Arabian Journal of Chemistry (2013) 6, 35–48

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

1st Cancer Update

Synthesis, method optimization, anticancer activity of 2,3,7-trisubstituted Quinazoline derivatives and targeting EGFR-tyrosine kinase by rational approach

Malleshappa N. Noolvi *, Harun M. Patel

Department of Pharmaceutical Chemistry, ASBASJSM College of Pharmacy, Bela (Ropar), 140111 Punjab, India

Received 6 December 2010; accepted 29 December 2010
Available online 1 January 2011

KEYWORDS

Synthesis 3-(benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3*H*)-one;
Fusion;
QSAR;
Rational design;
Anticancer activity

Abstract A novel 3-(substituted benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3*H*)-one (**7**–**27**) has been synthesized and characterised by IR, ^{1}H NMR, ^{13}C NMR spectroscopy, and elemental analysis. We changed the methodology for the synthesis of 3-amino 7-chloro-2-phenyl quinazolin-4(3*H*)-one **6** to fusion reaction at 250 °C, instead of using solvent, to avoid the problem of ring opening, which is commonly observed while synthesizing quinazolines from benzoxazinone. NCI selected, 7-chloro-3-{[(4-chlorophenyl) methylidene] amino}-2-phenylquinazolin-4(3*H*)-one **12**, with GI_{50} value of −5.59 M, TGI value of −5.12 M, and LC_{50} value of −4.40 M showed remarkable activity against CNS SNB-75 Cancer cell line. Rational approach and QSAR techniques enabled the understanding of the pharmacophoric requirement for 2,3,7-tri substituted quinazoline derivatives to inhibit EGFR-tyrosine kinase as antitumor agents and could be used as an excellent framework in this field that may lead to discovery of potent anti tumor agent.

1. Introduction

Protein tyrosine kinases are enzymes involved in many cellular processes such as cell proliferation, metabolism, survival, and apoptosis. Several protein tyrosine kinases are known to be activated in cancer cells and to drive tumor growth and progression. Blocking tyrosine kinase activity therefore represents a rational approach to cancer therapy. Protein kinases (PTKs) catalyze the phosphorylation of tyrosine and serine/threonine residues in various proteins involved in the regulation of all functions (Jordan et al., 2000). They can be broadly classified as receptor such as EGFR, or non-receptor kinases. Inappropriate or uncontrolled activation of many of these kinases, by

* Corresponding author. Tel.: +91 9417563874; fax: +91 1881263655.
E-mail addresses: mnoolvi@yahoo.co.uk, mallesh7301@rediffmail.com (M.N. Noolvi), hpatel_38@yahoo.com (H.M. Patel).

Peer review under responsibility of King Saud University.
doi:10.1016/j.arabjc.2010.12.031

Production and hosting by Elsevier

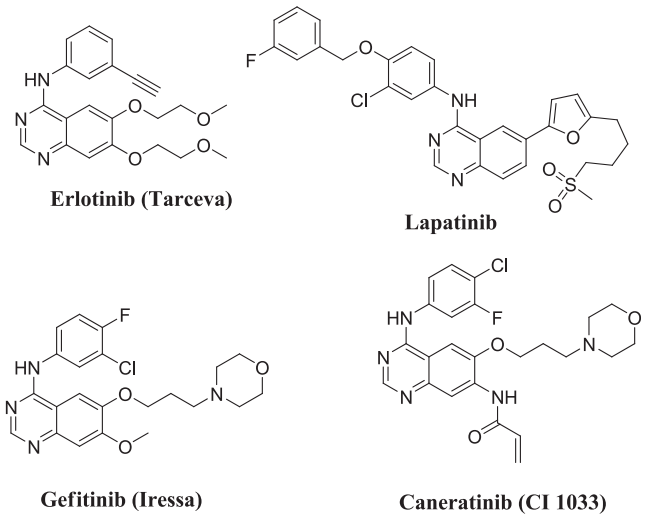


Chart 1 EGFR-tyrosine kinase inhibitors.

over-expression, constitutive activation, or mutation, has been shown to result in uncontrolled cell growth (Blume-Jensen and Hunter, 2001). Overexpression of these receptors was found in a number of cancers (e.g., breast, ovarian, colon, and prostate), their expression levels often correlate with vascularity, and is associated with poor prognosis in patients (Slichenmeyer et al., 2001; Fricker, 2006). Inhibitors of the EGFR PTK are therefore expected to have great therapeutic potential in the treatment of malignant and nonmalignant epithelial diseases. Drug discovery efforts have targeted this aberrant kinase activity in cancer, asthma, psoriasis, and inflammation (Cohen, 2002). Recent advances in the identification of erbB family kinase inhibitors have created hope for the modulation of uncontrolled cell growth in cancer therapy for solid tumors (Gschwind et al., 2004).

This strongly suggests that these targets represent drug intervention opportunities due to pivotal role in governing cellular proliferation, survival, and metastasis. A great number of different structural classes of tyrosine kinase inhibitors have been reported and reviewed (Adams, 2001; Yarden and

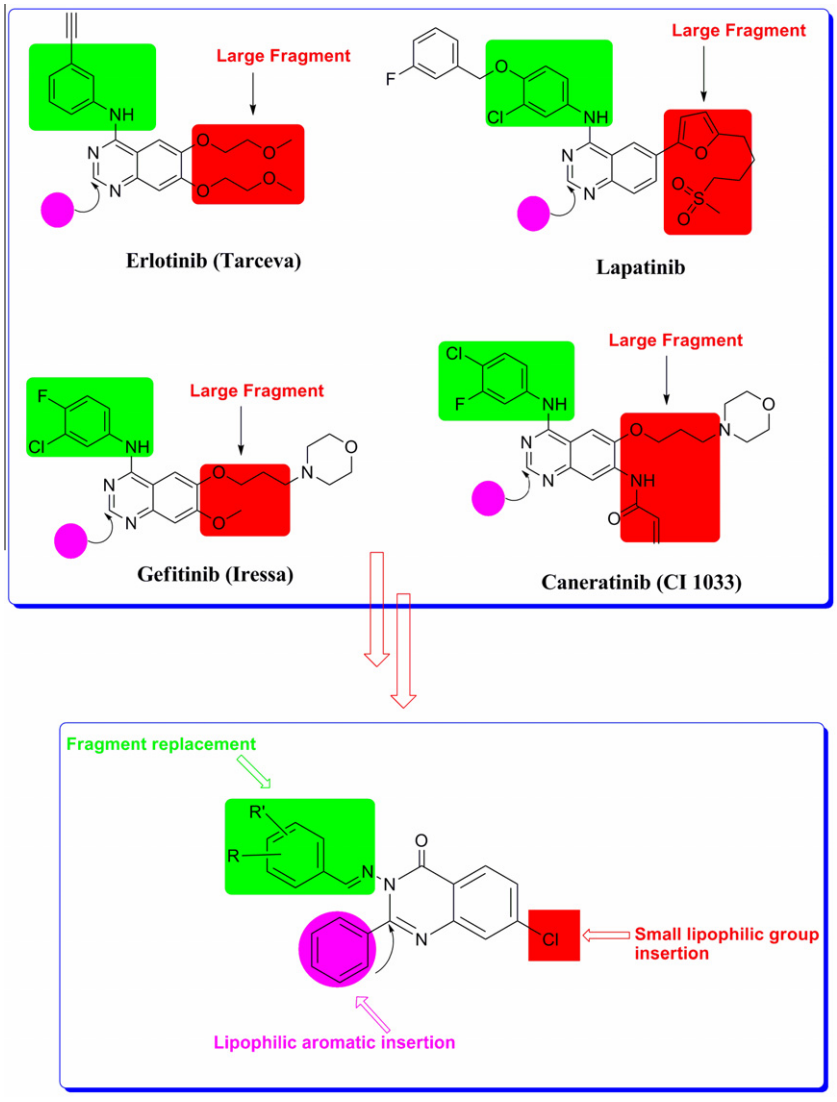


Figure 1 Reported and proposed antitumor quinazoline derivatives.

Sliwkowski, 2001; Dumas, 2001). The most promising small molecule selective EGFR-TK inhibitors include quinazolines. Chart 1 includes some examples that are currently approved drugs or in clinical trials (Fricker, 2006).

In view of previous rational and in continuation of our research for newer anti-cancer agents (Manjula et al., 2009; Badiger et al., 2006) in the present study new series of 2,3,7-tri substituted quinazoline have been optimized and synthesized (Fig. 1). Quinazoline ring opening is a common phenomenon in the synthesis of quinazoline from benzoxazinone. We changed the methodology for the synthesis of 3-amino 7-chloro-2-phenyl quinazolin-4(3*H*)-one **6** to fusion reaction at 250 °C, instead of using solvent, to avoid the problem of ring opening, which is commonly observed while synthesizing quinazolines from benzoxazinone. By using this new methodology the preparation of 3-amino 7-chloro-2-phenyl quinazolin-4(3*H*)-one **6** is simple, time-saving and eco-friendly process without solvent and also avoid the problem of ring opening. Thus, it is a step towards green chemistry.

