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Synthesis, Method Optimization, In-Vitro Antitumor Activity Of Some New Substituted Quinazoline And Quinazolin-4-One Derivatives: Search For Anticancer Agent

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Abstract

The essence of work is to synthesize some New 2,4 disubstituted Quinazoline and 2,3 disubstituted Quinazolin-4-one derivatives and to test them for their In-vitro anticancer activity. Two different starting materials namely Anthranilamide and Anthranilic acid were used to obtain two different intermediate one Benzamide intermediate and the second one Benzoxazine-4-one as an intermediate which lead to two different novel Quinazoline and Quinazolin-4-one derivatives and its further substitution gave a series of novel compounds. In the light of above, 2nd and 4thposition of quinazoline ring nucleus and 2nd and 3rdposition of quinazolin-4-one were explored to obtain different derivatives. Novel 4-Anilino Quinazoline derivatives and Schiff bases were prepared from reaction scheme 1 and various 2-substituted amino thiadiazole quinazoline-4-one derivatives were synthesized from reaction scheme 2.We have applied the methodology of fusion for the synthesis of 2-substituted amino thidadiazole-2-(4-nitrophenyl) quinazoline 4(3H)-one **6(a-d)** at 250-260 ^{0}C, instead of using organic solvent, to by pass the problem of ring opening, which is usually observed while synthesizing quinazolines from bentranil or benzooxazin-4-one. Fusion method is easy, more expedient, eco-friendly process and time saving method. It is a pace towards green chemistry.

To summarize, we have prepared 06 new 2,4 disubstituted quinazoline derivatives from reaction scheme 1 and 04 new 2,3 disubstituted Quinazolin-4-one derivatives from reaction scheme 2 successfully and evaluated them for their anticancer activity.

Keywords: Quinazoline, Quinazolin-4-one, 2-substituted amino thiadiazole, Schiff bases and Anticancer.

1. Introduction

Cancer is the second leading cause of death in the world. Now a days, chemotherapy, surgery, radiotherapy and endocrine therapy have been the standard treatment available for patients. It is an urgent need to develop newer more effective therapies to improve patient outcome. Protein tyrosine kinases have emerged as new promising targets for cancer therapy [1]. Cancer is a disease present in people and animals in which the structure and normal function of body tissues are disrupted. The exact etiology of most types of cancer is unknown. However, it is well known that infections, environmental factors (chemical substances, foreign particles, radiation), and genetic factors can induce transformation of normal cells to neoplastic cells, i.e. those that multiply and function abnormally. Cancer can be characterized by the following parameters: Cells begin to divide uncontrollably because the mechanisms that control growth are disrupted. Cells cease to differentiate. Cells begin to exhibit invasiveness and gain the ability to metastasize, i.e. to appear in tissues separate from the place of initial localization. Cells begin to intensely synthesize macromolecules from nucleosides and amino acids [2].

Cancer is a collective term used for a group of diseases that are characterized by the loss of control of the growth, division and spread of a group of cells, leading to a primary tumor that invades and destroys adjacent tissues. It may also spread to other regions of the body through a process known as metastasis, which is the cause of 90% of cancer deaths [3]. 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 [4]. They can be broadly classified as receptor such as VEGFR, EGFR or non-receptor kinases. Inappropriate or uncontrolled activation of many of these kinases, by over-expression, constitutive activation, or mutation, has been shown to result in uncontrolled cell growth [5].The importance of tyrosine kinase enzymes in health and disease is further underscored by the existence of aberration in tyrosine kinase enzymes signaling occurring in inflammatory diseases and diabetes. Inhibitors of tyrosine kinase as a new kind of effective anticancer drug are important mediators of cellular signal transduction that affects growth factors and oncogene on cell proliferation [6,7]. The development of tyrosine kinase inhibitors has therefore become an active area of research in pharmaceutical chemistry. The therapeutic potential of targeting tumor endothelium and vascular supply is now widely recognized to treat different diseases. One such disease is cancer; where endothelial cells are actively proliferating to support the tumor growth. Solid tumors cannot grow beyond the size of a few millimeters without inducing the proliferation of endothelium and formation of new blood vessels. Hence it is crucial to search for new agents that selectively block tumor blood supply. These include anti-angiogenic molecules, vascular disrupting agents or endothelial disrupting agents. The antiangiogenic molecules such as monoclonal antibodies and tyrosine kinase inhibitors disrupt endothelial cell survival mechanisms and new blood vessel formation and vascular disrupting agents for instance ligand-directed and small molecules can be used to disrupt the already existing abnormal vasculature that support tumors by targeting their dysmorphic endothelial cells. The recent advances in this area of research have identified a variety of investigational agents which are currently in clinical development at various stages and some of these candidates are already approved in cancer treatment [8]. **(As shown in Chart 1)**Vascular endothelial growth factor (VEGF) is a potent mitogen that is highly specific for vascular endothelial cell [9] and is a highly potent angiogenic agent that increases vessel permeability and enhances endothelial cell growth, proliferation, migration, and differentiation [10]. Angiogenesis plays an important role in the pathogenesis of a variety of disorders, such as cancer, proliferative retinopathies and rheumatoid arthritis [11,12,13]VEGF isoforms and their cognate tyrosine kinase receptors (VEGFRs) have been especially attractive targets for the inhibition of angiogenesis [14,15]. The biological effects of VEGF are mediated by two receptor tyrosine kinases (RTKs), VEGFR-1 kinase [16]and VEGFR-2 kinase (also known as kinase insert domain receptor kinase). It has been demonstrated that the inhibition of VEGF signaling not only blocks angiogenesis in tumors but can also change or destroy tumor vessel [17]. Therefore, VEGFR-2 is an attractive target for biological cancer therapies.

We have used the methodology of fusion for the synthesis of 2-substituted amino thidadiazole2-(4-nitrophenyl) quinazoline 4(3H)-one**S2 Q3 (a-d)** at 250-260 ^{0}C, instead of using solvent, to avoid the problem of ring opening, which is commonly observed while synthesizing quinazolines from bentranil or benzooxazin-4-one. Fusion method is simple, more convenient, eco-friendly process and time saving method. It is a pace towards green chemistry [18].

