6-diallyl amino **40**. The secondary amine **37** was subjected to reductive methylation using formaldehyde and $NaCNBH_3$ to produce the N-methylated tertiary amine, 6-allyl-(methyl)-amino-3-benzyl-2-methylthio-quinazolin-4(3*H*)-one (**41**), in good yield. Similarly, **17** and **24** was alkylated using cinnamyl bromide. Careful chromatographic purification of the obtained products allowed the separation of 3-benzyl-6-cinnamylamino-2-methylthio-quinazolin-4(3*H*)-one (**42**) along with the 6-dicinnamyl-amino analogue **43**, also 2-allylthio-3-benzyl-6-cinnamylamino-quinazolin-4(3*H*)-one (**44**) in addition to the 6-dicinnamyl-amino derivative **45** (Scheme 3).

3. Results and discussion

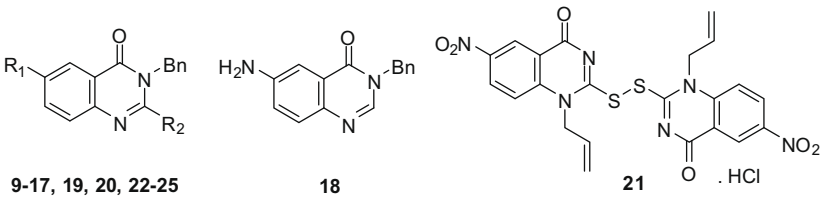
3.1. Dihydrofolate reductase (DHFR) inhibition

The synthesized compounds (**9–45**) were evaluated as inhibitors of bovine liver and human DHFR using reported procedure,[35] results were reported as IC_{50} values (Tables 1 and 2). Compounds **22**, **33–37**, **39–43**, and **45** proved to be the most active inhibitors of bovine liver DHFR with IC_{50} range of 0.4–1.0 μM. The analogy and similarity among bovine liver DHFR and hDHFR is almost 75%.[35] In this study, hDHFR enzyme was used to pursue and revaluate compounds proved to be bovine DHFR inhibitors. Under the assay conditions, no significant difference (P >0.05) was observed between bovine DHFR and hDHFR, using methotrexate (IC_{50} 0.08 μM) as a positive control.

3.1.1. Structure–activity relationship (SAR)

The type of substituent at positions 2- and 6- of the studied quinazolines manipulated the DHFR inhibition activity. *The 2-thioether function* affects the magnitude of DHFR inhibition. The order of activity is CH_2═CH–CH_2–S– or Ph–CH═CH–CH_2–S– (0.5–5.0 μM) > CH_3–S–, CH_3CH_2–S–, and Bn–S– (10.0–35.0 μM). The presence of 6-CH_3 group (0.5–2.0 μM) favors the activity rather than the 6-nitro (3.0–5.0 μM) or the 6-amino functions (8.0 μM). *In the 6-methyl series*, the existence of 2-cinnamylthio-function (**22**, IC_{50} 0.5 μM) favors the activity rather than 2-allylthio- (**19**, IC_{50} 2.0 μM). The combination of 6-methyl- and 2-cinnamylthio- (**22**, IC_{50} 0.5 μM) favors the activity rather than the combination of 6-nitro- and 2-cinnamylthio- (**23**, IC_{50} 5.0 μM). *In the 6-acrylamide series* (**31–33**), the order of activity is determined by the type of the 2-thioether function. Compounds bearing 2-cinnamylthio-function is 10-fold more active than the 2-allylthio, and 50-fold more active than the 2-methylthio-. *In the 6-cinnamamide series* (**34–36**), and in contrast to the 6-acrylamide series, the type of the 2-thioether moieties does not affect the magnitude of activity; all are in the remarkable side with IC_{50} values of 0.6–0.9 μM. The 6-cinnamamide moiety proved to contribute to activity more than the 6-acrylamide function. Another piece of evidence proving the

Table 1
DHFR inhibition (IC_{50}, μM), and antimicrobial activity results of compounds **9–25**



9-17, 19, 20, 22-25 **18** **21**

Compd	R_1	R_2	DHFR inhibition (IC_{50}, μM)		Inhibition zone (mm); MICs (μg/ml)				
			bDHFR	hDHFR	*S. aureus*	*B. subtilis*	*M. luteus*	*E. coli*	*P. aeuroginosa*
9	CH_3	SH	30.0	Nd	—	18 (4.0)	14 (Nd)	17 (4.0)	15 (Nd)
10	NO_2	SH	15.0	Nd	20 (4.0)	18 (8.0)	16 (16.0)	—	—
11	CH_3	S-CH_3	20.0	Nd	—	—	—	—	—
12	NO_2	S-CH_3	30.0	Nd	—	—	—	—	—
13	CH_3	S-Et	35.0	Nd	—	—	—	—	—
14	NO_2	S-Et	10.0	Nd	—	—	—	20 (8.0)	15 (Nd)
15	CH_3	S-Bn	10.0	Nd	—	—	—	—	—
16	NO_2	S-Bn	30.0	Nd	—	—	—	—	—
17	NH_2	S-CH_3	10.0	Nd	—	—	—	—	—
18	—	—	40.0	Nd	24 (2.0)	28 (2.0)	20 (4.0)	22 (4.0)	—
19	CH_3	S-CH_2–CH=CH_2	2.0	4.0	—	—	—	—	—
20	NO_2	S-CH_2–CH=CH_2	3.0	2.0	—	—	—	—	—
21	—	—	10.0	Nd	—	—	—	—	—
22	CH_3	S-CH_2–CH=CH–Ph	0.5	1.0	—	—	—	—	—
23	NO_2	S-CH_2–CH=CH–Ph	5.0	3.0	—	—	—	21 (8.0)	17 (16.0)
24	NH_2	S-CH_2–CH=CH_2	8.0	5.0	—	—	—	—	—
25	NH_2	S-CH_2–CH=CH–Ph	8.0	6.0	—	—	—	—	—
Gentamicin		27 (2.0)	25 (2.0)	18 (2.0)	21 (0.5)	19 (1.0)			
Sulfacetamide		20 (2.0)	22 (2.0)	18 (2.0)	28 (1.0)	25 (2.0)			

(—) Not active (8 mm), weak activity (8–12 mm), moderate activity (12–15 mm), strong activity (>15 mm). Solvent: DMSO (8 mm). MICs (μg/ml) showed in parentheses. Nd, not determined.

role of the 2-thioether function in determining the magnitude of activity appears in the 6-allylamino and the 6-cinnamylamino derivatives (**37–40**). *In the 6-allylamino series*, the effect of the 2-thioether group proved to be of minimal influence as presented by the almost equal potency of **37** and **39** (IC_{50} 0.6 and 0.8 μM, respectively). Replacing 6-allylamino by 6-diallylamino moiety enhanced the activity of the 2-allylthio-analogue **39** by twofold (**40**, IC_{50} 0.4 μM) and suppressed the potency of the 2-methylthio- analogue **37** by fivefold (**38**, IC_{50} 3.0 μM). *In the 6-cinnamylamino series*, the concept is reversed. Compound **42** bearing 2-methylthio- function (IC_{50} 0.5 μM) proved to be more active than the 2-allylthio-derivative **44** by 10-fold (IC_{50} 5.0 μM). Replacing the 6-cinnamylamino function by 6-dicinnamylamino moiety gave compounds **43** and **45** with almost equal potency (IC_{50} 0.6 and 0.5 μM, respectively).

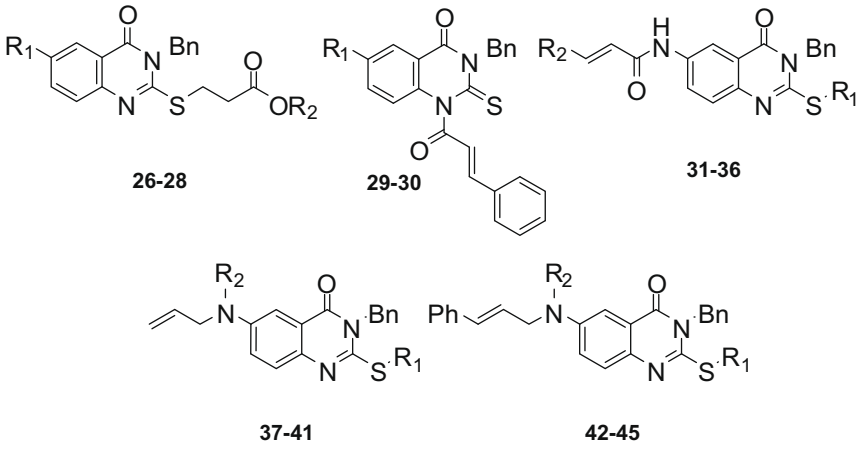
3.2. Antimicrobial activity

The synthesized compounds (**9–45**) were tested for their in vitro antimicrobial activity against a panel of standard strains of Gram-positive bacteria, Gram-negative bacteria, and yeast-like pathogenic fungus. The primary screen was carried out using the agar disc-diffusion method.[36,37] Gentamicin (100 μg/disc), sulfacetamide (100 μg/disc) and clotrimazole (100 μg/disc) were used as positive controls. The obtained results revealed that the tested compounds expressed varying degrees of activity against the tested microorganisms (Tables 1 and 2). Remarkable activity (>20 mm inhibition zone) against the Gram-positive bacteria was observed for compounds **10**, **18**, **28**, **45** against *Staphylococcus aureus*; **18**, **28**, **30**, **44**, **45** against *Bacillus subtilis*, and **18** against *Micrococcus luteus*. In case of Gram-negative bacteria, remarkable activity was observed for compounds **14**, **18**, **23**, and **30** against *Escherichia coli*. Strong activity (>15 mm inhibition zone) against the Gram-positive bacteria was observed for compounds **27**, **30**, **35**, **44** against *S. aureus*; **9**, **10**, **27**, **35**, **43** against *B. subtilis*; and **10**, **35**, **44** against *M. luteus*. In case of Gram-negative bacteria, strong activity was noticed for compounds **9**, **26**, **35**, **37**, **42–45** against *E. coli*; and compounds **9**, **14**, **23**, **26**, **30** against *Pseudomonas aeuroginosa*. Selectivity was noticed among the tested compounds. Compounds **14** and **23** possessed activity solely confined toward the used Gram-negative bacteria, while **10**, **27** and **28** showed activity solely confined toward the used Gram-positive bacteria. Compounds **18**, **30**, **35**, **44** and **45** showed a remarkable broad-spectrum activity against both Gram-positive and Gram-negative bacteria. Compound **18** proved to be the most active broad-spectrum antibiotic in this study with potency comparable to the known antibiotic gentamicin. There is no antifungal activity observed for all of the tested compounds. The minimal inhibitory concentrations (MICs) for the most active compounds was carried out using the micro-dilution susceptibility method as shown in Tables 1 and 2. The Gram-positive bacteria *B. subtilis* and the Gram-negative bacteria *E. coli* are sensitive to majority of the tested compounds. Comparing the potency of the active antibacterials and their DHFR inhibition revealed that compounds **23**, **35**, and **43–45** might exert their activity through DHFR inhibition.