2. Rational and design

In recent years, quinazolines have emerged as a versatile template for inhibition of a diverse range of receptor tyrosine kinases. The most widely studied of these is the epidermal growth factor receptor (EGFR), with the small-molecule inhibitor gefitinib being the first agent from this class to be approved for the treatment of Non-Small Cell Lung Cancer refractory to prior chemotherapeutic intervention (Peter et al., 2006; Ranson, 2004). Subsequent research aimed at further exploration of the SAR of this novel template has led to discovery of highly selective compounds that target EGFR. These compounds act via competing with ATP for binding at the catalytic domain of tyrosine kinase. Later on, a great structural variety of compounds of structurally diverse classes have proved to be highly potent and selective ATP-competitive inhibitors. The ATP binding site has the following features; Adenine region – contains two key hydrogen bonds formed by the interaction of N-1 and N-6 amino group of the adenine ring. Many potent inhibitors use one of these hydrogen bonds. Sugar region – a hydrophilic region, except a few e.g., EGFR. Hydrophobic pocket – though not used by ATP but plays an important role in inhibitor selectivity. Hydrophobic channels – it is not used by ATP and may be exploited for inhibitor specificity. Phosphate binding region – this is used for improving inhibitor selectivity (Fig. 2) (Fabbro et al., 2002). In this study, we present a new sub-family of compounds containing 2,3,7-tri substituted quinazoline core as EGFR inhibitors. Our strategy is directed toward designing a variety of ligands with diverse chemical properties hypothesizing that the potency of these molecules might be enhanced by adding alternative binding group such as phenyl ring at position 2-, imines at position 3-, and chloro group at position 7- of the quinazoline ring. In this way, such substitution pattern could target different regions of the ATP-binding site of the protein kinase domain to create differentially selective molecules. The design of our ligands was done based on previous quantitative structure–activity relationship (QSAR) of 4-anilinoquinazolines as EGFR inhibitor (Noolvi and Patel, 2010, Noolvi et al., 2011). We introduced larger moiety at 3 position of the quinazoline such as substituted arylidene moiety in a fashion similar

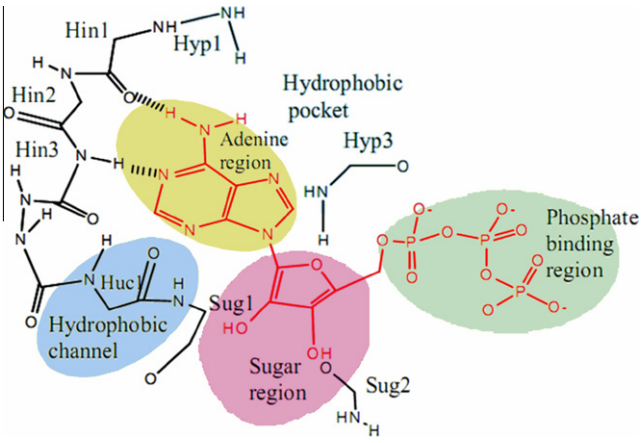


Figure 2 Model of the ATP-binding site of protein kinases. ATP is depicted in red; Sug1, Hyp1, and Hyc1 are residues lining the sugar region, hydrophobic pocket (Hyp), hydrophobic channel (Huc), and hinge region (Hin), respectively.

to lapatinib which binds in the ATP-binding cleft, so that the bulky group could be oriented deep in the back of the ATP binding site and makes predominantly hydrophobic interactions with the protein mimicking the 3′-chloro-4′-[(3-fluorobenzyl)oxy]aniline group of lapatinib (Fig. 3).

3. Materials and methods

3.1. Chemistry

4-Chloro 2-amino benzoic acid **1** reaction with benzoyl chloride **2** yielded 7-chloro 2-phenyl-4*H*-3, 1-benzoxazin-4 one **3** by N-acylation via dehydrative cyclization mechanism. Subsequently which was refluxed with hydrazine hydrate in dry pyridine in an attempt to obtain 3-amino 7-chloro-2-phenyl quinazolin-4(3*H*)-one, but we got mixture of 3-amino 7-chloro-2-phenyl quinazolin-4(3*H*)-one **5** and ring opened *N*-(5-chloro-2(hydrazine carbonyl) phenyl) benzamide **4**. Later we tried the same reaction at 250 °C by fusion without any solvent and this time we got the desired product 3-amino 7-chloro-2-phenyl quinazolin-4(3*H*)-one **6** without any ring opened structure (diamides). Fusion is more convenient and time saving method since it takes only 0.5 h, where conventional takes 3.0 h for the reaction. On the other hand ring opening is a common phenomenon in the synthesis of quinazoline from benzoxazinone. This problem can be avoided by fusing benzoxazinone at high temperature resulting in the synthesis of desired quinazoline otherwise one will get mixture of quinazoline and ring opened quinazoline (diamides), as shown mechanistically in Chart 2. Condensation of **6** with different substituted aromatic aldehydes gave corresponding arylidene derivatives of quinazoline **7**–**27** in glacial acetic acid (Scheme 1).

3.2. Anticancer screening at NIH, Bethesda, Maryland, USA

The tumor growth inhibition properties of compounds **12** with the NCI codes NSC D-753447/1 selected among **7**–**27** synthesized compounds (Scheme 1) by the National Cancer Institute (NCI), USA, were screened on human tumor cell lines at the NIH, Bethesda, Maryland, USA, under the drug discovery program of the NCI, for one and five dose anti-cancer assay.

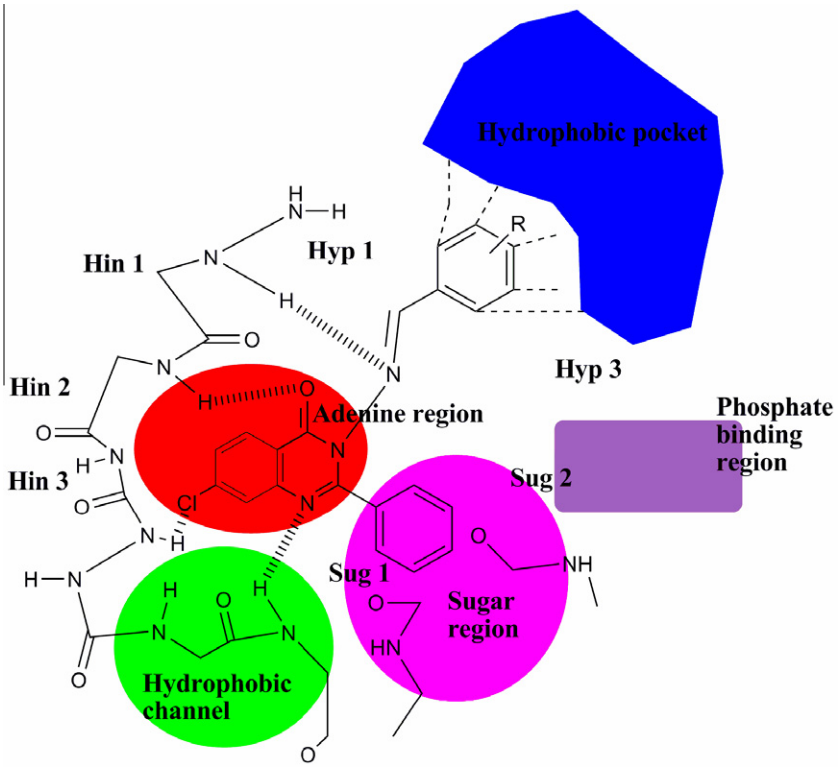


Figure 3 Proposed hypothetical model of the 2,3,7-trisubstituted quinazoline bound to ATP binding site of EGFR-protein tyrosine kinase.

The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10 μM. The output from the single dose screen is reported as a mean graph and is available for analysis by the COMPARE program. Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five concentration levels. The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96-well microtiter plates in 100 μl at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line are fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 μl of these different drug dilutions are added to the appropriate microtiter wells already containing 100 μl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed *in situ* by the gentle addition of 50 μl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μl) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 minutes at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

$$[(\mathrm{Ti} - \mathrm{Tz})/(\mathrm{C} - \mathrm{Tz})] \times 100 \text{ for concentrations for which } \mathrm{Ti} > / = \mathrm{Tz}$$
$$[(\mathrm{Ti} - \mathrm{Tz})/\mathrm{Tz}] \times 100 \text{ for concentrations for which } \mathrm{Ti} < \mathrm{Tz}$$

Three dose–response parameters are calculated for each experimental agent. Growth inhibition of 50% (GI_{50}) is calculated from $[(\mathrm{Ti} - \mathrm{Tz})/(\mathrm{C} - \mathrm{Tz})] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration