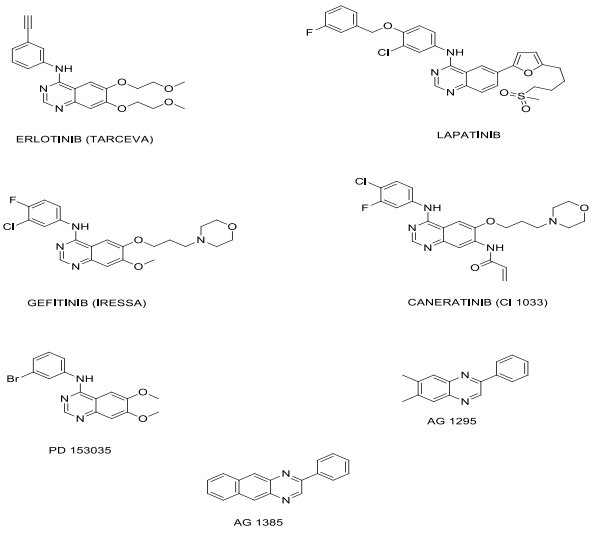


Chart 1. EGFR Tyrosine Kinase Inhibitors

2. Experimental

2.1. Synthesis

2.1.1. Materials and Methods

The chemicals employed in the synthetic work i.e. anthranilamide and p-nitrobenzoyl chloride were purchased from Sigma-Aldrich while all other chemicals i.e. anthranilic acid, pyridine, various anilines, aldehyde, DMF and $SOCl_2$ etc. were purchased from Spectrochem. All the solvents were used after distillation. Most of the solvents and chemicals used were of LR grade. The purity of the

compounds was confirmed by thin layer chromatography using precoated aluminum sheets and solvent system of Chloroform: Methanol (9:1). The spots were visualized using iodine chamber or UV cabinet. Infrared (IR) and ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds on SHIMADZU A400S8 (ν max in cm^{-1}) Spectrophotometer in KBr pellets and Bruker Model Advance (500 MHz, ^{1}H NMR) instrument, respectively. Chemical shifts are reported as δ parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. The physical data of synthesized compounds are given in Table 2.**(As shown in Table 2)**

2.1.2. N-(2-carbamoylphenyl)-4-nitrobenzamide (Q1)

Anthranilamide (0.01 mol,2.72gm) and P-Nitrobenzoyl chloride(0.01 mol,3.71gm) was added to a solution of triethylamine(2mL) and chloroform(81.6mL) and stirred at room temperature for 5 h. the resulting mixture was filtered to get Benzamide derivative. The solid crude product was recrystallized from absolute ethanol. Yield 77.15%, mp 206-210 ^{0}C, IR (KBr) cm^{-1}: 3435 (RCONHR'), 3334 (N-H), 1675 (C=O), 1653($CONH_2$), 1525 (Ar-NO_2).

2.1.3. 2-(4-nitrophenyl) quinazolin-4(3H)-one (Q2)

A mixture of Benzamide**3** (2.2gm7.0 mmol) in 10% aqueous KOH (50 mL) and EtOH (25 mL) was heated to reflux for 30 min. Ethanol was removed in vacuum, and the aqueous layer was extracted with ethyl acetate (50 mL*3). Yield 46.12%, mp 276-280 ^{0}C,IR (KBr) cm^{-1}: 3124 (str. CH), 1684 (C=O), 1566 (N=O), 1522 (Ar. NO_2).

2.1.4. 4-chloro-2-(4-nitrophenyl) quinazoline (Q3)

To a stirred solution of 2-(4-nitrophenyl) quinazolin-4(3H)-one **4** (1gm, 0.0042mole) and thionyl chloride (7.9 mL) was added dimethyl formamide (0.039mL). The reaction mixture was heated to reflux and stirred for about 5 hours. Excess of thionyl chloride is distilled off and the reaction mixture was quenched in ice with efficient stirring, solid precipitate was obtained which was filtered and washed with ice water. The obtain crude product was recrystallized using absolute ethanol. Yield 56.0%, mp 189-200 ^{0}C, IR (KBr) cm^{-1}: 3105 (Ar. CH), 1548 (N=O), 1512 (Ar. NO_2), 764(C-Cl).

2.1.5. N-(2,4-difluorophenyl)-2-(4-nitrophenyl) quinazolin-4-amine (Q4a)

A mixture of 4-chloro-2-(4-nitrophenyl) quinazoline (Q3), (0.1 mol) and 2,4-difluoroaniline (0.1 mol) in Isopropanol and 0.5mL con. HCl was heated to reflux for about 5h. The solid obtain was recrystallized using absolute ethanol. Yield 61.5%, mp 220-222 ^{0}C, IR (KBr) cm^{-1}: 3455 (N-H), 1681 (C=N), 1568 (Ar. NO_2), 1350 (C-F).

2.1.6. N-(3-chloro-4-fluorophenyl)-2-(4-nitrophenyl) quinazolin-4-amine (Q4b)

A mixture of 4-chloro-2-(4-nitrophenyl) quinazoline (Q3), (0.1 mol) and 3-Chloro-4-fluoroaniline (0.1 mol) in Isopropanol and 0.5mL con. HCl was heated to reflux for about 5h. The solid obtain was recrystallized using absolute ethanol. Yield 46.1 %, mp 210-215^{0}C, IR (KBr) cm^{-1}: 3414 (Str. N-H), 1629 (C=N), 1566 (Ar. NO_2), 1347 (C-F), 802 (C-Cl).

2.1.7. N-(2-fluorophenyl)-2-(4-nitrophenyl) quinazolin-4-amine (Q4c)

A mixture of 4-chloro-2-(4-nitrophenyl) quinazoline (Q3), (0.1mole) and 2-fluoroaniline (0.1mole) in Isopropanol and 0.5mL con. HCl was heated to reflux for about 5h. The solid obtain was recrystallized using absolute ethanol. Yield 58.3%, mp 230-232 ^{0}C, IR (KBr) cm^{-1}: 3359 (Str. N-H), 1681 (C=N), 1560 (Ar. NO_2), 1349 (C-F).

