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Research Article

Design, synthesis and *in vivo* antitumor activity of novel 3, 4 di-substituted quinazoline derivatives

Biswajit Dash*, Suvakanta Dash, Damiki Laloo and Jashabir Chakraborty

Department of Pharmaceutical Science, Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Hathkhowapara, Guwahati-781017, Assam, India

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***Correspondence Info:**
Biswajit Dash,
Assistant Professor,
Department of Pharmaceutical Science,
Girijananda Chowdhury Institute of Pharmaceutical Science,
Azara, Hathkhowapara, Guwahati-781017, Assam, India

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Abstract

Objective: The present investigation is designed to synthesize some new isomeric series of quinazoline-4-one/4-thione derivatives, depending upon on the pharmacophoric model of *in-vivo* anticancer activity by modifying the structures retaining the fundamental structural features for the activity and screened for their antitumor properties.

Methods: A new series of 7-chloro-3-[substituted (amino/phenyl amino)]-2-phenyl quinazolin-4 (3H)-one/thione derivatives and 1-(7-chloro-4-oxo/-2-phenylquinazoline-3 (4H-yl)) substituted urea derivatives were synthesized. The reaction scheme proceeds through 7-chloro-2-phenyl-4H-benzo [d] [1, 3] oxazin-4-one which is the intermediate one. The structures of the newly synthesized compounds were characterised from infrared (IR), H^1 nuclear magnetic resonance (NMR) and mass spectra (m/z) and elemental analysis. The *in-vivo* antitumor activity was evaluated by body weight analysis, mean survival time and percentage increase in life span methods in Swiss albino mice bearing Ehrilich ascites carcinoma (EAC).

Result: The physico-chemical and spectroscopic data established the synthesis of quinazoline derivatives with a common pharmacophore. The synthesized compounds were evaluated for their antitumor properties. Among the newly quinazoline derivatives screened, six compounds (IIh, IIi, IIj, IIIh, IIIi, IIIj)) have shown significant antitumor activity.

Conclusion: The quinazoline derivatives obtained from the present study indicates that the amino group at 3rd position and urea/thiourea group in phenyl hydrazine ring at 3rd position of quinzoline skeleton are essential for antitumor activity. Compounds IIh, IIi, IIj, IIIh, IIIi and IIIj were found to be biologically active which may be useful as potential resource for the discovery of anti-tumor compound having common quinazoline pharmacophore with lesser toxic effects.

Keywords: 7-chloro-2-phenyl-4H-benzo[d][1,3]oxazin-4-one, quinazoline derivatives, in-vivo anti- tumour activity.

1. Introduction

Cancer continues to pose an unprecedented public health problem and current treatment options have not been satisfactory. Several studies have demonstrated the inhibitory properties of varieties of synthetic drugs having anticancer potentiality [1]. Even though advancement in management of chemotherapeutic agents in some patients is made, but the obligation to the difficult task of discovering novel anticancer agent remains critically vital [2]. In the way of identifying numerous chemical substances which may be act as lead for the developments of anti-tumor agents, we are concerned in the present research work with quinazoline derivatives, which have been identified as a new class of anticancer agents with significant potency against solid tumours [3,4].

Quinazoline nucleus is a versatile lead molecule for the design of new bioactive compounds because of its broad spectrum of pharmacological activity [5]. Quinazoline and its derivatives are considered to be highly essential chemical moiety of various pharmacological activities. Quinazoline pharmacophore possess various

degree of biological activities including anti-hypertensive [6], anti-microbial [7], anti-oxidant [8], anti-inflammatory [9], anti-convulsant[10, 11] and anti-cancer activities[12, 13]. Predominantly quinazoline derivative compounds were effectively explored in the field of tyrosine kinase inhibitors for the discovery and development of new anticancer drugs. The best example was reported with erlotinib and geftinib as potential inhibitors of epidermal growth factor receptor (EGFR) [14]. Some quinazoline anticancer drugs show selective qualities which are becoming research focus of molecular targeted anticancer drug development [15].

2. Material and methods

2.1 General

The synthesis of the target compounds were accomplished as illustrated in the figure 2. The compounds were synthesized according to the procedure given in the respective literature[1,16,17]. All the reagents and solvents used in the study were of analytical grade purity and purchased from Sigma Aldrich Pvt. Ltd. (India). The progress of the reaction was monitored by thin layered chromatography with hexane: ethyl acetate (3:2) as the mobile phase and performed on silica gel 60 F_{254} aluminium sheets (Merck Ltd., Germany); the products were purified by recrystallization. Melting points were determined in open capillaries using Stuart SMP10 (Barloworld scientific Ltd., UK), electrothermal melting point apparatus. IR spectra were recorded on Shimadzu 8400S FTIR (Schimadzu Corporation, Japan) spectrophotometer using was recorded in cm^{-1}. H^1 NMR (400.13MHz) spectra were acquired on a Bruker Advance II-400 NMR spectrophotometer using tetra methyl silane (TMS) as the internal standard and the chemical shifts were recorded in δ. The mass spectrum was obtained on Water ZQ-4000 mass spectrophotometer. Elemental analysis for C, H and N were performed on Perkin Elmer 2400 Series-II CHN analyzer.

2.2 Chemical synthesis

2.2.1 General procedure

Synthesis of 7-chloro-2-phenyl-4H-benzo[d] [1, 3] oxazin-4-one (I): (Intermediate)

4-chloroanthranilic acid (0.01 mol) was dissolved in dry pyridine (30 ml) by stirring slowly at room temperature. The solution was cooled to 0°C and a solution of benzoyl chloride (0. 02 mole) in dry pyridine (30 ml) was added slowly with constant stirring. After that the reaction mixture was further stirred for half an hour at room temperature and kept at one side for 1 hour. The pasty mass obtained was diluted with water (50 ml) and treated with aqueous sodium bicarbonate solution. When the effervescence stopped, the precipitate obtained was filtered off and washed with water, dried and recrystallized from diluted ethanol [16].

General Procedure for the synthesis of compounds, IIa-IIj:

7-chloro-2-phenyl-4H-benzo[d][1,3]oxazin-4-one (0.01 mol) and substituted phenyl hydrazine derivatives /hydrazine hydrate/semicarbazide/thiosemicarbazide (0.01 mol) were refluxed for 3 h in presence of glacial acetic acid. The reaction mixture was allowed to cool at room temperature. The crude product was recrystallized using absolute alcohol to get the target compounds IIa-IIj[17].

***General Procedure for the synthesis of compounds, IIIa-IIIj*:**

A mixture of 7-chloro- (3-amino/substituted phenyl amino)-2-phenyl quinazoline-4 (3H)–one/1- (7-chloro-4-oxo-2-phenylquinazolin-3 (4H)-yl-urea/thiourea (10 mmol, 2.70 kg) and phosphorus penta sulphide (1 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 dimethyl sulphoxide (10 ml) and filtered. The clear filtrate was poured into ice water, dried and recrystallised from ethanol to get the target compounds IIIa-IIIj[1].