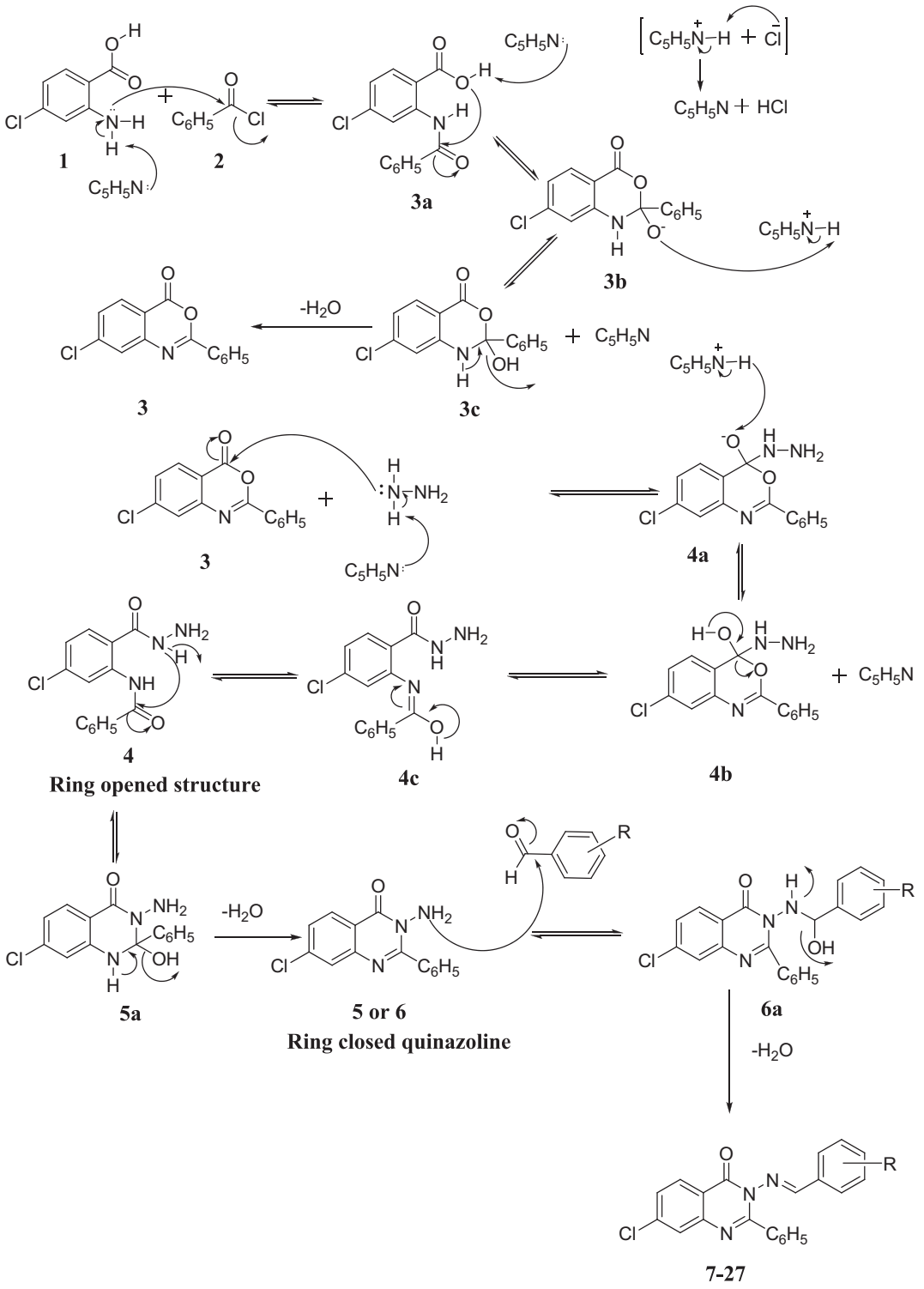
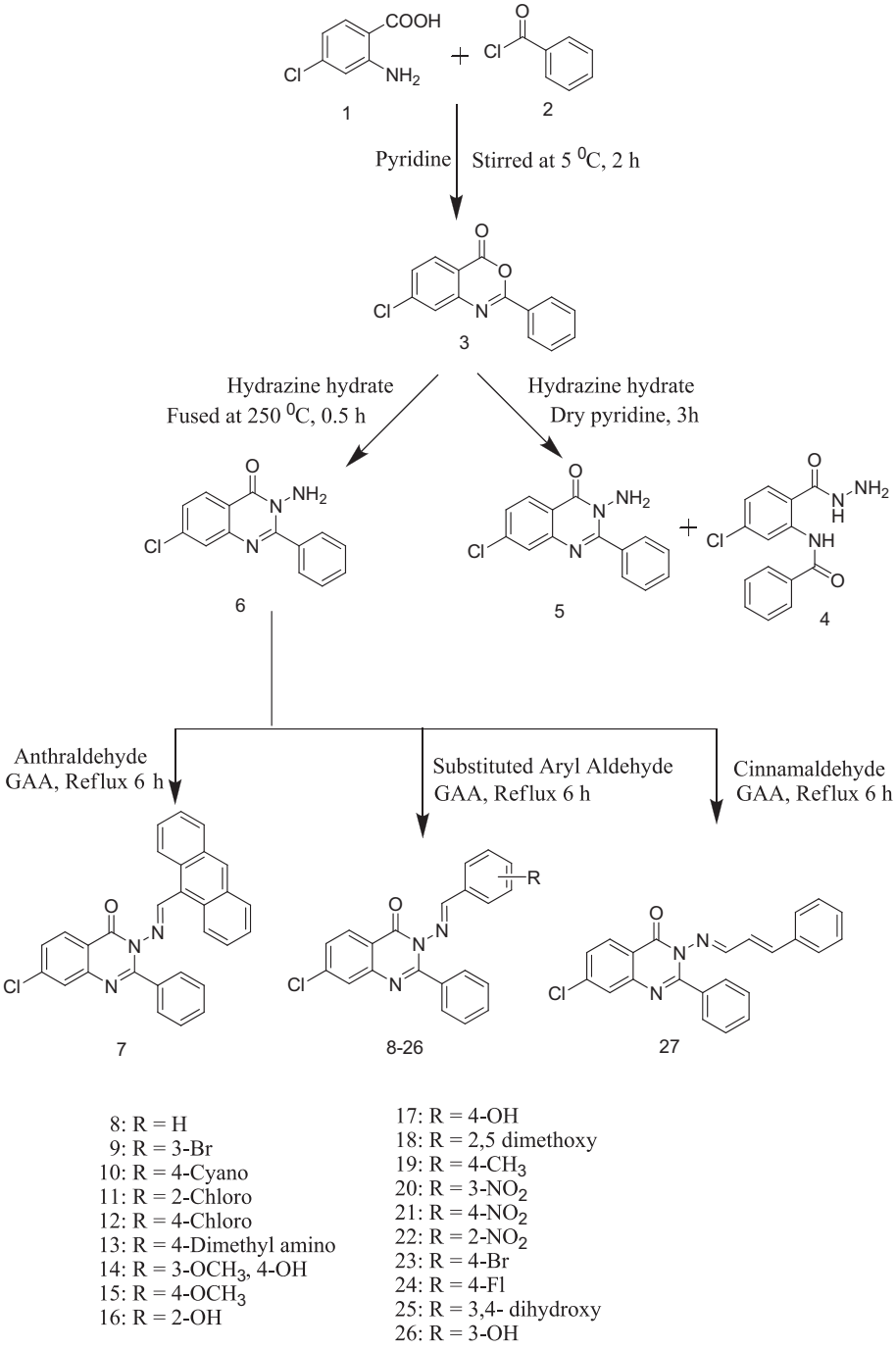


Chart 2 Reaction mechanism and ring opening phenomenon in the synthesis of target compound **7**–**27** (Scheme 1).

resulting in total growth inhibition (TGI) is calculated from Ti = Tz. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from $[(Ti - Tz)/Tz] \times 100 = -50$. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested (Alley et al., 1988; Grever et al., 1992; Boyd and Paul,1995).

4. Result and discussion

In the present work, 2,3,7-trisubstituted quinazolines were prepared according to reaction Scheme 1. The spectral data



Scheme 1 Synthetic route for the synthesis of quinazoline derivatives (**7**–**27**).

of **7**–**27** showed IR band at 1634–1678 cm^{-1} due to stretching vibration of C═O group of the quinazoline moiety and absorption band at 1372–1436 cm^{-1} is due to the C–N stretching vibration. C–Cl stretching vibration is appeared at 721–768. Further ^{1}H NMR of compounds **7**–**27** showed presence of a singlet between δ 8.77 and 9.34 ppm indicate the formation of imine (> HC═N–). Peak at δ 7.10–8.30 ppm showed presence of aromatic protons. Synthesis of **7**–**27** compounds was also confirmed by ^{13}C NMR data, peak at around δ 168.23 ppm has confirmed formation imine (> HC═N–).

The tumor growth inhibition properties of compounds **12** with the NCI codes NSC D-753447/1 selected among **7**–**27** synthesized compounds (Scheme 1) by the National Cancer Institute (NCI), USA, were screened on human tumor cell lines at the NIH, Bethesda, Maryland, USA, under the drug discovery program of the NCI, for one and five dose anti-cancer assay.

The tested quinazoline derivative showed a distinctive pattern of selectivity. With regard to sensitivity against individual cell lines (Fig. 4), compound **12** showed remarkably lowest cell growth promotion against Renal A-498 cancer cell line of −43.73, apart from this it also exhibited broad spectrum cell growth inhibition against Non-Small Cell Lung Cancer NCI-H522 (cell growth promotion 20.09%, inhibition 79.91%), Colon cancer HCT-116 (cell growth promotion 38.26%,

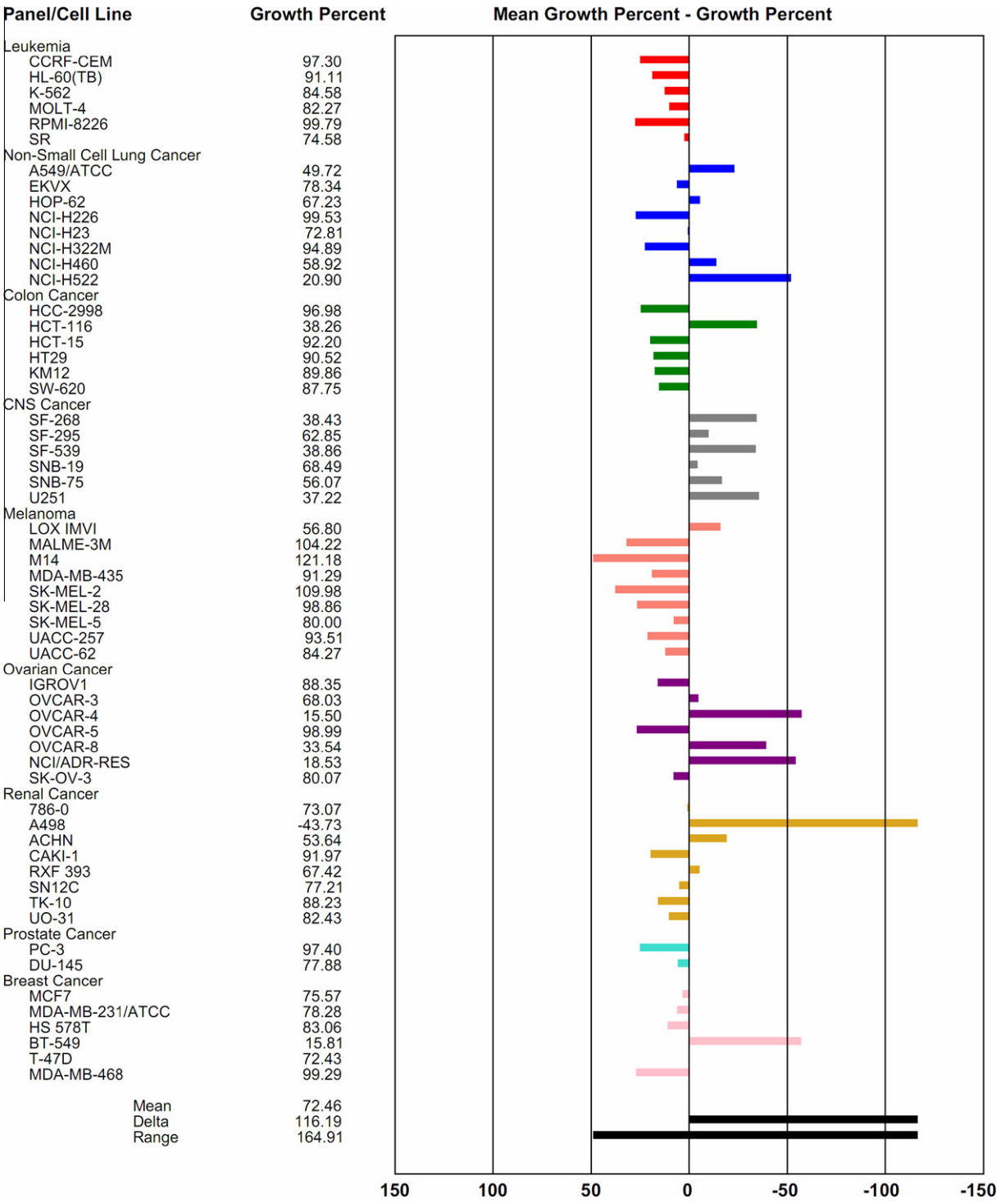


Figure 4 One dose mean graph of compound **12** at 10^{-5} M concentration.

inhibition 61.74%), CNS cancer U-251 (cell growth promotion 37.22%, inhibition 62.78%), and Ovarian OVCAR-8 cell line (cell growth promotion 33.54%, inhibition 66.46%) at concentration of 10^{-5} M in one dose primary assay.