2.1.7. N-(2, 4-difluorophenyl)-2-(4-nitrophenyl) quinazolin-4-amine (Q5a)

A mixture of Q4a (1gm), $SnCl_2$ (4gm) and 10mL con. HCl was dissolved in absolute ethanol (50mL), and heated to reflux for about 4h. Then water was added to the reaction mixture and filtered off, the filtrate was basified with 10%NaOH. The obtain precipitate was filtered off and crystallized from absolute ethanol. Yield 77.7 %, mp 258-260 ^{0}C, IR (KBr) cm^{-1}: 3601 (RNH_2), 3346 (NH str.), 1609 (C=C str.), 1357 (Ar-F).

2.1.8. 2-(4-aminophenyl)-N-(3-chloro-4-fluorophenyl) quinazolin-4-amine (Q5b)

A mixture of Q4b (1gm), $SnCl_2$ (4gm) and 10mL con. HCl was dissolved in absolute ethanol (50mL), and heated to reflux for about 4h. Then water was added to the reaction mixture and filtered off, the filtrate was basified with 10%NaOH. The obtain precipitate was filtered off and crystallized from absolute ethanol. Yield 67.5 %, mp 199-201

^{0}C, IR (KBr) cm^{-1}: 3325 (RNH_2), 3204 (N-H), 1606 (C=N), 1499 (Ar. C-C), 1210 (C-F), 759 (C-Cl).

2.1.9. 2-(4-aminophenyl)-N-(2-fluorophenyl) quinazolin-4-amine (Q5c)

A mixture of Q4c (1gm), $SnCl_2$ (4gm) and 10mL con. HCl was dissolved in absolute ethanol (50mL), and heated to reflux for about 4h. Then water was added to the reaction mixture and filtered off, the filtrate was basified with 10%NaOH. The obtain precipitate was filtered off and recrystallized from absolute ethanol. Yield 65.5 %, mp 240-242 ^{0}C, IR (KBr) cm^{-1}: 3587 (R-NH_2), 3487 (N-H), 1545 (Ar. NO_2), 1334 (C-F).

2.1.10.2-(4-(benzylideneamino) phenyl)-N-(2,4- difluorophenyl) quinazoline 4-amine (SBa)

A mixture of Q5a(1gm) and Benzaldehyde (1mL) was dissolved in absolute ethanol, the reaction mixture was catalyzed with 2mL of glacial acetic acid, and refluxed for 5h.,absolute ethanol was concentrated and then the obtain solid was collected and recrystallized using absolute ethanol. Yield 58.3 %, mp 140-142 ^{0}C, IR (KBr) cm^{-1}: 3299 (N-H), 1630 (C=N), 1331 (C-F), ^{1}H NMR (δppm):8.53 1H (s, NH); 6.65-8.0516H (m,Ar); 8.38 1H (s, CH).

2.1.11. 2-(4-(benzylideneamino) phenyl)-N-(3-chloro-4 fluorophenyl) quinazolin-4-amine (SBb)

A mixture of Q5b(1gm) and Benzaldehyde(1mL) was dissolved in absolute ethanol, the reaction mixture was catalyzed with 2mL of glacial acetic acid, and refluxed for 5h.,absolute ethanol was concentrated and then the obtain solid was collected and recrystallized using absolute ethanol. Yield 64.5 %, mp 158-162 0C, IR (KBr) cm^{-1}: 3212 (NH str.), 1632 (N=C), 1265 (C-F), 760(C-Cl), ^{1}H NMR (δppm):8.53 1H (s, NH); 6.65-8.0516H (m,Ar); 8.38 1H (s, CH).

2.1.12. 2-(4-(benzylideneamino) phenyl)-N-(2-fluorophenyl) quinazolin-4-amine (SBc)

A mixture of Q5c(1gm) and Benzaldehyde(1mL) was dissolved in absolute ethanol, the reaction mixture was catalyzed with 2mL of glacial acetic acid, and refluxed for 5h.,absolute ethanol was concentrated and then the obtain solid was collected and recrystallized using absolute ethanol. Yield 58.3%, mp 210-212 ^{0}C, IR (KBr) cm^{-1}: 3346 (NH str.), 1626 (C=N), 1357 (C-F), 760(C-Cl), ^{1}H NMR(δppm):8.53 1H (s, NH), 9.10 1H (s, NH);6.65-8.0516H(m,Ar); 8.38 1H (s, CH).

2.1.13.2-(4-(2,4,6-trimethoxybenzylideneamino) phenyl)-N-(2,4-difluorophenyl) quinazolin-4-amine (SB'a)

A mixture of Q5a(0.1 mol) and 2,4,6-trimethoxy Benzaldehyde(0.1 mol) was dissolved in absolute ethanol, the reaction mixture was catalyzed with 2mL of glacial acetic acid, and refluxed for 5h.,absolute ethanol was concentrated and then the obtain solid was collected and recrystallized using absolute ethanol. Yield 56.4 %, mp 140-142 ^{0}C, IR (KBr) cm^{-1}: 3346 (NH str.), 1626 (C=N), 1357 (C-F), 760(C-Cl), ^{1}H NMR (δppm):3.04-3.449H (m, CH_3); 6.49-7.9313H (m, Ar); 8.76 1H (s, CH); 9.10 1H (s, NH).

2.1.14.2-(4-(2,4,6-trimethoxybenzylideneamino) phenyl)-N-(3-chloro-4-fluorophenyl) quinazolin -4-amine (SB'b)

A mixture of Q5b(0.1 mol) and 2,4,6-trimethoxy Benzaldehyde(0.1 mol) was dissolved in absolute ethanol, the reaction mixture was catalyzed with 2mL of glacial acetic acid, and refluxed for 5h.,absolute ethanol was concentrated and then the obtain solid was collected and recrystallized using absolute ethanol. Yield 47.2 %, mp 105-107 ^{0}C, IR (KBr) cm^{-1}: 3346 (NH str.), 1626 (C=N), 1357 (C-F), 760(C-Cl), ^{1}H NMR (δppm):3.04-3.449H (m, CH_3); 6.49-7.9313H(m, Ar); 8.76 1H (s, CH); 9.10 1H (s, NH).