***Compound I* (7-chloro-2-phenyl-4H-benzo[d] [1, 3] oxazin-4-one):**

White powder (methanol); 4-chloroanthranilic acid (0.01 mol) was dissolved in dry pyridine (30 ml) by stirring slowly at room temperature. The solution was cooled to 0°C and a solution of benzoyl chloride (0.02 mol) in dry pyridine (30 ml) was added slowly with constant stirring. After that the reaction mixture was further stirred for half an hour at room temperature and kept at one side for 1 h. The pasty mass obtained was diluted with water (50 ml) and treated with aqueous sodium bicarbonate solution. When the effervescence stopped, the precipitate obtained was filtered off and washed with water, dried and recrystallized from diluted ethanol as white solid. (Yield: 61.2%); m.p 156-158°C; IR (cm^{-1}) ν_{max}: Ar-CHstretch (3072 cm^{-1}), C=O (1751.33 cm^{-1}), C=N (1592 cm^{-1}), cyclic C-O-Cstretch (1060.47cm^{-1}), C-Cl (680.72cm^{-1}); ^{1}H NMR (DMSO-d6, 400.13MHz): δ7. 31-7.69 (m, 5H, Ar-H,), 7.52-8.2 (t, 5H, Ar-H); MS m/z: 262.12 (M+), C_{14} H_8ClNO_2 (Calcd. 257.67); Anal calcd. (%) C, 65.26; H, 3.13; N, 5.44; Found: C, 65.67; H, 3.52; N, 5.84.

***Compound II_a* (7-chloro-2-phenyl-3-(phenylamino) quinazolin-4(3H)-one):**

Reddish brown crystalline solid (methanol); 7-chloro-2-phenyl-4H-benzo[d] [1, 3] oxazin-4-one (0.01 mol) and phenyl hydrazine derivatives hydrate (0.01 mol) were refluxed for 3 h in presence of glacial acetic acid. The reaction mixture was allowed to cool at room temperature. The crude product was recrystallized using absolute alcohol as reddish brown solid. (Yield:87.2%); m.p. 135-145^{0}C; IR

(cm^{-1}) ν_{max} Ar-$CH_{stretch}$ (3262 cm^{-1}), C=O (1664.49 cm^{-1}), C=N (1595 cm^{-1}), N-$NH_{stretch}$ (3343.75 cm^{-1}), C-Cl (689.04cm^{-1}), ^{1}H NMR (DMSO-d_6, 400.13MHz), δ 7.491-7.601 (m, 5H, Ar-H,), 7.781-8.115 (m, 5H, Ar-H), 7.907-8.723 (t, 3H, Ar-H), 3.466 (s, 1H, N-H); MS m/z: 365.11 (M+); $C_{20}H_{14}ClN_3O$ (Calcd. 347.8); Anal calcd. (%) C, 69.07; H, 4.06; N, 12.08; Found: C, 69.48;H, 4.48;N, 12.48.

***Compound II_b* (7-chloro-2-phenyl-3- (o-chloro-phenylamino) quinazolin-4 (3H)-one):**

White crystalline solid (methanol); 7-chloro-2-phenyl-4H-benzo[d] [1, 3] oxazin-4-one (0. 01 mol) and 2-chloro phenyl hydrazine (0.01 mol) were refluxed for 3 h in presence of glacial acetic acid. The reaction mixture was allowed to cool at room temperature. The crude product was recrystallized using absolute alcohol as white crystalline solid. (Yield: 57. 14%); m.p 168-170^{0}C; IR (cm^{-1}) ν_{max} Ar-$CH_{stretch}$ (3323.43 cm^{-1}), C=O (1665.77 cm^{-1}), C=N (1592 cm^{-1}), N-$NH_{stretch}$ (3307.746cm^{-1}), C-Cl (676.27cm^{-1}), ^{1}H NMR (DMSO-d_6, 400. 13MHz), δ 6.753-7.181 (m, 4H, Ar-H,), 7.305-7.72 (t, 5H, Ar-H)**,** 7.52-7.907 (t, 3H, Ar-H), 4.56 (s, 1H, N-H), MS, m/z:382.02 (M+); $C_{20}H_{13}C_{l2}N_3O$ (Calcd. 382.24); Anal calcd. (%)C, 62.84;H, 3.43;N, 10.99; Found: C, 63.24;H, 3.85;N, 11.35.

***Compound II_c* (7-chloro-2-phenyl-3- (o-methyl-phenylamino) quinazolin-4 (3H)-one):**

Light brown crystalline solid (methanol); 7-chloro-2-phenyl-4H-benzo[d] [1, 3] oxazin-4-one (0. 01 mol) and 2-methyl phenyl hydrazine (0.01 mol) were refluxed for 3 h in presence of glacial acetic acid. The reaction mixture was allowed to cool at room temperature. The crude product was recrystallized using absolute alcohol as Light brown crystalline solid. (Yield: 67%); m.p 163-166^{0}C; IR (cm^{-1}) ν_{max} Ar-$CH_{stretch}$ (3242.17cm^{-1}), C=O (1677.66 cm^{-1}), C=N (1654 cm^{-1}), N-$NH_{stretch}$ (3309.67 cm^{-1}), C-CH_3 (2911.79cm^{-1}), ^{1}H NMR (DMSO-d6, 400.13MHz), δ 6.85-7.02 (m, 4H, Ar-H), 7.282-7.621 (m, 5H, Ar-H), 7.653-7.91 (t, 3H, Ar-H), 3.818 (s, 1H, N-H), 2.51 (s, 1H, Ar-CH3), MS, m/z: 361.23 (M+); $C_{20}H_{13}Cl_2N_3O$ (Calcd. 361.82); Anal calcd. (%) C, 69.71; H, 4.46; N, 11.61; Found: C, 70.12; H, 4.87; N, 12.04.

***Compound II_d* (7-chloro-2-phenyl-3- (p-chloro-phenylamino) quinazolin-4(3H)-one):**

Brown crystalline solid (methanol); 7-chloro-2-phenyl-4H-benzo[d] [1, 3] oxazin-4-one (0.01 mol) and p-chloro phenyl hydrazine (0.01 mol) were refluxed for 3 h in presence of glacial acetic acid. The reaction mixture was allowed to cool at room temperature. The crude product was recrystallized using absolute alcohol as brown crystalline solid. (Yield: 91.67%); m. p 171-174^{0}C; IR (cm^{-1}); ν_{max} Ar-$CH_{stretch}$ (3010.02 cm^{-1}), C=O (1665.38 cm^{-1}), C=N (1594.74 cm^{-1}), N-$NH_{stretch}$ (3240.41 cm^{-1}), C-Cl (698cm^{-1}), 1H NMR (DMSO-d6, 400.13MHz), δ 6. 743-7.23 (m, 4H, Ar-H,), 7. 352-7.68 (t, 4H, Ar-H)**,** 7.54-7.907 (t, 3H, Ar-H), 4.43 (s, 1H, N-H), MS, m/z:382. 07 (M+); $C_{20}H_{13}Cl_2N_3O$ (Calcd. 382.24); Anal calcd. (%) C, 62.84; H, 3.42; N, 10.99; Found: C, 63.14; H, 3.82; N, 11.12.

***Compound II_e* (7-chloro-2-phenyl-3- (p-bromo-phenylamino) quinazolin-4(3H)-one):**

Brown crystalline solid (methanol); 7-chloro-2-phenyl-4H-benzo[d] [1, 3] oxazin-4-one (0.01 mol) and p-bromo phenyl hydrazine (0.01 mol) were refluxed for 3 h in presence of glacial acetic acid. The reaction mixture was allowed to cool at room temperature. The crude product was recrystallized using absolute alcohol as brown crystalline solid. (Yield: 91.67%); m.p 172-175^{0}C; λmax (nm) 274; IR (cm^{-1}); νmax Ar-$CH_{stretch}$ (3271.23 cm^{-1}), C=O (1691.23 cm^{-1}), C=N (1645.30 cm^{-1}), N-$NH_{stretch}$ (3332.01 cm^{-1}), C-Br (693.97cm^{-1}), ^{1}H NMR (DMSO-d6, 400.13MHz), δ 6. 52-7.24 (m, 5H, Ar-H), 7. 43-7.62 (m, 4H, Ar-H)**,** 7.42-7.85 (m, 3H, Ar-H), 4.42 (s, 1H, N-H), MS, m/z: 412.85 (M+); $C_{20}H_{13}$ Br ClN_3O (Calcd. 426.69); Anal calcd. (%) C, 56.3; H, 3.07; N, 9.85; Found: C, 56.72; H, 3.48; N, 10.25.