The same compound was further screened for 5-log dose range as it has shown prominent cell growth inhibition at 10^{-5} M concentration against verity of cell lines. Three response parameters, median growth inhibition (GI_{50}), total growth inhibition (TGI), and median lethal growth inhibition (LC_{50}) were calculated for each cell line (Skehan et al., 1990). It showed remarkable activity against CNS SNB-75 cancer cell line with GI_{50} value of −5.59 and TGI value of −5.12 apart from this it also exhibited broad spectrum cell growth inhibition against: Non-Small Cell Lung Cancer Lines; HOP-62 (GI_{50} value −5.13 and TGI value −4.51), NCI-H226 (GI_{50} value −5.40 and TGI value −4.70), NCI-H23 (GI_{50} value −5.08

Table 1 National Cancer Institute developmental therapeutics program in-vitro testing results of compound **12**.

NSC: D-753447/1 Experiment ID: 1009 NS13 Units: Molar
COMI: SIH6 (96135) Strain reagent: SRB Dual Pass SSPL: 0XXS

Parent cell line	Time		Mean optical densities					Percent growth					GI_{50}	TGI	LC_{50}
	Zero	Control	−8.0	−7.0	−6.0	−5.0	−4.0	−8.0	−7.0	−6.0	−5.0	−4.0			
Leukemia															
CCRFCEM	0.356	1.727	1.680	1.633	1.696	1.628	1.559	97	93	98	93	88	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4
MOLT-4	0.439	1.652	1.712	1.741	1.718	1.628	1.520	105	107	105	98	89	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4
RPMI-8226	0.795	2.712	2.696	2.643	2.631	2.033	1.772	99	96	96	65	51	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4
SR	0.240	1.406	1.428	1.455	1.447	0.743	0.547	102	104	104	43	26	7.70 E−6	> 1.00 E−4	> 1.00 E−4
Non-Small Cell Lung Cancer															
A549/ATCC	0.253	1.319	1.262	1.286	1.293	0.664	0.699	95	97	98	39	42	6.39 E−6	> 1.00 E−4	> 1.00 E−4
EKVX	0.720	1.673	1.653	1.674	1.611	1.288	1.158	98	100	94	60	46	5.05 E−5	> 1.00 E−4	> 1.00 E−4
HOP-62	0.421	1.062	1.027	1.005	1.004	0.703	0.226	94	91	91	44	−46	7.44 E−6	3.06 E−5	> 1.00 E−4
HOP-92	1.396	2.139	1.989	1.969	1.916	1.721	1.468	80	77	70	44	10	5.77 E−6	> 1.00 E−4	> 1.00 E−4
NCI-H226	0.630	1.349	1.341	1.347	1.353	0.743	0.397	99	100	101	16	−37	3.94 E−6	1.98 E−5	> 1.00 E−4
NCI-H23	0.514	1.382	1.406	1.388	1.432	0.909	0.444	103	101	106	45	−14	8.40 E−6	5.88 E−5	> 1.00 E−4
NCI-H322M	0.715	1.092	1.171	1.117	1.152	0.807	0.725	121	106	116	24	3	5.23 E−6	> 1.00 E−4	> 1.00 E−4
NCI-H460	0.154	1.363	1.434	1.455	1.455	0.141	0.148	106	108	108	−9	−4	3.13 E−6	8.41 E−6	> 1.00 E−4
NCI-H522	0.607	1.456	1.487	1.552	1.545	0.449	0.447	104	111	110	−26	−21	2.77 E−6	6.45 E−6	> 1.00 E−4
Colon cancer															
COLO 205	0.266	1.128	1.184	1.205	1.201	1.116	1.068	107	109	109	99	93	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4
HCC-2998	0.474	1.476	1.465	1.511	1.541	1.095	1.027	99	103	106	62	55	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4
HCT-116	0.171	1.540	1.590	1.536	1.486	0.230	0.130	104	100	96	4	−24	3.18 E−6	1.42 E−5	> 1.00 E−4
HCT-15	0.193	1.375	1.367	1.331	1.311	1.073	0.924	99	96	95	74	62	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4
HT-29	0.155	0.883	0.941	0.960	0.995	0.783	0.729	108	111	115	86	79	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4
KM 12	0.415	1.793	1.855	1.848	1.826	1.480	1.333	104	104	102	77	67	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4
SW-620	0.124	0.842	0.854	0.886	0.871	0.629	0.455	102	106	104	70	46	6.90 E−5	> 1.00 E−4	> 1.00 E−4
CNS cancer															
SF-268	0.346	1.231	1.271	1.264	1.230	0.395	0.360	104	104	100	6	9	3.38 E−6	> 1.00 E−4	> 1.00 E−4
SF-295	0.701	1.779	1.730	1.697	1.717	1.242	0.895	95	92	94	50	18	1.01 E−5	> 1.00 E−4	> 1.00 E−4
SF-539	0.492	1.757	1.742	1.711	1.690	0.707	0.269	99	96	95	17	−45	3.76 E−6	1.87 E−5	> 1.00 E−4
SNB-19	0.417	1.200	1.200	1.226	1.256	0.666	0.334	100	103	107	32	−20	5.73 E−6	4.12 E−5	> 1.00 E−4
SNB-75	0.662	1.136	1.049	1.108	1.108	0.574	0.172	82	94	94	−13	−74	2.57 E−6	7.51 E−6	4.02 E−5
U251	0.279	1.391	1.388	1.364	1.378	0.531	0.240	100	98	99	23	−14	4.37 E−6	4.12 E−5	> 1.00 E−4
Melanoma															
LOX IMVI	0.141	0.945	0.912	0.939	0.838	0.582	0.429	96	99	87	55	36	1.78 E−5	> 1.00 E−4	> 1.00 E−4
M14	0.284	1.199	1.199	1.164	1.236	1.291	0.865	100	96	104	110	63	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4
MDA-MB-435	0.340	1.243	1.221	1.237	1.257	0.981	0.971	98	99	101	71	70	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4
SK-MEL-2	0.821	2.277	2.389	2.405	2.415	1.322	0.849	108	109	110	34	9	6.20 E−6	> 1.00 E−4	> 1.00 E−4
SK-MEL-28	0.485	1.120	1.157	1.144	1.216	1.034	0.797	106	104	115	86	49	9.47 E−5	> 1.00 E−4	> 1.00 E−4
SK-MEL-5	0.316	1.746	1.763	1.736	1.689	1.062	0.749	101	99	96	52	30	1.25 E−5	> 1.00 E−4	> 1.00 E−4
UACC-257	0.466	1.075	1.065	1.047	1.051	0.876	0.800	98	95	96	67	55	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4
UACC-62	0.598	1.775	1.770	1.823	1.807	1.076	0.152	100	104	103	41	−75	7.05 E−6	2.25 E−5	6.12 E−5
Ovarian cancer															
IGROV1	0.499	1.085	1.175	1.184	1.203	0.532	0.377	115	117	120	6	−24	4.10 E−6	1.54 E−5	> 1.00 E−4
OVCAR3	0.282	0.859	0.917	0.918	0.907	0.212	0.077	110	110	108	−25	−73	2.74 E−6	6.51 E−6	3.36 E−5
OVCAR4	0.468	0.864	0.875	0.901	0.908	0.370	0.345	103	109	111	−21	−26	2.90 E−6	6.93 E−6	> 1.00 E−4
OVCAR5	0.433	1.386	1.343	1.318	1.334	1.142	0.932	95	93	95	74	52	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4
OVCAR8	0.253	1.037	1.078	1.054	1.007	0.337	0.267	105	102	96	11	2	3.47 E−6	> 1.00 E−4	> 1.00 E−4
NCI-RES	0.442	1.482	1.559	1.533	1.496	0.636	0.518	107	105	101	19	7	4.18 E−6	> 1.00 E−4	> 1.00 E−4
SK-OV-3	0.579	1.289	1.307	1.289	1.313	0.886	0.412	103	100	103	43	−29	7.70 E−6	3.97 E−5	> 1.00 E−4
Renal cancer															
786-0	0.582	1.943	1.856	1.893	1.781	0.955	0.518	94	96	88	27	−11	4.25 E−6	5.15 E−5	> 1.00 E−4
A498	0.798	1.312	1.305	1.340	1.315	1.035	0.758	98	105	100	46	−5	8.47 E−6	7.98 E−5	> 1.00 E−4
ACHN	0.213	1.255	1.303	1.297	1.235	0.626	0.401	105	104	98	40	18	6.63 E−6	> 1.00 E−4	> 1.00 E−4
CAKI-1	0.570	1.525	1.565	1.510	1.367	1.057	1.184	104	98	83	51	64	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4
RXF-393	0.464	1.040	1.037	1.072	1.032	0.844	0.413	99	106	99	66	−11	1.61 E−5	7.20 E−5	> 1.00 E−4
SN12C	0.431	1.502	1.562	1.588	1.540	1.009	0.686	106	108	104	54	24	1.35 E−5	> 1.00 E−4	> 1.00 E−4

Table 1 (*continued*)

NSC: D-753447/1 Experiment ID: 1009 NS13 Units: Molar
COMI: SIH6 (96135) Strain reagent: SRB Dual Pass SSPL: 0XXS