3.3. Antitumor screening

The active DHFR inhibitors (**22**, **30**, **34–37** and **42**) were subjected to the NCI's in vitro anticancer screen. Compounds **34** and **36** passed the primary anticancer assay at an arbitrary concentration of 100 μM. Consequently, those active compounds were carried over and tested against a panel of 60 different tumor cell lines at a 5-log dose range.[38–41] Three response parameters, GI_{50}, TGI, and LC_{50} were calculated for each cell line, using 5-fluorouracil (5-FU) as a positive control. The tested quinazolinone analogues

Table 2
DHFR inhibition (IC_{50}, μM), and antimicrobial activity results of compounds **26–45**



Compd	R_1	R_2	DHFR inhibition (IC_{50}, μM)		Inhibition zone (mm); MICs (μg/ml)				
			bDHFR	hDHFR	*S. aureus*	*B. subtilis*	*M. luteus*	*E. coli*	*P. aeuroginosa*
26	NO_2	Et	25.0	Nd	–	14 (Nd)	–	16 (Nd)	16 (Nd)
27	CH_3	H	50.0	Nd	18 (4.0)	16 (4.0)	14 (Nd)	–	–
28	NO_2	H	10.0	Nd	20 (4.0)	24 (2.0)	–	–	–
29	CH_3	–	5.0	3.0	–	–	–	–	–
30	NO_2	–	10.0	Nd	18 (8.0)	20 (4.0)	14 (Nd)	20 (4.0)	18 (8.0)
31	CH_3	H	50.0	Nd	–	–	–	–	–
32	CH_2–CH=CH_2	H	10.0	Nd	–	–	–	–	–
33	CH_2–CH=CH_2–Ph	H	1.0	2.0	15 (Nd)	–	–	–	–
34	CH_3	Ph	0.6	1.0	–	–	–	–	–
35	CH_2–CH=CH_2	Ph	0.7	0.5	16 (8.0)	18 (8.0)	18 (8.0)	18 (8.0)	–
36	CH_2–CH=CH_2–Ph	Ph	0.9	0.5					
37	CH_3	H	0.6	0.8	–	–	–	17 (Nd)	–
38	CH_3	CH_2–CH=CH_2	3.0	1.0	–	–	–	–	–
39	CH_2–CH=CH_2	H	0.8	1.0	–	–	–	–	–
40	CH_2–CH=CH_2	CH_2–CH=CH_2	0.4	0.9	–	–	–	–	–
41	CH_3	CH_3	1.0	2.0	–	–	–	–	–
42	CH_3	H	0.5	2.0	–	–	–	15 (Nd)	–
43	CH_3	CH_2–CH=CH–Ph	0.6	0.8	–	18 (8.0)	–	16 (16.0)	–
44	CH_2–CH=CH_2	H	5.0	3.0	19 (8.0)	20 (4.0)	16 (16.0)	18 (8.0)	–
45	CH_2–CH=CH_2	CH_2–CH=CH–Ph	0.5	0.7	22 (4.0)	20 (4.0)	–	16 (16.0)	–

(–) Not active (8 mm), weak activity (8–12 mm), moderate activity (12–15 mm), strong activity (>15 mm). Solvent: DMSO (8 mm). MICs (μg/ml) showed in parentheses. Nd, not determined.

showed a distinctive potential pattern of selectivity as well as a broad-spectrum antitumor activity. With regard to sensitivity against individual cell lines, compound **34** showed GI_{50} effectiveness against breast MDA-MB-468 cancer cell line at concentration of 2.2 μM. Compound **36** showed GI_{50} activity against colon HT29, and ovarian OVCAR-4 cell lines at concentration of 0.6 and 0.4 μM, respectively. Compounds **34** and **36** showed GI_{50} (MG-MID) values of 11.2, and 24.2 μM, and TGI (MG-MID) values of 86.2 and 81.9 μM, respectively; against nine tumor subpanel cell lines (Table 3). Compound **34** is almost twofold more active than the positive control 5-FU. When a full panel GI_{50} mean-graph (MG-MID) divided by a particular subpanel GI_{50} mean-graph, certain ratio will be obtained which help to predict the selectivity of this compound toward this cell lines subpanel. Ratios of 3–6 are considered mod-

Table 3
Median growth (GI_{50}) and total growth (TGI) inhibitory concentrations, (μM) of in vitro subpanel tumor cell lines

Compd	Subpanel tumor cell lines[a]										MG-MID[b]
	Activity	I	II	III	IV	V	VI	VII	VIII	IX	
34	GI_{50}	51.6	1.7	34.5	1.9	2.4	1.1	2.9	1.6	3.0	11.2
	TGI	[c]	80.9	[c]	68.4	26.9	[c]	[c]	[c]	[c]	86.2
36	GI_{50}	[c]	2.2	1.9	3.0	2.2	3.3	2.6	[c]	2.5	24.2
	TGI	[c]	76.4	[c]	37.0	86.9	[c]	68.0	[c]	68.6	81.9
5-FU	GI_{50}	15.1	[c]	8.4	72.1	70.6	61.4	45.6	22.7	76.4	22.6
	TGI	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]

[a] I, leukemia; II, non-small cell lung cancer; III, colon cancer; IV, CNS cancer; V, melanoma; VI, ovarian cancer; VII, renal cancer; VIII, prostate cancer; IX, breast cancer.
[b] GI_{50} full panel mean-graph midpoint (μM).
[c] Compounds showed values >100 μM. Median lethal concentration (LC_{50}, μM) of in vitro subpanel tumor cell lines and LC_{50} full panel mean-graph midpoint (μM) are > 100 μM.

erately selective and those with ratios of 6 or more are taken as selective.[41] Compound **34** proved to be selective toward non-small cell lung, ovarian and prostate cancer cell lines with selectivity ratio of 6.6, 10.2 and 7.0, respectively; while **36** proved to be selective toward non-small cell lung, colon, CNS, melanoma, ovarian, renal and breast cancer cell lines with selectivity ratio of 11.0, 12.7, 8.1, 11.0, 7.3, 9.3 and 9.7, respectively.

3.4. Molecular dynamic study

The investigated compounds were subjected to molecular modeling study to evaluate their recognition profile at hDHFR binding-pocket, in comparison to the dihydrofolate inhibitor 6-(5-quinolylamino-methyl)-2,4-diamino-5-methylpyrido[2,3-*d*]pyrimidine (LIH). The tertiary complex of hDHFR, NADPH and LIH was used as a reference for modeling and docking.[42–45] At hDHFR pocket, LIH formed bifurcated H-bonding with the key residue Glu30, besides trifurcated H-bonds with the 'catalytic triad' residues Val8, Tyr121 and Val115 (Fig. 1).[42–46] Molecular dynamic study indicated that the tested quinazoline's recognition with the key amino acid Glu30 and Ser59 is essential for binding and biological activity. The amino acid Ser59 is not one of the key residues of recognition of the parent ligand LIH but it plays a crucial role in the recognition of the tested compounds. Figure 2 showed the binding mode and residues involved in the recognition of the most inactive compound **31** (IC_{50} 50.0 μM) and the active compound **40** (IC_{50} 0.4 μM) docked and minimized in the hDHFR binding pocket.

Since atomic-level detailed structure of the relevant receptors are often not available, 3D flexible alignment (superposition) of putative ligands can be used to deduce structural requirements for biological activity.[47–49] To probe similarity among 3D structures of the most active hDHFR inhibitors **22**, **34–36**, **42** and **45** with minimal bias; MOE/MMFF94 flexible alignment was employed.[50,51] A common feature of the MOE-generated alignments is the superposition of

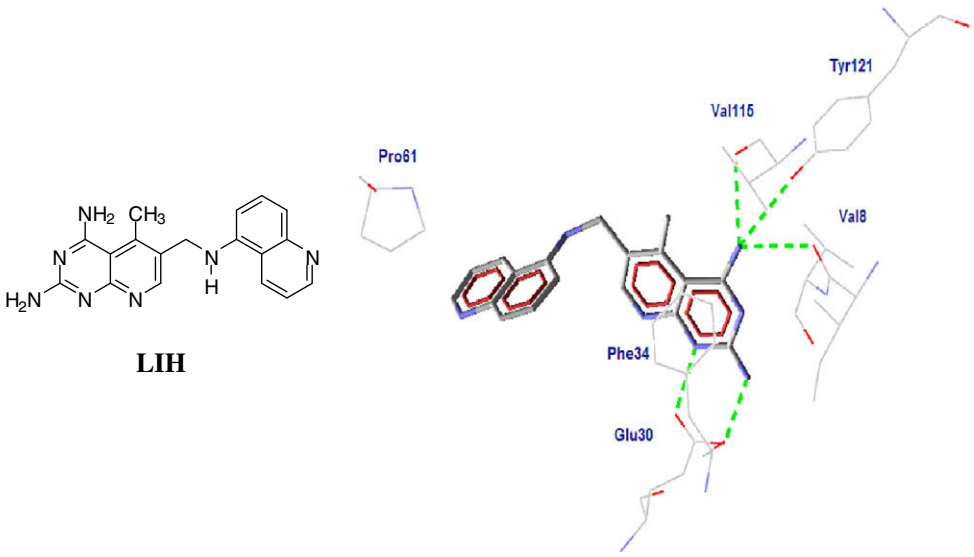


Figure 1. Binding mode for LIH docked and minimized in the hDHFR binding pocket, showing residues involved in its recognition.