***Compound II_f* (7-chloro-2-phenyl-3- (p-nitro-phenylamino) quinazolin-4(3H)-one):**

Reddish brown solid (methanol); 7-chloro-2-phenyl-4H-benzo[d] [1, 3] oxazin-4-one (0.01 mol) and p-nitro phenyl hydrazine (0.01 mol) were refluxed for 3 h in presence of glacial acetic acid. The reaction mixture was allowed to cool at room temperature. The crude product was recrystallized using absolute alcohol as brown crystalline solid. Yield: 80%); m.p 171-173°C; IR (cm^{-1}); ν_{max} Ar-$CH_{stretch}$ (3250 cm^{-1}), C=O (1650.49 cm^{-1}), C=N (1592 cm^{-1}), N-$NH_{stretch}$ (3341.75 cm^{-1}), C-Cl (682.04cm^{-1}), ^{1}H NMR (DMSO-d_6, 400.13MHz), δ 6. 94-7.31 (t, 5H, Ar-H), 6.96-8.12 (m, 5H, Ar-H), 7.5-7.81 (t, 3H, Ar-H), 3.9 (s, 1H, N-H), MS, m/z: 378.15 (M+); $C_{20}H_{13}ClN_4O_3$ (Calcd. 392.8); Anal calcd. (%) C, 61.16; H, 3.34; N, 14.26; Found: C, 62.12; H, 4.15; N, 14.67.

***Compound II_g* (7-chloro-2-phenyl-3- (p-methoxy-phenylamino) quinazolin-4(3H)-one):**

Brownish yellow solid (methanol); 7-chloro-2-phenyl-4H-benzo[d] [1, 3] oxazin-4-one (0. 01mol) and p-methoxy phenyl hydrazine (0. 01 mol) were refluxed for 3 h in presence of glacial acetic acid. The reaction mixture was allowed to cool at room temperature. The crude product was recrystallized using absolute alcohol as brown yellow solid. Yield: 92%); m. p 170-173°C; IR (cm^{-1}) ν_{max} Ar-$CH_{stretch}$ (3114.98 cm^{-1}), C=O (1752.81 cm^{-1}), C=N (1664.02 cm^{-1}), N-$NH_{stretch}$ (3310.40 cm^{-1}), OCH_3-$CH_{stretch}$ (3008.64cm^{-1}), Ar-$CH_{stretch}$ (3271.23 cm^{-1}), 1H NMR (DMSO-d6, 400.13MHz), δ 6.5-6.7 (m, 4H, Ar-H), 7.28-7.62 (m, 5H, Ar-H), 7.46-7.921 (t, 3H, Ar-H), 4.1 (s, 1H, N-H), 3.78 (s, 1H, Ar-OCH_3), MS, m/z: 345.12 (M+); $C_{21}H_{16}ClN_3O$ (Calcd. 361.82); Anal Calcd. (%) C, 66.76; H, 4.27; N, 11.12; Found: C, 67.16; H, 4.67; N, 11.54.

***Compound II_h* (3-amino-7-chloro-2-phenyquinazolin-4-(3H)-one):**

Brown crystalline solid (methanol); 7-chloro-2-phenyl-4H-benzo[d][1,3]oxazin-4-one (0.01mol) and hydrazine hydrazine hydrate (0.01 mol) were refluxed for 3 h in presence of glacial acetic acid. The reaction mixture was allowed to cool at room temperature. The crude product was recrystallized using absolute alcohol as brown yellow solid. (Yield: 92%); m. p 170-173°C; λmax (nm) 278; IR (cm^{-1}); νmax Ar-$CH_{stretch}$ (3126.37 cm^{-1}), C=O (1598 cm^{-1}), C=N (1552.02 cm^{-1}), N-$NH_{stretch}$ (3283.49cm^{-1}), C-Cl (696 cm^{-1}), 1H NMR (DMSO-d6, 400.13MHz), δ 7.21-7.56 (m, 5H, Ar-H), 7.42-7.87 (m, 3H, Ar-H), 2.4 (s, 1H, N-H), MS, m/z: 272.12 (M+); C_{14} $H_{10}ClN_3O$ (Calcd. 271.7); Anal calcd. (%) C, 61.89; H, 3.71; N, 15.47; Found: C, 62.29; H, 4.12; N, 15.87.

***Compound II_i* (1- (7-chloro-4-oxo-2-phenylquinazoline-3(4H-yl)) urea):**

White crystalline solid (methanol); 7-chloro-2-phenyl-4H-benzo[d] [1, 3] oxazin-4-one (0. 01 mol) and semicarbazide (0. 01 mol) were refluxed for 3 h in presence of glacial acetic acid. The reaction mixture was allowed to cool at room temperature. The crude product was recrystallized using absolute alcohol as white crystalline solid. (Yield: 60%); m.p 165-167°C; IR (cm^{-1}) ν_{max} Ar-$CH_{stretch}$ (3199.89 cm^{-1}), C=O (1671.85 cm^{-1}), C=N (1593.8 cm^{-1}), N-$NH_{stretch}$ (3027.51 cm^{-1}), C-Cl (692.51cm^{-1}), ^{1}H NMR (DMSO-d6, 400.13MHz), δ 7.25-7.58 (m, 5H, Ar-H), 7.37-7.86 (m, 3H, Ar-H), 5.8 (s, 1H, N-H), MS, m/z: 312.45 (M^+); C_{15} $H_{11}ClN_4O_2$ (Calcd. 314.72); Anal calcd. (%) C, 57.24; H, 3.52; N, 17.8; Found: C, 57.46; H, 3.94 ; N, 18.21.

***Compound II_j* (1- (7-chloro-4-oxo-2-phenylquinazoline-3(4H-yl)) thiourea):**

White amorphous solid (methanol); 7-chloro-2-phenyl-4H-benzo[d] [1, 3] oxazin-4-one (0.01 mol) and thiosemicarbazide (0.01 mol) were refluxed for 3 h in presence of glacial acetic acid. The reaction mixture was allowed to cool at room temperature. The crude product was recrystallized using absolute alcohol as white crystalline solid. (Yield: 70%); m. p 162-165°C; IR (cm-1); ν_{max} Ar-$CH_{stretch}$ (3126.71 cm^{-1}), C=O (1644.7 cm^{-1}), C=N (1552.32 cm^{-1}), N-$NH_{stretch}$ (3432.52 cm^{-1}), C-Cl (755.72cm^{-1}) ^{1}H NMR (DMSO-d6, 400.13MHz), δ 7.24-7.5 (m, 5H, Ar-H), 7.32-7.78 (m, 3H, Ar-H), 2.3 (s, 1H, N-H), MS, m/z: 332. 15 (M+); $C_{15}H_{11}ClN_4OS$ (Calcd. 330.79); Anal calcd. (%) C, 54.46; H, 3.35; N, 16.94; Found: C, 54.86; H, 3.35; N, 16.94.