Parent cell line	Time		Mean optical densities					Percent growth					GI_{50}	TGI	LC_{50}
	Zero	Control	−8.0	−7.0	−6.0	−5.0	−4.0	−8.0	−7.0	−6.0	−5.0	−4.0			
TK-10	0.544	1.369	1.391	1.398	1.447	0.979	0.490	103	104	109	53	−10	1.11 E−5	6.92 E−5	> 1.00 E−4
UO-31	0.679	1.233	1.184	1.164	1.200	0.792	0.669	91	87	94	20	−2	3.95 E−6	8.50 E−5	> 1.00 E−4
Prostate cancer															
PC-3	0.413	1.309	1.284	1.300	1.265	0.619	0.534	97	99	95	23	14	4.22 E−6	> 1.00 E−4	> 1.00 E−4
DU-145	0.298	1.204	1.243	1.214	1.194	0.765	0.566	104	101	99	51	30	1.17 E−5	> 1.00 E−4	> 1.00 E−4
Breast cancer															
MCF7	0.288	1.086	1.176	1.076	1.106	0.607	0.417	111	99	102	40	16	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4
MDA-MB-231	0.503	1.182	1.203	1.195	1.207	0.925	0.684	103	102	104	62	27	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4
HS-578T	0.636	1.257	1.272	1.291	1.271	0.956	0.733	102	105	102	51	16	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4
BT-549	0.866	1.733	1.743	1.689	1.729	0.994	0.555	101	95	100	15	−36	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4
T-47D	0.575	1.094	.055	1.088	1.071	0.647	0.610	93	99	96	14	7	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4
MDA-MB-468	0.462	1.057	1.036	1.097	1.069	0.896	0.720	96	105	102	73	43	> 1.00 E−4	> 1.00 E−4	> 1.00 E−4

and TGI value −4.23), NCI-H460 (GI_{50} value −5.51 and TGI −5.08), and NCI-H522 (GI_{50} value −5.56 &and TGI value −5.19), Colon HCT-116 cancer cell lines (GI_{50} value of −5.50 and TGI value −4.85), CNS cancer cell lines; SNB-19 (GI_{50} value −5.24 and TGI value −4.39), SF-539 (GI_{50} value −5.43 and TGI value −4.73), and U251 (GI_{50} value −5.36 and TGI value −4.38), Melanoma UACC-62 cancer cell line (GI_{50} value −5.15 and TGI value −4.65), Ovarian cancer cell lines; IGROV1 (GI_{50} value −5.31 and TGI value −4.81), OVCAR 3 (GI_{50} value −5.56 and TGI value −5.19), and SK-OV-3 (GI_{50} value −5.11 and TGI value −4.40), Renal cancer cell lines; 786-0 (GI_{50} value −5.37 and TGI value −4.29), A498 (GI_{50} value −5.07 and TGI value −4.10), RXF-393 (GI_{50} value −4.79 and TGI value −4.14), TK-10 (GI_{50} value −4.96 and TGI value −4.16), and UO-31 (GI_{50} value −5.40 and TGI value −4.07), Breast BT-549 cancer cell line with GI_{50} value of −5.42 and TGI value of −4.71. It was also found to be active at median lethal concentration against CNS SNB-75 (LC_{50} value −4.40), Melanoma UACC-62 (LC_{50} value −4.21), and Ovarian OVCAR 3 cancer cell line (LC_{50} value −4.47) at 5-log dose range as shown in Tables 1 and 2. The highest activity of this compound might be because of its structural resemblance with lapatinib since it contain 4-chloro benzylideneamine group at C-3 position of quinazoline which suppose to block the hydrophobic pocket of tyrosine kinase (Fig. 3).

5. Conclusion

A novel 3-(substituted benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3*H*)-one (**7**–**27**) has been synthesized. We changed the methodology for the synthesis of 3-amino 7-chloro-2-phenyl quinazolin-4(3*H*)-one **6** to fusion reaction at 250 °C, instead of using solvent, to avoid the problem of ring opening, which is commonly observed while synthesizing quinazolines from benzoxazinone. Fusion is more convenient and time saving method since it takes only 0.5 h, where conventional takes 3.0 h for the reaction. By using this new methodology the preparation of 3-amino 7-chloro-2-phenyl quinazolin-4(3*H*)-one **6** is simple, time-saving and eco-friendly process without solvent and also avoid the problem of ring opening. NCI selected, 7-chloro-3-{[(4-chlorophenyl) methylidene] amino}-2-phenylquinazolin-4(3*H*)-one **12**, with GI_{50} value of −5.59 M, TGI value of −5.12 M, and LC_{50} value of −4.40 M showed remarkable activity against CNS SNB-75 cancer cell line. Rational approach and QSAR techniques enabled the understanding of the pharmacophoric requirement for quinazoline derivatives. The overall outcome of this model revealed that: (i) the quinazoline ring is satisfactory backbone for antitumor activity, (ii) the presence of hydrophobic group at 3 position of quinazoline enhances the activity. These preliminary encouraging results of biological screening of the tested compounds could offer an excellent framework in this field that may lead to discovery of potent antitumor agent.

6. Experimental

All chemicals and solvents were supplied by Merck, S.D. Fine Chemical Limited, Mumbai. All the solvents were distilled and dried before use. The reactions were monitored with the help of thin-layer chromatography using pre-coated aluminum sheets with GF_{254} silica gel, 0.2 mm layer thickness (E. Merck). Melting points of the synthesized compounds were recorded on the Veego (VMP-MP) melting point apparatus. IR spectrum was acquired on a Shimadzu Infra Red Spectrometer, (model FTIR-8400S). Both ^{1}H NMR (DMSO) spectra of the synthesized compounds were performed with Bruker Avance-II 400 NMR Spectrometer operating at 400 MHz in SAIF, Punjab University (Chandigarh). Chemical shifts were measured relative to internal standard TMS (δ: 0). ^{13}C NMR spectra were recorded at 67.8 MHz on the same instrument with internal TMS (δ: 0, DMSO). Chemical shifts are reported in δ scale (ppm). Elemental analyses were performed at the Microanalytical Laboratory of the SAIF, Punjab University.

*6.1. Synthesis of 7-chloro 2-phenyl-benzo[d] [1,3] oxazine-4-one (**3**)*

4-Chloro 2-amino benzoic acid **1** (0.1 mole) was dissolved in minimum volume of dry pyridine (30 ml) by shaking. To this

Table 2 National Cancer Institute developmental therapeutics program in-vitro mean testing results of compound **12**.

NSC: D-753447/1 Experiment ID: 1009 NS13 Units: Molar
COMI: SIH6 (96135) Strain reagent: SRB Dual Pass SSPL: 0XXS

Cell Lines	$\log_{10}$ GI$_{50}$	$\log_{10}$ TGI	$\log_{10}$ LC$_{50}$
Leukemia			
CCRFCEM	> −4.00	> −4.00	> −4.00
MOLT-4	> −4.00	> −4.00	> −4.00
RPMI-8226	> −4.00	> −4.00	> −4.00
SR	−5.11	> −4.00	> −4.00
Non-Small Cell Lung Cancer			
A549/ATCC	−5.19	> −4.00	> −4.00
EKVX	−4.30	> −4.00	> −4.00
HOP-62	−5.13	−4.51	> −4.00
HOP-92	−5.24	> −4.00	> −4.00
NCI-H226	−5.40	−4.70	> −4.00
NCI-H23	−5.08	−4.23	> −4.00
NCI-H322M	−5.28	> −4.00	> −4.00
NCI-H460	−5.51	−5.08	> −4.00
NCI-H522	−5.56	−5.19	> −4.00
Colon cancer			
COLO 205	> −4.00	> −4.00	> −4.00
HCC-2998	> −4.00	> −4.00	> −4.00
HCT-116	−5.50	−4.85	> −4.00
HCT-15	> −4.00	> −4.00	> −4.00
HT-29	> −4.00	> −4.00	> −4.00
KM 12	> −4.00	> −4.00	> −4.00
SW-620	−4.16	> −4.00	> −4.00
CNS cancer			
SF-268	−5.47	> −4.00	> −4.00
SF-295	−4.99	> −4.00	> −4.00
SF-539	−5.43	−4.73	> −4.00
SNB-19	−5.24	−4.39	> −4.00
SNB-75	−5.59	−5.12	−4.40
U251	−5.36	−4.38	> −4.00
Melanoma			
LOXIMVI	−4.75	> −4.00	> −4.00
M14	> −4.00	> −4.00	> −4.00
MDA-MB-435	> −4.00	> −4.00	> −4.00
SK-MEL-2	−5.21	> −4.00	> −4.00
SK-MEL-28	−4.02	> −4.00	> −4.00
SK-MEL-5	−4.90	> −4.00	> −4.00
UACC-257	> −4.00	> −4.00	> −4.00
UACC-62	−5.15	−4.65	−4.21
Ovarian cancer			
IGROV1	−5.31	−4.81	> −4.00
OVCAR3	−5.56	−5.19	−4.47
OVCAR4	−5.54	> −4.00	> −4.00
OVCAR5	> −4.00	> −4.00	> −4.00
OVCAR8	−5.46	> −4.00	> −4.00
NCI-RES	−5.38	> −4.00	> −4.00
SK-OV-3	−5.11	−4.40	> −4.00
Renal cancer			
786-0	−5.37	−4.29	> −4.00
A498	−5.07	−4.10	> −4.00
ACHN	−5.18	> −4.00	> −4.00
CAKI-1	> −4.00	> −4.00	> −4.00
RXF-393	−4.79	−4.14	> −4.00
SN12C	−4.87	> −4.00	> −4.00
TK-10	−4.96	−4.16	> −4.00
UO-31	−5.40	−4.07	> −4.00
Prostate cancer			
PC-3	−5.37	> −4.00	> −4.00
DU-145	−4.93	> −4.00	> −4.00
Breast cancer			
MCF7	−5.16	> −4.00	> −4.00
MDA-MB-231	−4.66	> −4.00	> −4.00
HS-578T	−4.96	> −4.00	> −4.00
BT-549	−5.42	−4.71	> −4.00
T-47D	−5.44	> −4.00	> −4.00
MDA-MB-468	−4.23	> −4.00	> −4.00

solution benzoyl chloride **2** (0.2 mole) taken in dry pyridine (30 ml), was added slowly with constant stirring. When the addition was completed (the operation of addition required half an hour), the resultant solution was subjected to vigorous stirring for one hour mechanically subsequently, it was left as such for one hour at room temperature and treated with a solution of sodium bicarbonate (10%). Addition of sodium bicarbonate solution was continued till the effervescence due to the evolution of carbon-dioxide ceased. The separated solid was allowed to settle down and filtered off. It was washed with cold water repeatedly till there was no smell of pyridine and unreacted benzoyl chloride. The crude benzo-oxazine was dried in vacuum overnight and recrystallization from diluted ethanol afforded analytically pure sample of 2-phenyl-benzo[*d*] [1,3] oxazin-4-one **3** as white crystalline mass. Yield 82%; mp 195–198 °C. IR (KBr, ν_{max}, cm^{-1}): 3062 (C–H), 1745 (C═O), 1510 (C═N), 1480 (C═C), 1364 (C–N), 771 (C–Cl). ^{1}H NMR (DMSO-d_6) δ ppm: 7.60–8.21 (m, 8H, Ar-H). ^{13}C NMR (DMSO-d_6) δ ppm: 164.2, 158.4, 156.2, 140.4, 136.2, 134.6, 130.8, 128.6, 128.4, 126.6, 124.4, 114.4. Anal. Calcd for $C_{14}H_8ClNO_2$: C, 65.26; H, 3.13; N, 5.44. Found: C, 65.42; H, 3.04; N, 5.65%.

