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Original article

Design, synthesis and biological evaluation of novel quinazoline derivatives as potential antitumor agents: Molecular docking study

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ABSTRACT

Novel derivatives of quinazoline (**1**–**27**) have been synthesized and tested for their antitumor activity against three tumor cell lines among these cell lines the human breast carcinoma cell line (MCF-7) in which EGFR is highly expressed. All tested compounds showed potent and selective activity against breast cancer (MCF-7) with IC_{50} range of 3.35–6.81 μg/ml. With regarding broad-spectrum activity compounds **5**, **9**, **15**, **18** and **20** exploited potent antitumor against human liver cell line (HEPG2), human breast cell line (MCF-7) and human cervix cell line (HELA) with IC_{50} range of 3.35–5.59 μg/ml. Virtual screening was carried out through docking the designed compounds into the ATP binding site of epidermal growth factor receptor (EGFR) to predict if these compounds have analogous binding mode to the EGFR inhibitors.

1. Introduction

Cancer is continuing to be a major health problem in developing as well as undeveloped countries [1–9]. Surpassing heart diseases, it is taking the position number one killer due to various worldwide factors. Although major advances have been made in the chemotherapeutic management of some patients, the continued commitment to the laborious task of discovering new anticancer agents remains critically important. In the course of identifying various chemical substances which may serve as leads for designing novel antitumor agents, we are particularly interested in the present work with quinazoline derivatives which have been identified as a new class of cancer chemotherapeutic agents with significant therapeutic efficacy against solid tumors [7–9]. It is well known that quinazoline derivatives are potent inhibitors of epidermal growth factor receptor (EGFR) [10–18]. The epidermal growth factor receptor (EGFR) is cellular trans-membrane tyrosine kinases that is over-expressed in a significant number of human tumors (e.g., breast, ovarian, colon, and prostate), their expression levels often correlate with vascularity, and is associated with poor prognosis in patients [19–22]. Inhibitors of the EGFR PTK are therefore expected to have great therapeutic potential in the treatment of malignant and nonmalignant epithelial diseases. A number of small molecule EGFR kinase inhibitors have been evaluated in cancer clinical trials [10–22]. For example (Fig. 1), anilinoquinazoline-containing compounds Gefitinib (*Iressa*™) [10–12], Erlotinib (*Tarceva*™) [13], Lapatinib (*Tykerb*™, also known as GW-572016) and Vandetanib (*Zactima*™) were recently approved for the treatment of HER2-positive metastatic breast cancer [14–18]. Many more compounds are still under evaluation in clinical trials for the treatment of cancer [20–22].

In view of the previous rationale and in continuation of an ongoing program aiming at finding new structure leads with potential chemotherapeutic activities [7–9], new series of 4-substituted 6-chloro-2-*p*-tolylquinazolin (**1**–**27**) have been synthesized and screened for antitumor activity (Fig. 1). These series comprise the derived 4-substituted quinazoline pharmacophores that are structurally related to Erlotinib and Lapatinib (Fig. 1). The

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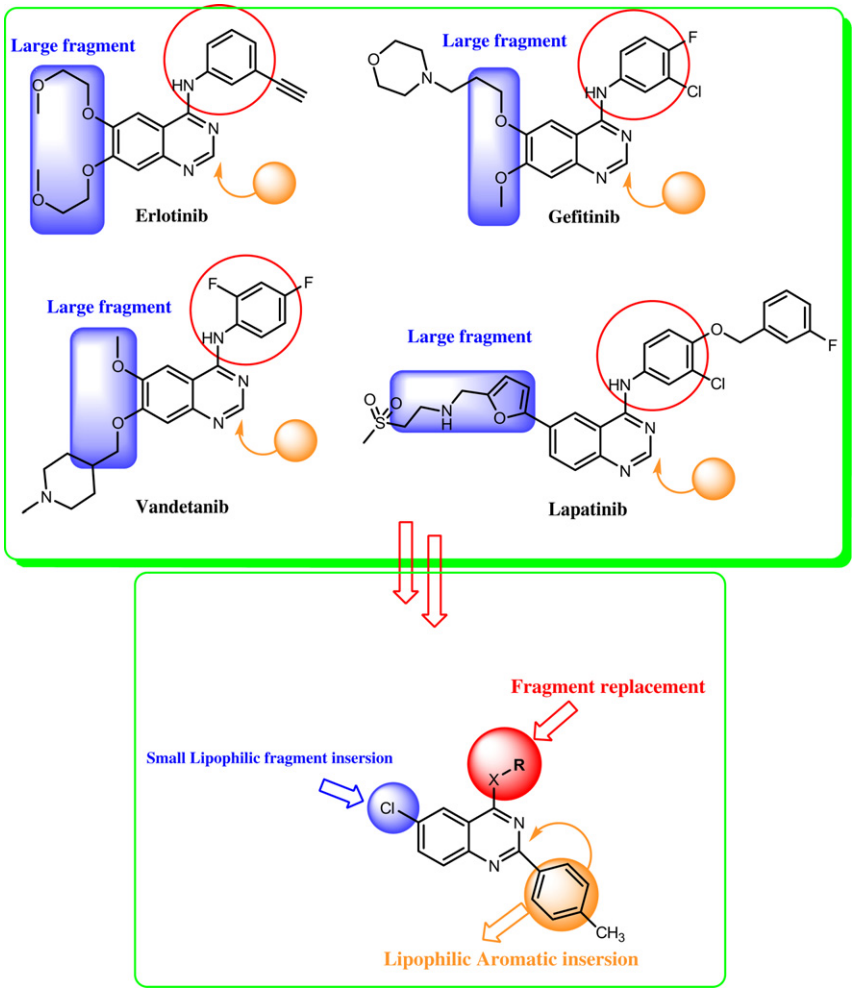


Fig. 1. Reported and proposed antitumor quinazoline derivatives.

thrust of efforts in the derivatization of such type of compounds focused mainly on the aryl moiety of the 4-substituted quinazoline. In the present study, the substitution pattern at the 4-substituted quinazoline pharmacophores was selected so as to confer different electronic environment that would affect the lipophilicity, and hence the activity of the target molecules. The objective of forming these hybrids is an attempt to reach an active antitumor agent with potentiated activity and selectivity toward cancerous cells. Moreover drug-likeness and molecular docking methodology were used to identify the structural features required for the antitumor properties of these new series. However the results of this molecular docking could support the postulation that our active compounds may act on the same enzyme target where EGFR inhibitor acts confirming the molecular design of the reported class of antitumor agents.