***Compound III_a* (7-chloro-2-phenyl-3- (phenylamino)-quinazolin-4(3H)-thione):**

Light brown crystalline solid (methanol) A mixture of 7-chloro-2-phenyl-3- (phenylamino) quinazolin-4 (3H)-one (10 mmol, 2.70 g) and phosphorus penta sulphide (1 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 dimethyl sulphoxide (10 ml) and filtered. The clear filtrate was poured into ice water, dried and recrystallized from ethanol to form light brown crystalline solid. (Yield: 68%); m.p. 165-168°C; IR (cm-1) ν_{max} Ar-$CH_{stretch}$ (3025 cm^{-1}), C-N (1150.39 cm^{-1}), C=N (1666.18 cm^{-1}), C=S (1262.41 cm^{-1}), C-Cl (735.15 cm^{-1}), N-NH (bend) (3057.18 cm^{-1}), ^{1}H NMR (DMSO-d6, 400.13MHz), δ 6.5-7.4 (m, 5H, Ar-H), 6.42-7.71 (m, 5H, Ar-H), 7.15-7.5 (m, 3H, Ar-H), 3.8 (s, 1H, N-H), MS, m/z: 362.15 (M^+); $C_{20}H_{14}ClN_3S$ (Calcd. 363.86); Anal calcd. (%) C, 66.02; H, 3.88; N, 11.55; Found: C, 66.42; H, 4.28; N, 11.96.

***Compound III_b* (7-chloro-2-phenyl-3- (o-chlorophenylamino)-quinazolin-4(3H)-thione):**

Brown crystalline solid (methanol); A mixture of 7-chloro-2-phenyl-3-(o-chloro-phenylamino) quinazolin-4 (3H)-one (10 mmol, 2.70 g) and phosphorus penta sulphide (1 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 dimethyl sulphoxide (10 ml) and filtered. The clear filtrate was poured into ice water, dried and recrystallized from ethanol to form brown crystalline solid. (Yield: 67%); m.p. 140-142°C; IR (cm^{-1}); ν_{max} Ar-$CH_{stretch}$ (3010.02 cm^{-1}), C=S (1665.38 cm^{-1}), C=N (1594.74 cm^{-1}), N-$NH_{stretch}$ (3240.41 cm^{-1}), C-Cl (698cm^{-1}), ^{1}H NMR (DMSO-d6, 400.13MHz), δ 6.62-7.35 (m, 5H, Ar-H), 6.45-7.68 (m, 5H, Ar-H), 7.2-7.48 (m, 3H, Ar-H), 4.1 (s, 1H, N-H), MS, m/z: 396.14 (M+); $C_{14}H_{10}ClN_3S$ (Calcd. 398.31); Anal calcd. (%) C, 60.31; H, 3.29; N, 10.55; Found: C, 60.71; H, 3.59; N, 10.97.

***Compound III_c* (7-chloro-2-phenyl-3- (o-methylphenylamino)-quinazolin-4(3H)-thione):**

Brownish yellow crystalline solid (methanol); A mixture of 7-chloro-2-phenyl-3- (o-methyl-phenylamino) quinazolin-4 (3H)-one (10 mmol, 2.70 g) and phosphorus penta sulphide (1 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 dimethyl sulphoxide (10 ml) and filtered. The clear filtrate was poured into ice water, dried and recrystallised from ethanol to form brownish yellow crystalline solid. (Yield: 71%); m. p 172-175°C; IR (cm^{-1}); ν_{max} Ar-$CH_{stretch}$ (3114.98 cm^{-1}), C=S (1264.56 cm^{-1}), C=N (1664.02 cm^{-1}), C-N (1099.72 cm^{-1}) N-$NH_{stretch}$ (3310.40 cm^{-1}), CH_3-$CH_{stretch}$ (3008.64cm^{-1}), ^{1}H NMR (DMSO-d6, 400.13MHz), δ 6.5-7.57 (m, 5H, Ar-H), 6.67-7.58 (m, 5H, Ar-H), 7.31-7.35 (t, 3H, Ar-H), 3.9 (s, 1H, N-H), 2.34 (s, 1H, Ar-CH3), MS, m/z: 378.19 (M+) $C_{20}H_{13}Cl_2N_3S$ (Calcd.

377.89); Anal calcd. (%) C, 66.75; H, 4.27; N, 10.55; Found: C, 60.71; H, 3.47; N, 11.44.

***Compound III_d* (7-chloro-2-phenyl-3- (p-chlorophenylamino)-quinazolin-4 (3H)-thione):**

Dark brown crystalline solid (methanol); A mixture of 7-chloro-2-phenyl-3-(p-chloro-phenylamino) quinazolin-4 (3H)-one (10 mmol, 2.70 g) and phosphorus penta sulphide (1 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 dimethyl sulphoxide (10 ml) and filtered. The clear filtrate was poured into ice water, dried and recrystallized from ethanol to form dark brown crystalline solid. (Yield: 72%); m. p 162-163°C; IR (cm^{-1}) ν_{max} Ar-$CH_{stretch}$ (3029 cm^{-1}), C-N (1171.25 cm^{-1}), C=N (1676.63 cm^{-1}), C=S (1263.99 cm^{-1}), C-Cl (761.37 cm^{-1}), N-NH (bend) (3196.89 cm^{-1}), ^{1}H NMR (DMSO-d6, 400.13MHz), δ 6.58-7.24 (m, 5H, Ar-H), 6. 7-7.34 (m, 5H, Ar-H), 7.25-7.42 (m, 3H, Ar-H), 4.2 (s, 1H, N-H), MS, m/z: 398.05 (M+); $C_{20}H_{13}Cl_2N_3S$ (Calcd. 398.31); Anal calcd. (%) C, 60.31; H, 3.29; N, 11.12; Found: C, 67.12; H, 4.67; N, 11.02.

***Compound III_e* (3- (4-bromophenylamino)-7-chloro-2-phenyl-quinazolin-4(3H)-thione):**

Brownish yellow crystalline solid (methanol); A mixture of 7-chloro-2-phenyl-3-(p-bromo-phenylamino) quinazolin-4 (3H)-one (10 mmol, 2.70 g) and phosphorus penta sulphide (1 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 dimethyl sulphoxide (10 ml) and filtered. The clear filtrate was poured into ice water, dried and recrystallized from ethanol to form brownish yellow crystalline solid. (Yield: 69%); m.p. 170-173°C; IR (cm^{-1}); ν_{max} Ar-$CH_{stretch}$ (2918.13 cm^{-1}), C=S (1233.04 cm^{-1}), C=N (1665.31 cm^{-1}), N-$NH_{stretch}$ (3060.79 cm^{-1}), C-Br (683.52cm^{-1}), ^{1}H NMR (DMSO-d6, 400.13MHz), δ 6.53-7.58 (m, 5H, Ar-H,), 7.43-7.62 (m, 4H, Ar-H), 7.24-7.41 (m, 3H, Ar-H), 4.2 (s, 1H, N-H), MS, m/z:445.82 (M+); $C_{20}H_{13}BrClN_3S$ (Calcd. 442.76); Anal calcd. (%) C, 54.25; H, 2.96; N, 9.49; Found: C, 54.65; H, 3.37; N, 9.59.

***Compound III_f* (7-chloro-2-phenyl-3- (p-nitrophenylamino)-quinazolin-4(3H)-thione):**

Reddish brown crystalline solid (methanol); A mixture of 7-chloro-2-phenyl-3- (p-nitro-phenylamino) quinazolin-4 (3H)-one (10 mmol, 2.70 g) and phosphorus penta sulphide (1 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 dimethyl sulphoxide (10 ml) and filtered. The clear filtrate was poured into ice water, dried and recrystallized from ethanol to form reddish brown crystalline solid. (Yield: 70%); m. p 171-174°C; IR (cm^{-1}) ν_{max} Ar-$CH_{stretch}$ (3255 cm^{-1}), C=O (1648.49 cm^{-1}), C=N (1594 cm^{-1}), N-$NH_{stretch}$ (3342.75 cm^{-1}), C-Cl (692.04cm^{-1}), ^{1}H NMR (DMSO-d_6, 400.13MHz), δ 7.12-7.56 (t, 5H, Ar-H,), 6.95-8.2 (m, 5H, Ar-H), 7.31-7.42 (t, 3H, Ar-H), 3.89 (s, 1H, N-H), MS, m/z:407.16 (M+); $C_{20}H_{13}ClN_4O_2S$ (Calcd. 408.86); Anal calcd. (%) C, 58.75;H, 3.20; N, 13.7; Found: C, 59.12; H, 3.62; N, 14.12.