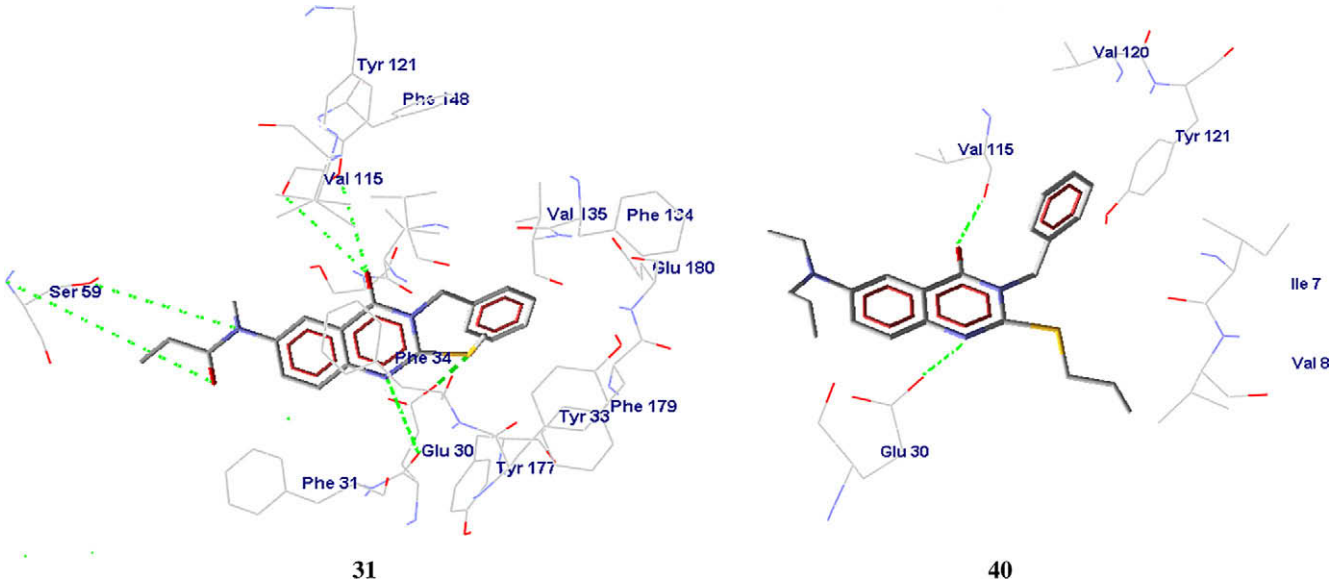


Figure 2. The binding mode and residues involved in the recognition of the most inactive compound **31** (IC_{50} 50.0 μM) and the most active compound **40** (IC_{50} 0.4 μM) docked and minimized in the hDHFR binding pocket.

the quinazolinone core of all compounds (Fig. 3a), in addition to six points of similarities; namely, 4-carbonyl unit, N-1 of the quinazoline core, a hydrophilic area at position 6-, the π-system represented by aryl group and/or olefinic function located at positions 2- and 6- and the 3-benzyl fragment. This alignment suggests the possible existence of a region on the receptor suited for specific recognition of such features. This hypothesis is supported by biological results and docking studies. Adopting the same analogy, the most active DHFR inhibitors **22**, **34**, **42** and the least active compounds **26** and **27** were subjected to flexible alignments (Fig. 3b). Analyses of the least active molecule will help to understand the essential features for a given activity. Compounds **26** and **27** were flexibly aligned in a different fashion at position 2- of the quinazoline core, and the hydrophilic propionic acid moiety; in addition to their lower lipophilicity as compared to the most active compounds explaining their low biological activity.

In an attempt to figure out the reason behind the diminished DHFR inhibition activity of compounds **26** and **27**, electrostatic and hydrophobic mapping was carried out for the lowest energy conformers, to examine the similarity and dissimilarity in the electronic, electrostatic binding characteristics of the molecule surface and the conformational properties (Fig. 4). Comparison of the electrostatic mapping of the most active compounds, represented by compound **40** (Fig. 4; right panel), showed a common feature, which are negative charged hydrogen bond acceptor–donor region located on the 4-carbonyl, N-1, 6-NH (in red); and non-polar area located on the aryl moiety attached to the quinazoline core (in green). Such results indicated the structure and hence biological similarity among those active analogues. On the contrary, the low activity of the other compounds, represented by **26** (Fig. 4; right panel), may be attributed to the difference in electrostatic mapping in which the red hydrogen bond area was mainly located on the carboxylic group. Similarly, hydrophobic mapping study of the most active compounds showed a common features, which are hydrophobic region (in green) distributed on both sides of the aryl parts; and hydrophilic region (in red) located on 4-carbonyl, N-1, 6-NH of the quinazoline core (**40**, Fig. 4; left panel). Those hydrophobic parts are responsible for the interaction with the key amino acid residues inside the enzyme active pocket. On the other hand, the hydrophobic distributions of the least active compounds, (**26**, Fig. 4; left panel), lack such aromatic moieties and hence the required lipophilicity, while the hydrophilic red region was located on the carboxylic acid fragment. The obtained charge distribution, electrostatic, hydrophobic mapping and conformation of the active agents suggest a similar interaction of the molecules with the potential protein-binding site.

Compound **36** served as the reference to which all conformations of each analogue were aligned For pharmacophore generation and prediction.[26,42,49] All structures were built de novo using 2D/3D editor sketcher in MOE.[50] The generated models for compound **36** and its analogues **22**, **42** (Fig. 5, left panel) showed pharmacophoric elements consists of aryl moieties at positions 2-, 3- and 6- as a hydrophobic triangle, in addition to a hydrophilic triangle; in the middle of this hydrophobic area. The hydrophilic triangle consists of two hydrogen bond acceptors, (4-carbonyl and N-1 of the quinazoline core); and a hydrogen bond donor at the basic 6-NH. On the contrary, models for compounds **26** and **27** (Fig. 5; right panel) showed common elements only in the quinazoline core area which attached to the non-favorable propionic acid fragment. Since the two triangle elements of **36** plays an important role in the binding mode, it was considered as the representative pharmaophore map of DHFR inhibitors. Accordingly, the minimal structure requirements for DHFR inhibitors of this class of compounds consist of at least two aryl moieties or their equivalent π-systems

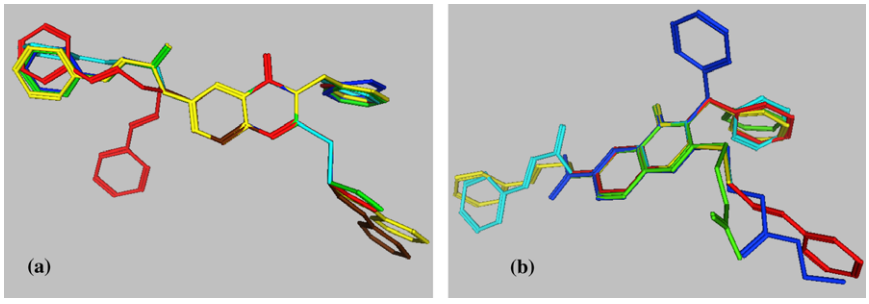


Figure 3. (a) Superposition of the most active compounds **36** (in brown), **34** (in blue), **35** (in green), **36** (in yellow), **42** (in cyan) and **45** (in red) using flexible alignments. (b) Flexible alignment of the most active compounds **22** (red), **34** (cyan), **42** (yellow) and the least active compound **26** (blue) and **27** (green).

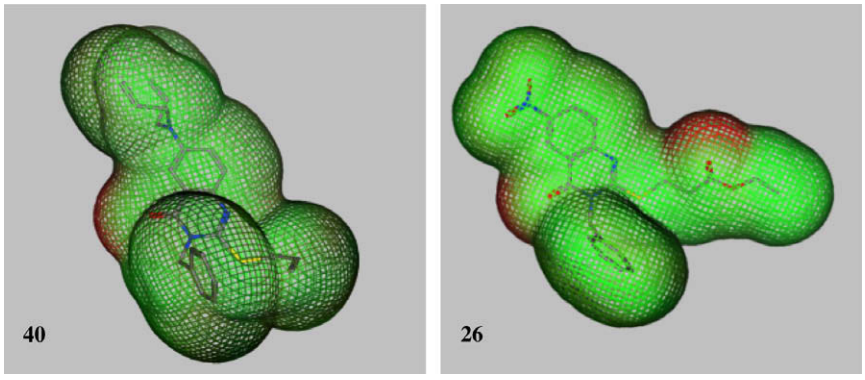


Figure 4. Electrostatic and hydrophobic map of the lowest energy conformers for the most active compound **40** and the least active compound **26**, maps are color coded: red for a hydrogen bond and a hydrophilic region, and green for a hydrophobic region.

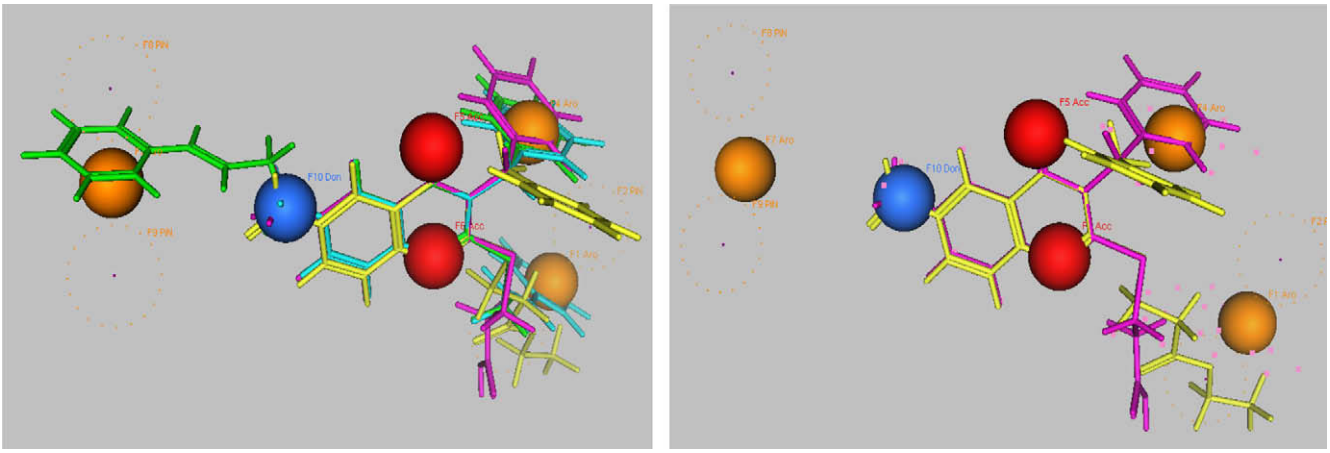


Figure 5. The least active compounds **26** (yellow) and **27** (pink), mapped to the pharmacophore model for DHFRs (right panel). The left panel showed the most active compounds **22** (cyan), **42** (green) and the least active **26** (yellow), **27** (pink) mapped to the pharmacophore features. Pharmacophore features are color coded: orange for hydrophobics aromatic, blue for a hydrogen bonds donor, and red for a hydrogen bonds acceptor feature.

attached to the quinazoline ring which are essential for hydrophobic interaction with the enzyme's hydrophobic pocket and at least two H-bonding regions located in the quinazoline core which are essential for hydrophilic interaction inside the receptor pocket. For most of the active molecules, reasonable non-bonded spatial distances among the hydrophilic triangle as well as the non-bonded distances among the plane of the hydrophobic triangle is needed. This pharmacophoric assumption was consistent with the biological results and docking studies.

