

Design, Synthesis and Preliminary Pharmacological Screening (antimicrobial, analgesic and anti-inflammatory activity) of Some Novel Quinazoline Derivatives

Biswajit Dash^{1*}, Suvakanta Dash¹, Damiki Laloo¹, Chitrani Medhi²

¹Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Hathkhowapara, Guwahati-781017, Assam, India.

²Department of Chemistry, Gauhati University, Gopinath Bordoloi Nagar, Guwahati-781014, Assam, India.

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ABSTRACT

Objectives: The present research work is designed to synthesize some new series of quinazoline-4-one/4-thione derivatives by modifying the structures retaining the fundamental structural features for the biological activity and screened for their anti-microbial, analgesic and anti-inflammatory properties.

Material and methods: A series of 7-chloro-3-[substituted (amino/phenyl amino)]-2-phenyl quinazolin-4(3H)-one/thione derivatives and 1-(7-chloro-4-oxo/-2-phenylquinazolin-3-(4H-yl))-substituted urea derivatives were prepared and characterized from different spectra and elemental analysis. The anti-microbial, analgesic and anti-inflammatory activity were investigated by agar diffusion cup plate method, tail immersion method and carrageenan-induced paw oedema method respectively.

Results: The physico-chemical and spectroscopic data confirmed the synthesis of quinazoline derivatives. Five compounds (**IIc**, **IIg**, **IIh**, **IIi** and **IIj**) showed good activity against microbes and two compounds (**IIc** and **IIg**) showed good activity profile against both pain and inflammation. Three compounds (**IIh**, **IIi** and **IIj**) shows good therapeutic activity only against inflammation.

Conclusion: The quinazoline derivatives obtained indicates that the methyl/methoxy group in phenyl hydrazine ring, amine, urea and thiourea substitution at 3rd position of quinazolin ring are essential for anti-microbial, analgesic and anti-inflammatory activity. Compounds **IIc**, **IIg**, **IIh**, **IIi** and **IIj** were found to be potent which may be effective as potential source for the development of anti-microbial, analgesic and anti-inflammatory compound.

INTRODUCTION

Microbial infections cause pain and inflammation in the body. Generally two groups of agents are given for normal practice simultaneously (anti-microbial, analgesic and anti-inflammatory effect). Compound with all three properties are not very common. The commercially available antimicrobial agents are having many adverse effects (Alagarsamy *et al.*, 2005)

and increased risk of antibiotic (β -lactam antibiotic like penicillin and broad spectrum antibiotic like tetracycline) resistance among pathogenic bacteria has become a serious problem for the clinical management of infectious diseases and hence the treatment of bacterial infections remains a challenging therapeutic problem (Lokhandwala and Patel, 2013; Chavan *et al.*, 2014; Sarvanan *et al.*, 2015). Pain is associated with inflammation. Inflammation is a biological response to a series of biochemical reactions whose major function is to protect the body from infections and tissue damage due to injury.

But it is an ill defined, unpleasant feeling caused by nociceptive agents. In the last few decades, first generation non-

* Corresponding Author

Biswajit Dash, Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Hathkhowapara, Guwahati-781017, Assam, India.
Email: biswajit.itbhu@gmail.com

steroidal anti-inflammatory drugs (NSAIDs) had become well established in the treatment of pain and inflammation. Research was going on in these areas which lead to the discovery of second and third generation NSAIDs. From that study, some quinazoline derivative drugs are evolved as third generation NSAIDs such as proquazone, fluproquazone etc. It has been found that the pharmacological profile (anti-inflammatory response) of proquazone is quiet remarkable as compared to indomethacin (Chandrika *et al.*, 2008). But the chronic uses of NSAIDs cause various adverse effects such as bleeding, gastro-intestinal lesion and nephrotoxicity as revealed through literature (Hemlatha and Girija, 2011).

The application of heteroaromatic compounds in the field of medicinal chemistry has been recognized and has been studied experimentally due to potent pharmacological activity. Heterocyclic compounds containing nitrogen performs a broad spectrum of biological activity (El-Gazzar *et al.*, 2009). Hence the search of our interest is based on the undiscovered synthetically accessible heterocyclic template which is capable of bearing potential pharmacophoric group to elicit and enhance pharmacological activity such as chemotherapeutic, analgesic and anti-inflammatory principle.