***Compound III_g* (7-chloro-2-phenyl-3- (p-methoxyphenylamino)-quinazolin-4 (3H)-thione):**

Light brown crystalline solid (methanol); A mixture of 7-chloro-2-phenyl-3- (p-methoxy-phenylamino) quinazolin-4 (3H)-one (10 mmol, 2.70g) and phosphorus penta sulphide (1 mmol, 2.43 g) was refluxed 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 dimethyl sulphoxide (10 ml) and filtered. The clear filtrate was poured into ice water, dried and recrystallized from ethanol to form light brown crystalline solid. (Yield: 72%); m. p 172-174°C; IR (cm^{-1}) ν_{max} Ar-$CH_{stretch}$ (3242.17cm^{-1}), C=S (1261.65 cm^{-1}), C=N (1677.66 cm^{-1}), C-N (1193.01 cm^{-1}), N-$NH_{stretch}$ (3309.67 cm^{-1}), C-CH_3 (2911.79cm^{-1}), ^{1}H NMR (DMSO-d6, 400.13MHz), δ 6.54-7.52 (m, 5H, Ar-H), 6.7-7.58 (m, 5H, Ar-H), 7.24-7.35 (m, 3H, Ar-H), 4.2 (s, 1H, N-H), MS, m/z: 338.15 (M+); $C_{21}H_{16}ClN_3S$ (Calcd. 393.89); Anal calcd. (%) C, 58.75; H, 3.20; N, 13.7; Found: C, 59.12; H, 3.62; N, 14.12.

***Compound III_h* (3-amino-7-chloro-2-phenyl-quinazoline-4(3H)-thione):**

Reddish brown crystalline solid (methanol); A mixture of 3-amino-7-chloro-2-phenyquinazolin-4- (3H)-one (10 mmol, 2.70g) and phosphorus penta sulphide (1 mmol, 2.43 g) was 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 dimethyl sulphoxide (10 ml) and filtered. The clear filtrate was poured into ice water, dried and recrystallized from ethanol to form reddish brown crystalline solid. (Yield: 65%); m.p. 172-175°C; IR (cm^{-1}) ν_{max} Ar-$CH_{stretch}$ (3262.75 cm^{-1}), C-N (1152.59 cm^{-1}), C=N (1664.49 cm^{-1}), C=S (1274.92 cm^{-1}), C-Cl (747.66 cm^{-1}), N-NH (bend) (3343.75 cm^{-1}), ^{1}H NMR (DMSO-d6, 400.13MHz), δ 7.24-7.62 (m, 5H, Ar-H), 7.23-7.41 (m, 3H, Ar-H), 2.3 (s, 1H, N-H). MS, m/z: 288.12 (M+); $C_{21}H_{16}ClN_3OS$ (Calcd. 287.77); Anal calcd. (%) C, 58.43; H, 3.5; N, 14.6; Found: C, 58.89; H, 3.82; N, 15.05.

***Compound III_i* (1- (7-chloro-2-phenyl-4-thioxoquinazoline-3-(4H)-urea) :**

White crystalline solid (methanol); A mixture of 1-(7-chloro-4-oxo-2-phenylquinazoline-3 (4H-yl)) urea (10 mmol, 2.70 g) and phosphorus penta sulphide (1 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 dimethyl sulphoxide (10 ml) and filtered. The clear filtrate was poured into ice water, dried and recrystallized from ethanol to form white crystalline solid. (Yield: 64%); m. p 162-165°C; IR (cm^{-1}) ν_{max} Ar-$CH_{stretch}$ (3126.37 cm^{-1}), C=S (1243.35 cm^{-1}), C=N (1598.62 cm^{-1}), C-N (1193.95 cm^{-1}) N-$NH_{stretch}$ (3283.49cm^{-1}), ^{1}H NMR (DMSO-d6, 400.13MHz), δ 7.24-7.63 (m, 5H, Ar-H), 7.24-7.42 (m, 3H, Ar-H), 6.2 (s, 1H, N-H). MS, m/z: 332.12 (M+); $C_{15}H_{11}ClN_4OS$ (Calcd. 330.79); Anal calcd. (%)C, 54.46; H, 3.35; N, 16.94; Found: C, 54.86; H, 3.85; N, 17.32.

***Compound IIIj* (1- (7-chloro-2-phenyl-4-thioxoquinazoline-3(4H)-thiourea):**

White crystalline solid (methanol); A mixture of 1-(7-chloro-4-oxo-2-phenylquinazoline-3 (4H-yl)) thiourea (10 mmol, 2.70 g) and phosphorus penta sulphide (1 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 dimethyl sulphoxide (10 ml) and filtered. The clear filtrate was poured into ice water, dried and recrystallized from ethanol to form white crystalline solid. (Yield: 75%); m.p. 163-165^{0}C; IR (cm^{-1}) νmax Ar-$CH_{stretch}$ (3199.89 cm^{-1}), C=S (1266.56 cm^{-1}), C=N (1671.85 cm^{-1}), N-$NH_{stretch}$ (3027.51 cm^{-1}), C-Cl (692.51cm^{-1}), ^{1}H NMR (DMSO-d6, 400.13MHz), δ 7.25-7.54 (m, 5H, Ar-H), 7.31-7.42 (m, 3H, Ar-H), 2.3 (s, 1H, N-H). MS, m/z: 345.12 (M+); $C_{15}H_{11}ClN_4S_2$ (Calcd. 346.86); Anal calcd. (%) C, 51.94; H, 3.2; N, 16.15; Found: C, 52.34; H, 3.46; N, 16.55.

2.3 Pharmacological activity

2.3.1 Drug and chemicals

The synthesized compounds were suspended in a 0.25% w/v carboxy methyl cellulose (CMC) solution just prior to administration and administered intraperitoneally (i.p). Gefitinib (Natco Pharma, Hyderabad, India) was prepared as suspension in 0.25% CMC. All the other laboratory chemicals used in this research work were procured from Sigma-aldrich Ltd (India). The newly synthesized compounds (IIa-IIIj) were evaluated for their antitumor activity.

2.3.2 Animals

The animal activity screening was carried out using Swiss albino mice weighing 20 ± 5 g. They were taken from the animal house of GIPS. The mice were grouped, housed in polyacrylic cages and maintained properly under standard laboratory conditions (temperature 25 ± 2^o C) with light/ dark cycle (12/12 h). They were given a proper dry pellet diet and water *ad libitum*. The animals were accustomed to laboratory conditions for 10 days before beginning of the experiment. All the experimental works were done after getting authorization from the institutional animal ethics committee, GIPS, Guwahati, India (**GIPS/IAEC/09)**.

2.3.3 Tumor Cell

The EAC induced mice were originally obtained from Institute of Advanced Research in Science and Technology (IASST). The EAC cells were maintained in Swiss albino mice by intraperitoneal (i.p.) transplantation of 1×10^6 cells / mouse after every 10 days [18].

2.3.4 Determination of maximum tolerable dose

Maximum tolerable dose of the synthesized compound were determined by following the OECD (Organisation for Economic Cooperation and Development) guidelines-2001. In concise, Swiss albino mice were deprived of food for 18 h, were administered various doses of synthesized quinazoline derivatives and observed for any symptoms of toxic effects continuously for 4 h, then after 24 h and finally the number of survivors was noted after a period of 72 h. Depending on the results obtained, the therapeutic dose for further studies was selected.