2. Results and discussion

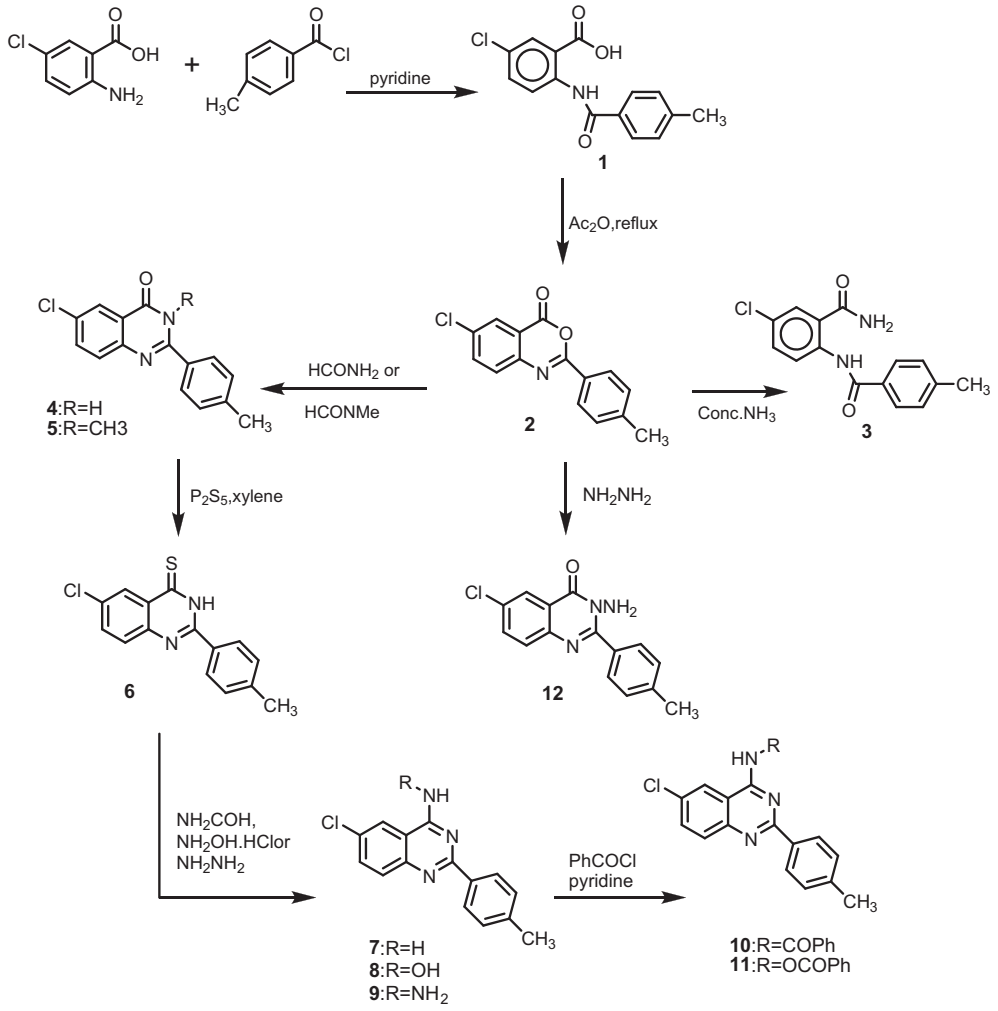
2.1. Chemistry

2.1.1. Synthesis of compounds **1**–**12** (Scheme 1)

Scheme 1 outlines the synthetic pathway used to obtain compounds **1**–**12** (series-**I**). The starting material 6-choro-2-*p*-tolyl-4*H*-benzo[*d*][1,3]oxazin-4-one (**2**) was prepared by the reaction of 5-chloroanthranilic acid with 4-methylbenzoyl chloride in pyridine to afford the 5-chloro-2-(4-methylbenzamido)-benzoic acid (**1**), which was boiled with acetic anhydride to give compound **2**. Upon stirring of compound **2** with concentrated ammonia solution at room temperature, the corresponding diamide **3** was obtained in relatively good yield. Moreover the treatment of **2** with formamide or methylformamide produced 6-chloro-2-*p*-tolylquinazolin-4(3*H*)-one (**4**) and 6-chloro-3-methyl-2-*p*-tolylquinazolin-4(3*H*)-one (**5**) in 73% and 68% yields, respectively. On the other hand, when compound **2** was reacted with hydrazine hydrate gave the corresponding 3-amino-4(3*H*)quinazolinone (**12**) in 88% yield. The reaction of compound **4** with P_2S_5 in anhydrous xylene afforded 6-chloro-2-*p*-tolylquinazolin-4(3*H*)-thione (**6**). 4-Substituted aminoquinazolines (**7**–**9**) were obtained by the reaction of compound **6** with formamide, hydroxylamine hydrochloride and/or hydrazine hydrate. *N*-(6-chloro-2-*p*-tolylquinazolin-4-yl) benzamide (**10**) and O-benzoyl-*N*-(6-chloro-2-*p*-tolylquinazolin-4-yl)hydroxylamine (**11**) were obtained in a relatively good yield (69% and 71%, respectively) via the reaction of compound **7** or **8** with benzoyl chloride in pyridine.

2.1.2. Synthesis of compounds **13**–**27** (Scheme 2)

Scheme 2 outlines the synthetic pathway used to obtain compounds **13**–**27** (series-**II**). The reaction of **2** with P_2S_5 in anhydrous xylene afforded 6-chloro-2-p-tolyl-4H-benzo[d][1,3]-oxazin-4-thione (**13**) which was converted into compound **6** by heating with formamide. Interaction of **6** with various halogenated compounds in acetone containing K_2CO_3 at room



Scheme 1. Synthesis of the proposed antitumor 4-quinazolone and 4-aminoquinazoline derivatives (series-**I**).

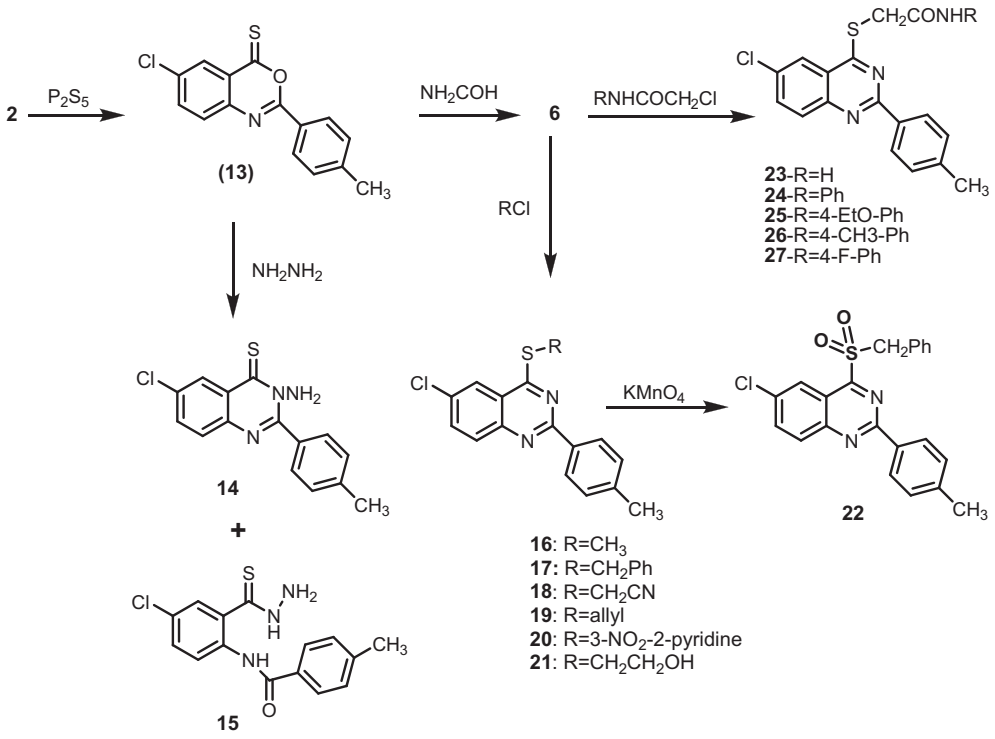
temperature furnished 6-chloro-4-(substitutedthio)-2-*p*-tolylquinazolines (**16**–**21**) in 80–95% yield. The oxidation of 6-chloro-4-(benzylthio)-2-*p*-tolylquinazoline (**16**) with $KMnO_4$ in acetic acid afforded 4-(benzylsulfonyl)-6-chloro-2-*p*-tolylquinazoline (**22**) in a 63% yield. Moreover 2-(6-chloro-2-*p*-tolylquinazolin-4-ylthio) substituted acetamides (**23**–**27**) were produced by heating of compound **6** with various chloroacetanilides in acetone containing K_2CO_3 in 84–90% yield. Finally, the reaction of **13** with hydrazine hydrate afforded 3-amino-6-chloro-2-*p*-tolylquinazolin-4(3*H*)-thione (**14**) and *N*-(4-chloro-2-(hydrazinecarbonothioylphenyl)-4-methylbenzamide (**15**) in 46% and 43% yields respectively.

2.2. Biological activity

2.2.1. In vitro antitumor evaluation

The antitumor activity of all compounds against HEPG2 human liver cell line, MCF-7 cell line and HELA cervix cell line was determined using Sulphorhodamine-B assay and Doxorubicin (DOX) as a reference drug control [23]. Each cell line was incubated with four concentrations (6–62 μg/ml) for each compound and was used to create compound concentration versus survival fraction curves. The response parameter (IC_{50}) was calculated for each cell line (Table 1). The IC_{50} value corresponds to the compound's concentration causing a net 50% loss of initial cells at the end of the incubation period (48 h). The antitumor drug discovery screen has been designed to distinguish between broad-spectrum antitumor compounds and tumor selective agents [24].

In present study, the active analogs showed a distinctive potential pattern of selectivity as well as broad-spectrum antitumor activity. With regard to selectivity against individual cell lines, most of the compounds showed effectiveness against cell line human breast cancer MCF-7 with IC_{50} values range of 3.35–6.81 μg/ml comparative to DOX IC_{50} (3.76 μg/ml) (Table 1). HEPG2 human liver cell line proved to be sensitive toward compounds **5**, **9**, **15**, **18**, **20** and **25** with IC_{50} concentration range of 4.17–5.99 μg/ml comparative to DOX IC_{50} (4.57 μg/ml). Regarding HELA cervix cell line, higher sensitivity was observed with compounds **5**, **6**, **7**, **9**, **11**, **15**, **18**, **20** and **23** with IC_{50} concentration range of 3.56–5.39 μg/ml comparative to DOX IC_{50} (3.64 μg/ml). With regard to broad-spectrum antitumor activity, compounds **5**, **9**, **15**, **18** and **20** showed IC_{50} concentration range of 3.35–5.59 μg/ml against the three cell lines (Table 1). On the other hand compounds **6**, **7**, **11** and **23** showed higher cytotoxic activity against MCF-7 cell line and HELA cervix cell line with low activity against HEPG2 human liver cell line. Moreover compounds **1**, **2**, **10**, **14** and **27** proved to be ineffective against the three cell lines.



Scheme 2. Synthesis of the proposed antitumor quinazoline-4-thione and 4-substituted mercaptoquinazoline derivatives (series-**II**).

Table 1
In vitro antitumor activity of the designed quinazoline deriviatives.

Compd no.	IC_{50} (μg/ml)[a]		
	MCF-7[b]	HELA[c]	HEPG2[d]
1	14.7	18.9	15.95
2	10.5	8.23	22.5
3	5.39	8.23	21
4	4.98	10.1	9.86
5	3.35	4.37	4.17
6	3.35	4.57	7.01
7	3.76	3.56	14.5
8	6.81	12.7	23.1
9	3.76	3.98	4.17
10	16.1	16.2	17.5
11	5.59	5.18	12.1
12	5.39	12.1	18.4
13	5.18	13.3	11.1
14	9.25	14.01	17
15	3.35	4.98	5.59
16	8.64	14.7	13.3
17	9.66	11.3	18.4
18	3.35	3.76	4.37
19	8.03	9.66	23.9
20	3.56	5.39	5.59
21	3.76	9.25	9.04
22	3.96	7.01	7.21
23	5.59	4.98	9.45
24	4.78	11.7	14.3
25	15.8	10.1	5.99
26	5.79	12.3	18.8
27	14.7	10.5	18.4
DOX	3.76	3.64	4.57

[a] IC_{50}, compound concentration required to inhibit tumor cell proliferation by 50%.
[b] Human breast cell line (MCF-7).
[c] Human cervix cell line (HELA).
[d] Human liver cell line (HEPG2).