In the recent years, the chemistry of quinazoline and its derivatives has received considerable attention owing to their synthetic and effective biological importance. The connection between a wide spectrum of biological activities and compounds containing quinazoline moiety has been known and is well documented in the literature. So with proven pharmacological significance, quinazoline scaffold has taken wide attention for the investigators and their efforts were quite significant as mentioned in the literature and it clearly demonstrate the remarkable potential of quinazoline derivatives as source of useful pharmacophore for the new drug discovery.

In the literature, it has been reported that the quinazoline and its derivatives possesses a wide range of biological activities such as anti-convulsant (Adel S *et al.*, 2012; Srivastava and Kumar, 2004; Jatav *et al.*, 2008; Sarvanan *et al.*, 2012), Central nervous system (CNS) depressant (Kashaw *et al.*, 2009; Jatav *et al.*, 2008; Kashaw *et al.*, 2008), anti-cancer (Adel *et al.*, 2010), anti-tubercular (Mosaad *et al.*, 2004) and anti-malarial activity (Jiang *et al.*, 2005). By analyzing the structural activity relationship (SAR) of synthesized quinazoline derivatives from literature survey, it revealed that substitution of different heterocyclic moieties at 2nd or 3rd position of quinazoline nucleus alters the biological activity (Patel and Patel, 2011). Quinazoline-4(3H)-one with substituted phenyl ring, bridged phenyl rings at position 3 was reported to be linked with anti-microbial activity (Abdel-Rahman *et al.*, 1997; Hassan *et al.*, 1991). It has also been found that substitution at 2 and 3 position of quinazoline derivative is having significant analgesic and anti-inflammatory activity (Hemlatha and Girija, 2011). In the present study, we attempt to design and synthesize isomeric new series of quinazoline-4-one/4-thione derivatives by structural modifications retaining the essential structural features for the activity and evaluated for their

anti-microbial, analgesic and anti-inflammatory activity in order to identify new candidates that may be potent and selective with lesser toxic effects.

MATERIAL AND METHODS

Chemicals and Instrumentation

The synthesis of the target compounds was accomplished as illustrated in the Figure 2. The compounds were synthesized according to the procedure given in the respective literature (Hemlatha and Girija, 2011; Adel *et al.*, 2010; Ilangoan *et al.*, 2010). All the reagents and solvents used in the study were of analytical grade purity and procured 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 F₂₅₄ aluminium sheets (Merck Ltd., Germany) and 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 KBr pallets and were recorded in cm⁻¹. ¹H NMR (400.13MHz) spectra were acquired on a Bruker Avance II-400 NMR Spectrophotometer using 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.

EXPERIMENT

Method of synthesis

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

4-chloroanthranilic acid (0.01 mole) 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 this addition, the reaction mixture was further stirred for half an hour at room temperature and set aside for 1hr. The pasty mass obtained was diluted with water (50 ml) and treated with aqueous sodium bicarbonate solution. When the effervescence ceased, the precipitate obtained was filtered off and washed with water, dried and recrystallized from diluted ethanol (Ilangoan *et al.*, 2010)

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 hrs 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 afford the target compounds IIa-IIj (Hemlatha *et al.*, 2011).

General Procedure for the synthesis of compounds, IIIa-IIIj

A mixture of 7-chloro-(3-amino/substituted phenyl amino)-2-phenyl quinazolin-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 hrs. 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 afford the target compounds IIIa-IIIj (Adel *et al.*, 2010).