2.3.5 *In vivo* antitumor activity against EAC model in mice

EAC cells were collected from EAC tumor bearing mice using a 23 gauge needle into a sterile syringe. The ascitic fluid was properly diluted in normal saline or sterile phosphate buffered saline (PBS) to get a concentration of 10×10^6 cells/ml of cell suspension. From this stock suspension, 0.25 ml (2.5 million cells) was administered intraperitoneally to each mouse. After 24 h of tumor inoculation, the tumor bearing mice were divided at random into 22 groups of six animals each and treated the test compounds as follows:

Group-1: The EAC bearing mice (Control).

Group-2: The EAC bearing mice treated with standard (Gefitinib) 10 mg/kg, i.p.

Group-3-22: The EAC bearing mice treated with compound IIa-IIIj at 30 mg/kg, i.p.

Standard and the test compounds were administered intraperitoneally for 10 days. The control group was treated with 0.25% CMC. Every third day, animals weighed to measure the tumor growth.

2.3.6 Determination of % increase in body weight

The % increase in growth was calculated using the following formula [19]

$$\% \text{ Increase in Weight} = \frac{\text{Animal weight on respective day- Animal weight on day 0}}{\text{Animal weight on day 0}} \times 100$$

2.3.7 Determination of mean survival time:

Mortality of animals was recorded to calculate mean survival time (MST).The percentage increase in life span (ILS) was calculated by the formula as follows [20]

$$\% \text{ Increase of life span} = \frac{\text{MST of treated group - MST of control group}}{\text{MST of control group}} \times 100$$

$$\text{Where MST} = \frac{\text{Total survival time (days) of each mice in a group}}{\text{Total number of mice}}$$

2.3.8 Effect of synthesized quinazoline derivatives on haematological parameters of EAC bearing mice

To study the effect of synthesized compounds on haematological parameters, blood was collected from similar set animals on 15th day from retro orbital plexus. The blood was collected into a micro centrifuge tubes containing EDTA and to determine RBC count, WBC count and haemoglobin count by using an automatic analyzer[21].

2.3.9 Determination of tumor volume

The growth of tumor in animals was determined by measuring the diameter of tumor growth in two perpendicular planes using Vernier callipers. The tumor volume was calculated using the formula **V=4/3πa2b/2** Where a is minor diameter and b is major diameter[22].

2.3.10 Determination of Tumor weight

At the end of the fifth week, animals were sacrifice under anaesthesia using diethyl ether, tumor extirpated and weighed [23].

2.3.11 Effect of synthesized quinazoline derivatives on viable and non viable cell count of EAC bearing mice

The viability and non viability of the cells were checked for the above groups by tryphan blue assay. The cells were stained with tryphan blue (0.4 % in normal saline) dye. Upon staining, the viable cells did not take the stain while the non viable cells were stained blue and counted by using invitrogen auto cell counter [24].

2.4 Statistical Analysis

Statistical significance (*p*) was calculated by one-way ANOVA between the treated groups and the EAC control group followed by Dunnett's multiple comparison tests of significance where, $p<0.05$, considered being significant respectively. All data are expressed as mean ± S.E.M (n = 6 mice per group)[25].

3. Result and discussion

3.1 Physico chemical and spectral characterisation

A new series of 7-chloro-3-[substituted (amino/phenyl amino)]-2-phenyl quinazolin-4 (3H)-one/thione derivatives and 1-(7-chloro-4-oxo/-2-phenylquinazoline-3(4H-yl)) substituted urea derivatives were synthesized as shown in the figure 2 and the characterisation were done by using TLC, IR, ^{1}H-NMR and mass spectroscopy. The synthesized compounds were soluble in methanol. The spectral data of compound I shows that Ar-$CH_{stretch}$ (3072 cm^{-1}), C=O (1751.33 cm^{-1}), C=N (1592 cm^{-1}), cyclic C-O-$C_{stretch}$ (1060.47cm^{-1}), C-Cl (680.72cm^{-1}). Compounds IIa-IIj mainly characterised by the absorption width at the range 1752-1598cm^{-1} which confirmed the presence of ketonic group (C=O) in the quinazoline scaffold. Compounds IIa-IIIj showed the spectral range of 3323-3010 cm^{-1} which confirmed the presence of (C-H) group in aromatic ring. The spectral range of 3432-3027 cm^{-1} indicated the presence of N-NH stretching which also attributes the quinazoline skeleton in the respective compound. The presence of C-Cl group is shown by the spectral range of 735-676 cm^{-1}. The presence of C=S is illustrated by the spectral range 1274-1233 cm^{-1}. In addition all the compounds displayed C_4-H deformation. The mass spectra of the compounds were studied and the molecular ion peaks (M+), which were remain same for all the compounds. The elemental analyses were within ± % of the theoretical values. ^{1}H NMR spectra of IIa – IIIj illustrated different spectral ranges in which each appears as multiplet and triplet due to the presence of non magnetically equivalent proton. The aromatic protons show at the peak at δ 6.5-8.725 ppm. Appearance of singlet protons around δ 3.46-4.5 ppm for single protons in the ^{1}H NMR spectra might be assigned to -NH- group. Appearance of singlet proton δ 2.34-2.51 ppm for three protons in its ^{1}H NMR spectra which might be assigned to aromatic methyl group confirms the formation of IIc/IIIc. The structures of the compounds are established from the characteristics of the results obtained from analytical techniques.

3.2 Antitumor activity

Antitumor activity of the synthesized quinazoline derivatives against EAC tumor bearing mice were screened by different parameters such as percentage increase in body weight, MST, percentage increase in life span, tumour weight, tumor volume as shown in the table 1 and the haematological parameters, viable and non-viable cell count as shown in the table 2.

All the compounds showed significant effect as compared to control by studying all the *in vivo* screening parameters. Out of all the compounds, compound **IIh, IIi, IIj, IIIh, IIIi, IIIj** showed very good antitumor potentiality. Increased life span is key factor in the cancer treatment, because most of the anticancer drugs have not increased significant life span and also it produced more side effects. Hence the synthesized compounds showed increase in life span when compared to control. Tumor bearing mice possessed increased ascites fluid and cancer cell counts. Since the ascites fluid provides the essential nutrients for the growth of cancer cells, increase in fluid volume directly linked with tumor growth. The newly synthesized quinazoline derivatives lowered the ascites fluid volume as well as peritoneal cell counts. The efficiency of compound IIh, IIi, IIj, IIIh, IIIi and IIIj in enhancing life span of tumor bearing animal was comparable to that of standard. Considerable increased in body weight was observed in EAC inoculated control mice with a maximum gain (32.78±0.39). Standard drug significantly reduced body weight (16.12±0.53) as compared to control. Compound IIh, IIi, IIj, IIIh, IIIi, IIIj when treated with a dose of 30 mg/kg caused significant reduction in the body weight as shown in the table 1. All the haematological parameter studies were analysed and the newly synthesized

compounds were compared with control. The red blood cells (RBCs) counts were not increased in a normal way but were significantly increased in quinazoline treated animals as compared to control. Compound IIh, IIi, IIj, IIIh, IIIi and IIIj improve the RBC count but not as efficiently as standard. The white blood cells (WBCs) levels were lowered by the groups treated with quinazoline derivative compounds. The haemoglobin levels in mice treated with newly synthesized compounds was not increased significantly. The viable cells were decreased in the compound treated groups. The non viable cells are increased in the quinazoline treated groups as compared to control. This shows that after treating the animals with the quinazoline derivatives, the spreading of EAC tumor cells are decreased. Quinazoline derivative compounds significantly decreased the tumor weight and volume in mice at respective dose as compared to control.