2.2.2. Structural–activity relationship, Lipinski rule of five and drug-likeness profile

2.2.2.1. Structural–activity relationship. The activity of the tested compounds could be correlated to structure variation and modifications. By investigating the variation in selectivity of the tested compounds over the three cell lines, it was revealed that nearly all of the compounds belonging to series-**I** and series-**II** (Schemes 1 and 2) showed significant inhibition for the breast cancer cell line (MCF-7). This great inhibition at the mentioned concentration indicates a great potency for the compound with a strong lethal effect over (MCF-7) breast cancer cells. Moreover the IC_{50} of cyctotoxic activity for HEPG2 human liver cell line and HELA cervix cancer cell reached 3.56 μg/ml in a number of the tested derivatives (Table 1). However, distinguished selectivity was observed between the first series-**I** (compounds **1**–**12**), and the second series-**II** (compounds **13**–**27**). Both series-**I** and series-**II** showed significant and selective inhibition for (MCF-7) breast cancer (IC_{50} value of 3.35 μg/ml). On the other hand series-**I** were more selective for HELA cervix cancer cell than HEPG2 human liver cell line as observed in Table 1. The agreement between the two series in the inhibition of (MCF-7) breast cancer cells could be correlated to a similar inhibitory mechanism related to the common structural feature in the two series (the quinazoline fragment), while the variation in selectivity over HEPG2 human liver cell line and HELA cervix cancer cell line is probably caused by the differences in the substitution pattern in the two series. These variations could be also correlated to the lipophilic difference substitution pattern of the quinazoline cores of the two series.

Briefly the obtained screening results showed that, among the tested compounds, compounds **5**–**7** and **9** from series-**I** and compounds **15**, **18**, **20**–**22** from series-**II** are the most active members with IC_{50} values of 3.35–3.96 μg/ml. Regarding two series, upon cyclization of compound **1** afforded compound **2** with little increase in activity while replacement of the carboxylic moiety of compound **1** with amide group as in **3** this resulted in

Table 2
Solubility and calculated Lipinski's rule of five for the most active compounds over breast cancer (MCF-7) cell line.

Compd no.	IC_{50}[a]	log S[b]	Parameter				
			log P[c]	PSA[d]	MW[e]	*n*ON[f]	*n*OHNH[g]
5	3.35	−3.92	3.58	34.89	284.75	3	0
6	3.35	−4.22	4.16	28.68	286.78	2	1
7	3.76	−5.58	4.73	51.81	269.73	3	2
9	3.76	−5.66	4.27	63.83	284.75	4	3
15	3.35	−4.50	2.66	67.15	319.82	4	4
18	3.35	−6.33	4.92	49.57	325.82	3	0
20	3.56	−6.37	5.46	84.50	408.87	6	0
21	3.76	−5.67	4.42	46.01	330.84	3	1
22	3.96	−7.10	4.92	59.92	408.91	4	0

[a] Data taken from Table 1.
[b] Solubility parameter.
[c] Calculated lipophilicity.
[d] Polar surface area ($Å^2$).
[e] Molecular weight.
[f] Number of hydrogen bond acceptor.
[g] Number of hydrogen bond donor.

a remarkable increase in the magnitude of antitumor activity against MCF-7-and HELA cell lines with IC_{50} value 5.39 and 8.23 μg/ml respectively. On the other hand, introduction of NH or N-CH_3 moieties at position-3 of compound **2**, afforded **4** and **5** with a remarkable increase in the antitumor activity against breast cancer, IC_{50} values, 4.98 and 3.25 μg/ml respectively. Moreover, also compound **4** showed moderate activity toward HELA and HEPG2 cancer cell lines with IC_{50} values, 10.1 and 9.86 μg/ml respectively. Upon thiation of compound **4** to the corresponding quinazoline-thione, compound **6** was obtained with a dramatic broad-spectrum antitumor activity. In case of compound **2**, the presence of NH_2 moiety at position-3 favoured the antitumor activity against breast cancer MCF-7 and retains the activity against HELA and HEPG2 cancer cell lines. Interestingly the addition of such NH_2 group at position-4 rather than the position-3 resulted in an increase in antitumor activity against both breast cancer and HELA cell lines, while replacement of such group with NHOH moiety lead to decrease in cytotoxic activity against all cell lines. On the other hand insertion of $-NHNH_2$ fragment at the same position-4 resulted in sharp increase in antitumor activity with broad-spectrum effect. In addition, an increase in antitumor activity over breast cancer was observed when the benzoxazine-4-one (**2**) (IC_{50} value; 10.5 μg/ml) was converted to benzoxazine-4-thione (**13**) (IC_{50} value; 5.18 μg/ml). On contrary to conversion of **2** (IC_{50} value; 10.5 μg/ml) to **12** (IC50 value; 5.39 μg/ml) with increased in antitumor activity over breast cancer, conversion of benzoxazine-4-thione (**13**) (IC_{50} value; 5.18 μg/ml) into 3-aminoquinazoline-4-thione (**14**) resulted in two fold decrease in activity (IC_{50} value; 9.25 μg/ml). The ring opening of 3-aminoquinazoline-4-thione (**14**) resulted in the formation of benzothiohydrazide (**15**) with broad-spectrum antitumor agent with IC_{50} values of 3.35 μg/ml, 4.49 μg/ml and 5.59 μg/ml against MCF-77, HELA and HEPG2 cell lines, respectively. More interestingly, S-substituted compound **6** resulted in derivative with variant activity against the three cell line such as compounds **18** and **20** were broad-spectrum antitumor agents with IC_{50} range of 3.35–5.59 μg/ml. The high activity over breast cancer cell line of such two compounds **18** (3.35 μg/ml) and **20** (3.56 μg/ml) over similar analogs **16** (8.64 μg/ml), **17** (9.66 μg/ml) and **19** (8.03 μg/ml) may be attributed to the occurrence of CN and NO_2 moieties which may be an important fragments for hydrogen bonding formation at the receptor site as described in docking section.

2.2.2.2. Lipinski rule of five and drug-likeness profile. In this work, we submitted the most active compounds over MCF-7 cell line to the analysis of Lipinski rule of five that indicates if a chemical compound could be an orally active drug in humans [25]. Our results showed that all active compounds (**5**–**7**, **9**, **15**, **18** and **20**–**22**) fulfilled this rule (molecular weight = 269.73–408.91, *c* log *P* = 2.66–4.92, *n*ON = 2–6, and *n*OHNH = 0–4) (Table 2), similarly to clinically used drugs (i.e. Tarceva, Iressa, Vandetanib and Lapatinib) (data not shown). Currently there are many approaches that assess a compound drug-likeness based on topological descriptors, fingerprints of molecular drug-likeness structure keys or other properties such as *c* log *P* and molecular weight [26]. In the Osiris program (http://www.organic-chemistry.org/prog/peo) the occurrence frequency of each fragment is determined within the collection created by shreddering 3300 traded drugs as well as 15,000 commercially available chemicals (Fluka) yielding a complete list of all available fragments. In this work, we used the Osiris program for calculating the fragment based drug-likeness of the active compounds also comparing them with Tarceva, Iressa, Vandetanib and Lapatinib (Fig. 2). Interestingly, the derivatives **5**–**7**, **9**, **15**, **18** and **20**–**21** presented better drug-likeness values (from 2.22 to −5.54) than Tarceva (−6.73) and similar results to Iressa, Vandetanib and Lapatinib (−2.62, 3.51 and −4.8 respectively). In this study we also verified the drugscore [27]. Our theoretical data showed that compounds **5**–**7**, **9**, **15**, **18** and **20**–**21** presented values once again higher than Lapatinib (Fig. 2). Moreover, we used the Osiris program (Fig. 2) to predict the overall toxicity of the most active derivatives as it may point to the presence of some fragments generally responsible for the irritant, mutagenic, tumorigenic, or reproductive effects in these molecules.

Compd No.	Toxicity effects			
	M	T	I	R
5	Low	Low	Low	Low
6	Low	Low	Low	Low
7	Medium	Low	Low	Low
9	Medium	Low	Low	Low
15	Low	Low	Low	Low
18	Medium	Low	Low	Low
20	Medium	Medium	Low	Medium
21	Medium	Low	Medium	Low
22	Medium	Low	Low	Low
Erlotinib	Low	Low	Low	Low
Gefitinib	Low	Low	Low	Low
Vandetanib	Low	Low	Low	Low
Lapatinib	Low	Low	Low	Medium

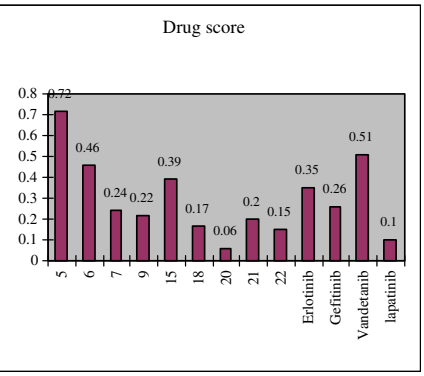


Fig. 2. *In silico* toxicity risks (left panel) and drugscore (right panel) of the reported and the most active antitumor quinazoline derivatives over breast cancer (M, mutagenic; T, tumorigenic; I, irritant; R, reproductive).

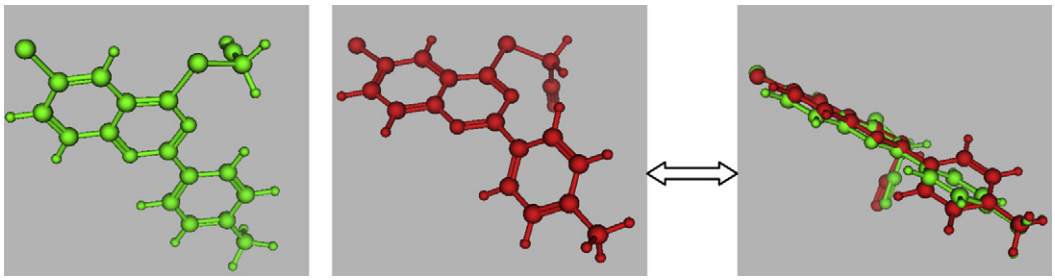


Fig. 3. Lowest energy (green color) and bioactive conformers (red color) of the most active compound **18** as representative example and their superposition (left panel).