*6.2. Synthesis of isomers of N-(5-chloro-2-(hydrazine carbonyl) phenyl) benzamide (**4**) and 3-amino 7-chloro-2-phenyl quinazolin-4(3H)-one (**5**)*

A mixture of 7-chloro 2-phenyl-benzo[*d*] [1,3] oxazine-4-one **3** (0.01 mol) and hydrazine hydrate (0.01 mol) in dry pyridine (50 ml) was heated under reflux for 3 h. Subsequently, mixture was poured into water (containing few drops of HCl) solid thus separated was filtered, washed repeatedly with water. It was dried and crystallized from ethanol. Isomers were separated by column chromatography using benzene: acetone in 7:3 ratio.

*6.2.1. N-(5-Chloro-2-(hydrazine carbonyl) phenyl) benzamide (**4**)*

This compound was prepared, purified, and separated as per the above mentioned procedure: yield 35%; mp 215–217 °C. IR (KBr, ν_{max}, cm^{-1}): 3212 (s), 1560 (b) (NH), 3057 (C–H), 1665 (C═O), 1484 (C═C), 748 (C–Cl). 1H NMR (DMSO-d_6) δ ppm: 6.21 (s, 2H, NH_2), 7.14–8.60 (m, 8H, Ar-H),

10.19 (s, 1H, –NHCO–), 9.12 (s, 1H, –CONH–). 13C NMR (DMSO-d_6) δ ppm: 168.6, 168.2, 145.6, 142.7, 136.6, 132.1, 130.3, 128.8, 126.8, 122.4, 120.5, 118.4. Anal. Calcd for $C_{14}H_{12}ClN_3O_2$: C, 58.04; H, 4.17; N, 14.50. Found: C, 58.18; H, 4.42; N, 14.81%.

*6.2.2. 3-Amino 7-chloro-2-phenyl quinazolin-4(3H)-one (**5**)*

This compound was prepared, purified and separated as per the above mentioned procedure: yield 41%; mp 206–209 °C. IR (KBr, ν_{max}, cm^{-1}): 3270 (s), 1582 (b) (NH_2), 3078 (CH), 1666 (C═O), 1564 (C═N), 1491 (C═C), 1371 (C–N), 762 (C–Cl). ^{1}H NMR (DMSO-d_6) δ ppm: 5.25 (s, 2H, NH_2), 7.14–8.18 (m, 8H, Ar-H). ^{13}C NMR (DMSO-d_6) δ ppm: 164.8, 158.4, 154.6, 138.6, 132.6, 132.6, 130.8, 130.6, 128.6, 127.6, 120.8. Anal. Calcd for $C_{14}H_{10}ClN_3O$: C, 61.89; H, 3.71; N, 15.47. Found: C, 61.66; H, 3.78; N, 15.68%.

*6.2.3. Optimized method for the synthesis of 3-amino 7-chloro-2-phenyl quinazolin-4(3H)-one (**6**)*

A mixture of 7-chloro 2-phenyl-benzo[*d*] [1,3] oxazine-4-one **3** and hydrazine hydrate was fused together at 250 °C in an oil bath for 0.5 h. The mixture was cooled and methanol was added to the mixture. The separated solid was collected by filtration, washed with methanol, dried, and crystallized from ethanol. Yield 68%; mp 206–209 °C. IR (KBr, ν_{max}, cm^{-1}): 3270 (s), 1582 (b) (NH_2), 3078 (CH), 1666 (C═O), 1564 (C═N), 1491 (C═C), 1371 (C–N), 762 (C–Cl). ^{1}H NMR (DMSO-d_6) δ ppm: 5.25 (s, 2H, NH2), 7.14–8.18 (m, 8H, Ar-H). ^{13}C NMR (DMSO-d_6) δ ppm: 164.8, 158.4, 154.6, 138.6, 132.6, 132.6, 130.8, 130.6, 128.6, 127.6, 120.8. Anal. Calcd for $C_{14}H_{10}ClN_3O$: C, 61.89; H, 3.71; N, 15.47. Found: C, 61.46; H, 3.85; N, 15.66%.

*6.3. General procedure for the synthesis of 3-(substituted benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**7–27**)*

A mixture of 3-amino-7-chloro-2-phenyl-3*H*-quinazoline-4-one **6** and different aldehydes in equimolar quantities in glacial acetic acid was heated under reflux for six hours. The solution was cooled and poured carefully into crushed ice. A solid separated out which was allowed to settle down. It was filtered off, washed successively with water and dried. The solid thus obtained was on recrystallization from diluted ethanol afforded white crystalline solid mass.

*6.3.1. 3-(Anthracene-9-yl methyleneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**7**)*

This compound was prepared and purified as per the above mentioned procedure: yield 62%; mp 288–291 °C. IR (KBr, ν_{max}, cm^{-1}): 3052 (CH), 1650 (C═O), 1545 (C═N), 1495 (C═C), 1384 (C–N), 752 (C–Cl). 1H NMR (DMSO-d_6) δ ppm: 6.54–8.28 (m, 17H, Ar-H), 8.77 (s, 1H, N═CH). 13C NMR (DMSO-d_6) δ ppm: 170.5, 168.7, 156.6, 154.5, 146.8, 140.8, 132.8, 131.2, 131.1, 129.9, 129.8, 129.2, 129.0, 125.6, 124.6, 123.8, 122.2, 118.0. Anal. Calcd for $C_{29}H_{18}ClN_3O$: C, 75.73; H, 3.94; N, 9.14. Found: C, 75.64; H, 3.64; N, 9.06%.

*6.3.2. 3-(Benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**8**)*

This compound was prepared and purified as per the above mentioned procedure: yield 68%; mp 234–236 °C. IR (KBr, ν_{max}, cm^{-1}): 3089 (CH), 1654 (C═O), 1552 (C═N), 1488 (C═C), 1386 (C–N), 724 (C–Cl). ^{1}H NMR (DMSO-d_6) δ ppm: 7.41–8.28 (m, 13H, Ar-H), 9.29 (s, 1H, N═CH). 13CNMR (DMSO-d_6) δ ppm: 170.7, 168.6, 160.4, 156.4, 154.2, 140.4, 135.7, 132.0, 131.2, 131.1, 130.6, 129.8, 129.2, 125.6, 122.6, 118.0. Anal. Calcd for $C_{21}H_{14}ClN_3O$: C, 70.10; H, 3.92; N, 11.68. Found: C, 70.24; H, 3.68; N, 11.51%.

*6.3.3. 3-(3-Bromo benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**9**)*

This compound was prepared and purified as per the above mentioned procedure: yield 71%; mp 248–251 °C. IR (KBr, ν_{max}, cm^{-1}): 3065 (CH), 1634 (C═O), 1556 (C═N), 1494 (C═C), 1389 (C–N), 735 (C–Cl), 685 (C–Br). 1H NMR (DMSO-d_6) δ ppm: 7.05–8.36 (m, 12H, Ar-H), 8.98 (s, 1H, N═CH). 13C NMR (DMSO-d_6) δ ppm: 170.7, 168.6, 156.2, 154.6, 152.5, 140.2, 136.9, 134.9, 132.7, 131.2, 131.1, 130.2, 129.8, 129.2, 125.1, 123.7, 122.4, 118.0. Anal. Calcd for $C_{21}H_{13}BrClN_3O$: C, 57.49; H, 2.99; N, 9.58. Found: C, 57.36; H, 2.91; N, 9.45%.

*6.3.4. 3-(4-Cyano benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**10**)*

This compound was prepared and purified as per the above mentioned procedure: yield 82%; mp 222–225 °C. IR (KBr, ν_{max}, cm^{-1}): 3071 (CH), 2245 (cyano), 1645 (C═O), 1534 (C═N), 1488 (C═C), 1392 (C–N), 765 (C–Cl). 1H NMR (DMSO-d_6) δ ppm: 7.41–8.28 (m, 12H, Ar-H), 9.29 (s, 1H, N═CH). 13C NMR (DMSO-d_6) δ ppm: 170.7, 168.3, 160.8, 156.4, 154.5, 141.2, 140.2, 135.3, 131.2, 131.1, 129.8, 125.4, 126.3, 122.6, 118.0, 116.6, 114.6. Anal. Calcd for $C_{22}H_{13}ClN_4O$: C, 68.67; H, 3.41; N, 14.56. Found: C, 68.75; H, 3.24; N, 14.39%.

*6.3.5. 3-(2-Chloro benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**11**)*

This compound was prepared and purified as per the above mentioned procedure: yield 83%; mp 211–214 °C. IR (KBr, ν_{max}, cm^{-1}): 3058 (CH), 1647 (C═O), 1536 (C═N), 1490 (C═C), 1421 (C–N), 756 (C–Cl). ^{1}H NMR (DMSO-d_6) δ ppm: 7.12–8.28 (m, 12H, Ar-H), 9.12 (s, 1H, N═CH). ^{13}C NMR (DMSO-d_6) δ ppm: 170.8, 168.4, 156.3, 154.2, 146.6, 140.1, 136.7, 135.9, 132.4, 131.1, 129.8, 129.2, 125.4, 125.2, 126.9, 122.4, 118.0. Anal. Calcd for $C_{21}H_{13}Cl_2N_3O$: C, 63.98; H, 3.32; N, 10.66. Found: C, 63.88; H, 3.27; N, 10.63%.