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 this addition, the reaction mixture was further stirred for half an hour at room temperature and set aside for 1hr. The pasty mass obtained was diluted with water (50 ml) and treated with aqueous sodium bicarbonate solution. When the effervescence ceased, the precipitate obtained was filtered off and washed with water, dried and recrystallized from diluted ethanol as a white solid. (Yield: 61.2%); m.p 156-158°C; IR (KBr cm^{-1}) ν_{max} : Ar-CH stretch (3072 cm^{-1}), C=O (1751 cm^{-1}), C=N (1592 cm^{-1}), cyclic C-O-C stretch (1060 cm^{-1}), C-Cl (680 cm^{-1}); $^1\text{H NMR}$ (DMSO- d_6 , 400.13 MHz): δ 7.31-7.69 (m, 5H, Ar-H), 7.52-8.20 (t, 5H, Ar-H); MS m/z: 262.12 (M+), $\text{C}_{14}\text{H}_8\text{ClNO}_2$ (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 hrs 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 a reddish brown solid. (Yield:87.2%); m.p. 135-145°C; IR (KBr cm^{-1}) ν_{max} : Ar-CH_{stretch} (3262 cm^{-1}), C=O (1664 cm^{-1}), C=N (1595 cm^{-1}), N-NH_{stretch} (3343 cm^{-1}), C-Cl (689 cm^{-1}), $^1\text{H NMR}$ (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+); $\text{C}_{20}\text{H}_{14}\text{ClN}_3\text{O}$ (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) have been refluxed for 3 hrs 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°C; IR (KBr cm^{-1}) ν_{max} : Ar-CH_{stretch} (3323 cm^{-1}), C=O (1665 cm^{-1}), C=N (1592 cm^{-1}), N-NH_{stretch} (3307 cm^{-1}), C-Cl (676 cm^{-1}), $^1\text{H NMR}$ (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+); $\text{C}_{20}\text{H}_{13}\text{Cl}_2\text{N}_3\text{O}$ (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) have been refluxed for 3 hrs 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°C; IR (KBr cm^{-1}) ν_{max} : Ar-CH_{stretch} (3242 cm^{-1}), C=O (1677 cm^{-1}), C=N (1654 cm^{-1}), N-NH_{stretch} (3309 cm^{-1}), C-CH₃ (2911 cm^{-1}), $^1\text{H NMR}$ (DMSO- d_6 , 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+); $\text{C}_{20}\text{H}_{13}\text{Cl}_2\text{N}_3\text{O}$ (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 hrs 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°C; IR (cm^{-1}); ν_{max} Ar-CH_{stretch} (3010 cm^{-1}), C=O (1665 cm^{-1}), C=N (1594 cm^{-1}), N-NH_{stretch} (3240 cm^{-1}), C-Cl (698 cm^{-1}), $^1\text{H NMR}$ (DMSO- d_6 , 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+); $\text{C}_{20}\text{H}_{13}\text{Cl}_2\text{N}_3\text{O}$ (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 (0.01mol) and *p*-bromo phenyl hydrazine (0.01 mol) were refluxed for 3 hrs 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°C; λ_{max} (nm) 274; IR (KBr cm^{-1}) ν_{max} : Ar-CH_{stretch} (3271

cm^{-1}), C=O (1691 cm^{-1}), C=N (1645 cm^{-1}), N-NH_{stretch} (3332 cm^{-1}), C-Br (693 cm^{-1}), ¹H 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⁺); C₂₀H₁₃BrClN₃O (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 (0.01mol) and p-nitro phenyl hydrazine (0.01 mol) were refluxed for 3 hrs 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 (KBr cm^{-1}) v_{max}: Ar-CH_{stretch} (3250 cm^{-1}), C=O (1650 cm^{-1}), C=N (1592 cm^{-1}), N-NH_{stretch} (3341 cm^{-1}), C-Cl (682 cm^{-1}), ¹H NMR (DMSO-d₆, 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), 3.90 (s, 1H, N-H), MS, m/z: 378.15 (M⁺); C₂₀H₁₃ClN₄O₃ (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 (0.01 mol) and p-methoxy phenyl hydrazine (0.01 mol) were refluxed for 3 hrs 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 (KBr cm^{-1}) v_{max}: Ar-CH_{stretch} (3114 cm^{-1}), C=O (1752 cm^{-1}), C=N (1664 cm^{-1}), N-NH_{stretch} (3310 cm^{-1}), OCH₃-CH_{stretch} (3008 cm^{-1}), Ar-CH_{stretch} (3271 cm^{-1}), ¹H NMR (DMSO-d₆, 400.13MHz), δ 6.50-6.70 (m, 4H, Ar-H), 7.28-7.62 (m, 5H, Ar-H), 7.46-7.921 (t, 3H, Ar-H), 4.10 (s, 1H, N-H), 3.78 (s, 1H, Ar-OCH₃), MS, m/z: 345.12 (M⁺); C₂₁H₁₆ClN₃O (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-phenylquinazolin-4 (3H)-one)