Interestingly, most of the active derivatives presented a low *in silico* toxicity risk profile, similar to Tarceva, Iressa, Vandetanib and Lapatinib (Fig. 2). These theoretical data reinforced the cytotoxicity experimental data described in this work pointing these compounds as lead compounds with low toxicity risk profile.

2.3. Molecular modeling study

Modeling studies are required in order to construct molecular models that incorporate all experimental evidences reported. These models are necessary to obtain a consistent and more precise picture of the biological active molecules at the atomic level and furthermore, provide new insights that can be used to design novel therapeutic agents.

2.3.1. Conformational analysis

As an attempt to gain a better insight into the molecular structures of the most active compounds **5**–**7**, **9**, **15**, **18** and **20**–**22**, conformational analysis has been performed by use of the MM+ force-field [28] (calculations in vacuo, bond dipole option for electrostatics, Polak–Ribiere algorithm, and RMS gradient of 0.01 kcal/Å mol) as implemented in HyperChem 5.1 [29]. The most stable conformer was fully optimized with AM1 semi-empirical molecular orbital calculation [30]. The global minimum was confirmed as true minimum not saddle point by the absence of negative eigen value of the Hessian through frequency calculation [3,31]. The calculation results showed that the lowest energy-minimized structures of the compounds under investigation exhibited a common arrangement of the 2-phenyl moiety, such group arranged in a coplanar form to the quinazoline core. On the other hand the bioactive conformation resulted from conformational analysis in the vicinity of receptor site showed that 2-phenyl moiety attached to quinazoline core was out of the plane of quinazoline ring by 30.7–51.9° (Fig. 3).

2.3.2. Electrostatic and hydrophobic mappings [9,31]

In an attempt to understand the lowest activity of compound **17** and the highest antitumor activity of **18** as a representative example of the same series, electrostatic and hydrophobic mappings have been carried out for the lowest energy conformers, to examine the similarity and dissimilarity in electronic, electrostatic binding characteristics of the surface of the molecules and conformational properties (Fig. 4; left panel). Comparison of the electrostatic mappings of **17** and **18** show that compound **18** possesses an increased negative charge regions located on the CN groups representing hydrogen bond regions (in red). In contrast,

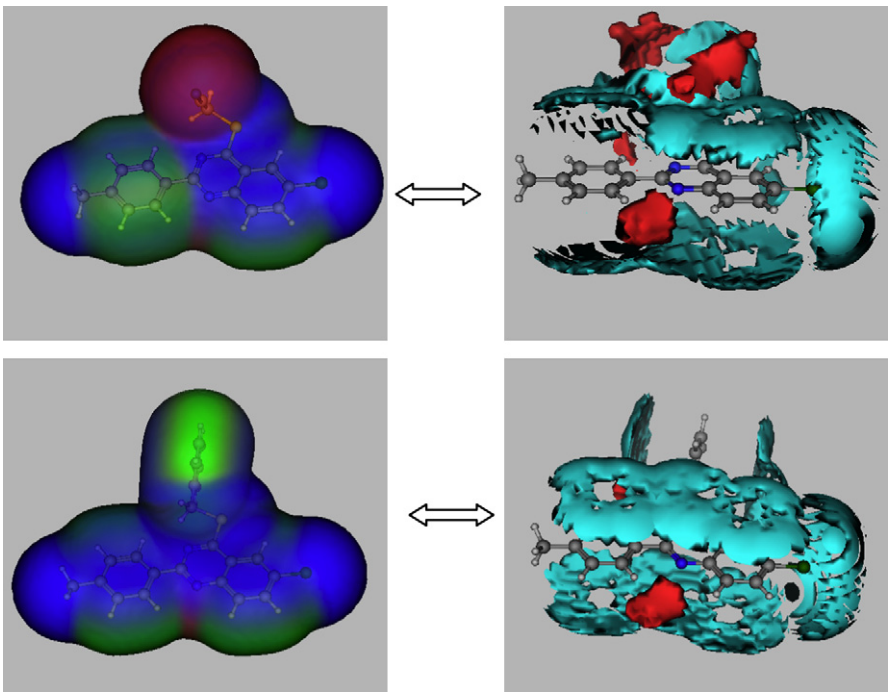


Fig. 4. Electrostatic maps (left panels) and hydrophobic maps (right panels) of the lowest energy conformers for the most active compound **18** (upper panel) and the least active compound **17** (lower panel); maps are color coded: red for a hydrogen bond and a hydrophilic region, green for a medium polar region, and blue or cyan for a hydrophobic region.

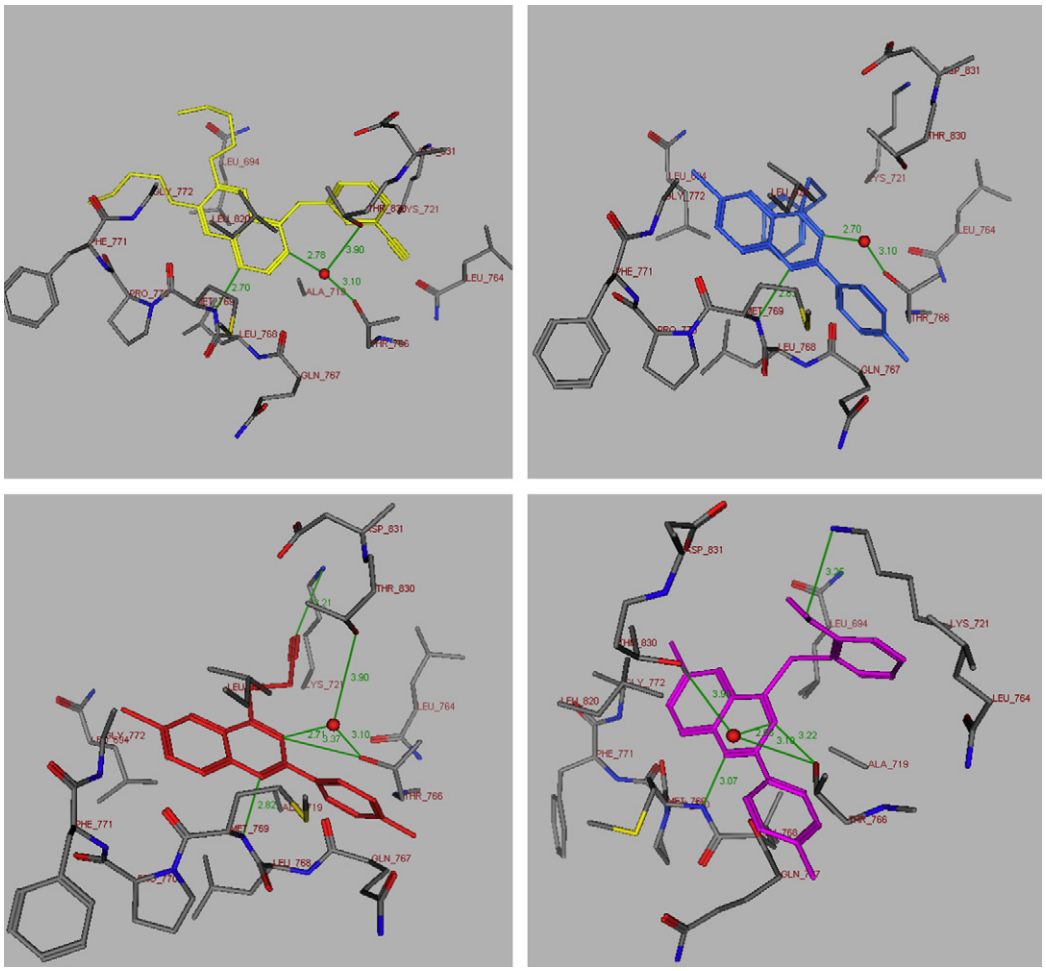


Fig. 5. Docking of compounds **17** (upper right panel), **18** (lower left panel) and **20** (lower right panel) into the active site of epidermal growth factor receptor. Upper left panel showed the Erlotinib inhibitor/EGFR complex. Hydrogen bonds are shown in green.

the lowest activity of compound **17** maybe attribute to the difference in electrostatic mapping with lack of the red hydrogen bond area on such site which may be important for receptor interaction (Fig. 4; lower left panel).

Similarly, hydrophobic mappings of the most active compound **18** showed that the hydrophobic region in cyan was distributed on both side of 2-phenyl fragment and the quinazoline core while the hydrophilic region in red was located mainly on CN fragments at 4 position of quinazoline core (Fig. 4; upper right panel). On the other hand the hydrophobic distributions of the compound **17** occupy additional hydrophobic benzyl fragment at position-4 of quinazoline core and lacking of hydrophilic region at such position indicating dissimilarity of such compound (Fig. 4; lower right panel).

It is clear that the related charge distribution, electrostatic and hydrophobic mappings suggest a similar activity of the molecules. As a result, we find that compounds **5**–**7**, **9**, **15**, **18** and **20**–**22** all can adopt conformations that have identical distances and orientations and that show sufficient agreement of the overall structure and of the electrostatic potential pattern.