Compounds IIh and IIIh showed significant *in vivo* antitumor activity which may be due to the presence of NH_3 group at the 3rd position of quinazoline skeleton. Literature survey shows that compound with quinazoline scaffold with ammonia group at 3rd position is having good anticancer activity [1]. Similarly, the *in vivo* screening of compounds **IIi** and **IIj** showed improved in activities which are having urea and thio urea substitution in the 3rd position of quinazoline pharamcophore. Compound **IIIi** and **IIIj** are also having urea and thio urea substitution but there is isosteric replacement of ketonic group substitution with the thio group at 4th position of quinazoline skeleton which probably have additive effect on antitumor activity along with the main substituents.

In compounds **IIb, IId, IIe and IIf**, there is introduction of electron withdrawing group i.e. 2-chloro, 4-chloro, 4-bromo and 4-nitro group which shows significant results as compared to control but the activity is less as compared to standard. Similar case happened in case of compounds **IIIb, IIId, IIIe and IIIf**. Compounds **IIc, IIIc, IIg, and IIIg** are having electron releasing group i.e. 2-methyl and 4-methoxy group respectively. Introduction of these groups in the quinazoline skeleton causes mild to moderate antitumor activity. The main problem of cancer chemotherapy is decline in RBC count and myelosupression. Haematological parameters such as RBC, WBC, and haemoglobin levels are frequently affected by the cancer chemotherapy. Most of modern antineoplastic agents produce anaemia due to their cytotoxic effect. The lowering level of RBC count and haemoglobin content of tumor bearing animals are may be due to aplastic or megaloblastic anaemia or break down of RBC [26,27]. Treatment with different synthesized quinazoline derivatives normalizes the abnormalities found in the haematological parameters study. This clearly shown the haemopoietic protective role of quinaozline scaffold. Compound IIh, IIi, IIj, IIIh, IIIi, IIIj decreased viable cell count and increased nonviable cell count. It revealed that the quinazoline derivatives having direct relationship with tumor cells because these anticancer agents cause the destruction of the cells by direct cytotoxic mechanism.

Table 1: Effect of synthesized quinazoline derivatives on % increase in weight, MST, % increase in life span, tumour weight and tumor volume of EAC bearing mice

Sl. No	Compound	Dose (Mg/Kg)	% Increase in body weight	MST (days)	% Increase in life span	Tumor weight (g)	Tumour volume (ml)
1	Control	30	32.78±0.39	22.25±0.35	16.14±0.24	9.12±0.14	7.43±0.054
2	Std (Gefitinib)	10	16.12±0.53	38.42 ± 0.27	30.75±0.62	2.24±0.19	1.87 ±0.023
3	IIa	30	25.45±0.64***	28.34±0.24***	23.12±0.58***	6.34±0.21***	3.57 ± 0.014***
4	IIb	30	25.54±0.62***	27.23±0.15***	24.23±0.37***	5.74±0.32***	3.87 ± 0.018***
5	IIc	30	26.48±0.61***	27.56±0.21***	23.15±0.68***	6.15±0.45***	3.72 ± 0.021***
6	IId	30	25.52±0.68***	28.04±0.25***	23.45±0.52***	5.84±0.37***	3.63 ± 0.017***
7	IIe	30	26.23±0.61***	27.95±0.18***	23.48±0.47***	5.75±0.48***	3.92 ± 0.012***
8	IIf	30	26.32±0.65***	27.89±0.16***	24.36±0.59***	6.24±0.64***	3.73 ± 0.018***
9	IIg	30	25.76±0.64***	27.34±0.24***	23.12±0.17***	6.52±0.27***	3.65 ± 0.023***
10	**IIh**	30	20.32±0.56***	31.24±0.25***	25.14±0.23***	4.31±0.18***	3.08 ± 0.024***
11	**IIi**	30	19.52±0.59***	32.52±0.38***	25.95±0.37***	3.92±0.27***	3.17 ± 0.012***
12	**IIj**	30	19.78±0.54***	32.78±0.46***	25.98±0.67***	3.72±0.36***	3.35 ± 0.015***
13	IIIa	30	27.12±0.621***	28.92±0.28***	22.24±0.15***	6.12±0.52***	3.42 ± 0.034***
14	IIIb	30	26.81±0.695***	27.37±0.19***	23.64±0.74***	5.98±0.28***	3.54 ± 0.027***
15	IIIc	30	25.76±0.674***	27.32±0.18***	22.98±0.82***	6.08±0.74***	3.86 ± 0.026***
16	IIId	30	26.22±0.602***	28.76±0.23***	23.07±0.19***	5.89±0.23***	3.73 ± 0.043***
17	IIIe	30	27.17±0.628***	28.71±0.25***	23.08±0.21***	6.46±0.87***	3.91 ± 0.014***
18	IIIf	30	26.74±0.624***	28.62±0.27***	23.57±0.29***	6.34±0.64***	3.71± 0.042***
19	IIIg	30	26.82±0.675***	28.83±0.29***	22.47±0.24***	6.78±0.52***	3.84 ± 0.029***
20	**IIIh**	30	19.94±0.627***	30.51±0.26***	25.43±0.31***	4.25±0.34***	3.53 ± 0.021***
21	**IIIi**	30	18.97±0.618***	32.32±0.27***	26.54±0.32***	3.74±0.58***	3.62 ± 0.028***
22	**IIIj**	30	19.02±0.654***	32.54±0.29***	26.64±0.42***	3.92±0.48***	3.27 ± 0.016***

All the values are mean ± SEM of six mice, where *** $p<0.05$ compared to control. All data are analyzed by one way ANOVA followed by Dunnett's Multiple Comparison Tests.

Table 2: Effect of synthesized quinazoline derivatives on haematological parameters, cell viability and of EAC treated mice

Sl. No	Treatment	RBC count (1×10^6cells/mm^3)	WBC count (1×10^3cells/mm^3)	Haemoglobin (g/dl)	Viable cells (1×10^7cells/mm^3)	Non viable cells (1×10^7cells/mm^3)
1	Control	3.23 ± 0.032	18.67 ± 0.267	3.82 ± 1.265	7.72 ± 0.032	1.98 ± 0.054
2	Standard (Gefitinib)	8.76 ± 0.078	7.98 ± 0.012	13.27 ± 0.086	2.60 ± 0.043	5.32 ± 0.98
3	IIa	5.23 ± 0.143***	9.42 ± 0.021***	8.28 ± 0.256***	4.83± 0.132***	3.24 ± 0.0112***
4	IIb	5.61 ± 0.145***	10.12 ± 0.018***	9.02 ± 0.232***	3.52 ± 0.114***	3.48 ± 0.0124***
5	IIc	5.72 ± 0.137***	9.89 ± 0.023***	8.76 ± 0.264***	3.76 ± 0.104***	3.68 ± 0.0134***
6	IId	5.45 ± 0.127***	9.78 ± 0.017***	8.56 ± 0.258***	3.72 ± 0.112***	3.47 ± 0.0142***
7	IIe	5.59 ± 0.138***	9.25 ± 0.012***	8.42 ± 0.274***	3.79 ± 0.119***	3.41 ± 0.0139***
8	IIf	5.62 ± 0.135***	9.84 ± 0.021***	8.32 ± 0.257***	3.73 ± 0.126***	3.53 ± 0.0142***
9	IIg	5.43 ± 0.152***	9.32 ± 0.029***	8.37 ± 0.268***	3.82 ± 0.127***	3.51 ± 0.0138***
10	**IIh**	6.04 ± 0.145***	8.27 ± 0.018***	9.42 ± 0.254***	3.05 ± 0.123***	4.03 ± 0.0131***
11	**IIi**	6.45 ± 0.158***	8.92 ± 0.025***	9.25 ± 0.264***	3.12 ± 0.134***	4.25 ± 0.0128***
12	**IIj**	6.54 ± 0.167***	8.87 ± 0.031***	9.32 ± 0.271***	3.25 ± 0.128***	4.34 ± 0.0125***
13	IIIa	5.53 ± 0.148***	9.12 ± 0.016***	8.12 ± 0.262***	3.94 ± 0.124***	3.25 ± 0.0115***
14	IIIb	5.15 ± 0.128***	9.29 ± 0.036***	8.24 ± 0.231***	3.85 ± 0.129***	3.14 ± 0.0117***
15	IIIc	5.63 ± 0.175***	9.42 ± 0.023***	8.25 ± 0.238***	3.35 ± 0.117***	3.49 ± 0.0132***
16	IIId	5.76 ± 0.164***	9.95 ± 0.019***	8.67 ± 0.258***	3.45 ± 0.127***	3.47 ± 0.0131***
17	IIIe	5.19 ± 0.118***	8.72 ± 0.034***	8.12 ± 0.256***	3.52 ± 0.125***	3.37 ± 0.0123***
18	IIIf	5.29± 0.136***	8.45 ± 0.027***	8.28 ± 0.263***	3.82 ± 0.134***	3.39 ± 0.0127***
19	IIIg	5.57 ± 0.139***	8.79 ± 0.037***	8.53 ± 0.252***	3.56 ± 0.143***	3.76 ± 0.0134***
20	**IIIh**	6.43 ± 0.145***	8.52 ± 0.028***	9.48 ± 0.262***	3.25 ± 0.114***	4.27 ± 0.0126***
21	**IIIi**	6.31 ± 0.146***	8.37 ± 0.024***	9.52 ± 0.256***	3.67 ± 0.126***	4.32 ± 0.0134***
22	**IIIj**	6.23 ± 0.135***	8.28 ± 0.017***	9.17 ± 0.234***	3.26± 0.119***	4.49 ± 0.0152***