Brown crystalline solid (methanol); 7-chloro-2-phenyl-4H-benzo[d][1,3]oxazin-4-one (0.01mol) and hydrazine hydrate (0.01 mol) were refluxed for 3 hrs 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; λ_{max} (nm) 278; IR (KBr cm^{-1}) v_{max}: Ar-CH_{stretch} (3126 cm^{-1}), C=O (1598 cm^{-1}), C=N (1552 cm^{-1}), N-NH_{stretch} (3283 cm^{-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), 2.40 (s, 1H, N-H), MS, m/z: 272.12 (M⁺); C₁₄ H₁₀ClN₃O (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 (0.01mol) and semicarbazide (0.01 mol) were refluxed for 3 hrs 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 (KBr cm^{-1}) v_{max}: Ar-CH_{stretch} (3199 cm^{-1}), C=O (1671 cm^{-1}), C=N (1593 cm^{-1}), N-NH_{stretch} (3027 cm^{-1}), C-Cl (692 cm^{-1}), ¹H NMR (DMSO-d₆, 400.13MHz), δ 7.25-7.58 (m, 5H, Ar-H), 7.37-7.86 (m, 3H, Ar-H), 5.80 (s, 1H, N-H), MS, m/z: 312.45 (M⁺); C₁₅ H₁₁ClN₄O₂ (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 (0.01mol) and thiosemicarbazide (0.01 mol) were refluxed for 3 hrs 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 (KBr cm^{-1}) v_{max}: Ar-CH_{stretch} (3126 cm^{-1}), C=O (1644 cm^{-1}), C=N (1552 cm^{-1}), N-NH_{stretch} (3432 cm^{-1}), C-Cl (755 cm^{-1}) ¹H NMR (DMSO-d₆, 400.13MHz), δ 7.24-7.50 (m, 5H, Ar-H), 7.32-7.78 (m, 3H, Ar-H), 2.30 (s, 1H, N-H), MS, m/z: 332.15 (M⁺); C₁₅H₁₁ClN₄OS (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 hrs. 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 light brown crystalline solid. (Yield: 68%); m.p. 165-168°C; IR (KBr cm^{-1}) v_{max}: Ar-CH_{stretch} (3025 cm^{-1}), C-N (1150 cm^{-1}), C=N (1666 cm^{-1}), C=S (1262 cm^{-1}), C-Cl (735 cm^{-1}), N-NH (bend) (3057 cm^{-1}), ¹H NMR (DMSO-d₆, 400.13MHz), δ 6.50-7.40 (m, 5H, Ar-H), 6.42-7.71 (m, 5H, Ar-H), 7.15-7.50 (m, 3H, Ar-H), 3.8 (s, 1H, N-H), MS, m/z: 362.15 (M⁺); C₂₀H₁₄ClN₃S (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 hrs. 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 brown crystalline solid. (Yield: 67%); m.p. 140-142°C; IR (KBr cm^{-1}) ν_{max} : Ar-CH_{stretch} (3010 cm^{-1}), C=S (1665 cm^{-1}), C=N (1594 cm^{-1}), N-NH_{stretch} (3240 cm^{-1}), C-Cl (698 cm^{-1}), ¹H 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), 4.10 (s, 1H, N-H), MS, m/z: 396.14 (M⁺); C₁₄H₁₀ClN₃S (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, 2.70g) and phosphorus penta sulphide (1 mmol, 2.43 g) was heated under reflux in anhydrous xylene (100 ml) for 12 hr.