2.3.3. Molecular docking studies

The level of antitumor activities of the proposed compounds over MCF-7 breast cancer cell, in which EGFR is highly expressed, prompted us to perform molecular docking into the ATP binding site of epidermal growth factor receptor (EGFR) to predict if these compounds have analogous binding mode to the EGFR inhibitors. Compounds **18** (3.35 μg/ml; MCF-7), **17** (9.66 μg/ml; MCF-7) and **20** (3.56 μg/ml; MCF-7) were used for docking study as representative examples of the most active and moderate active compounds of the same series. All the calculations were performed using MOE 2008.10 software [32] installed on 2.0G Core 2 Duo. The crystal structure of epidermal growth factor receptor with Erlotinib (*Tarceva*™) (1M17) was obtained from protein data bank PDB (Fig. 5, upper left panel) [33–35]. The automated docking program of MOE 2008.10 was used to dock compounds **17**, **18** and **20** into ATP binding site of EGFR. The complexes were energy-minimized with a MMFF94 force-field [36] till the gradient convergence 0.01 kcal/mol was reached. The binding energies of −15.30, −25.66 and −23.79 kcal/mol were obtained for **17**, **18** and **20**, respectively (Fig. 5). These docking studies have revealed that the quinazoline ring binds to a narrow hydrophobic pocket in the N-terminal domain of EGFR-TK where N-1 of the quinazoline ring interacts with the backbone NH of Met-769 via a hydrogen bond, and similarly, a water (HOH-10) molecule-mediated hydrogen bonding interaction is observed between the N-3 of the quinazoline ring and the Thr-830 side chain. These interactions revealed the importance of both nitrogen atoms for binding and the subsequent inhibitory capacity.

Compounds **18** and **20** complex with EGFR-TK showed the occurrence of four hydrogen bonds with Met-769 (2.82 Å, 3.07 Å respectively), Thr-766 (3.37 Å, 3.22 Å respectively), Lys-721 (3.21 Å, 3.25 Å respectively) and HOH-10 mediated hydrogen bonding

interaction with Thr-830 side chain (2.71 Å, 2.66 Å respectively). The lower interaction energy observed for **17** (Fig. 5, upper right panel) rationalizes the insufficient binding into the EGFR-TK active site than that of the compounds **18** and **20** (Fig. 5, lower panels). The insufficient binding can be explained in terms of the occurrence of only two weak hydrogen bonds between the N-1 and N-3 of quinazoline ring system and Met-769 (2.83 Å) and HOH-10 mediated hydrogen bonding interaction with Thr-766 side chain (3.10 Å). In short, Fig. 5 demonstrates binding models of quinazoline in the ATP binding site. In each case, the N-1 atom of the quinazoline is hydrogen bonded to the backbone nitrogen of Met-769, while the N-3 atom is hydrogen bonded to the side chain hydroxyl of Thr-830 via a water molecule, and in case of compounds **18** and **20** we noticed that an additional hydrogen bond is formed between N-3 and the side chain hydroxyl of Thr-766 and hydrogen bond between CN of compound **18** and NO_2 of compound **20** with the backbone nitrogen of Lys-721. The results of this molecular docking could support the postulation that our active compounds may act on the same enzyme target where EGFR inhibitor acts confirming the molecular design of the reported class of antitumor agents [7–9,11–16,37].

3. Conclusion

The present work led to the development of novel antitumor molecules containing 4-substituted quinazoline pharmacophore. Three cell lines including human liver cell line (HEPG2), human breast cell line (MCF-7) and human cervix cell line (HELA) were used to measure cytotoxic activity of the proposed quinazoline derivatives. Compounds **5**, **9**, **15**, **18** and **20** exploited broad-spectrum and potent antitumor activity with IC_{50} range of 3.35–5.59 μg/ml. All tested compounds showed potent and selective activity against breast cancer (MCF-7) with IC_{50} range of 3.35–6.81 μg/ml. Since breast cells are known to overexpress EGFR, which leads to continuous activation of the EGFR pathway involved in cell proliferation, therefore, molecular docking studies further helps in understanding the antitumor selectivity over MCF-7 cell line and thereby helps to design novel potent inhibitors. Molecular docking studies further supported the strong inhibitory activity of **18** and **20** compared to **17** of the same series and further help understanding the various interactions between the ligands and enzyme active sites in detail and thereby help to design novel potent inhibitors.

4. Experimental

4.1. Chemistry

Melting points (uncorrected) were recorded on Barnstead 9100 Electrothermal melting apparatus. IR spectra were recorded on a Perkin–Elmer spectrometer. ^{1}H NMR and ^{13}C NMR was recorded in DMSO-d_6 and/or $CDCl_3$ on a Jeol 500 MHz instrument using TMS as internal standard (chemical shifts in δ ppm). Microanalytical data (C, H, and N) were performed on Perkin–Elmer 240B analyzer and they agreed with proposed structures within ±0.4% of the calculated values. Mass spectra were recorded on a Shimadzu PQ-5000 GC–MS apparatus. Solvent evaporation was performed under reduced pressure using Buchan Rotatory Evaporator unless otherwise stated. T.L.C. was performed on precoated silica gel plates (60-F254, 0.2 mm), manufactured by E.M. Sciences, Inc, and shortwave UV (254) nm was used to detect the U.V. absorbing compounds (CH_2Cl_2, EtOH 10:1).

4.1.1. 5-Chloro2-(4-methylbenzamido)benzoic acid (*1*)

5-Chloroanthranilic acid (20 mmol, 3.43 g) and 4-methylbenzoyl chloride (22 mmol, 3.40 g) were stirred at room temperature in pyridine (80 ml) for 6 h. The solvent was removed under reduced pressure; the obtained residue was washed with acidulated water, filtered, washed with water, dried, recrystallized from ethanol, mp 284–286 °C in 94% yield.

^{1}H NMR (DMSO-d_6): δ 12.07 (s, 1H, NHCO), 8.71 (s, 1H, Ar.), 7.95 (d, 1H, $J = 2.0$ Hz), 7.82 (d, 2H, $J = 7.5$ Hz), 7.68–7.61 (m, 2H), 7.34 (d, 2H, $J = 8.0$ Hz), 2.35 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 169.2, 165.0, 143.4, 140.5, 134.4, 131.9, 130.8, 130.0, 127.5, 126.8, 122.1, 118.8, 21.4. MS: (M − 1, 288, 6%).

Anal. calcd. for $C_{15}H_{12}ClNO_3$ (%): C, 62.19; H, 4.17; N, 4.83. Found: C, 62.43; H, 4.12; N, 4.53.

4.1.2. 6-Chloro-2-p-tolyl-4H-benzo[d][1,3]oxazin-4-one (*2*)

A benzoic acid derivative **1** (20 mmol, 5.79 g) was heated under reflux in acetic anhydride (100 ml) for 3 h. The solid obtained was filtered while hot and dried, mp 199–201 °C in 89% yield.

IR (KBr, cm^{-1}) ν: 1763 (CO); ^{1}H NMR ($CDCl_3$): δ 8.47 (s, 1H, Ar.), 8.23 (d, 1H, $J = 7.5$ Hz), 8.06 (s, 1H), 7.71–7.60 (m, 3H), 7.40–7.38 (m, 1H), 2.53 (s, 3H, CH_3). MS: M (271, 30.84%), M + 2 (273, 10.34%). Anal. calcd. for $C_{15}H_{10}ClNO_2$ (%): C, 66.31; H, 3.71; N, 5.16. Found: C, 66.70; H, 4.02; N, 5.32.

4.1.3. 5-Chloro-2-(4-methylbenzamido)benzamide (*3*)

Benzoxazine (**2**) (2 mmol, 543 mg) was stirred at room temperature in conc. ammonia solution (10 mL) for 6 h. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol, mp 229–231 °C in 78% yield.

^{1}H NMR (DMSO-d_6): δ 12.77 (s, 1H, NHCO), 8.67 (d, 2H, $J = 6.5$ Hz), 7.93–7.59 (m, 5H), 7.34 (s, 2H, $CONH_2$), 2.35 (s, 3H, CH_3). MS: (M − 1, 287). Anal. calcd. for $C_{15}H_{13}ClN_2O_2$ (%): C, 62.40; H, 4.54; N, 9.70. Found: C, 62.67; H, 4.32; N, 9.47.

4.1.4. 6-Chloro-2-p-tolylquinazolin-4(3H)-one (*4*)

Benzoxazine (**2**) (10 mmol, 2.71 g) was heated under reflux in formamide (50 ml) for 3 h. The solid obtained was filtered while hot and dried, mp 300–302 °C in 73% yield.

IR (KBr, cm^{-1}) ν: 3179 (NH), 1668 (CO); ^{1}H NMR (DMSO-d_6): δ 12.41 (s, 1H, NHCO), 8.11 (s, 1H, Ar.), 8.04 (d, 1H, $J = 1.5$ Hz), 7.82 (d, 2H, $J = 8.0$ Hz), 7.68 (d, 1H, $J = 8.5$ Hz), 7.37–7.22 (m, 2H), 2.33 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 167.7, 160.2, 147.9, 146.3, 143.4, 134.9, 129.9, 129.8, 128.2, 124.3, 125.3, 127.5, 21.6. MS: (M − 28, 252). Anal. calcd. for $C_{15}H_{11}ClN_2O$ (%): C, 66.55; H, 4.10; N, 10.35. Found: C, 66.65; H, 4.34; N, 9.98.

4.1.5. 6-Chloro-3-methyl-2-p-tolylquinazolin-4(3H)-one (*5*)

Benzoxazine (**2**) (10 mmol, 2.71 g) was heated under reflux in *N*-methylformamide (50 ml) for 3 h. The solid obtained was filtered while hot and dried, mp 146–148 °C in 68% yield.