*6.3.6. 3-(4-Chloro benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**12**)*

This compound was prepared and purified as per the above mentioned procedure: yield 63%; mp 228–230 °C. IR (KBr, ν_{max}, cm^{-1}): 3052 (CH), 1657 (C═O), 1530 (C═N), 1495 (C═C), 1412 (C–N), 725 (C–Cl). 1H NMR (DMSO-d_6) δ ppm: 7.34–8.61 (m, 12H, Ar-H), 9.23 (s, 1H, N═CH). 13C NMR (DMSO-d_6) δ ppm: 170.6, 168.3, 160.4, 156.7, 154.5, 140.1, 136.6, 132.8, 131.6, 131.2, 131.1, 129.9, 129.8, 129.2, 125.4, 122.4, 118.0. Anal. Calcd for: $C_{21}H_{13}Cl_2N_3O$ C, 63.98; H, 3.32; N, 10.66. Found: C, 63.82; H, 3.36; N, 10.58%.

*6.3.7. 3-(4-Dimethylamino benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**13**)*

This compound was prepared and purified as per the above mentioned procedure: yield 52%; mp 218–220 °C. IR (KBr,

ν_{max}, cm^{-1}): 3059 (CH), 1659 (C═O), 1538 (C═N), 1478 (C═C), 1423 (C–N), 734 (C–Cl). 1H NMR (DMSO-d_6) δ ppm: 2.48 (s, 6H, CH_3), 7.39–8.39 (m, 12H, Ar-H), 9.34 (s, 1H, N═CH). ^{13}C NMR (DMSO-d_6) δ ppm: 170.8, 168.3, 160.8, 156.6, 156.1, 154.2, 140.1, 131.2, 131.1, 129.8, 129.3, 129.2, 125.4, 123.6, 122.4, 118.0, 114.8, 41.6. Anal. Calcd for $C_{23}H_{19}ClN_4O$: C, 68.57; H, 4.75; N, 13.91. Found: C, 68.52; H, 4.78; N, 13.63%.

*6.3.8. 3-(3-Methoxy 4-hydroxy benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**14**)*

This compound was prepared and purified as per the above mentioned procedure: yield 78%; mp 260–263 °C. IR (KBr, ν_{max}, cm^{-1}): 3410 (OH), 3058 (CH), 1678 (C═O), 1562 (C═N), 1485 (C═C), 1435 (C–N), 710 (C–Cl). ^{1}H NMR (DMSO-d_6) δ ppm: 3.94 (s, 3H, OCH_3), 7.51–8.34 (m, 11H, Ar-H), 8.90 (s, 1H, N═CH), 10.12 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ ppm: 170.8, 168.7, 156.2, 154.5, 153.8, 152.4, 150.1, 140.8, 131.9, 131.2, 131.1, 129.8, 129.2, 125.4, 122.8, 122.3, 118.0, 116.6, 114.6. Anal. Calcd for $C_{22}H_{16}ClN_3O3$: C, 65.11; H, 3.97; N, 10.35. Found: C, 65.18; H, 3.63; N, 10.21%.

*6.3.9. 3-(4-Methoxy benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**15**)*

This compound was prepared and purified as per the above mentioned procedure: yield 62%; mp 251–254 °C. IR (KBr, ν_{max}, cm^{-1}): 3055 (CH), 1664 (C═O), 1540 (C═N), 1489 (C═C), 1414 (C–N), 714 (C–Cl). 1H NMR (DMSO-d_6) δ ppm: 3.96 (s, 3H, OCH_3), 7.10–8.26 (m, 12H, Ar-H), 9.11 (s, 1H, N═CH). 13C NMR (DMSO-d_6) δ ppm: 170.4, 168.2, 164.1, 160.4, 156.2, 154.5, 140.8, 131.2, 131.1, 129.8, 129.2, 125.4, 126.0, 122.4, 118.0, 116.4, 54.4. Anal. Calcd for $C_{22}H_{16}ClN_3O_2$: C, 67.78; H, 4.14; N, 10.78. Found: C, 67.81; H, 4.05; N, 10.67%.

*6.3.10. 3-(2-Hydroxy benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**16**)*

This compound was prepared and purified as per the above mentioned procedure: yield 69%; mp 210–213 °C. IR (KBr, ν_{max}, cm^{-1}): 3390 (OH), 3088 (CH), 1656 (C═O), 1648 (C═C), 1510 (C═N), 1498 (C═C), 1445 (C–N), 724 (C–Cl). 1H NMR (DMSO-d_6) δ ppm: 7.56–8.30 (m, 12H, Ar-H), 9.10 (s, 1H, N═CH), 11.99 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ ppm: 170.4, 168.3, 160.6, 156.1, 154.2, 146.1, 140.4, 134.4, 131.2, 131.1, 129.8, 129.2, 125.5, 125.4, 122.2, 121.1, 118.0, 114.4, 112.4. Anal. Calcd for $C_{21}H_{14}ClN_3O_2$: C, 67.12; H, 3.75; N, 11.18. Found: C, 67.32; H, 3.72; N, 11.05%.

*6.3.11. 3-(4-Hydroxy benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**17**)*

This compound was prepared and purified as per the above mentioned procedure: yield 72%; mp 244–247 °C. IR (KBr, ν_{max}, cm^{-1}): 3418 (OH), 3079 (CH), 1644 (C═O), 1524 (C═N), 1478 (C═C), 1405 (C–N), 768 (C–Cl) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ ppm: 7.16–8.70 (m, 12H, Ar-H), 9.16 (s, 1H, N═CH), 11.99 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ ppm: 170.4, 168.6, 164.8, 160.3, 156.6, 154.2, 140.1, 131.6, 131.2, 131.1, 129.8, 129.2, 125.4, 126.5, 122.4, 118.0, 114.2. Anal. Calcd for $C_{21}H_{14}ClN_3O_2$: C, 67.12; H, 3.75; N, 11.18. Found: C, 67.04; H, 3.67; N, 11.26%.

*6.3.12. 3-(2,5-Dimethoxy benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**18**)*

This compound was prepared and purified as per the above mentioned procedure: yield 78%; mp 251–254 °C. IR (KBr, ν_{max}, cm^{-1}): 3071 (CH), 1646 (C═O), 1522 (C═N), 1491 (C═C), 1428 (C-N), 745 (C–Cl). 1H NMR (DMSO-d_6) δ ppm: 3.96 (s, 6H, OCH_3), 7.10–8.64 (m, 11H, Ar-H), 8.88 (s, 1H, N═CH). 13C NMR (DMSO-d_6) δ ppm: 170.4, 168.3, 156.6, 156.1, 154.4, 150.9, 146.2, 140.2, 131.2, 131.1, 129.8, 129.2, 125.4, 122.6, 118.0, 116.9, 116.6, 114.6, 112.4, 54.2. Anal. Calcd for $C_{23}H_{18}ClN_3O_3$: C, 65.79; H, 4.32; N, 10.01. Found: C, 65.98; H, 4.21; N, 10.14%.

*6.3.13. 3-(4-Methyl benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**19**)*

This compound was prepared and purified as per the above mentioned procedure: yield 62%; mp 223–226 °C. IR (KBr, ν_{max}, cm^{-1}): 3078 (CH), 1662 (C═O), 1512 (C═N), 1498 (C═C), 1434 (C–N), 724 (C–Cl). 1H NMR (DMSO-d_6) δ ppm: 2.48 (s, 3H, CH_3), 7.12–8.87 (m, 12H, Ar-H), 8.90 (s, 1H, N═CH). ^{13}C NMR (DMSO-d_6) δ ppm: 170.8, 168.3, 160.2, 156.4, 154.2, 142.7, 140.2, 131.7, 131.2, 131.1, 130.5, 129.8, 129.2, 125.4, 126.1, 122.4, 118.0, 22.4. Anal. Calcd for $C_{22}H_{16}ClN_3O$: C, 70.68; H, 4.31; N, 11.24. Found: C, 70.66; H, 4.12; N, 11.65%.

*6.3.14. 3-(3-Nitro benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**20**)*

This compound was prepared and purified as per the above mentioned procedure: yield 70%; mp 246–250 °C. IR (KBr, ν_{max}, cm^{-1}): 3076 (CH), 1648 (C═O), 1518 (C═N), 1545, 1354 (NO_2), 1496 (C═C), 1421 (C–N), 752 (C–Cl). 1H NMR (DMSO-d_6) δ ppm: 7.18–8.33 (m, 12H, Ar-H), 9.28 (s, 1H, N═CH). 13C NMR (DMSO-d_6) δ ppm: 170.1, 169.2, 168.3, 156.4, 154.2, 150.4, 140.2, 136.8, 132.4, 131.2, 131.1, 130.2, 129.8, 129.2, 125.4, 123.6, 122.1, 118.0. Anal. Calcd for $C_{21}H_{13}ClN_4O_3$: C, 62.31; H, 3.24; N, 13.84. Found: C, 62.16; H,3.32; N, 13.63%.

*6.3.15. 3-(4-Nitro benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**21**)*

This compound was prepared and purified as per the above mentioned procedure: yield 71%; mp 232–234 °C. IR (KBr, ν_{max}, cm^{-1}): 3072 (CH), 1676 (C═O), 1528 (C═N), 1554, 1348 (NO_2), 1486 (C═C), 1428 (C–N), 760 (C–Cl). ^{1}H NMR (DMSO-d_6) δ ppm: 7.65–8.53 (m, 12H, Ar-H), 9.45 (s, 1H, N═CH). ^{13}C NMR (DMSO-d_6) δ ppm: 170.8, 169.4, 168.3, 160.2, 156.4, 154.1, 144.7, 140.2, 131.2, 130.1, 129.8, 129.2, 125.7, 125.4, 122.6, 121.1, 118.0. Anal. Calcd for $C_{21}H_{13}ClN_4O_3$: C, 62.31; H, 3.24; N, 13.84. Found: C, 62.29; H, 3.21; N, 13.88%.