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: 71%); m. p 172-175°C; IR (KBr cm^{-1}) ν_{max} : Ar-CH_{stretch} (3114 cm^{-1}), C=S (1264 cm^{-1}), C=N (1664 cm^{-1}), C-N (1099 cm^{-1}) N-NH_{stretch} (3310 cm^{-1}), CH₃-CH_{stretch} (3008 cm^{-1}), ¹H NMR (DMSO-d₆, 400.13 MHz), δ 6.50-7.57 (m, 5H, Ar-H), 6.67-7.58 (m, 5H, Ar-H), 7.31-7.35 (t, 3H, Ar-H), 3.90 (s, 1H, N-H), 2.34 (s, 1H, Ar-CH₃), MS, m/z: 378.19 (M⁺) C₂₀H₁₃Cl₂N₃S (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 hrs. 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 (KBr cm^{-1}) ν_{max} : Ar-CH_{stretch} (3029 cm^{-1}), C-N (1171 cm^{-1}), C=N (1676 cm^{-1}), C=S (1263 cm^{-1}), C-Cl (761 cm^{-1}), N-NH (bend) (3196 cm^{-1}), ¹H NMR (DMSO-d₆, 400.13MHz), δ 6.58-7.24 (m, 5H, Ar-H), 6.70-7.34 (m, 5H, Ar-H), 7.25-7.42 (m, 3H, Ar-H), 4.20 (s, 1H, N-H), MS, m/z: 398.05 (M⁺); C₂₀H₁₃Cl₂N₃S (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 hrs. 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 (KBr cm^{-1}) ν_{max} : Ar-CH_{stretch} (2918 cm^{-1}), C=S (1233 cm^{-1}), C=N (1665 cm^{-1}), N-NH_{stretch} (3060 cm^{-1}), C-Br (683 cm^{-1}), ¹H 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), 4.20 (s, 1H, N-H), MS, m/z:445.82 (M⁺); C₂₀H₁₃BrClN₃S (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 hrs. 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 (KBr cm^{-1}) ν_{max} : Ar-CH_{stretch} (3255 cm^{-1}), C=O (1648 cm^{-1}), C=N (1594 cm^{-1}), N-NH_{stretch} (3342 cm^{-1}), C-Cl (692 cm^{-1}), ¹H NMR (DMSO-d₆, 400.13MHz), δ 7.12-7.56 (t, 5H, Ar-H,), 6.95-8.20 (m, 5H, Ar-H), 7.31-7.42 (t, 3H, Ar-H), 3.89 (s, 1H, N-H), MS, m/z:407.16 (M⁺); C₂₀H₁₃ClN₄O₂S (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, 2.70 g) and phosphorus penta sulphide (1 mmol, 2.43 g) was refluxed in anhydrous xylene (100 ml) for 12 hrs. 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 (KBr cm^{-1}) ν_{max} : Ar-CH_{stretch} (3242 cm^{-1}), C=S (1261 cm^{-1}), C=N (1677 cm^{-1}), C-N (1193 cm^{-1}), N-NH_{stretch} (3309 cm^{-1}), C-CH₃ (2911 cm^{-1}), ¹H NMR (DMSO-d₆, 400.13MHz), δ 6.54-7.52 (m, 5H, Ar-H), 6.70-7.58 (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-phenylquinazolin-4-(3H)-one (10 mmol, 2.70 g) and phosphorus penta sulphide (1 mmol, 2.43 g) was reflux in anhydrous xylene (100 ml) for 12 hrs. 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: 65%); m.p. 172-175°C; IR (KBr cm^{-1}) v_{max}: Ar-CH_{stretch} (3262 cm^{-1}), C-N (1152 cm^{-1}), C=N (1664 cm^{-1}), C=S (1274 cm^{-1}), C-Cl (747 cm^{-1}), N-NH (bend) (3343 cm^{-1}), ¹H NMR (DMSO-d₆, 400.13MHz), δ 7.24-7.62 (m, 5H, Ar-H), 7.23-7.41 (m, 3H, Ar-H), 2.30 (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 hrs. 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: 64%); m.p 162-165°C; IR (KBr cm^{-1}) v_{max}: Ar-CH_{stretch} (3126 cm^{-1}), C=S (1243 cm^{-1}), C=N (1598 cm^{-1}), C-N (1193 cm^{-1}) N-NH_{stretch} (3283 cm^{-1}), ¹H NMR (DMSO-d₆, 400.13MHz), δ 7.24-7.63 (m, 5H, Ar-H), 7.24-7.42 (m, 3H, Ar-H), 6.20 (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 III_j (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 hrs. 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: 75%); m.p. 163-165°C; IR (KBr cm^{-1}) v_{max}: Ar-CH_{stretch} (3199 cm^{-1}), C=S (1266 cm^{-1}), C=N (1671 cm^{-1}), N-NH_{stretch} (3027 cm^{-1}), C-Cl (692 cm^{-1}), ¹H NMR (DMSO-d₆, 400.13MHz), δ 7.25-7.54 (m, 5H, Ar-H), 7.31-7.42 (m, 3H, Ar-H), 2.30 (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.

Pharmacological activity

The present biological study was approved by the institute animal ethics committee (GIPS/IAEC/9). All the chemicals and solvents used for the pharmacological activity were purchased from Sigma-Aldrich. The newly synthesized compounds (IIa-IIIj) were tested for their anti-microbial, analgesic and anti-inflammatory activity.

Anti-microbial activity (Disk diffusion method)

The antimicrobial activities were performed by disk diffusion method (Table-1). The synthesized compounds were dissolved in dimethyl formamide (DMF) at 100 µg/mL. Anti-bacterial activity against *Staphylococcus aureus*, *Bacillus Subtilis* (gram positive), *Pseudomonas aeruginosa*, *Escheria coli*, (gram negative). Anti-fungal activity was carried out against *Aspergillous niger* and *Candida albicans* under aseptic conditions. Ciprofloxacin and fluconazole (50 µg/ml) were used as standard drug for anti-bacterial and anti-fungal activity. The zone of inhibition was compared with the standard drug after 24 hrs of incubation at 25°C for anti-bacterial activity and 48 hrs at 32°C for anti-fungal activity (Maddeshiya and Agarwal, 2013).