2.1.15. 2-(4-(2,4,6-trimethoxybenzylideneamino) phenyl)-N-(2 fluorophenyl) quinazolin-4-amine (SB'c)

A mixture of Q5c(0.1 mol) and 2,4,6-trimethoxy Benzaldehyde(0.1 mol) was dissolved in absolute ethanol, the reaction mixture was catalyzed with 2mL of glacial acetic acid, and refluxed for 5h.,absolute ethanol was concentrated and then the obtain solid was collected and recrystallized using absolute ethanol. Yield 60.0 %, mp 160-162 ^{0}C, IR (KBr) cm^{-1}: 3346 (NH str.), 1626 (C=N), 1357 (C-F), 760(C-Cl), ^{1}H NMR(δppm):3.04-3.449H(m,CH_3);6.49-7.9313H(m,Ar);8.38 1H (s, CH);8.76 1H (s, CH); 9.10 1H (s, NH).

2.1.16. 2-(4-nitrobenzamido) benzoic acid (S2Q1)

Para-nitro benzoyl chloride II (0.01 mol) was added slowly to a stirred solution of Anthranilic acid I (0.01 mol) in pyridine (50 mL) and the reaction mixture was stirred at room temperature for 3h. The

reaction mixture was poured into cold 5% dil. HCl solution (100 mL). The solid obtained was filtered, washed several time with water, dried and crystallized from ethanol. Yield 65.1 %, mp 183-185 ^{0}C, IR (KBr) cm^{-1}: 3322 (Ar. NH), 1769 (C=O), 1599 (Ar. NO_2), 1533(RCONHR).

2.1.17. 2-(4-nitrophenyl)-4H-benzo[d] [1,3] oxazin- 4-one (S2Q2)

A mixture of S2Q1 (0.01 mol) and Acetic anhydride (0.1 mol) was heated under reflux for 4h.The solvent was removed under pressure. The residue was triturated with pet. Ether (40-60), the separated solid was collected by filtration and crystallized from ethanol. Yield 85.7 %, mp 192-195 ^{0}C,IR (KBr) cm^{-1}: 3110 (C=C), 1768 (C=O), 1698 (C=N), 1597 (Ar. NO_2), 1111 (RCOOR').

2.1.18.3-(5-(1-(5-methoxynaphthalen-2-yl) ethyl)-1,3,4-thiadiazol-2-yl)-2-(4nitrophenyl) quinazolin-4(3H)-one(S2Q3a)

Equimolar amount of S2Q2 and 5-(1-(5-methoxynaphthalen-2-yl) ethyl)-1,3,4-thiadiazol-2-amine was fused together at 250 °C in an oil bath for 6h. The mixture was cooled and crystallized from glacial acetic acid. Yield 36.8 %, mp 250-252 ^{0}C,IR (KBr) cm^{-1}: 2892 (CH str.), 1690 (C=O), 1522 (Ar. NO_2). ^{1}H NMR (δ ppm): 1.91 3H (s, CH_3); 3.82 3H (s, OCH_3); 4.06 1H (s, CH); 7.22-8.42 14H (m,Ar.).

2.1.19.3-(5-(benzo[d][1,3]dioxol-5-yl)-1,3,4-thiadiazol-2-yl)-2-(4-nitrophenyl)quinazolin-4(3H)-one (S2Q3b)

Equimolar amount of S2Q2 and 5-(benzo[d][1,3]dioxol-5-yl)-1,3,4-thiadiazol-2-amine was fused together at 250 °C in an oil bath for 6h. The mixture was cooled and crystallized from glacial acetic acid. Yield 41.1 %, mp 236-238 ^{0}C,IR (KBr) cm^{-1}: 2892 (CH str.), 1690 (C=O), 1522 (Ar. NO_2). ^{1}H NMR (δ ppm): 6.07 2H (s, CH_2); 7.30-7.79 11H (m,Ar.).

2.1.20.3-(5-(1-(4-isobutylphenyl) ethyl)-1,3,4-thiadiazol-2-yl)-2-(4-nitrophenyl)quinazolin-4(3H)-one (S2Q3c)

Equimolar amount of S2Q2 and 5-(1-(4-isobutylphenyl)ethyl)-1,3,4-thiadiazol-2-amine was fused together at 250 °C in an oil bath for 6h. The mixture was cooled and crystallized from glacial acetic acid. Yield 63 %, mp 208-210 ^{0}C,IR (KBr) cm^{-1}: 2892 (CH str.), 1690 (C=O), 1522 (Ar. NO_2). ^{1}H NMR (δ ppm): 1.05 3H (s, CH_3); 2.0-2.5 2H (s, CH_2); 4.35 1H (s, CH); 6.18-8.14 19H (m,Ar.).

2.1.21.3-(5-((5-methyl-7-oxo-7,8-dihydronaphthalen-2-yloxy)methyl)-1,3,4-thiadiazol-2-yl)-2-(4-nitrophenyl)quinazolin-4(3H)-one (S2Q3d)

Equimolar amount of S2Q2 and 7-((5-amino-1,3,4-thiadiazol-2-yl)methoxy)-4-methyl-2H-chromen-2-one was fused together at 250 °C in an oil bath for 6h. The mixture was cooled and crystallized from glacial acetic acid. Yield 53 %, mp 155-158 ^{0}C,IR (KBr) cm^{-1}: 2892 (CH str.), 1690 (C=O), 1522 (Ar. NO_2). ^{1}H NMR (δ ppm): 1.91 3H (s, CH_3); 4.31 2H (s, CH_2); 5.84 2H (s, CH_2); 6.13-8.3 12H (m,Ar).