4. Conclusion

Compounds **22**, **33–37**, **39–43**, and **45** proved to be the most active inhibitors of DHFR with IC_{50} range of 0.4–1.0 μM. Structure–activity relationship studies revealed that, the type of substituent at positions 2- and 6- of the studied quinazolin-4-ones manipulate the DHFR inhibition activity. Compound **18** showed broad-spectrum antimicrobial potency comparable to the known antibiotic gentamicin. Compounds **23**, **35**, and **43–45** exert their antibacterial activity through DHFR inhibition. Compounds **34** and **36** showed antitumor activity at GI_{50} (MG-MID) concentration of 11.2, and 24.2 μM, respectively, and TGI (MG-MID) concentration of 86.2 and 81.9 μM, respectively. Compound **34** is almost twofold more active than the used positive control 5-FU. Molecular modeling study was performed for the investigated compounds to evaluate their recognition profiles at hDHFR binding-pocket. It is concluded that recognition with key amino acid Glu30 and Ser59 are essential for binding and biological activities. Flexible alignment; electrostatic, hydrophobic mappings; and pharmacophore prediction study were performed. Two main triangles featured a pharmacophore model. This model justifies the importance of the main pharmacophoric groups (the 4-carbonyl fragment, the basic nitrogen atom at N-1, and the hydrophobic π-system regions) as well as of their relative spatial distances. The substitution pattern and spatial considerations of the π-systems in regard to the quinazoline nucleus proved to be critical for biological activity. Therefore, the obtained pharmacophoric model could be useful for the development of new DHFR inhibitors.

5. Experimental

Melting points (°C) were determined on Mettler FP80 melting point apparatus and are uncorrected. Microanalyses were performed on a Perkin–Elmer 240 elemental analyzer at the Central Research Laboratory, College of Pharmacy, King Saud University. All of the new compounds were analyzed for C, H and N and agreed with the proposed structures within ±0.4% of the theoretical values. ^1H, ^{13}C NMR and other 2-D spectra were recorded on a Varian XL 500 MHz FT spectrometer; chemical shifts are expressed in δ ppm with reference to TMS. Mass spectral (MS) data were obtained on a Perkin–Elmer, Clarus 600 GC/MS and Joel JMS-AX 500 mass spectrometers. Thin layer chromatography was performed on precoated (0.25 mm) silica gel GF_{254} plates (E. Merck, Germany), compounds were detected with 254 nm UV lamp. Silica gel (60–230 mesh) was employed for routine column chromatography separations. DHFR inhibition activity experiments were performed at Pharmacology Department, College of Pharmacy, King Saud University. Bovine liver DHFR enzyme, hDHFR enzyme, and methotrexate (MIX) were used in the assay (Sigma Chemical Co, USA). Compounds **9–12** and **15–17** were previously reported.[24–26] The in vitro antimicrobial testing was performed at Department of Microbiology, Faculty of Pharmacy, Mansoura University, Egypt. The agar disc-diffusion method and a panel of standard strains (*S. aureus* IFO 3060, *B. subtilis* IFO 3007, *M. luteus* IFO 3232, *E. coli* IFO 3301, *P. aeuroginosa* IFO 3448, and *Candida albicans* IFO 0583) were employed**.** In vitro antitumor activity was conducted at the NCI, Bethesda, MD. All modeling experiments were conducted with Hyperchem 6.03 package from HYPERCUBE and POWERFIT1.0 program from 'Micro Simulations' running on a PC computer. The docking of the candidates into hDHFR pocket was performed with PowerFit software. Starting coordinate of hDHFR enzyme in tertiary complex with reduced-nicotinamide adenine dinucleotide phosphate (NADPH) and LIH, code ID 1DLS, was obtained from the Protein Data Bank of Brookhaven National Laboratory (Fig. 1).[44–46]

5.1. General procedure for preparation of 3-benzyl-2-ethylthio-6-methyl or nitro-quinazolin-4(3*H*)-one (13 and 14)

A mixture of the 2-thioxo-quinazoline analogues **9** or **10** (0.01 mol), ethyl iodide (5 ml) and anhydrous potassium carbonate (2 g) in acetone (50 ml) was heated under reflux for 8 h. The separated solid was filtered while hot, washed with acetone, the filtrate was then evaporated and the obtained residue was dried and recrystallized from the suitable solvents.

5.1.1. 3-Benzyl-2-ethylthio-6-methyl-quinazolin-4(3*H*)-one (13)

The crude product was recrystallized from EtOH (65%): mp 134–135 °C; ^1H NMR ($CDCl_3$) δ 1.42 (t, 3H, J = 7.5 Hz, CH_2CH_3),

2.48 (s, 3H, CH_3Ph), 3.26–3.30 (q, 2H, J = 7.5 Hz, –CH_2CH$_3$), 5.41 (s, 2H, CH_2Ph), 7.28–7.39 (m, 5 h, ArH), 7.48–7.54 (m, 2H, ArH), 8.07 (s, 1H, ArH). ^{13}C NMR δ 13.9, 21.2, 26.8, 47.2, 119.1, 126, 126.5, 127.6, 127.7, 128.5, 135.7, 135.8, 135.9, 145.7, 155.8, 162.1; MS m/e (310, 12%). Anal. ($C_{18}H_{18}N_2OS$) C, H, N.

5.1.2. 3-Benzyl-2-ethylthio-6-nitro-quinazolin-4(3*H*)-one (14)

The crude product was recrystallized from EtOH (72%): mp 149–150 °C; ^{1}H NMR (CDCl3) δ 1.45 (t, 3H, J = 7.5 Hz, two eclipsed triplets for the two isomers S–CH$_2$CH_3 and N–CH$_2$CH_3), 3.30–3.34 (q, 2H, J = 7.5 Hz, for the S–CH_2CH$_3$ isomer), 3.47–3.51(q, 2H, J = 7.5 Hz, for the N–CH_2CH$_3$ isomer), 5.41 (s, 2H, –CH_2Ph), 7.28–7.39 (m, 5H, ArH), 7.65 (d, 1H, J = 9 Hz, ArH), 8.47–8.50 (dd, 1H, J = 2.5, 9 Hz, ArH), 9.13 (d, 1H, J = 2.5 Hz, 1H). ^{13}C NMR δ 8.2 (N-isomer), 13.8, 27.3, 47.7, 53.3 (N-isomer), 119.2, 124.2, 127.5, 127.7, 128.0, 128.6, 128.7, 134.8, 144.6, 151.2, 160.8, 161.9; MS m/e (341, 18%). Anal. ($C_{17}H_{15}N_3O_3S$) C, H, N.

5.2. 6-Amino-3-benzyl-2-methylthio-quinazolin-4(3*H*)-one (17)

A mixture of the 3-benzyl-2-methylthio-6-nitro-quinazolin-4(3*H*)-one (**12**; 3.3 g, 0.01 mol), Fe powder pre-washed with dilute HCl and water (0.5 g), concentrated HCl (10 ml), in ethanol (50 ml), was heated under reflux for 0.5 h. The reaction mixture was cooled and treated with concentrated ammonia (5 ml) to precipitate Fe salts. The resulting mixture was filtered through Celite. Filtrate was concentrated to give the crude products which were chromatographed on silica gel using 0.5% MeOH/CH_2Cl_2 as eluant. Pure sample of the reported compound **17**[26] was obtained, along with a material identified to be compound **18.**

5.3. 6-Amino-3-benzyl-quinazolin-4(3*H*)-one (18)

The product was recrystallized from EtOH to yield brownish crystals (15%): mp 168–169 °C; ^{1}H NMR ($CDCl_3$) δ 3.95 (br s, 2H, NH_2), 5.10 (s, 2H, –CH_2Ph), 7.00–7.02 (m, 1H, ArH), 7.19–7.26 (m, 5H, ArH), 7.42–7.45 (m, 2H, ArH), 7.84 (s, 1H, ArH). ^{13}C NMR δ 49.5, 109.2, 122.8, 123.3, 127.9, 128.1, 128.8, 128.9, 136.1, 140.8, 142.9, 146.1, 160.9; MS m/e (251, 11%). Anal. ($C_{15}H_{13}N_3O$) C, H, N.

5.4. 2-Allylthio-3-benzyl-6-methyl-quinazolin-4(3*H*)-one (19)

A mixture of 3-benzyl-2-mercapto-6-methyl-quinazolin-4(3*H*)-one (**9**; 2.8 g, 0.01 mol), allyl bromide (1.8 g, 1.3 ml, 0.015 mol) and anhydrous potassium carbonate (2 g) in DMF (50 ml) was heated under reflux for 7 h. Solvent was then removed under reduced pressure, the obtained residue was dissolved in CH_2Cl_2 and washed with 10% NaOH solution, then water. The organic layer was separated, dried and evaporated. The obtained residue was chromatographed on silica gel using 25% EtOAc/hexane as eluant. An analytical pure sample of **19** was obtained and recrystallized from EtOAc/hexane (89%): mp 109–10 °C; ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3H, CH_3Ph), 3.94 (d, 2H, J = 6.5 Hz, –CH_2–CH=CH$_2$), 5.18–5.38 (dd, 2H, J = 10 Hz, –CH$_2$–CH=CH_2), 5.39 (s, 2H, –CH_2Ph), 5.96–6.03 (m, 1H, –CH$_2$–CH=CH$_2$), 7.27–7.48 (m, 7H, ArH), 8.08 (m, 1H, ArH); MS m/e (322, 8%). Anal. ($C_{19}H_{18}N_2OS$) C, H, N.

5.5. 2-Allylthio-3-benzyl-6-nitro-quinazolin-4(3*H*)-one (20)

A mixture of 3-benzyl-2-mercapto-6-nitro-quinazolin-4(3*H*)-one (**10**; 3.1 g, 0.01 mol), allyl bromide (1.8 g, 1.3 ml, 0.015 mol) and anhydrous potassium carbonate (2 g) in DMF (50 ml) was heated under reflux for 12 h. Solvent was then removed under reduced pressure, the obtained residue was dissolved in CH_2Cl_2 and washed with 10% NaOH solution, then water. The organic layer was separated, dried and evaporated. The obtained residue was chromatographed on silica gel using 20% EtOAc/hexane as eluant to afford **20** and the disulfide **21**. An analytical pure sample of **20** was obtained and recrystallized from EtOAc (82%): mp 128–130 °C; ^{1}H NMR ($CDCl_3$) δ 3.99 (d, 2H, J = 12 Hz, CH_2–CH=CH$_2$), 5.23–5.41 (dd, 2H, J = 9.5 Hz, –CH$_2$–CH=CH_2), 5.42 (s, 2H, –CH_2Ph), 5.95–6.03 (m, 1H, –CH$_2$–CH=CH$_2$), 7.28–7.40 (m, 5H, ArH), 7.66 (d, 1H, J = 9 Hz, ArH), 8.48–8.51 (dd, 1H, J = 2.5, 9 Hz, ArH), 9.14 (d, 1H, J = 2.5 Hz, ArH). ^{13}C NMR δ 35.6, 47.8, 119.2, 119.6, 124.2, 127.5, 127.8, 128.1, 128.6, 128.7, 131.8, 134.7, 144.8, 151.0, 160.8, 161.2; MS m/e (353, 10%). Anal. ($C_{18}H_{15}N_3O_3S$) C, H, N.