Analgesic activity: (Tail immersion method)

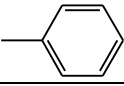
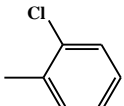
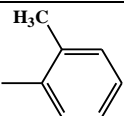
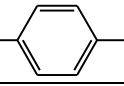
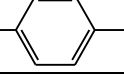
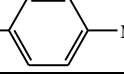
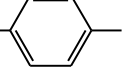
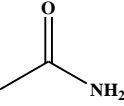
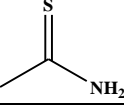
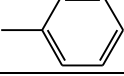
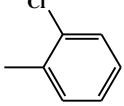
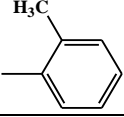

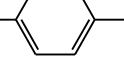
The analgesic activity was determined by the tail-immersion method (Hemlatha and Girija, 2011). Swiss mice (n=6) of either sex selected by random sampling technique. Diclofenac sodium at the dose of 20 mg/kg (I.P.) was used as a standard drug. The test compounds at a dose of 100 mg/kg in dimethylsulphoxide (DMSO) were administered i.p. The animals were held in position by a suitable restrainer with the tail extending out and the tail (up to 5 cm) was taken dipped in a beaker of water maintained at 55±0.5°C.

The time in sec taken to withdraw the tail clearly out of the water was taken as the reaction time. The first reading 0 min was taken immediately after the administration of the test compound and the subsequent reaction time was recorded at 15, 30, 60 and 120 min after the administration of compounds. A cut off point 15 sec was observed to prevent the tail damage. The percentage analgesic activity was calculated using the following formula and the results are presented in the table 2.

$$PAA = \frac{T_2 - T_1}{T_2} \times 100$$

Where T₁ is the reaction time (in sec) before treatment, T₂ is the reaction time (in sec) after treatment and PAA is the percentage analgesic activity.

Table 1: Antimicrobial activity.

Name of compound with their structural modification			Antibacterial activity (Zone of inhibition in mm)			Antifungal activity (Zone of inhibition in mm)		
Compound Name	R/R ¹	X	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>A. niger</i>	<i>C. albicans</i>
Standard Ciprofloxacin (50 µg/mL)	----	----	17.45	13.5	15.64	18.26	---	---
Standard Fluconazole (50 µg/mL)	----	----	----	----	----	----	21.76	16.42
IIa (100 µg/mL)		O	12.24	8.28	10.24	12.24	14.43	10.27
IIb (100 µg/mL)		O	12.45	11.12	11.42	13.32	13.97	11.29
IIc (100 µg/mL)		O	12.56	10.42	11.08	14.24	20.58	15.78
II d (100 µg/mL)		O	13.25	8.76	10.51	13.52	14.72	10.56
IIe (100 µg/mL)		O	14.12	9.1	9.24	12.88	13.86	11.57
II f (100 µg/ml)		O	11.28	9.23	10.12	12.95	14.75	12.98
IIg (100 µg/mL)		O	16.54	8.75	14.76	14.57	20.42	15.24
IIh	NH ₂	O	13.42	9.32	14.82	10.74	13.54	13.75
IIi (100 µg/mL)		O	12.34	10.23	9.82	14.43	20.81	14.95
IIj (100 µg/mL)		O	11.67	10.12	11.32	14.64	20.56	15.82
IIIa (100 µg/mL)		S	10.24	9.85	10.91	11.12	13.74	12.87
IIIb (100 µg/mL)		S	12.58	10.57	11.25	14.24	12.91	13.74
IIIc (100 µg/mL)		S	13.14	10.32	10.87	14.35	14.72	12.82
III d (100 µg/mL)		S	12.78	8.92	9.54	12.08	14.56	12.64
IIIe (100 µg/mL)		S	12.61	9.24	10.34	11.75	14.91	10.51