Table 2: Physical Data of Synthesized Compounds

Comp code	Mol formula	Mol Wt gm/mole	Tlc solvent	Recrystallization solvent	M.P °c	% yeild	Rf value
SBa	$C_{27}H_{18}F_2N_4$	436.36	Chloroform:Methanol (9:1)	Ethanol	140-142	58.3	0.38
SBb	$C_{27}H_{18}ClFN_4$	452.91	Chloroform:Methanol (9:1)	Ethanol	158-162	64.5	0.48
SBc	$C_{27}H_{19}FN_4$	418.46	Chloroform:Methanol (9.5:0.5)	Ethanol	210-212	58.3	0.39
SB'a	$C_{30}H_{24}F_2N_4O_3$	525.53	Chloroform:Methanol (9.5:0.5)	Ethanol	140-142	56.4	0.57
SB'b	$C_{30}H_{24}ClFN_4O_3$	542.99	Chloroform:Methanol (9.5:0.5)	Ethanol	105-107	47.2	0.50
SB'c	$C_{30}H_{25}FN_4O_3$	508.54	Chloroform:Methanol (9.5:0.5)	Ethanol	160-162	60.0	0.53
S2Q3a	$C_{29}H_{21}N_5O_4S$	525.57	Chloroform:Methanol (9:1)	Glacial acetic acid	250-252	36.8	0.47
S2Q3b	$C_{23}H_{13}N_5O_5s$	471.44	Chloroform:Methanol (9.5:0.5)	Glacial acetic acid	236-238	41.1	0.40
S2Q3c	$C_{28}H_{25}N_5O_3s$	522.59	Chloroform:Methanol (9:1)	Glacial acetic acid	208-210	63.0	0.68
S2Q3d	$C_{28}H_{19}N_5O_5S$	537.55	Chloroform:Methanol (9:1)	Glacial acetic acid	155-158	53.0	0.64

2.2. Biological activity

2.2.1. In vitro anticancer activity

NCI-60 DTP HUMAN TUMOR CELL LINE SCREEN [20]

The screening is a two stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10μM. 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 further evaluated against the 60 cell panel at five concentration level.

Methodology of the in vitro cancer screen:

The human cancer cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum at 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100μL at plate densities ranging from 5,000 to 40,000 cells/well depending upon the doubling time of individual cell lines. After cell inoculation, the micro

titerplatesareincubatedat37°C, 5% CO2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell lines 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 50µg/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 100µl of these different drug dilutions are added to the appropriate microtiter wells already containing 100µl of medium, resulting in the required final drug concentration. Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO2, 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 50µl 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 (SRB) solution (100µl) 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 10mM 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 by the wells by gentle adding 50µl of 80% TCA (final concentration 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C) and the test growth in the presence of drug at five concentration levels (Ti), the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

[(Ti-Tz)/(C-Tz)] X 100 for concentrations for which Ti ≥Tz

[(Ti-Tz)/Tz] X 100 for concentrations for which Ti<Tz

Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50%(GI50) is calculated from [(Ti-Tz)/(C-Tz)] X 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 resulting in total growth inhibition (TGI) is calculated from Ti=Tz. The LC50 (concentration of the drug resulting in 50% reduction in the measured protein at the end of the drug treatment as compared from [(Ti-Tz)/Tz] X 100 =-50. Values are calculated for each of these 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.

The output from the one dose screen is reported as a mean graph and is available for analysis by the compare program as in figure below **(As shown in**