5.6. Bis(1-allyl-6-nitro-quinazolin-4(1*H*)-one-2-yl)-disulfide hydrochloride (21)

A light yellow oil, which was converted to its salt by the use of ethereal HCl, identified to be the disulfide compound **21** (10%): ^{1}H NMR ($CDCl_3$) δ 4.86 (d, 4H, J = 12 Hz, –CH_2–CH=CH$_2$), 5.31–5.47 (ddd, 4H, J = 18.5, 1 Hz, –CH$_2$–CH=CH_2), 6.03–6.11 (m, 2H, –CH$_2$–CH=CH$_2$), 6.45 (s, 1H, exchangeable), 6.69 (d, 2H, J = 9 Hz, ArH), 8.15–8.17 (dd, 2H, J = 2.5, 9 Hz, ArH), 8.89 (d, 2H, J = 2.5 Hz, ArH). ^{13}C NMR δ 65.8, 109.3, 116.21, 116.23, 119.0, 128.9, 129.3, 131.7, 137.5, 154.8, 166.5; MS m/e (524, 10%). Anal. ($C_{22}H_{17}ClN_6O_6S_2$) C, H, N.

5.7. General procedure for preparation of 3-benzyl-2-cinnamylthio-6-(methyl or nitro)-quinazolin-4(3*H*)-one (22 and 23)

A mixture of 2-thioxo-quinazoline analogues **9** or **10** (0.01 mol), cinnamyl bromide (3.0 g, 0.015 mol) and anhydrous potassium carbonate (2 g) in DMF (50 ml) was heated under reflux for 8 h. Solvent was then removed under reduced pressure. The obtained residue was dissolved in CH_2Cl_2 and washed with 10% NaOH solution, then water. The organic layer was separated, dried and evaporated. The obtained residue was chromatographed on silica gel using 25% EtOAc/hexane as eluant.

5.7.1. 3-Benzyl-2-cinnamylthio-6-methyl-quinazolin-4(3*H*)-one (22)

Analytical pure gummy material of **22** was obtained (87%): ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3H, CH_3Ph), 4.13 (d, 2H, J = 7.5 Hz, CH_2–CH=CHPh), 5.43 (s, 2H, –CH_2Ph), 6.33–6.39 (m, 1H, –CH$_2$–CH=CH–Ph), 6.72 (d, 1H, J = 15.5 Hz, –CH$_2$–CH=CH–Ph), 7.25–7.56 (m, 12H, ArH), 8.10 (s, 1H, ArH). ^{13}C NMR δ 21.3, 35.1, 47.3, 113.9, 119.2, 123.8, 126.0, 126.4, 126.6, 127.6, 127.62, 128.4, 134.1, 134.7, 135.8, 135.8, 135.9, 136.6, 145.6, 155.2, 162.1; MS m/e (398, 3%). Anal. ($C_{25}H_{22}N_2OS$) C, H, N.

5.7.2. 3-Benzyl-2-cinnamylthio-6-nitro-quinazolin-4(3*H*)-one (23)

The product was recrystallized from EtOAc/hexane to yield **23** (79%): mp 125–126 °C; ^{1}H NMR (DMSO-d_6) δ 4.18 (d, 2H, J = 10 Hz, –CH_2–CH=CH–Ph), 5.37 (s, 2H, –CH_2Ph), 6.33–6.45 (m, 1H, CH$_2$–CH=CHPh), 6.78 (d,1H, J = 15 Hz, –CH$_2$–CH=CH–Ph), 7.21–7.50 (m, 10H, ArH), 7.87 (d,1H, J = 10 Hz, ArH), 8.57–8.59 (dd, 1H, J = 2.5, 10 Hz, ArH), 8.83 (d, 1H, J = 2.5 Hz, ArH). ^{13}C NMR δ 34.6, 47.3, 118.8, 123.0, 123.5, 126.3, 126.8, 127.5, 127.8, 128.6, 128.9, 134.0, 135.0, 136.1, 144.2, 150.6, 160.2, 161.4; MS m/e (M+2; 431, 10%). Anal. ($C_{24}H_{19}N_3O_3S$) C, H, N.

5.8. General procedure for preparation of 2-(allylthio or cinnamylthio)-6-amino-3-benzyl-quinazolin-4(3*H*)-one (24 and 25)

A mixture of 0.01 mol of 2-Allylthio-3-benzyl-6-nitro-quinazolin-4(3*H*)-one (**20**), or 3-benzyl-2-cinnamylthio-6-nitro-quinazolin-4(3*H*)-one (**23**), Fe powder pre-washed with dilute HCl and

water (0.5 g), concentrated HCl (10 ml), in ethanol (50 ml), was heated under reflux for 0.5 h. The reaction mixture was cooled and treated with concentrated ammonia (5 ml) to precipitate Fe salts. The resulting mixture was filtered through Celite. Filtrate was concentrated to give the crude products, which were chromatographed on silica gel using 0.5% MeOH/CH_2Cl_2 as eluant.

5.8.1. 2-Allylthio-6-amino-3-benzyl-quinazolin-4(3*H*)-one (24)

The product was recrystallized from MeOH to give **24** (45%): mp 136–137 °C; ^{1}H NMR ($CDCl_3$) δ 3.91–3.93 (m, 4H, NH_2 & CH_2–CH=CH_2), 5.16–5.36 (dd, 2H, J = 10 Hz, –CH_2–CH=CH_2), 5.39 (s, 2H, –CH_2Ph), 5.95–6.03 (m, 1H, –CH_2–CH=CH_2), 7.09–7.11 (m, 1H, ArH), 7.27–7.37 (m, 5H, ArH), 7.43–7.46 (m, 2H, ArH); MS *m*/*e* (323, 52%). Anal. ($C_{18}H_{17}N_3OS$) C, H, N.

5.8.2. 6-Amino-3-benzyl-2-cinnamylthio-quinazolin-4(3*H*)-one (25)

The product was recrystallized from MeOH/H_2O to afford **25** (52%): mp 157–158 °C; ^{1}H NMR ($CDCl_3$) 3.93 (br s, 2H, NH_2), 4.10 (d, 2H, J = 7 Hz, –CH_2–CH=CH–Ph), 5.41 (s, 2H, CH_2Ph), 6.32–6.38 (m, 1H, –CH_2–CH=CH–Ph), 6.69 (d, 1H, J = 15.5 Hz, –CH_2–CH=CH–Ph), 7.10–7.12 (m, 1H, ArH), 7.25–7.37 (m, 10H, ArH), 7.47–7.49 (m, 2H, ArH); MS *m*/*e* (399, 10%). Anal. ($C_{24}H_{21}N_3OS$) C, H, N.

5.9. Ethyl 3-(3-benzyl-6-nitro-4-oxo-3,4-dihydro-quinazolin-2-yl-thio)-propanoate (26)

To a mixture of 3-benzyl-2-mercapto-6-nitro-quinazolin-4(3*H*)-one (**10**, 3.1 g, 0.01 mol), and Et_3N (2 ml), in CH_2Cl_2 (30 ml), stirred in ice bath, acryloyl chloride (3.2 ml, 0.04 mol) was added dropwise over a period of 15 min. Stirring was continued in ice bath for 1 h then at room temperature overnight. Solvent was then removed under reduced pressure; the obtained residue was dissolved in CH_2Cl_2 and washed with 10% NaOH solution, water, separated and dried over $MgSO_4$ then evaporated in vacuo. The obtained residue was chromatographed on silica gel using 10% EtOAc/hexane as eluant. An analytical pure sample of **26** was obtained and recrystallized from hexane/CH_2Cl_2 (94%): mp 100–2 °C;. ^{1}H NMR ($CDCl_3$) δ 1.19 (t, 3H, J = 7.5 Hz, –CH_2CH_3), 2.78 (t, 2H, J = 7.5 Hz, –S–CH_2–CH_2–CO–), 3.47 (t, 2H, J = 7.5 Hz, –S–CH_2–CH_2–CO–), 4.08–4.12 (q, 2H, J = 7.5 Hz, C$H_2$$CH_3$), 5.30 (s, 2H, –C$H_2$Ph), 7.22–7.29 (m, 5H, ArH), 7.57 (d, 1H, J = 9 Hz, ArH), 8.40–8.42 (dd, 1H, J = 2.5, 9 Hz, ArH), 9.05 (d, 1H, J = 2.5 Hz, ArH). ^{13}C NMR δ 14.2, 27.7, 33.7, 47.7, 60.9, 119.3, 124.2, 127.5, 127.8, 128.1, 128.6, 128.7, 134.6, 144.8, 151.0, 160.7, 161.2, 171.4 (ester C=O). MS *m*/*e* (M+2; 415, 3%). Anal. ($C_{20}H_{19}N_3O_5S$) C, H, N.

5.10. General procedure for preparation of 3-(3-benzyl-6-(methyl or nitro)-4-oxo-3,4-dihydro-quinazolin-2-yl-thio)-propanoic acid (27 and 28)

2-Thioxo-quinazoline analogues **9** or **10** (0.01 mol) was dissolved in toluene (30 ml), stirred in ice bath, and acryloyl chloride (3.2 ml, 0.04 mol) was added dropwise over a period of 15 min. Stirring was continued in ice bath for 1 h then at room temperature overnight. Solvent was then removed under reduced pressure; the obtained residue was suspended in water and pH was adjusted to neutrality using 10% ammonia solution, and then extracted with CH_2Cl_2, washed with water, separated and dried over $MgSO_4$, then evaporated in vacuo. The obtained residue was chromatographed on silica gel using 0.5% MeOH/CH_2Cl_2 as eluant.

5.10.1. 3-(3-Benzyl-6-methyl-4-oxo-3,4-dihydro-quinazolin-2-yl-thio)-propanoic acid (27)

The product was recrystallized from EtOAc/hexane to afford **27** (72%): mp 146–147 °C; ^{1}H NMR (CDCl3) δ 2.48 (s, 3H, CH_3PH), 2.94 (t, 2H, J = 6.5 Hz, –S–CH_2–CH_2–CO–), 3.51 (t, 2H, J = 6.5 Hz, –S–CH_2–CH_2–CO–), 5.39 (s, 2H, –CH_2Ph), 7.28–7.38 (m, 5H, ArH), 7.49–7.55 (m, 2H, ArH), 8.08 (s, 1H, ArH), 11.10 (br s, 1H, exchangeable OH). ^{13}C NMR δ 21.2, 26.3, 30.9, 47.3, 119.1, 126.0, 126.6, 127.6, 127.7, 128.4, 135.6, 136.0, 136.1, 145.5, 154.9, 162.1, 177.3. MS *m*/*e* (354, 1%). Anal. ($C_{19}H_{18}N_2O_3S$) C, H, N.