All the values are mean ± SEM of six mice, where *** $p<0.05$ compared to control. All data are analyzed by one way ANOVA followed by Dunnett's Multiple Comparison Tests.

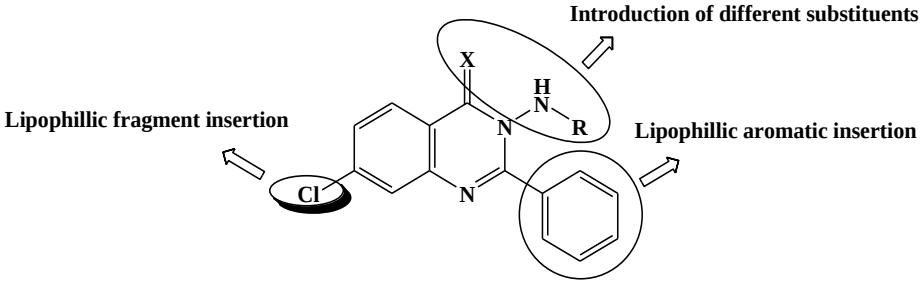

Figure 1: Scaffold of the designed quinazoline derivatives

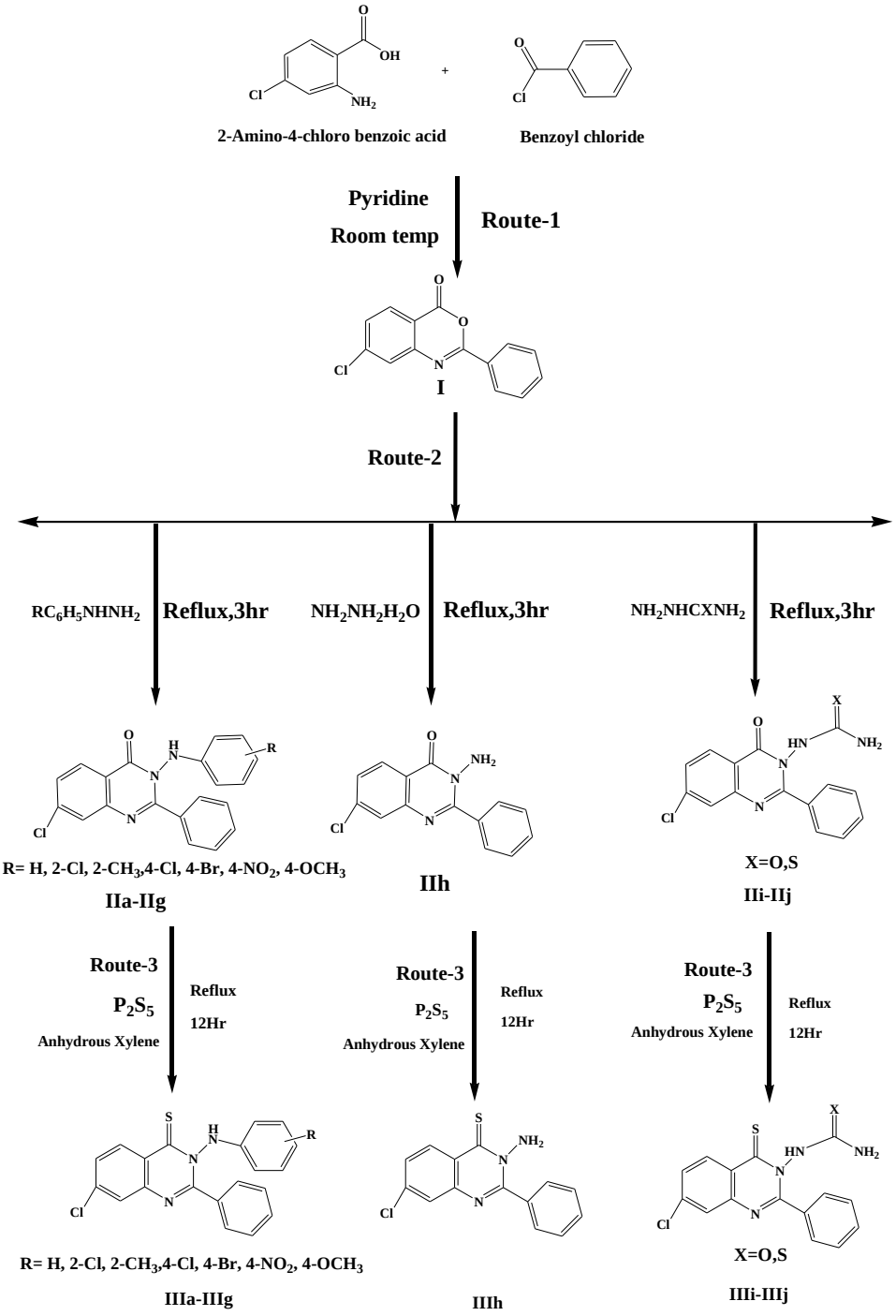

Figure 2: Scheme for the synthesis of 7-chloro-3-[substituted (amino/ phenyl amino)]-2-phenyl quinazolin-4 (3H)-one/thione derivatives and 1- (7-chloro-4-oxo/-2-phenylquinazoline-3 (4H-yl)) substituted urea

4. Conclusion

A new isomeric series of quinazoline derivatives with a common chemical moiety were synthesised by replacing different substituted hydrazine/phenyl hydrazine/urea/thiourea derivatives at 3rd position of quinazoline scaffold by suitable techniques. The quinazoline derivatives obtained from this research work indicates that the amino group at 3rd position and urea/thiourea group in phenyl hydrazine ring at 3rd position of quinzoline scaffold are crucial for antitumor activity. Compounds IIh, IIi, IIj, IIIh, IIIi and IIIj were established as therapeutically potent compound which may be useful as potential source for the development of antitumor compound having common quinazoline scaffold with lesser toxic effects. Research activities are going on for *in-vitro* antitumor study of the newly synthesized and pharmacologically potent quinazoline molecules.

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