Fig. 2 & 3) Anticancer activity data of compound S2Q3b

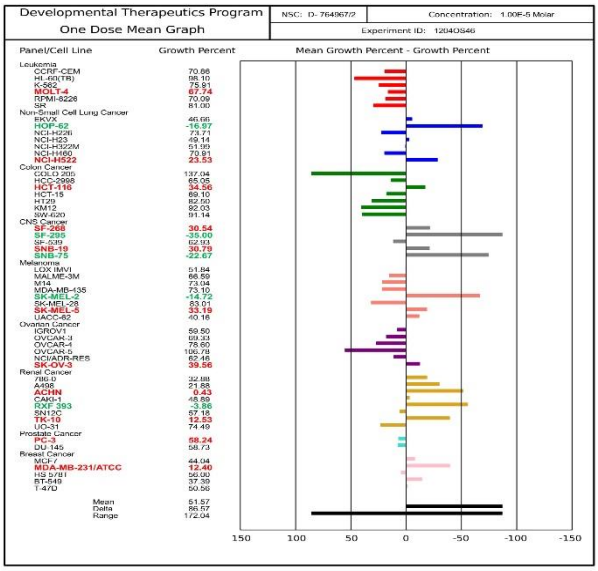


Figure 2: One dose mean graph of compound S2Q3b at 10^{-5} M concentration

Anticancer activity data of compoundS2Q3c

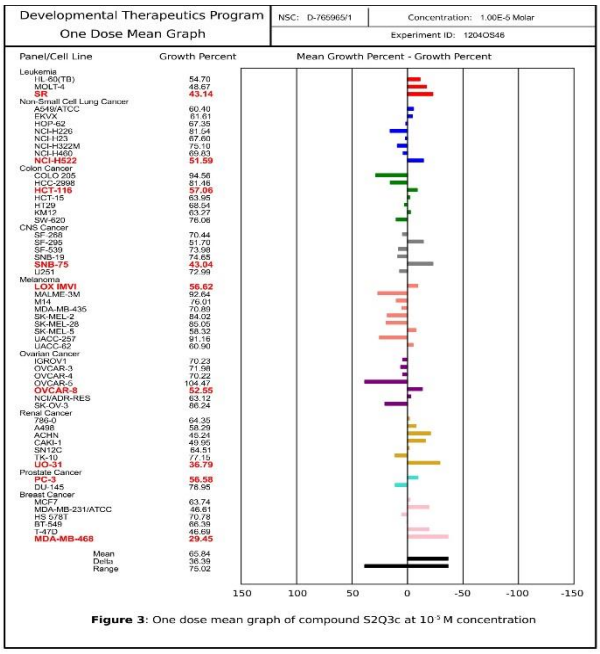


Figure 3: One dose mean graph of compound S2Q3c at 10^{-5} M concentration

3. Result and Discussion

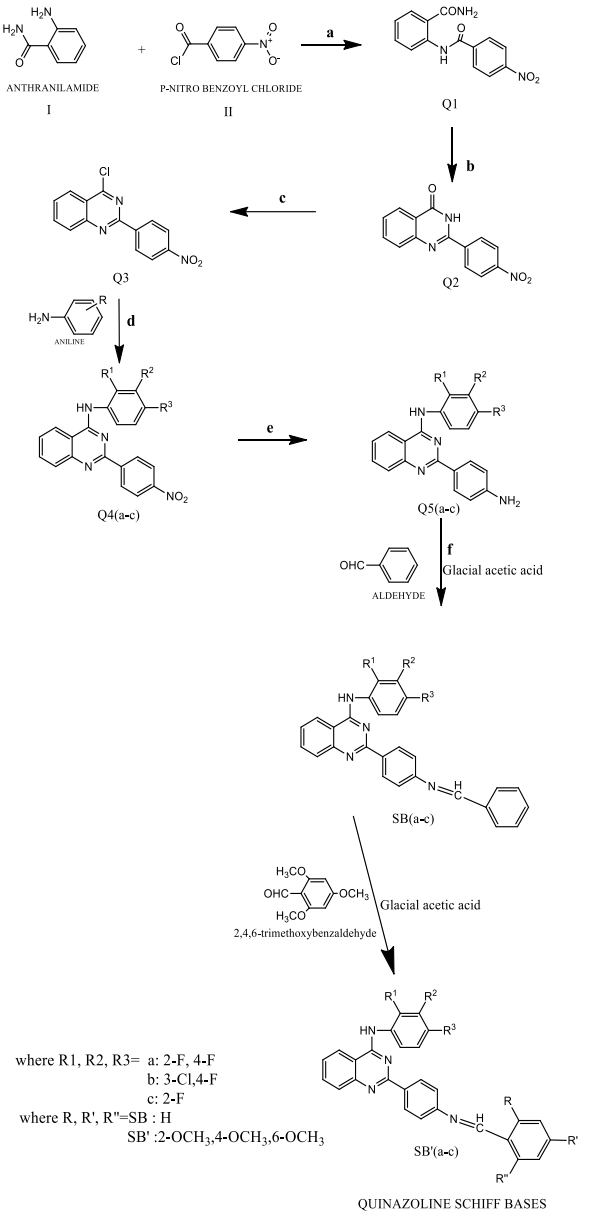
3.1. Chemistry

The reaction anthranilamide **I** and para-nitro benzoyl chloride **II** afforded the Benzamide analog **Q1**which was refluxed with aq. Potassium hydroxide and ethanol to obtain the key intermediate 2-(4-nitrophenyl) quinazolin-4(3H)-one **Q2**(scheme 1). The derivative **Q2**was further reacted with thionyl chloride to obtain 4- chloro-2-(4-nitrophenyl) quinazoline **Q3**. Replacement of chlorine with various substituted aromatic anilines afforded compounds **Q4 (a-c)**. Further reduction of compounds **Q4a-Q4c** using stannous chloride yielded compounds **Q5 (a-c)**. Compounds **Q5 (a-c)** were further treated with different aromatic aldehydes to yield a Schiff bases **SB (a-c) andSB' (a-c).(Scheme 1& Table 1)**

Condensation of anthranilic acid **I** with para-nitro benzoyl chloride **II** afforded 2-(4-nitrobenzamido) benzoic acid **S2Q1**,which was further cyclized in presence of acetic anhydride to 2-(4-nitrophenyl)-4H-benzo[d][1,3]oxazin-4-one **S2Q2**. The reaction of 2-(4-nitrophenyl)-4H- benzo[d][1,3]oxazin-4-one **S2Q2**with various substituted 2-amino thiadiazole **Th(a-d)** derivatives by fusion at high temperature afforded various 2-substituted amino thiadiazole 2-(4-nitrophenyl) quinazolin-4(3H)-one**S2Q3(a-d).(Scheme 2)**

Scheme I

1. Synthesis of Benzamide derivative.
2. Synthesis of Quinazoline-4-one derivative.
3. Synthesis of 4-chloro-2-(4-nitrophenyl) quinazoline.
4. Synthesis of substituted 2-(4-nitrophenyl) quinazolin-4-amine.
5. Synthesis of substituted 2-(4-aminophenyl) quinazolin-4-amine.
6. Synthesis of substituted 2-(4-(benzylideneamino) phenyl quinazolin-4-amine
7. Synthesis of Quinazoline Schiff bases



Reagents: (a) $CHCl_3$, TEA; **(b)** KOH, EtOH; **(c)** $SOCl_2$, DMF; **(d)** Isopropanol, Sub.Aniline; **(e)** $SnCl_2$, Con.HCl; **(f)** Glacial Acetic acid, Ar. Aldehyde

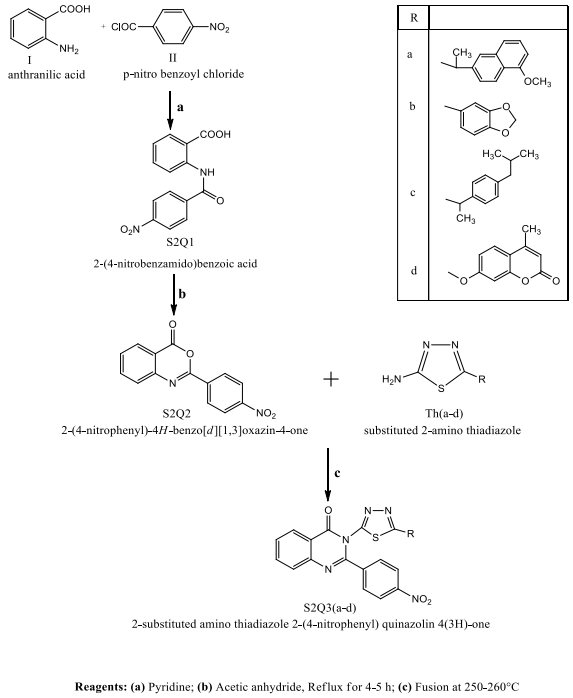
Scheme 1 Synthetic route for the synthesis of substituted Quinazoline derivatives