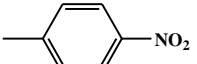
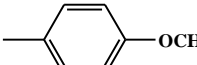
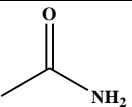
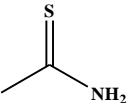
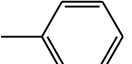
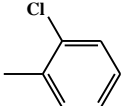
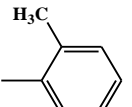
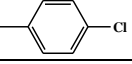
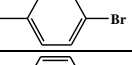
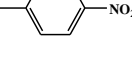
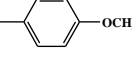
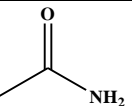
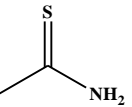
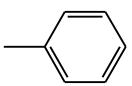
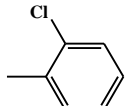
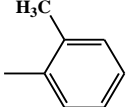
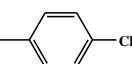
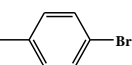
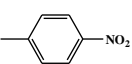
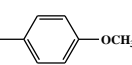
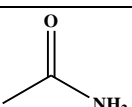
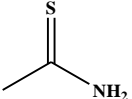
III _f (100 µg/mL)		S	13.53	8.57	11.28	10.91	14.82	11.28
III _g (100 µg/mL)		S	13.75	9.26	12.25	14.56	14.54	12.48
III _h (100 µg/mL)	NH ₂	S	14.28	9.54	10.76	13.81	15.72	12.72
III _i (100 µg/mL)		S	12.86	9.07	11.51	11.78	14.21	14.89
III _j (100 µg/mL)		S	12.23	10.21	10.76	16.13	14.85	13.78

Table 2: Analgesic activity.

Compound	R/R ¹	X	Basal Reaction Time in minutes									
			0 min		15 min		30 min		60 min		120 min	
			Mean±SEM	Mean±SEM	%	Mean±SEM	%	Mean±SEM	%	Mean±SEM	%	
Control	---	---	1.25±0.01	1.14±0.02	---	1.15±0.01	---	1.6±0.03	---	1.56±0.03	---	
Standard (20 mg/kg)	---	---	1.78±0.07	5.35±0.34	66.72	8.5±0.15	78.39	9.64±0.14	81.53	4.34±0.3	58.98	
II _a (100 mg/kg)		O	1.31±0.03	2.85±0.02	54.03	4.25±0.16	69.17	4.68±0.12***	72.00	2.64±0.12* **	50.37	
II _b (100 mg/kg)		O	1.34±0.04	1.2±0.02	57.05	4.85±0.15	72.37	5.32±0.6***	74.81	2.72±0.14* **	50.73	
II _c (100 mg/kg)		O	1.56±0.02	4.15±0.25	62.4	6.42±0.12	75.7	7.95±0.15***	80.37	3.48±0.12* **	55.17	
II _d (100 mg/kg)		O	1.32±0.03	3.14±0.4	57.96	4.72±0.17	72.03	5.26±0.16***	74.90	2.68±0.14* **	50.74	
II _e (100 mg/kg)		O	1.35±0.05	3.15±0.18	57.14	4.45±0.15	69.66	5.36±0.16***	74.81	2.72±0.05* **	50.36	
II _f (100 mg/kg)		O	1.28±0.04	2.62±0.03	51.14	4.21±0.15	69.59	4.54±0.11***	71.8	2.59±0.14* **	50.57	
II _g (100mg/kg)		O	1.52±0.04	4.21±0.23	63.89	6.44±0.14	76.39	7.85±0.14***	80.89	3.45±0.13* **	55.94	
II _h (100 mg/kg)	NH ₂	O	1.30±0.02	2.51±0.04	48.20	4.26±0.12	69.48	4.48±0.15***	70.98	2.56±0.11* **	49.21	
II _i (100mg/kg)		O	1.34±0.04	2.62±0.14	48.85	4.45±0.12	69.88	4.52±0.15***	70.35	2.62±0.05* **	48.8 5	
II _j (100mg/kg)		O	1.32±0.03	2.85±0.15	53.68	4.25±0.19	68.94	4.75±0.07***	72.21	2.57±0.04* **	48.63	
III _a (100mg/kg)		S	1.35±0.04	2.52±0.02	46.42	4.28±0.15	68.45	4.46±0.12***	69.73	2.48±0.13* *	45.56	