5.10.2. 3-(3-Benzyl-6-nitro-4-oxo-3,4-dihydro-quinazolin-2-yl-thio)-propanoic acid (28)

The product was recrystallized from EtOAc/hexane to afford **28** (80%): mp 198–199 °C; ^{1}H NMR (DMSO-d_6) δ 2.76 (t, 2H, J = 6.5 Hz, –S–CH_2–CH_2–CO–), 3.45 (t, 2H, J = 6.5 Hz, S–CH_2–CH_2–CO–), 5.32 (s, 2H, –CH_2Ph), 7.28–7.33 (m, 5H, ArH), 7.72 (d, 1H, J = 9 Hz, ArH), 8.08 (dd, 1H, J = 2.5, 9 Hz ArH), 8.78 (d, 1H, J = 2.5 Hz, ArH), 12.45 (br s, 1H, exchangeable OH). ^{13}C NMR δ 27.9, 33.6, 47.7, 119.2, 123.4, 127.3, 128.0, 128.2, 129.1, 129.3, 135.4, 144.6, 151.1, 160.6, 162.2, 173.1. MS *m*/*e* (M-1; 384, 1%). Anal. ($C_{18}H_{15}N_3O_5S$) C, H, N.

5.11. General procedure for preparation of 3-benzyl-1-cinnamoyl-6-(methyl or nitro)-2-thioxo-2,3-dihydro-quinazolin-4(1*H*)-one (29 and 30)

2-Thioxo-quinazoline analogues **9** or **10** (0.01 mol) was dissolved in toluene (30 ml), stirred in ice bath, and cinnamoyl chloride (6.7 g, 0.04 mol) was added dropwise over a period of 15 min. Stirring was continued in ice bath for 1 h then boiled under reflux for 2 h. Solvent was then removed under reduced pressure; the obtained residue was dissolved in CH_2Cl_2 and washed with 10% NaOH solution, then water, separated and dried over $MgSO_4$, then evaporated in vacuo. The obtained residue was chromatographed on silica gel using 0.5% MeOH/CH_2Cl_2 as eluant.

5.11.1. 3-Benzyl-1-cinnamoyl-6-methyl-2-thioxo-2,3-dihydro-quinazolin-4(1*H*)-one (29)

The product was recrystallized from hexane/CH_2Cl_2 to afford **29** (79%): mp 185–186 °C; ^{1}H NMR (CDCl3) δ 2.44 (s, 3H, CH_3PH), 5.82 (s, 2H, –CH_2Ph), 6.89 (d, 1H, J = 15.5 Hz, –CO–CH=CH–Ph), 7.00 (d, 1H, J = 7.5 Hz, ArH), 7.29–7.34 (m, 3H, ArH), 7.42–7.46 (m, 4H, ArH), 7.58–7.59 (m, 4H, ArH), 7.86 (d,1H, J = 15.5 Hz, –CO–CH=CH–Ph), 8.07 (s, 1H, ArH). ^{13}C NMR 20.8, 48.8, 114.6, 116.5, 121.8, 127.4, 127.5, 128.4, 128.7, 128.8, 129.1, 131.6, 133.8, 135.6, 135.7, 163.1, 136.6, 147.9, 159.2, 166.1, 173.5 (C=S). MS *m*/*e* (M+1; 413, 1%). Anal. ($C_{25}H_{20}N_2O_2S$) C, H, N.

5.11.2. 3-Benzyl-1-cinnamoyl-6-nitro-2-thioxo-2,3-dihydro-quinazolin-4(1*H*)-one (30)

The product was recrystallized from hexane/CH_2Cl_2 to afford **30** (61%): mp 220–222 °C; ^{1}H NMR (CDCl3) δ 5.81 (s, 2H, CH_2Ph), 6.86 (d, 1H, J = 15.5 Hz, –CO–CH=CH–Ph), 7.22 (d, 1H, J = 9 Hz, ArH), 7.33–7.36 (m, 3H, ArH), 7.44–7.50 (m, 3H, ArH), 7.56–7.61 (m, 4H, ArH), 7.93 (d,1H, J = 15.5 Hz, –CO–CH=CH–Ph), 8.45 (d, 1H, J = 9 Hz, ArH), 9.13 (s, 1H, ArH). ^{13}C NMR; 49.2, 115.8, 116.8, 120.8, 125.7, 128.1, 128.5, 128.9, 129.0, 129.2, 130.0, 132.1, 133.4, 135.2, 141.0, 144.3, 149.3, 157.7, 165.3, 174.5 (C=S). MS *m*/*e* (443, 1%). Anal. ($C_{24}H_{17}N_3O_4S$) C, H, N.

5.12. General procedure for preparation of N-(3-benzyl-2-substituted-thio-4-oxo-3,4-dihydro-quinazolin-6-yl)-acrylamides (31–33)

6-Amino-quinazolin-4(3*H*)-one (**17**, **24** or **25**, 0.01 mol), was dissolved in CH_2Cl_2 (30 ml). Et_3N (2 ml) was added and the mixture was stirred in ice bath. Acryloyl chloride (3.2 ml, 0.04 mol) was added dropwise over a period of 15 min. Stirring was continued in ice bath for 4 h. The CH_2Cl_2 solution was then washed succes-

sively with water and 10% NaOH solution. The organic layer was then separated, dried over $MgSO_4$, and then evaporated in vacuo. The obtained residue was flash chromatographed on silica gel using 30% CH_2Cl_2/hexane as eluant.

5.12.1. *N*-(3-Benzyl-2-methylthio-4-oxo-3,4-dihydro-quinazolin-6-yl)-acrylamide (31)

The product was recrystallized from CH_2Cl_2/hexane to afford **31** (42%): mp 236–237 °C; ^{1}H NMR ($CDCl_3$) δ 2.64 (s, 3H, S–CH_3), 5.43 (s, 2H, CH_2Ph), 5.82 (d, 1H, J = 9.5 Hz, CH_2=CH–CO), 6.27–6.32 (dd, 1H, J = 9.5 Hz, CH_2=CH–CO), 6.44 (d, 1H, J = 16.5 Hz, CH_2=CH–CO), 7.28–7.39 (m, 5H, ArH), 7.53 (br s, 1H, –CONH–), 7.62 (d, 1H, J = 8.5 Hz, ArH), 8.10–8.11 (m, 1H, ArH), 8.32–8.46 (m, 1H, ArH). MS m/e (351, 10%). Anal. ($C_{19}H_{17}N_3O_2S$) C, H, N.

5.12.2. *N*-(2-Allylthio-3-benzyl-4-oxo-3,4-dihydro-quinazolin-6-yl)-acrylamide (32)

The product was recrystallized from CH_2Cl_2/hexane to afford **32** (56%): mp 186–7 °C; ^{1}H NMR ($CDCl_3$) δ 3.94 (d, 2H, J = 6 Hz, –S–CH_2–CH=CH_2), 5.19–5.38 (dd, 2H, J = 10 Hz, S–CH_2–CH=CH_2), 5.39 (s, 2H, –CH_2Ph), 5.58 (d, 1H, J = 10 Hz, CH_2=CH–CO), 5.96–6.04 (m, 1H, –S–CH_2–CH=CH_2), 6.16–6.22 (m, 1H, –CH_2=CH–CO), 6.40 (d, 1H, J = 17 Hz, –CH_2=CH–CO), 7.29–7.30 (m, 5H, ArH), 7.56 (d, 1H, J = 9 Hz, ArH), 8.13 (s, 1H, ArH), 8.50 (br s, 1H, –CONH–), 8.60 (s, 1H, ArH). ^{13}C NMR δ 35.3, 47.4, 116.5, 119.0, 119.4, 127.1, 127.2, 127.6, 127.7, 127.9, 128.6, 131.0, 132.5, 135.3, 136.4, 144.2, 155.1, 162.0, 163.9. MS m/e (M+1; 378, 1%). Anal. ($C_{21}H_{19}N_3O_2S$) C, H, N.

5.12.3. (*E*)-*N*-(3-Benzyl-2-cinnamylthio-4-oxo-3,4-dihydro-quinazolin-6-yl)-acryl-amide (33)

The product was recrystallized from CH_2Cl_2/hexane to afford **33** (38%): mp 221–222 °C; ^{1}H NMR (DMSO-d_6) δ 4.10 (d, 2H, J = 7 Hz, S–CH_2–CH=CH–Ph), 5.33 (s, 2H, –CH_2Ph), 5.80 (d, 1H, J = 6 Hz, –CH_2=CH–CO), 6.28–6.51 (m, 3H, acryloyl and cinnamyl-H), 6.75 (d, 1H, J = 9.5 Hz, –CH_2=CH–CO), 7.23–7.40 (m, 10H, ArH), 7.67 (d, 1H, J = 9 Hz, ArH), 8.03–8.11 (s, 1H, J = 9 Hz, ArH), 8.53 (s, 1H, ArH), 10.51 (br s, 1H, –CONH–). MS m/e (453, 1%). Anal. ($C_{27}H_{23}N_3O_2S$) C, H, N.

5.13. General procedure for preparation of *N*-(3-benzyl-2-substituted-thio-4-oxo-3,4-dihydro-quinazolin-6-yl)-cinnamamides (34–36)

6-Amino-quinazolin-4(3H)-one (**17**, **24** or **25**, 0.01 mol), was dissolved in CH_2Cl_2 (30 ml). Potassium carbonate (1.0 g) was added and the mixture was stirred in ice bath. Cinnamoyl chloride (6.7 g, 0.04 mol) was added dropwise over a period of 15 min. Stirring was continued in ice bath for 8 h. The CH_2Cl_2 solution was the washed successively with water, and 10% NaOH solution. The organic layer was then separated, dried over $MgSO_4$, and then evaporated in vacuo. The obtained residue was flash chromatographed on silica gel using 30% CH_2Cl_2/hexane as eluant.

5.13.1. *N*-(3-Benzyl-2-methylthio-4-oxo-3,4-dihydro-quinazolin-6-yl)-cinnamamide (34)

The product was recrystallized from EtOH to afford **34** (68%): mp 141–142 °C; ^{1}H NMR (DMSO-d_6) δ 2.59 (s, 3H, S–CH_3), 5.35 (s, 2H, –CH_2Ph), 6.86 (d, 1H, J = 15.5 Hz, Ph–CH=CH–CO), 7.25–7.34 (m, 5H, ArH), 7.43–7.47 (m, 3H, ArH), 7.60–7.72 (m, 4H, ArH), 8.06–8.08 (m, 1H, ArH), 8.57 (s, 1H, ArH), 10.58 (br s, 1H, –CONH–). ^{13}C NMR δ 14.6, 46.8, 115.2, 118.9, 121.9, 126.6, 126.7, 126.7, 127.3, 127.8, 128.5, 129.0, 129.8, 134.6, 135.7, 137.1, 140.6, 143.1, 155.9, 160.8, 163.7. MS m/e (427, 10%). Anal. ($C_{25}H_{21}N_3O_2S$) C, H, N.