Table 1: Substituted Quinazoline derivatives

No.	R1	R2	R3	R	R'	R''
Q4a	F	-	F	-	-	-
Q4b	-	Cl	F	-	-	-
Q4c	F	-	-	-	-	-
Q5a	F	-	F	-	-	-
Q5b	-	Cl	F	-	-	-
Q5c	F	-	-	-	-	-
SBa	F	-	F	-	-	-
SBb	-	Cl	F	-	-	-
SBc	F	-	-	-	-	-
SB'a	F	-	F	OCH3	OCH3	OCH3
SB'b	-	Cl	F	OCH3	OCH3	OCH3
SB'c	F	-	-	OCH3	OCH3	OCH3

Scheme II

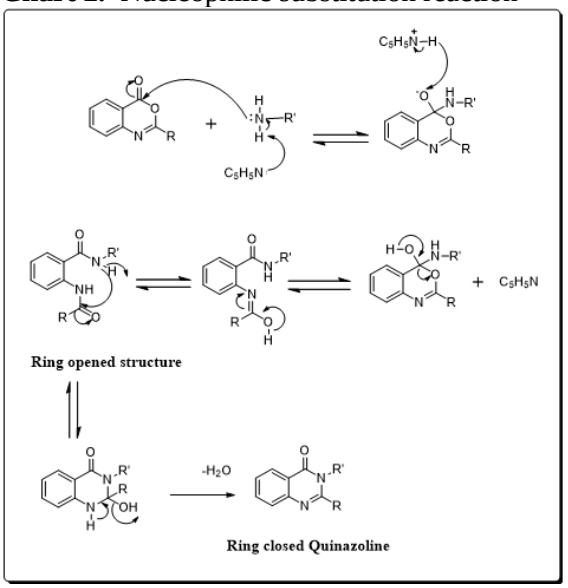
1. Synthesis of 2-(4-nitrobenzamido) benzoic acid.
2. Synthesis of benzooxazin-4-one derivative.
3. Synthesis of various 2-substituted amino thiadiazole 2-(4-nitrophenyl) quinazolin-4(3H)-one.



Scheme 2 Synthetic route for the synthesis of Substituted Quinazolinone

3.2. Reaction Mechanism

Chart 2. Nucleophilic substitution reaction



Electron rich primary amino group attacks on electron deficient carbonyl carbon in presence of pyridine, which leads to reversible ring opening and ring closing mechanism. After that when removal of water molecule takes place, a stable quinazoline ring was formed. **(As shown in chart 2).**

4. Discussion

In the present work, I have prepared 06 new 2,4 disubstituted quinazoline derivatives from reaction scheme 1 and 04 new 2,3 disubstituted Quinazolin-4-one derivatives from reaction scheme 2 successfully and evaluated them for their In-vitro anticancer activity at National Cancer Institute (NCI), USA, was screened on human tumor cell lines at the NIH, Bethesda, Maryland, USA. There are 06 new Schiff bases were prepared according to reaction scheme1.Substituted-1,3,4-thiadiazol-2-yl-2-(4-nitrophenyl)quinazolin-4(3H)-one were prepared according to reaction Scheme 2. The spectral data of SB(a-c) showed IR band atIR (KBr) cm^{-1}: 3346 (NH str.), 1626 (C=N), 1357 (C-F), 760(C-Cl), Further 1H NMR of compounds showed^{1}H NMR(δppm):3.04-3.449H(m,CH_3)6.65-8.0516H(m,Ar); 8.38 1H (s, CH); 8.53 1H (s, NH); 9.10 1H (s, NH) and SB'(a-c) showed IR band atIR (KBr) cm^{-1}: 2892 (CH str.), 1690 (C=O), 1522 (Ar. NO_2).Further 1H NMR of compounds showed^{1}H NMR (δ ppm): 1.91 3H (s, CH_3); 3.82 3H (s, OCH_3); 4.06 1H (s, CH); 7.22-8.42 14H (m,Ar.).

The spectral data of **S2Q3 (a-d)** showed IR band at 2892 (CH str.), 1690 (C=O), 1522 (Ar. NO_2). Further 1H NMR of compounds showed ^{1}H NMR (δ ppm): 1.91 3H (s, CH_3); 3.82 3H (s, OCH_3); 4.06 1H (s, CH); 7.22-8.42 14H (m,Ar.).

The tumor growth inhibition properties of compounds **S2Q3b** 3-(5-(benzo[d][1,3]dioxol-5-yl)-1,3,4- thiadiazol-2-yl)-2-(4-nitrophenyl) quinazolin-4(3H)-one with the NCI codes NSC D-764967/2 and **S2Q3c**3-(5-(1-(4-isobutylphenyl)ethyl)-1,3,4-thiadiazol-2-yl)-2-(4-nitrophenyl)quinazolin-4(3H)-one with the NCI codes NSC D-765965/2 selected among **S2Q3(a-d)** synthesized compounds **(Scheme 2)** 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. 2)**, compound **S2Q3b** showed remarkably lowest cell growth promotion against **CNS Cancer**, SF-295 cancer cell line of -35.00, SNB-75 cancer cell line of -22.67, **Non Small Cell Lung Cancer** (NSCLC), HOP-62 cancer cell line of -16.97, **Melanoma Cancer**, SK-MEL-2 cancer cell line of -14.72 and **Renal Cancer,** RXF-393 cancer cell line of -3.86 apart from this it also **exhibited broad spectrum**