^{1}H NMR (DMSO-d_6): δ 8.56 (d, 1H, $J = 8.5$ Hz), 8.44 (d, 1H, $J = 8.5$ Hz), 8.23 (d, 1H, $J = 8.5$ Hz), 7.48–7.42 (m, 2H), 7.18 (d, 2H, $J = 7.5$ Hz), 2.35 (s, 3H, CH_3), 2.16 (s, 3H, NCH_3). ^{13}C NMR (DMSO-d_6): δ 167.7, 160.2, 147.9, 146.3, 143.4, 134.9, 129.9, 129.8, 128.2, 124.3, 125.3, 127.5, 24.8, 21.6, MS: (M, 284). Anal. calcd. for $C_{16}H_{13}ClN_2O$ (%): C, 67.49; H, 4.60; N, 9.84. Found: C, 67.65; H, 4.36; N, 9.88.

4.1.6. 6-Chloro-2-p-tolylquinazolin-4(3H)-thione (*6*)

Method-A

A mixture of 6-chloro-2-*p*-tolylquinazolin-4(3*H*)-one (**4**) (10 mmol, 2.70 g) and phosphorous pentasulfide (11 mmol, 2.43 g) was heated under reflux in anhydrous xylene (100 ml) for 12 h. The

reaction mixture was filtered while hot, the solvent was evaporated, and the residue was triturated with dimethylsulfoxide (10 ml) and filtered. The clear filtrate was poured into ice water and stirred; the solid obtained was filtered, washed with water, dried and recrystallized from ethanol, mp 258–260 °C in 77% yield.

Method-B

6-Chloro-2-*p*-tolyl-4*H*-benzo[*d*][1,3]oxazin-4-thione (**13**) (5 mmol, 1.44 g) was heated under reflux in formamide (25 ml) for 3 h. The solid obtained was filtered while hot and dried (75% yield).

IR (KBr, cm^{-1}) ν: 3188 (NH), 1206 (CS); ^{1}H NMR (DMSO-d_6): δ 13.98 (s, 1H, Ar.), 8.49 (d, 1H, $J = 1.5$ Hz), 8.06 (d, 2H, $J = 7.5$ Hz), 7.87–7.83 (m, 1H), 7.75–7.69 (m, 1H), 7.33 (d, 2H, $J = 7.5$ Hz), 2.38 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 187.2, 161.8, 152.3, 147.9, 143.6, 135.7,132.6, 129.5, 128.9, 128.3, 126.4, 122.6, 21.5. MS: (M, 286). Anal. calcd. for $C_{15}H_{11}ClN_2S$ (%): C, 62.82; H, 3.87; N, 9.77. Found: C, 62.91; H, 3.60; N, 9.55.

*4.1.7. 6-Chloro-2-p-tolylquinazolin-4-amine (**7**)*

6-Chloro-2-*p*-tolylquinazolin-4(3*H*)-thione **(6)** (2 mmol, 773 mg) was heated under reflux in formamide (15 ml) for 3 h. The solid obtained was filtered while hot, dried and recrystallized from ethanol, mp 193–195 °C in 68% yield.

IR (KBr, cm^{-1}) ν: 3413, 3287 (NH); ^{1}H NMR (DMSO-d_6): δ 8.39 (s, 1H, Ar.), 8.34 (d, 2H, $J = 8.0$ Hz), 7.84 (s, 2H, D_2O exchangeable, NH_2), 7.78–7.73 (m, 2H), 7.29 (d, 2H, $J = 8.0$ Hz), 2.36 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 161.8, 160.7, 149.7, 140.3, 136.0, 133.7, 130.2, 129.7, 129.3, 128.4, 123.4, 114.5, 21.4. MS: (M, 269). Anal. calcd. for $C_{15}H_{12}ClN_3$ (%): C, 66.79; H, 4.48; N, 15.58. Found: C, 67.02; H, 4.50; N, 15.56.

*4.1.8. N-(6-chloro-2-p-tolylquinazolin-4-yl)hydroxylamine (**8**)*

6-Chloro-2-*p*-tolylquinazolin-4(3H)-thione (**6**) (2 mmol, 773 mg) and hydroxylamine hydrochloride (2.1 mmol, 146 mg) was heated under reflux for 12 h in pyridine (15 ml). The reaction mixture was cooled and the solvent was removed under reduced pressure; the residue was triturated with water and filtered. The solid obtained was dried and recrystallized from ethanol, mp 227–229 °C in 72% yield.

IR (KBr, cm^{-1}) ν: 3429 (NH), 3104 (OH); ^{1}H NMR (DMSO-d_6): δ 10.51 (s, 1H, OH), 7.98 (m, 2H), 7.76 (s, 1H, Ar.), 7.53 (d, 1H, $J = 8.0$ Hz), 7.44 (d, 1H, $J = 8.0$ Hz), 7.34 (d, 2H, $J = 8.0$ Hz), 2.39 (s, 3H, CH_3). MS: (M, 285). Anal. calcd. for $C_{15}H_{12}ClN_3O$ (%): C, 63.05; H, 4.23; N, 14.71. Found: C, 63.08; H, 4.41; N, 14.47.

*4.1.9. 6-Chloro-4-hydrazino-2-p-tolylquinazoline (**9**)*

6-Chloro-2-*p*-tolylquinazolin-4(3H)-thione (**6**) (2 mmol, 773 mg) was heated under reflux in (5 ml) hydrazine hydrate for 3 h The solid obtained was filtered while hot, dried and recrystallized from ethanol, mp 273–275 °C in 76% yield.

^{1}H NMR (DMSO-d_6): δ 9.69 (s, 1H), 8.45 (d, 2H, $J = 8.0$ Hz), 8.32 (s, 1H), 7.74 (d, 2H, $J = 11.0$ Hz), 7.29 (d, 2H, $J = 8.0$ Hz), 4.92 (s, 2H), 2.37 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 160.2, 159.5, 148.8, 140.4, 136.0, 133.3, 130.1, 129.4, 129.2, 128.6, 122.2, 114.0, 21.5. MS: (M + 1, 285). Anal. calcd. for $C_{15}H_{13}ClN_4$ (%): C, 63.27; H, 4.60; N, 19.68. Found: C, 63.27; H, 4.39; N, 19.45.

*4.1.10. N-(6-chloro-2-p-tolylquinazolin-4-yl)benzamide (**10**)*

A mixture 6-chloro-2-*p*-tolylquinazolin-4-amine (**7**) (2 mmol, 539 mg) and benzoyl chloride (2.1 mmol, 295 mg) in pyridine (10 ml) was heated under reflux for 12 h. The reaction mixture was cooled and the solvent was removed under reduced pressure; the residue was triturated with water and filtered. The solid obtained was dried and recrystallized from ethanol, mp 191–193 °C in 69% yield.

IR (KBr, cm^{-1}) ν: 3170 (NH), 1705 (CO); ^{1}H NMR (DMSO-d_6): δ 8.14 (s, 1H, NHCO), 8.04 (d, 1H, $J = 2.0$ Hz), 7.92–7.89 (m, 2H), 7.83–7.81 (m, 1H), 7.62–7.55 (m, 1H), 7.54–7.47 (m, 4H), 7.42 (d, 1H, $J = 7.5$ Hz), 7.30–7.25 (m, 2H), 2.35 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 161.9, 141.9, 135.9, 135.2, 134.0, 133.9, 133.0, 132.8, 131.2, 129.9, 129.7, 129.2, 128.8, 128.5, 127.5, 126.1, 124.1, 21.5. MS: (M, 373). Anal. calcd. for $C_{22}H_{16}ClN_3O$ (%): C, 70.68; H, 4.31; N, 11.24. Found: C, 70.58; H, 4.68; N, 11.34.

*4.1.11. O-Benzoyl-N-(6-chloro-2-p-tolylquinazolin-4-yl) hydroxylamine (**11**)*

A mixture of *N*-(6-chloro-2-*p*-tolylquinazolin-4-yl)hydroxylamine (**8**) (2.0 mmol, 571 mg) and benzoyl chloride (2.1 mmol, 295 mg) in pyridine (10 ml) was heated under reflux for 12 h. The reaction mixture was cooled and the solvent was removed under reduced pressure; the residue was triturated with water and filtered. The solid obtained was dried and recrystallized from ethanol, mp 196–198 °C in 71% yield.

^{1}H NMR (DMSO-d_6): δ 9.43 (s, 1H, NHCO), 8.42 (d, 1H, $J = 8.5$ Hz), 8.03 (dd, 2H, $J = 3.0$ Hz), 7.89 (d, 1H, $J = 2.0$ Hz), 7.86–7.63 (m, 3H), 7.64 (dd, 1H, $J = 2.0$ Hz), 7.44–7.39 (m, 3H), 7.20 (d, 1H, $J = 7.5$ Hz), 2.23 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): 166.6, 161.1, 155.2,148.3, 137.4, 136.8, 135.4, 134.7, 131.7, 131.6 129.7, 128.1, 127.9, 124.3, 124.2, 124.0, 119.9, 21.9. MS: (M, 391). Anal. calcd. for $C_{22}H_{16}ClN_3O2$ (%): C, 67.78; H, 4.14; N, 10.78. Found: C, 67.97; H, 4.42; N, 11.02.

*4.1.12. 3-Amino-6-chloro-2-p-tolylquinazolin-4(3H)-one (**12**)*

Benzoxazine (**2**) (2 mmol, 543 mg) was heated under reflux in hydrazine hydrate (5 ml) for 3 h. The solid obtained was filtered while hot and dried, mp 208–210 °C in 88% yield.

IR (KBr, cm^{-1}) ν: 3266, 3121 (NH), 1680 (CO); ^{1}H NMR (DMSO-d_6): δ 8.10 (d, 1H, $J = 2.5$ Hz), 7.84 (dd, 1H, $J = 2.5$ Hz), 7.74–7.71 (m, 3H), 7.29 (d, 2H, $J = 8.0$ Hz), 5.70 (s, 2H, NH_2), 2.39 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 160.7, 156.6, 145.9, 139.9, 134.8, 132.1, 131.2, 130.2, 130.1, 128.4, 125.3, 121.6, 21.4. MS: M (285, 60%), M + 2 (287, 20%). Anal. calcd. for $C_{15}H_{12}ClN_3O$ (%): C, 63.05; H, 4.23; N, 14.71. Found: C, 63.41; H, 4.22; N, 14.77.