*6.3.16. 3-(2-Nitro benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**22**)*

This compound was prepared and purified as per the above mentioned procedure: yield 62%; mp 262–265 °C. IR (KBr, ν_{max}, cm^{-1}): 3064 (CH), 1676 (C═O), 1510 (C═N), 1561, 1352 (NO_2), 1484 (C═C), 1436 (C–N), 736 (C–Cl). 1H NMR (DMSO-d_6) δ ppm: 7.65–8.43 (m, 12H, Ar-H), 9.12 (s, 1H, N═CH). 13C NMR (DMSO-d_6) δ ppm: 170.3, 168.8, 168.4, 156.3, 154.1, 146.1, 140.8, 136.8, 132.8, 130.2, 130.2, 130.1, 130, 129.8, 129.2, 125.4, 124.6, 122.6, 121.1, 118.0. Anal.

Calcd for $C_{21}H_{13}ClN_4O_3$: C, 62.31; H, 3.24; N, 13.84. Found: C, 62.38; H, 3.16; N, 13.98%.

*6.3.17. 3-(4-Bromo benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**23**)*

This compound was prepared and purified as per the above mentioned procedure: yield 68%; mp 268–270 °C. IR (KBr, ν_{max}, cm^{-1}): 3068 (CH), 1656 (C═O), 1538 (C═N), 1486 (C═C), 1398 (C–N), 764 (C–Cl), 671 (C–Br). 1H NMR (DMSO-d_6) δ ppm: 7.16–8.54 (m, 12H, Ar-H), 9.35 (s, 1H, N═CH). 13C NMR (DMSO-d_6) δ ppm: 170.6, 168.4, 160.5, 156.3, 154.2, 140.8, 134.7, 132.7, 131.2, 131.1, 129.8, 129.5, 129.2, 125.4, 124.4, 122.4, 118.0. Anal. Calcd for $C_{21}H_{13}BrClN_3O$: C, 57.49; H, 2.99; N, 9.58. Found: C, 57.64; H, 3.08; N, 9.45%.

*6.3.18. 3-(4-Fluro benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**24**)*

This compound was prepared and purified as per the above mentioned procedure: yield 58%; mp 224–226 °C. IR (KBr, ν_{max}, cm^{-1}): 3064 (CH), 1658 (C═O), 1544 (C═N), 1492 (C═C), 1368 (C–N), 1214 (C–Fl), 728 (C–Cl). ^{1}H NMR (DMSO-d_6) δ ppm: 7.55–8.64 (m, 12H, Ar-H), 9.14 (s, 1H, N═CH). 13C NMR (DMSO-d_6) δ ppm: 170.0, 168.7, 165.3, 160.6, 156.5, 154.2, 140.2, 131.8, 131.2, 131.1, 130.2, 129.8, 129.2, 125.4, 122.6, 118.0, 114.8. Anal. Calcd for $C_{21}H_{13}ClFN_3O$: C, 66.76; H, 3.47; N, 11.12. Found: C, 66.64; H, 3.62; N, 11.02%.

*6.3.19. 3-(3,4-Dihydroxy benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**25**)*

This compound was prepared and purified as per the above mentioned procedure: yield 52%; mp 236–241 °C. IR (KBr, ν_{max}, cm^{-1}): 3428 (OH), 3057 (CH), 1639 (C═O), 1515 (C═C), 1556 (C═N), 1494 (C═C), 1388 (C–N), 732 (C–Cl). ^{1}H NMR (DMSO-d_6) δ ppm: 7.16-8.51 (m, 11H, Ar-H), 8.91 (s, 1H, N═CH), 9.63 (s, 2H, OH). ^{13}C NMR (DMSO-d_6) δ ppm: 170.4, 168.6, 156.4, 154.4, 152.4, 150.8, 148.2, 140.6, 132.3, 131.2, 131.1, 129.8, 129.2, 125.4, 123.5, 122.5, 118.0, 116.2, 114.2. Anal. Calcd for $C_{21}H_{14}ClN_3O_3$: C, 64.37; H, 3.60; N, 10.72. Found: C, 64.56; H, 3.32; N, 10.62%.

*6.3.20. 3-(3-Hydroxy benzylideneamino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**26**)*

This compound was prepared and purified as per the above mentioned procedure: yield 48%; mp 214–218 °C. IR (KBr, ν_{max}, cm^{-1}): 3412 (OH), 3061 (CH), 1638 (C═O), 1532 (C═C), 1565 (C═N), 1489 (C═C), 1372 (C–N), 721 (C–Cl). 1H NMR (DMSO-d_6) δ ppm: 7.56–8.58 (m, 12H, Ar-H), 8.96 (s, 1H, N═CH), 9.32 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ ppm: 170.4, 168.4, 159.4, 156.2, 154.1, 152.2, 140.7, 131.2, 131.1, 129.8, 129.2, 125.4. Anal. Calcd for $C_{21}H_{14}ClN_3O_2$: C, 67.12; H, 3.75; N, 11.18. Found: C, 67.15; H, 3.89; N, 11.24%.

*6.3.21. 3-(3-Phenyl allyllidene amino)-7-chloro-2-phenyl quinazoline-4(3H)-one (**27**)*

This compound was prepared and purified as per the above mentioned procedure: yield 71%; mp 283–285 °C. IR (KBr) ν_{max} 3066 (CH), 2110 (C═C), 1651 (C═O), 1514 (C═N), 1482 (C═C), 1394 (C–N), 764 (C–Cl). 1H NMR (DMSO-d_6) δ ppm: 6.95–9.32 (a set of signals, 16H, Ar-H, olefinic CH═CH and N═CH). 13C NMR (DMSO-d_6) δ ppm: 170.6, 168.4, 156.2, 154.2, 140.6, 138.6, 136.2, 134.1, 131.2, 131.1, 129.8, 129.6, 129.5, 129.2, 125.9, 125.4, 122.4, 118.0. Anal. Calcd for $C_{23}H_{16}ClN_3O$: C, 71.59; H, 4.18; N, 10.89. Found: C, 71.68; H, 4.31; N, 10.61%.

Acknowledgements

The authors thank Director General, Department of Science and Technology, New Delhi for funding the project (Grant No. SR/FT/LS-0024/2008), Chairman, Captain M.P. Singh and Sardar Sangat Singh Longia, Secretary ASBASJSM College of Pharmacy for providing the necessary facilities.

References

Adams, J., 2001. Kinetic and catalytic mechanisms of protein kinases. Chem. Rev. 101, 2271.

Alley, M.C., Scudiero, D.A., Monks, P.A., Hursey, M.L., Czerwinski, M.J., Fine, D.L., Abbott, B.J., Mayo, J.G., Shoemaker, R.H., Boyd, M.R., 1988. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res. 48, 589–601.

Badiger, A.M., Noolvi, M.N., Nayak, P.V., 2006. QSAR study of Benzthiazole derivatives as p56 lck inhibitors. Lett. Drug Des. Discov. 3, 550–560.

Blume-Jensen, P., Hunter, T., 2001. Oncogenic kinase signaling. Nature 411, 355.

Boyd, M.R., Paul, K.D., 1995. Some practical considerations and applications of the National Cancer Institute in vitro anticancer drug discovery screen. Drug Dev. Res. 19, 91–109.

Cohen, P., 2002. Protein kinases-the major drug targets for the twenty first century. Nat. Rev. Drug Discov. 1, 309.

Dumas, J., 2001. Growth factor receptor kinase inhibitors; recent advances in clinical impact. Curr. Opin. Drug Discov. Devel. 4, 378.

Fabbro, D., Ruetz, S., Buchdunger, E., Cowan-Jacob, S.W., Fendrich, G., Liebetanz, J., O'Reilley, T., Traxler, P., Chaudhari, B., Fretz, H., Zimmermann, J., Meyer, T., Carvatti, G., Furet, P., Manley, P.W., 2002. Protein kinases as targets for anti-cancer agents: from inhibitors to useful drugs. Pharmacol. Ther. 93, 79–98.

Fricker, J., 2006. Tyrosine kinase inhibitors: The next generation. Lancet Oncol. 7, 621.

Grever, M.R., Schepartz, S.A., Chabner, B.A., 1992. The national cancer institute: cancer drug discovery and development program. Semin. Oncol. 19, 622–638.

Gschwind, A., Fischer, O.M., Ullrich, A., 2004. The discovery of receptor tyrosine kinase: targets for the cancer therapy. Nat. Rev. Cancer 4, 361.

Jordan, J.D., Landau, E.M., Iyengar, R., 2000. Signaling of networks: the origin of the multicellular multi-tasking. Cell 103, 193.

Manjula, S.N., Noolvi, M.N., Parihar, K.V., Manohara Reddy, S.A., Ramani, V., Gadad, A.K., Singh, G., Kutty, N.G., Rao, M., 2009. Synthesis and anti-tumour activity of optically active thiourea and their 2-aminobenzothiazole derivatives: A novel class of anticancer agents. Eur. J. Med. Chem. 44, 2923–2929.

Noolvi, M.N., Patel, H.M., 2010. 2D QSAR studies on a series of quinazoline derivatives as Tyrosine Kinase (EGFR) Inhibitor: An Approach to Design Anticancer Agents. Lett. Drug Des. Discov. 7, 556–586.

Noolvi, M.N., Patel, H.M., Bhardwaj, V.B., 2011. An approach to design anticancer agents by 2D QSAR studies on a series of quinazoline analogues as tyrosine kinase (erbB-2) inhibitors. Med. Chem. 7, in press.

Peter, B., Robert, H.B., Craig, S.H., Laurent, F.A.H., Mark, H., Jason, G.K., Jane, K., Teresa, K., Donald, J.O., Stuart, E.P., Emma, J.W.,

Ingrid, . Design, synthesis and in vitro antitumor activity of 4-amino quinoline and 4-amino quinazoline derivatives targeting EGFR tyrosine kinase. Bioorg. Med. Chem. Lett. 16, 4908.

Ranson, M., 2004. Epidermal growth receptor tyrosine kinase inhibitors. Br. J. Cancer 90, 2250.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMohan, J., Vistica, D., Warren, J.R., Bokesch, H., Kenny, S., Boyd, M.R., 1990. New colorimetric cytotoxicity assay for anti-cancer drug screening. J. Natl. Cancer Inst. 82, 1107–1112.

Slichenmeyer, W.J., Elliott, W.L., Fry, D.W., 2001. A pan erbB-tyrosine kinase inhibitors. Semin. Oncol. 28, 67.

Yarden, Y., Sliwkowski, M.X., 2001. Untangling the ErbB signaling network. Nat. Rev. 2, 127.