cell growth inhibition against Leukemia cancer MOLT-4 cell line (cell growth promotion 67.74%, inhibition 32.26%), Non-Small Cell Lung Cancer NCI-H522 cell line (cell growth promotion 23.53%, inhibition 76.47%), Colon cancer HCT-116 cell line (cell growth promotion 34.56%,inhibition 65.44%), CNS cancer SF-268 cell line (cell growth promotion 30.54%, inhibition 69.46%), Melanoma cancer SK-MEL-5 cell line (cell growth promotion 33.19%, inhibition 66.81%), Ovarian Cancer SK-OV-3 cell line (cell growth promotion 39.56%, inhibition 60.44%), Renal cancer ACHN cell line (cell growth promotion 00.43%, inhibition 99.57%) also TK-10 cell line (cell growth promotion 12.53%, inhibition 87.47%), Prostate Cancer PC-3 cell line (cell growth promotion 58.24%, inhibition 41.76%) and Breast cancer MDA-MB-231/ATCC cell line (cell growth promotion 12.40%, inhibition 87.60%) at concentration of 10^{-5} M in one dose primary assay.**(As shown in fig. 2)**Compound **S2Q3c** showed that, it cannot show lowest cell growth promotion against any human cancer line apart from this it also **exhibited broad spectrum cell growth inhibition against** Leukemia cancer SR cell line (cell growth promotion 43.14%, inhibition 56.86%), Non-Small Cell Lung Cancer NCI-H522 cell line (cell growth promotion 51.59%, inhibition 48.41%), Colon cancer HCT-116 cell line (cell growth promotion 57.06%,inhibition 42.94%), CNS cancer SNB-75 cell line (cell growth promotion 43.04%, inhibition 56.96%), Melanoma cancer LOX IMVI cell line (cell growth promotion 56.62%, inhibition 43.38%), Ovarian Cancer OVCAR-8 cell line (cell growth promotion 52.55%, inhibition 47.45%), Renal cancer UO-31 cell line (cell growth promotion 36.79%, inhibition 63.21%), Prostate Cancer PC-3 cell line (cell growth promotion 56.58%, inhibition 43.32%) and Breast cancer MDA-MB-468 cell line (cell growth promotion 29.45%, inhibition 70.55%) at concentration of 10^{-5} M in one dose primary assay.**(As shown in fig. 3)**

5. Conclusion

Conclusively, a variety of Quinazoline derivatives have been successfully synthesized in appreciable yields and screened for their In-vitro anticancer activity. Different Schiff bases have been successfully prepared from reaction scheme 1 and various 2-substituted amino thiadiazole 2-(4-nitrophenyl) quinazolin-4(3H)-one derivative have been always synthesized in appropriate yields from reaction scheme 2. All the newly synthesized compounds were characterized on the basis of their physical and spectral data. The IR spectra, NMR spectra of the representative compounds were analyzed, studied and ascertained. Among the all synthesized compounds, 5 compounds were selected for anticancer testing at NCI, U.S.A; two compounds from reaction scheme 1 and 3 compounds from reaction scheme 2 were selected and submitted. Results are obtain for 2 compounds **S2Q3b** and **S2Q3c** which were screened for In-vitro anticancer activity against 60 cell lines at NCI,U.S.A., both compounds showed good anticancer activity.

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7. References

[1] Madhusudan, S., Trivedi, S. G. (**2004**). Tyrosine kinase inhibitors in cancer therapy. Clinical Biochemistry, 37, 618-635.

[2] Vardanyan RS, Hruby VJ. Synthesis of Essential Drugs, Elsevier publication, **2006**;1:389.

[3] Avendano C, Menendez JC. Medicinal Chemistry of Anticancer drugs, Elsevier publication **2008**;1:1.

[4] Jordan JD, Landau EM, Iyengar R. Signaling networks: the origins of cellular multitasking, Cell, **2000**;103(2):193.

[5] Blume JP, Hunter T. Oncogenic kinase signaling, Nature, **2001**;411(6835):355.

[6] Oblak M, Randic M, Solmajer T.Quantitative Structure-Activity Relationship of Flavonoid Analogues.3. Inhibition of p56lck Protein Tyrosine Kinase, J. Chem. Inf. Comput. Sci, **2000**; 40:994.

[7] Naumann T, Matter T. Structural Classification of Protein Kinases Using 3D Molecular Interaction Field Analysis of TheirLigand Binding Sites: Target Family Landscapes, J. Med. Chem., **2002**; 45:2366.

[8] Maen A, Santhi K, Ruyaz B, Philip A, Cheryl H. Angiogenesis: An update and potential drug approaches (Review), Inter. J. of Onco., **2010**; 36:5.

[9] Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis, Am. J. Pathol.,**1995**;146:1029.

[10] Ferrara N, Gerber HP, LeCouter J, The biology of VEGF and its receptors, Nat. Med., **2003**; 9: 669.

[11] Folkman J, Shing Y, Angiogenesis, J. Biol. Chem., **1992**; 267:10931.
[12] Klagsbrun MD, Amore PA. Regulators of angiogenesis, Annu. Rev. Physiol., **1991**; 53: 217.
[13] Folkman J. What is the evidence that tumors are angiogenesis dependent? J. Natl. Cancer Inst., **1990**; 82:4.
[14] Traxler P. Tyrosine kinases as targets in cancer therapy -successes and failures, Exp. Opin. Ther. Targ., **2003**; 7:215.
[15] Shawver LK, Lipson KE, Fong TA, McMahon G,Plowman GD, Strawn LM,Receptor tyrosine kinases as targets for inhibition of angiogenesis, Drug Discov. Today **1997**; 2:50.
[16] De Vries C, Escobedo JA, Ueno H, Houck KN, Williams LT, The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor, Science, **1992**;255:989.
[17] Shalaby F, Rossant J,Yamaguchi TP, Gertsenstein M, Wu XF, Breitman ML, Schuh AC, Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice, Nature,**1995**;367:62.
[18] Noolvi, M. N., Patel, H. M. (2013). Synthesis, method optimization, anticancer activity of 2, 3,7-trisubstituted Quinazoline derivatives and targeting EGFR-tyrosine kinase by rational approach. Arabian Journal of Chemistry, 6, 35–48
[19] Kerbel R, Folkman J, Clinical translation of angiogenesis inhibitors, Nat. Rev. Cancer,**2002**; 2:727.
[20] Kapetanovic IM, Computer aided drug discovery in silico-chemico biological approach Chem. Biol. Interact., **2008**; 171:165.