*4.1.13. 6-Chloro-2-p-tolyl-4H-benzo[d][1,3]oxazin-4-thione (**13**)*

6-Chloro-2-*p*-tolyl-4*H*-benzo[*d*][1,3]oxazin-4-one (**2**) (5 mmol, 1.36 g) and phosphorous pentasulfide (5.5 mmol, 1.22 g) was heated under reflux in anhydrous xylene (150 ml) for 12 h. The reaction mixture was filtered while hot, the solvent was evaporated, and the residue was triturated with dimethylsulfoxide (10 ml) followed by filtration. The clear filtrate was poured into ice water and stirred; the solid obtained was filtered, washed with water and dried, mp 188–190 °C in 93% yield.

IR (KBr, cm^{-1}) ν: 1251 (CS); ^{1}H NMR (DMSO-d_6): δ 7.81 (d, 1H, $J = 8.5$), 7.44 (s, 1H), 7.41–7.30 (m, 3H), 6.82–6.75 (m, 2H), 2.11 (s, 3H). MS: M (287, 31%, M + 2, 289, 10%). Anal. calcd. for $C_{15}H_{10}ClNOS$ (%): C, 62.61; H, 3.50; N, 4.87. Found: C, 62.53; H, 3.36; N, 4.88.

*4.1.14. 3-Amino-6-chloro-2-p-tolylquinazolin-4(3H)-thione (**14**) and N-(4-chloro-2-(hydrazinecarbonothioylphenyl)-4-methylbenzamide (**15**)*

6-Chloro-2-*p*-tolyl-4*H*-benzo[*d*][1,3]oxazin-4-thione (**13**) (3 mmol, 863 mg) was heated under reflux in hydrazine hydrate (10 ml) for 5 h. The reaction mixture was cooled and the solvent was removed under reduced pressure; the solid obtained was dried and chromatographed with chloroform as fluent.

Compound **14**: yield 46%, mp 218–220 °C. IR (KBr, cm^{-1}) ν: 3455, 3339 (NH), 1220 (CS); ^{1}H NMR (DMSO-d_6): δ 8.10 (d, 1H, $J = 7.5$ Hz), 7.85–7.81 (m, 1H), 7.74–7.70 (m, 3H), 7.29 (d, 2H, $J = 8.5$ Hz), 5.70 (s, 2H, NH_2), 2.38 (s, 3H, CH_3). MS: (M, 301). Anal. calcd. for

$C_{15}H_{12}ClN_3S$ (%): C, 59.70; H, 4.01; N, 13.92. Found: C, 60.04; H, 4.01; N, 14.21.

Compound **15**: yield 43%, mp 140–142 °C. ^{1}H NMR (DMSO-d_6): δ 13.11 (s, 1H, SH), 8.71 (s, 1H, NHCO), 7.95 (d, 1H, $J = 2$ Hz), 7.82 (d, 2H, $J = 7.5$ Hz), 7.62 (d, 1H, $J = 2.0$ Hz), 7.34 (s, 2H, NH_2), 7.31–7.28 (m, 3H), 2.33 (s, 3H, CH_3). MS: (M − 35) 284. Anal. calcd. for $C_{15}H_{14}ClN_3OS$ (%): C, 56.33; H, 4.41; N, 13.14. Found: C, 56.68; H, 4.26; N, 12.88.

*4.1.15. 6-Chloro-4-(substitutedthio)-2-p-tolylquinazolines (**16**–**21**)*

A mixture of 6-chloro-2-*p*-tolylquinazolin-4(3*H*)-thione (**6**) (2 mmol, 773 mg) and the appropriate alkyl, allyl, aryl or aralkyl halides derivatives (2.1 mmol) in pyridine (10 mL) was heated under reflux for 10–12 h. The reaction mixture was cooled and the solvent was removed under reduced pressure; the residue was triturated with water and filtered. The solid obtained was dried and recrystallized.

*4.1.15.1. 6-Chloro-4-(methylthio)-2-p-tolylquinazoline (**16**).* Yield 86%, mp 207–209 °C (AcOH). ^{1}H NMR (DMSO-d_6): δ 8.30–8.28 (m, 2H), 8.16 (dd, 1H, $J = 7.5$ Hz), 7.49–7.46 (m, 2H), 7.41–7.40 (m, 2H), 2.35 (s, 3H, CH_3), 2.11 (s, 3H, SCH_3).

^{13}C NMR (DMSO-d_6): δ 161.6, 159.6, 151.9, 148.1, 136.6, 134.9, 131.6, 130.9, 129.7, 127.9, 124.1, 119.9, 21.4, 17.3. MS: (M, 300). Anal. calcd. for $C_{16}H_{13}ClN_2S$ (%): C, 63.89; H, 4.36; N, 9.31. Found: C, 63.88; H, 4.57; N, 9.60.

*4.1.15.2. 4-(Benzylthio)-6-chloro-2-p-tolylquinazoline (**17**).* Yield 95%, mp 152–154 °C (EtOH). ^{1}H NMR ($CDCl_3$): δ 8.46 (s, 1H, Ar.), 8.05–7.98 (m, 4H), 7.52–7.33 (m, 7H), 4.82 (s, 2H, CH_2Ph), 2.40 (s, 3H, CH_3). MS: (M, 376). Anal. calcd. for $C_{22}H_{17}ClN_2S$ (%): C, 70.11; H, 4.55; N, 7.43. Found: C, 70.47; H, 4.55; N, 7.12.

*4.1.15.3. 2-(6-Chloro-2-p-tolylquinazolin-4-ylthio)acetonitrile (**18**).* Yield 92%, mp 196–198 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 8.41 (d, 1H, $J = 8.0$ Hz), 8.17–8.07 (m, 1H), 8.00–7.95 (m, 2H), 7.73–7.55 (m, 1H), 7.42–7.25 (m, 2H), 5.62 (s, 2H, CH_2CN), 2.36 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 164.2, 159.4, 150.6, 141.8, `135.7, 130.3, 129.8, 128.7, 128.2, 125.3, 122.4, 116.6, 115.0, 52.2, 21.5. MS: (M-2, 323). Anal. calcd. for $C_{17}H_{12}ClN_3S$ (%): C, 62.67; H, 3.71; N, 12.90. Found: C, 62.41; H, 4.10; N, 12.13.

*4.1.15.4. 4-(Allylthio)-6-chloro-2-p-tolylquinazoline (**19**).* Yield 88%, mp 147–149 °C (AcOH). ^{1}H NMR (DMSO-d_6): δ 8.00 (d, 1H, $J = 1.0$ Hz), 7.52 (d, 2H, $J = 8.5$), 7.41–7.36 (m, 3H), 7.17–7.15 (m, 2H), 6.87 (d, 2H, $J = 8.5$ Hz), 4.81 (s, 2H), 2.36 (s, 3H, CH_3).

MS: (326, 4.3%). Anal. calcd. for $C_{18}H_{15}ClN_2S$ (%): C, 66.15; H, 4.63; N, 8.57. Found: C, 66.15; H, 4.45; N, 8.59.

*4.1.15.5. 6-Chloro-4-(2-nitropyridylthio)-2-p-tolylquinazoline (**20**).* Yield 90%, mp 214–216 °C (AcOH). ^{1}H NMR ($CDCl_3$): δ 8.19 (d, 1H, $J = 7.5$ Hz), 7.97 (d, 2H, $J = 7.0$ Hz), 7.77 (d, 1H, $J = 7.0$ Hz), 7.72–7.70 (m, 1H), 7.51–7.73 (m, 5H), 7.07 (d, 1H, $J = 7.0$ Hz), 2.30 (s, 3H, CH_3).

^{13}C NMR ($CDCl_3$): δ 160.6, 154.9, 143.9, 141.1, 136.4, 135.1, 133.9, 131.6, 129.7, 129.5, 129.0, 128.7, 128.0, 127.7, 127.6, 126.3, 126.1, 122.5, 23.8. MS: (M, 408). Anal. calcd. for $C_{20}H_{13}ClN_4O_2S$ (%): C, 58.75; H, 3.20; N, 13.70. Found: C, 58.64; H, 3.56; N, 13.74.

*4.1.15.6. 2-(6-Chloro-2-p-tolylquinazolin-4-ylthio)ethanol (**21**).* Yield 80%, mp 286–288 °C (AcOH). IR (KBr, cm^{-1}) ν: 3179 (OH); ^{1}H NMR (DMSO-d_6): δ 8.11(s, 1H), 8.04 (d, 1H, $J = 7.5$ Hz), 7.82 (d, 2H, $J = 7.5$ Hz), 7.68 (d, 1H, $J = 8.5$ Hz), 7.38–7.27 (m, 2H), 4.07 (s, 1H, OH), 3.50 (t, 2H, $J = 5$ Hz), 3.27 (t, 2H, $J = 5$ Hz), 2.29 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 166.6, 1601, 59.6, 151.9, 148.0, 136.6, 134.9, 131.8, 129.7, 127.9, 124.1, 119.9, 60.3, 41.7, 21.5. MS: (M, 330). Anal. calcd. for $C_{17}H_{15}ClN_2OS$ (%): C, 61.72; H, 4.57; N, 8.47. Found: C, 61.76; H, 4.87; N, 8.74.