5.13.2. *N*-(2-Allylthio-3-benzyl-4-oxo-3,4-dihydro-quinazolin-6-yl)-cinnamamide (35)

The product was recrystallized from EtOH to afford **35** (59%): mp 105–106 °C; ^{1}H NMR (DMSO-d_6) δ 3.93 (d, 2H, J = 6 Hz, S–CH_2–CH=CH_2), 5.15–5.39 (dd, 2H, J = 10 Hz, S–CH_2–CH=CH_2), 5.34 (s, 2H, CH_2Ph), 5.89–6.05 (m, 1H, –S–CH_2–CH=CH_2), 6.86 (d, 1H, J = 15 Hz, Ph–CH=CH–CO), 7.22–7.74 (m, 12H, ArH), 8.04–8.12 (m, 1H, ArH), 8.57 (s, 1H, ArH), 10.55 (br s, 1H, –CONH–). MS m/e (453, 10%). Anal. ($C_{27}H_{23}N_3O_2S$) C, H, N.

5.13.3. *N*-(3-Benzyl-2-cinnamylthio-4-oxo-3,4-dihydro-quinazolin-6-yl)-cinnamamide (36)

The product was recrystallized from EtOH to afford **36** (65%): mp 180–182 °C; ^{1}H NMR (DMSO-d_6) δ 4.11 (d, 2H, J = 7 Hz, S–CH_2–CH=CH–Ph), 5.34 (s, 2H, CH_2Ph), 6.31–6.44 (m, 1H, cinnamyl H), 6.68–6.90 (m, 3H, cinnamoyl and cinnamyl-H), 7.23–7.69 (m, 17H, ArH), 8.58 (s, 1H, ArH), 10.61 (br s, 1H, –CONH–). MS m/e (M-1; 528, 1%). Anal. ($C_{33}H_{27}N_3O_2S$) C, H, N.

5.14. General procedure for preparation of 6-(allylamino or diallylamino)-3-benzyl-2-methylthio-quinazolin-4(3*H*)-one (37 and 38)

A mixture of 6-amino-3-benzyl-2-methylthio-quinazolin-4(3H)-one (**17**, 3 g, 0.01 mol), allyl bromide (1.8 g, 1.3 ml, 0.015 mol) and anhydrous potassium carbonate (2 g) in DMF (50 ml) was heated under reflux for 10 h. Solvent was then removed under reduced pressure, the obtained residue was chromatographed on silica gel using 25% EtOAc/hexane as eluant. An analytical pure sample of **37** was obtained along with the 6-diallylamino- analogue **38**.

5.14.1. 6-Allylamino-3-benzyl-2-methylthio-quinazolin-4(3*H*)-one (37)

The product was recrystallized from CH_2Cl_2/hexane to afford **37**(35%): mp 143–144 °C; ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3H, S–CH_3), 3.78 (d, 2H, J = 5.5 Hz, –CH_2–CH=CH_2), 4.18 (br s, 1H, NH–), 5.10–5.24 (dd, 2H, J = 10.5 Hz, –CH_2–CH=CH_2), 5.31 (s, 2H, –CH_2Ph), 5.85–5.91 (m, 1H, –CH_2–CH=CH_2), 6.94–6.96 (dd, 1H, J = 2.5 Hz, ArH), 7.15–7.28 (m, 6H, ArH), 7.34 (d, 1H, J = 8.5 Hz, ArH). ^{13}C NMR δ 15.1, 46.5, 47.3, 106.0, 116.6, 120.3, 122.3, 127.2, 127.5, 127.53, 128.5, 134.7, 136.0, 140.4, 146.1, 152.5, 162.1. MS m/e (337, 22%). Anal. ($C_{19}H_{19}N_3OS$) C, H, N.

5.14.2. 3-Benzyl-6-diallylamino-2-methylthio-quinazolin-4(3*H*)-one (38)

A light brownish yellow gummy material of **38** was obtained (52%): ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 3H, S–CH_3), 3.83–3.84 (m, 4H, –CH_2–CH=CH_2), 5.02–5.06 (m, 4H, –CH_2–CH=CH_2), 5.21 (s, 2H, –CH_2Ph), 5.68–5.75 (m, 2H, –CH_2–CH=CH_2), 6.95–6.97 (m, 1H, ArH), 7.06–7.22 (m, 5H, ArH), 7.28–7.32 (m, 2H, ArH). ^{13}C NMR δ 15.1, 47.5, 53.0, 106.7, 116.5, 120.2, 120.7, 127.1, 127.5, 127.6, 128.5, 133.4, 136.2, 139.4, 146.8, 152.2, 162.2. MS m/e (377, 10%). Anal. ($C_{22}H_{23}N_3OS$) C, H, N.

5.15. General procedure for preparation of 6-(allylamino or diallylamino)-2-allylthio-3-benzyl-quinazolin-4(3*H*)-one (39 and 40)

A mixture of 2-allylthio-6-amino-3-benzyl-quinazolin-4(3H)-one (**24**, 3.2 g, 0.01 mol), allyl bromide (1.8 g, 1.3 ml, 0.015 mol) and anhydrous potassium carbonate (2 g) in DMF (50 ml) was heated under reflux for 10 h. Solvent was then removed under reduced pressure, the obtained residue was chromatographed on silica gel using 25% EtOAc/hexane as eluant. An analytical pure

sample of **39** was obtained, along with the 6-diallylamino- analogue **40**.

5.15.1. 6-Allylamino-2-allylthio-3-benzyl-quinazolin-4(3*H*)-one (39)

A light brownish yellow gummy material of **39** was obtained (58%): ^{1}H NMR ($CDCl_3$) δ 3.85–4.29 (m, 5H, –S–C*H*$_2$–CH=CH$_2$, –N*H*–C*H*$_2$–CH=CH$_2$), 5.17–5.42 (m, 6H, –C*H*$_2$Ph, –S–CH$_2$–CH=C*H*$_2$, –NH–CH$_2$–CH=C*H*$_2$), 5.84–6.04 (m, 2H, –S–CH$_2$–C*H*=CH$_2$, –NH–C*H*$_2$–C*H*=CH$_2$), 7.04–7.06 (m, 1H, ArH), 7.27–7.35 m, 6H, ArH), 7.53–7.56 (m, 1H, ArH). ^{13}C NMR δ 35.3, 46.5, 47.4, 105.6, 116.5, 119.0, 120.4, 122.4, 127.3, 127.5, 127.6, 128.5 132.5, 134.7, 136.0, 140.2, 146.3, 151.3, 162.2. MS *m*/*e* (363, 15%). Anal. ($C_{21}H_{21}N_3OS$) C, H, N.

5.15.2. 2-Allylthio-3-benzyl-6-diallylamino-quinazolin-4(3*H*)-one (40)

A light brownish yellow gummy material of **40** was obtained (29%): ^{1}H NMR ($CDCl_3$) δ 3.93–4.04 (m, 6H, –S–C*H*$_2$–CH=CH$_2$ & -N–C*H*$_2$–CH=CH$_2$), 5.13–5.44 (m, 8H, –C*H*$_2$Ph, S–CH$_2$–CH=C*H*$_2$ &-N–CH$_2$–CH=C*H*$_2$), 5.87–6.05 (m, 3H, S–CH$_2$–C*H*=CH$_2$ & –N–CH$_2$–C*H*=CH$_2$), 7.15–7.19 (m, 1H, ArH), 7.62–7.52 (m, 7H, ArH). ^{13}C NMR δ 35.2, 47.4, 53.0, 106.7, 116.7, 118.6, 120.3, 120.7, 127.1, 127.5, 127.6, 128.5, 133.0, 133.3, 136.1, 139.3, 146.9, 151.0, 162.3. MS *m*/*e* (403, 8%). Anal. ($C_{24}H_{25}N_3OS$) C, H, N.

5.16. 6-Allyl-(methyl)-amino-3-benzyl-2-methylthio-quinazolin-4(3*H*)-one (41)

To a solution of 6-allylamino-3-benzyl-2-methylthio-quinazolin-4(3*H*)-one (**37**, 1.7 g, 0.005 mol) in 25 ml of acetonitrile, formaldehyde (0.4 g, 0.011 mol) was added with constant stirring. $NaCNBH_3$ (0.5 g, 0.008 mol) was then added, and the pH of the mixture was adjusted to 2–3 using concentrated HCl. A bright yellow precipitate was obtained. The acetonitrile was evaporated under reduced pressure and the obtained residue was suspended in 10 ml of water, and neutralized using NH_4OH to afford **41**, which was filtered, dried and recrystallized from CH_2Cl_2/hexane (81%): mp 123–125 °C; ^{1}H NMR (DMSO-d_6) δ 2.55 (s, 3H, S–C*H*$_3$), 3.01 (s, 3H, N–C*H*$_3$), 4.05 (s, 2H, –N–C*H*$_2$–CH=CH$_2$), 5.10–5.16 (m, 2H, –N–CH$_2$–CH=C*H*$_2$), 5.32 (s, 2H, –C*H*$_2$Ph), 5.81–5.90 (m, 1H, –N–CH$_2$–C*H*=CH$_2$), 7.19 (d, 1H, *J* = 3 Hz, ArH), 7.19–7.36 (m, 6H, ArH), 7.46 (d, 1H, *J* = 9 Hz, ArH). ^{13}C NMR δ 15.0, 38.6, 47.1, 54.7, 105.8, 116.7, 119.9, 121.3, 127.2, 127.4, 127.7, 129.0, 133.9, 136.6, 138.9, 147.7, 152.4, 161.5. MS *m*/*e* (351, 30%). Anal. ($C_{20}H_{21}N_3OS$) C, H, N.

5.17. General procedure for preparation of 3-benzyl-6-(cinnamylamino or dicinnamylamino)-2-methylthio-quinazolin-4(3*H*)-one (42 and 43)

A mixture of 6-amino-3-benzyl-2-methylthio-quinazolin-4(3*H*)-one (**17**, 3 g, 0.01 mol), cinnamyl bromide (3.0 g, 0.015 mol) and anhydrous potassium carbonate (2 g) in DMF (50 ml) was heated under reflux for 10 h. Solvent was then removed under reduced pressure, the obtained residue was chromatographed on silica gel using 10% EtOAc/hexane as eluant. An analytical pure sample of **42** was obtained, along with the 6-dicinnamylamino analogue **43**.

5.17.1. 3-Benzyl-6-cinnamylamino-2-methylthio-quinazolin-4(3*H*)-one (42)

A gummy material of **42** was obtained (34%): ^{1}H NMR ($CDCl_3$) δ 2.61 (s, 3H, S–C*H*$_3$), 4.03–4.16 (m, 2H, C*H*$_2$–CH=CH–Ph), 4.2 (br s, 1H, N*H*), 5.42 (s, 2H, –C*H*$_2$Ph), 6.34–6.38 (m, 1H, –CH$_2$–C*H*=CH–Ph), 6.67 (d, 1H, *J* = 15.5 Hz, –CH$_2$–CH=C*H*–Ph), 7.08–7.10 (m, 1H, ArH), 7.26–7.48 (m, 12H, ArH). ^{13}C NMR δ 15.1, 46.2, 47.3, 106.0, 120.4, 122.4, 126.2, 126.4, 127.3, 127.6, 127.7, 128.5, 128.6, 128.8, 132.1, 136.0, 136.5, 140.5, 146.0, 152.6, 162.1. MS *m*/*e* (413, 1%). Anal. ($C_{25}H_{23}N_3OS$) C, H, N.

5.17.2. 3-Benzyl-6-dicinnamylamino-2-methylthio-quinazolin-4(3*H*)-one (43)

A light brownish yellow gum of **43** was obtained (45%): ^{1}H NMR ($CDCl_3$) δ 2.64 (s, 3H, S–C*H*$_3$), 4.20–4.29 (m, 4H, –C*H*$_2$–CH=CH–Ph), 5.46 (s, 2H, –C*H*$_2$Ph), 6.35–6.40 (m, 2H, –CH$_2$–C*H*=CH–Ph), 6.66 (d, 2H, *J* = 15.5 Hz, CH$_2$–CH=C*H*–Ph), 7.29–7.47 (m, 16H, ArH), 7.60–7.62 (m, 1H, ArH), 7.71–7.72 (m, 1H, ArH). ^{13}C NMR 14.4, 15.2, 21.1, 47.4, 52.4, 60.4, 106.9, 120.4, 121.1, 125.2, 126.6, 127.6, 128.6, 131.9, 136.2, 136.9, 139.7, 146.9, 152.5, 162.2, 171.1. MS *m*/*e* (529, 12%). Anal. ($C_{34}H_{31}N_3OS$) C, H, N.

5.18. General procedure for preparation of 2-allylthio-3-benzyl-6-(cinnamylamino or dicinnamylamino)-quinazolin-4(3*H*)-one (44 and 45)

A mixture of 2-allylthio-6-amino-3-benzyl-quinazolin-4(3*H*)-one (**24**, 3.2 g, 0.01 mol), cinnamyl bromide (3.0 g, 0.015 mol) and anhydrous potassium carbonate (2 g) in DMF (50 ml) was heated under reflux for 10 h. Solvent was then removed under reduced pressure, the obtained residue was chromatographed on silica gel using 10% EtOAc/hexane as eluant. An analytical pure sample of **44** was obtained, along with the 6-dicinnamylamino analogue **45**.

5.18.1. 2-Allylthio-3-benzyl-6-cinnamylamino-quinazolin-4(3*H*)-one (44)

A gummy material of **44** was obtained (39%): ^{1}H NMR ($CDCl_3$) δ 3.81–4.04 (m, 2H, NH–C*H*$_2$–CH=CH–Ph), 4.26–4.28 (m, 2H, –S–C*H*$_2$–CH=CH$_2$), 5.18–5.43 (m, 4H, C*H*$_2$Ph, –S–CH$_2$–CH=C*H*$_2$), 5.98–6.06 (m, 1H, –S–CH$_2$–C*H*=CH$_2$), 6.31–6.35 (m, 1H, CH$_2$–C*H*=CH–Ph), 6.61 (d, 1H, *J* = 16 Hz, –CH$_2$–CH=C*H*–Ph) 7.28–7.67 (m, 14H, ArH & NH). ^{13}C NMR δ 36.1, 47.3, 52.1, 106.9, 118.6, 120.4, 121.9, 125.1, 126.5, 127.4, 127.55, 127.6, 127.62, 128.55, 128.6, 131.9, 132.6, 136.1, 136.8, 139.5, 147.0, 151.3, 162.2. MS *m*/*e* (439, 10%). Anal. ($C_{27}H_{25}N_3OS$) C, H, N.

5.18.2. 2-Allylthio-3-benzyl-6-dicinnamylamino-quinazolin-4(3*H*)-one (45)

A light brownish yellow gum of **45** was obtained (37%): ^{1}H NMR ($CDCl_3$) δ 3.95–4.28 (m, 6H,S–C*H*$_2$–CH=CH$_2$ & N–C*H*$_2$–CH=CHPh), 5.19–5.44 (m, 4H, –C*H*$_2$Ph, –S–CH$_2$–CH=C*H*$_2$), 5.98–6.05 (m, 1H, –S–CH$_2$–C*H*=CH$_2$), 6.32–6.35 (m, 2H, –CH$_2$–C*H*=CH–Ph), 6.61 (d, 2H, *J* = 16 Hz, –CH$_2$–CH=C*H*–Ph), 7.15–7.61 (m, 18H, ArH), ^{13}C NMR 14.3, 29.8, 35.2, 47.4, 52.4, 106.9, 118.6, 121.0, 121.2, 125.1, 126.5, 127.4, 127.6, 128.56, 128.6,131.9, 132.9, 136.1, 136.8, 139.5, 147.0, 151.2,162.3. MS *m*/*e* (555, 15%). Anal. ($C_{36}H_{33}N_3OS$) C, H, N.

5.19. Dihydrofolate reductase (DHFR) inhibition assay

The assay mixture contained 50 μM Tris–HCl buffer (pH 7.4), 50 μM NADPH, 20 μl DMSO or the same volume of DMSO solution containing the test compounds to a final concentration of 10^{-11} to 10^{-5} M, and 0.02 units of bovine liver DHFR or Human DHFR, in a final volume of 2.0 ml. After addition of the enzyme, the mixture was incubated at room temperature for 2.0 min, and the reaction was initiated by adding 25 μM FH_2, the change in absorbance (ΔA/min) was measured at 340 nm. The activity under these conditions was linear for 10 min.[35] Results are reported as % inhibition of enzymatic activity calculated using the following formula:

$$\% \text{ Inhibition} = \left(1 - \frac{\Delta A/\text{min}_{\text{test}}}{\Delta A/\text{min}_{\text{DMSO}}}\right)$$

The % inhibition values were plotted versus drug concentration (log scale). The 50% inhibitory concentration (IC_{50}) of each compound was obtained using the GRAPH PAD PRISM program, version 3 (San Diego, CA).

5.20. Determination of in vitro antimicrobial activity

The primary screen was carried out using the agar disc-diffusion method[36] using Müller–Hinton agar medium. Sterile filter paper discs (8 mm diameter) were moistened with the compound solution in dimethylsulfoxide of specific concentration 200 μg/disc, the antibacterial antibiotic gentamicin, sulfacetamide (100 μg/disc) and the antifungal drug Clotrimazole (100 μg/disc) were carefully placed on the agar cultures plates that had been previously inoculated separately with the microorganisms. The plates were incubated at 37 °C, and the diameter of the growth inhibition zones were measured after 24 h in case of bacteria and at 25 °C for 48 h in case of *C. albicans*. The minimal inhibitory concentrations (MIC, μg/ml) for the test compounds against the same microorganisms used in the primary screening were carried out using the microdilution susceptibility method in Müller–Hinton Broth.[37] Test compounds, gentamicin, and Sulfacetamide were dissolved in dimethylsulfoxide at concentration of 64 μg/ml. The two-fold dilutions of the solution were prepared (64 till 0.5 μg/ml). The microorganism suspensions at 106 CFU/ml concentrations (colony forming unit/ml) were inoculated to the corresponding wells. The plates were incubated at 37 °C for 24 h. The MIC values were determined as the lowest concentration that completely inhibited visible growth of the microorganism as detected by unaided eye.

5.21. Docking and molecular dynamic simulations

Lowest energy conformer of each new analogue 'global-minima' was docked into the hDHFR enzyme-binding domain. For each of the quinazoline analogues, energy minimizations (EM) were performed using 1000 steps of steepest descent, followed by conjugate gradient minimization to a RMS energy gradient of 0.01 Kcal/mol Å. The active site of the enzyme was defined using a radius of 8.0 Å around LIH. MD simulations were carried out for each ligand, with the rest of the enzyme kept fixed. The cofactor (NADPH) as a part of the enzyme structure was not fixed during the simulations. MD simulation was performed using time steps of 0.001 pico-second (ps), a distance dependant dielectric of 4.00, and a non-bonded cut-off distance of 8.0 Å, at 300 K. Complexes were first equilibrated for 10 ps and then simulated for 40 ps. Trajectory frames were collected every 200 steps for detailed analysis on the basis of potential energy and hydrogen bond interactions. These selected frames were minimized until RMS deviation values of 0.01 kcal/mol Å were reached for the active-site residues. Energy of binding was calculated as the difference between the energy of the complex and individual energies of the enzyme and ligand:

$$E\text{binding} = E\text{Complex} - (E\text{Ligand} + E\text{enzyme}).$$

where *E*Complex is the energy of the ligand–enzyme complex, *E*Ligand is the energy of the ligand corresponding to the binding conformation and *E*enzyme is the energy of the enzyme.[43,44]

5.21.1. Conformational search

Initial structures for the investigated molecules were constructed using the HYPERCHEM program version 5.1.[52] The MM (calculations in vacuo, bond dipole option for electrostatics, PolakeRibiere algorithm, and RMS gradient of 0.01 kcal/mol) conformational searching in torsional space was performed using the multiconformer method.[53–55] Energy minima for investigated compounds were determined by a semi-empirical method AM1[56] (as implemented in HyperChem 5.1). The conformations thus obtained were confirmed as minima by vibrational analysis. Atom-centred charges for each molecule were computed from the AM1 wave functions (HyperChem 5.1) by the procedure of Orozco and Luque,[57] which provides derived charges that closely resemble those obtainable from ab initio 6-31G* calculations.

5.21.2. Flexible alignment and pharmacophore prediction

The investigated compounds were subjected to flexible alignment and pharmacophore prediction experiment using 'Molecular Operating Environment' software (MOE of Chemical Computing Group Inc., on a Core 2 duo 1.83 GHz workstation).[50] The molecules were built using the Builder module of MOE. Their geometry was optimized by using the MMFF94 forcefield followed by a flexible alignment using systematic conformational search. Lowest energy aligned conformation(s) were identified through the analysis module of DSV by Accelrys Inc.,[58] and the distances among the pharmacophoric elements were measured.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.03.019.

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