*4.1.16. 4-(Benzylsulfonyl)-6-chloro-2-p-tolylquinazoline (**22**)*

6-Chloro-4-(benzylthio)-2-*p*-tolylquinazoline (**17**) (1 mmol, 376 mg) was stirred with $KMnO_4$ (1.5 mmol, 235 mg) in acetic acid (5 ml) at room temperature for 2 h. The solvent was evaporated under reduced pressure; the solid obtained was washed with water, filtered, dried and recrystallized from acetic acid, mp 234–236 °C in quantitative yield.

^{1}H NMR ($CDCl_3$): δ 8.26 (d, 2H, $J = 8.5$ Hz), 7.92 (d, 2H, $J = 8.0$ Hz), 7.72 (d, 2H, $J = 8.0$ Hz), 7.41–7.32 (m, 4H), 7.18 (d, 2H, $J = 7.5$ Hz), 5.48 (s, 2H, CH_2Ph), 2.29 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 161.4, 154.0, 152.6, 147.6, 144.3, 138.9, 136.8, 135.3, 131.6, 129.6, 127.5, 126.6, 133.9, 122.3, 120.0, 117.2, 70.2, 24.3. MS: (M + 1, 409). Anal. calcd. for $C_{22}H_{17}ClN_2O_2S$ (%): C, 64.62; H, 4.19; N, 6.85. Found: C, 64.87; H, 4.60; N, 6.73.

*4.1.17. 2-(6-Chloro-2-p-tolylquinazolin-4-ylthio)substituted acetamides (**23**–**27**)*

A mixture of 6-chloro-2-*p*-tolylquinazolin-4(3*H*)-thione (**6**) (2 mmol, 773 mg) and the appropriate chloroacetanilide (2.1 mmol) in acetone (15 ml) containing anhydrous K_2CO_3 (2.5 mmol, 345 mg) was heated under reflux for 10–12 h. The reaction mixture was filtered while hot, the solvent was removed under reduced pressure; the solid obtained was dried and recrystallized.

*4.1.17.1. 2-(6-Chloro-2-p-tolylquinazolin-4-ylthio)acetamide (**23**).* Yield 90%, mp 266–268 °C (AcOH). IR (KBr, cm^{-1}) ν: 3366, 3180 (NH), 1639 (CO); ^{1}H NMR ($CDCl_3$): δ 8.02 (s, 1H, Ar.), 7.90 (d, 1H, $J = 8.0$ Hz), 7.44–7.38 (m, 4H), 7.30–7.16 (d, 1H, $J = 7.5$ Hz), 6.39 (s, 1H, NHCO), 4.72 (s, 2H, CH_2CO), 2.65 (s, 1H, NHCO), 2.21 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): 24.0, 70.1, 119.2, 120.9, 122.2, 127.0, 127.5, 129.8, 131.6, 135.5, 136.5, 151.7, 154.5, 161.1, 172.6. MS: (M, 343). Anal. calcd. for $C_{17}H_{14}ClN_3OS$ (%): C, 59.38; H, 4.10; N, 12.22. Found: C, 59.00; H, 4.47; N, 12.46.

*4.1.17.2. 2-(6-Chloro-2-p-tolylquinazolin-4-ylthio)phenylacetamide (**24**).* Yield 88%, mp 277–279 °C (AcOH). IR (KBr, cm^{-1}) ν: 3260 (NH), 1656 (CO); ^{1}H NMR ($CDCl_3$): δ 9.74 (s, 1H, exchangeable, NHCO), 7.95 (d, 1H, $J = 7.0$ Hz), 7.61–7.38 (m, 7H), 7.34 (s, 1H, Ar.), 7.03–6.89 (m, 3H), 4.81 (s, 2H, CH_2CO), 2.23 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 24.3, 70.1, 115.5, 115.7, 120.1, 121.5, 122.3, 126.6, 127.8, 129.8, 131.8, 136.5, 139.1, 152.5, 154.8, 158.6, 160.3, 161.0, 165.8. MS: (M + 1, 420). Anal. calcd. for $C_{23}H_{18}ClN_3OS$ (%): C, 65.78; H, 4.32; N, 10.01. Found: C, 65.41; H, 4.49; N, 10.06.

*4.1.17.3. Ethyl(6-chloro-2-p-tolylquinazolin-4-ylthio)ethyl-4-(2-acetamido)benzoate (**25**).* Yield 88%, mp 242–244 °C (EtOH). ^{1}H NMR ($CDCl_3$): δ 9.84 (s, 1H, exchangeable, NHCO), 8.00 (d, 1H, $J = 7.5$ Hz), 7.52 (d, 2H, $J = 8.5$ Hz), 7.46–7.37 (m, 5H), 7.16 (d, 1H, $J = 7.5$ Hz), 6.86 (d, 2H, 8.5), 4.83 (s, 2H, CH_2CO), 4.01 (q, 2H, $J = 7.0$ Hz), 2.23 (s, 3H, CH_3), 1.39 (t, 3H, $J = 7.0$ Hz). ^{13}C NMR ($CDCl_3$): δ 14.81, 24.1, 63.7, 71.1, 114.8, 121.2, 122.2, 122.3, 127.1, 127.8, 129.8, 130.4, 131.6, 135.2, 136.6, 139.1, 152.9, 154.7, 156.1, 161.0, 168.5. MS: (M − 1, 462). Anal. calcd. for $C_{25}H_{22}ClN_3O_2S$ (%): C, 64.72; H, 4.78; N, 9.06. Found: C, 64.78; H, 4.88; N, 9.06.

*4.1.17.4. 2-(6-Chloro-2-p-tolylquinazolin-4-ylthio)-p-tolyacetamide (**26**).* Yield 84%, mp 262–264 °C (AcOH). ^{1}H NMR ($CDCl_3$): δ 9.71 (s, 1H, exchangeable, NHCO), 8.02 (d, 1H, $J = 7.0$ Hz), 7.51–7.40 (m, 7H), 7.19–7.14 (m, 3H), 4.86 (s, 2H, CH_2CO), 2.33 (s, 3H, CH_3), 2.26 (s, 3H, CH_3). MS: (M, 433). Anal. calcd. for $C_{24}H_{20}ClN_3OS$ (%): C, 66.43; H, 4.65; N, 9.68. Found: C, 66.56; H, 4.90; N, 9.41.

*4.1.17.5. 2-(6-Chloro-2-p-tolylquinazolin-4-ylthio)-p-florophenylacetamide (**27**).* Yield 90%, mp 266–228 °C (AcOH). ^{1}H NMR ($CDCl_3$): δ 9.91 (s, 1H, NHCO), 8.01 (d, 1H, $J = 7.0$ Hz), 7.61–7.58 (m, 2H), 7.49–7.38 (m, 5H), 7.16 (d, 1H, $J = 7.5$ Hz), 7.04–7.00 (m, 2H), 4.85 (s, 2H, CH_2CO), 2.35 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 166.8, 161.0, 158.7, 154.8, 152.8, 139.2, 136.5, 135.2, 133.4, 131.7, 129.8, 127.8, 127.1, 122.3, 121.4, 120.3, 115.8, 71.1, 24.2. MS: (M, 437). Anal. calcd. for $C_{23}H_{17}ClFN_3OS$ (%): C, 63.08; H, 3.91; N, 9.60. Found: C, 63.41; H, 4.17; N, 9.29.

4.2. Biological evaluation

4.2.1. Materials

Doxorubicin vial was a generous gift from the National Cancer Institute drug store. The HEPG2 human liver cell line, MCF-7 cell line and HELA cervix cell line were obtained from the American Type Culture Collection (Rockville, MD, USA). RPMI medium, Fetal Calf Serum (FCS), antibiotics for cell culture, trypsin solution and tissue culture plasticware were purchased from Costar (Milan, Italy). All other chemicals were obtained from Sigma Chemical Company (St. Louis, MO, USA).

4.2.2. Evaluation of cellular cytotoxicity

The cytotoxic activity of compounds **1**–**27** against HEPG2 cells, MCF-7 cells and HELA cells was determined using Sulphorhodamine-B assay [23]. In brief, tumour cells were seeded into 96-well microtiter plates at a concentration of 5×10^4 cells/well in fresh medium and left to attach to the plate for 24 h. Cells were then incubated for 48 h in the absence (control) and in the presence of each compound at the noted concentrations (16–62 μg/ml). Following 48-h exposure to the compounds, cells were fixed with 50% cold TCA for 1 h, stained for 30 min with 0.4% Sulphorhodamine-B and then washed with 1% acetic acid. The plates were then air-dried and the optical density of each well was measured spectrophotometrically at 564 nm using the ELISA microplate reader (Meter tech.© 960, USA). Surviving fraction for each cell type was performed from which IC_{50} was calculated for each compound under investigation. Worth mentioning is that the cytotoxic activity of Doxorubicin, a standard and well known anticancer drug, against the three cell lines was performed at the same concentrations of tested compounds.

4.3. Molecular modeling methods

4.3.1. Conformational search

Initial molecular structures of the most active compounds **5**–**7**, **9**, **15**, **18** and **20**–**22** were constructed using the HyperChem program version 5.1. The MM+ (calculations in vacuo, bond dipole option for electrostatics, Polak–Ribiere algorithm, and RMS gradient of 0.01 kcal/mol) conformational searching in torsional space was performed using the multiconformer method [38]. Energy minima for compounds **5**–**7**, **9**, **15**, **18** and **20**–**22** were determined by a semi-empirical method AM1 (as implemented in HyperChem 5.1). The conformations thus obtained were confirmed as minima by vibrational analysis.

4.3.2. Molecular docking methodology

Docking studies have been performed using MOE 2008.10. With this purpose, crystal structure of Erlotinib (*Tarceva*™) was obtained from Protein Data Bank (PDB codes: 1M17) in order to prepare protein for docking studies. Docking procedure was followed using the standard protocol implemented in MOE 2008.10 and the geometry of resulting complexes was studied using the MOE's Pose Viewer utility.

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