IIIb (100mg/kg)		S	1.42±0.06	3.58±0.11	60.33	5.82±0.18	75.6	5.31±0.59***	73.12	3.38±0.07***	57.98
IIIc (100mg/kg)		S	1.29±0.06	3.02±0.4	57.28	4.54±0.16	71.58	5.15±0.15***	74.95	2.52±0.16***	48.8
III d (100mg/kg)		S	1.57±0.04	4.25±0.12	63.05	6.58±0.08	76.13	5.24±0.2***	70.03	3.52±0.14***	55.39
IIIe (100mg/kg)		S	1.36±0.02	2.43±0.04	44.03	4.31±0.15	68.44	4.38±0.14***	68.94	2.42±0.12***	43.80
III f (100mg/kg)		S	1.38±0.04	2.45±0.02	43.67	4.34±0.11	68.20	4.39±0.12***	68.56	2.44±0.16***	43.44
III g (100mg/kg)		S	1.37±0.02	2.49±0.05	44.97	4.38±0.13	68.72	4.53±0.14***	69.75	2.49±0.12***	44.97
III h (100mg/kg)	NH ₂	S	1.39±0.04	2.51±0.02	44.62	4.41±0.12***	68.48	4.57±0.16***	69.58	2.53±0.14***	45.05
III i (100mg/kg)		S	1.46±0.06	3.64±1.64	59.89	5.97±0.17***	75.54	4.53±0.14***	67.78	3.54±0.02***	58.75
II a (100mg/kg)		S	1.45±0.08	3.57±0.11	59.38	5.87±0.12***	75.29	4.8±0.05***	69.79	3.53±0.03***	58.92

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.

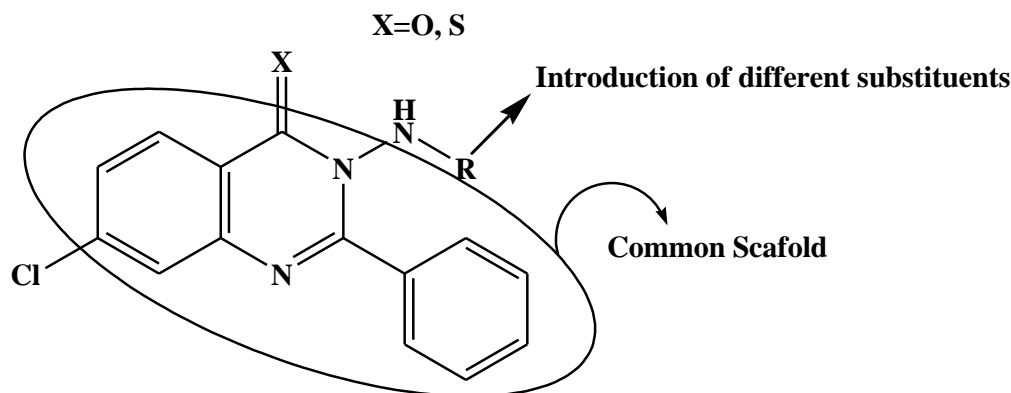
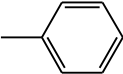
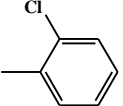
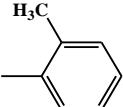
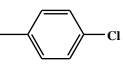
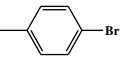
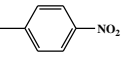
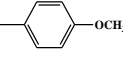
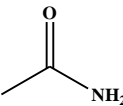
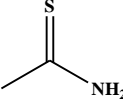
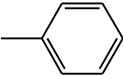
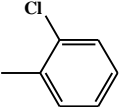
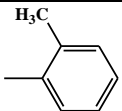
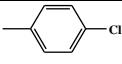
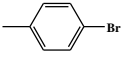
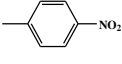
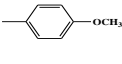
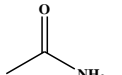


Fig. 1: Scaffold of the designed Quinazoline derivatives.

Table 3: Anti inflammatory activity.

Compound	R/R ¹	X	Time interval in minutes (Mean ± S.E.M) % inhibition						% inhibition
			0 min	15 min	30 min	60 min	120 min	180 min	
Control	----	----	0.18±0.006	0.2±0.0095	0.24±0.007	0.27±0.008	0.31±0.009	0.35±0.008	----
Standard Diclofenac (20mg/kg)	----	----	0.12±0.005	0.14±0.007	0.16±0.006	0.19±0.008	0.14±0.007	0.10±0.005 ^a	71.42
IIa (100mg/kg)		O	0.14±0.002	0.16±0.0017 ^a	0.18±0.0056 ^a	0.2±0.0021 ^a	0.17±0.0047 ^a	0.15±0.004 ^a	57.14
IIb (100mg/kg)		O	0.16±0.002	0.17±0.004 ^a	0.2±0.003 ^a	0.18±0.006 ^a	0.15±0.005 ^a	0.14±0.004 ^a	60
IIc (100mg/kg)		O	0.17±0.0042	0.18±0.0057 ^a	0.21±0.005 ^a	0.23±0.007 ^a	0.19±0.002 ^a	0.15±0.004 ^a	57.14
IId (100mg/kg)		O	0.17±0.0041	0.18±0.0052 ^a	0.21±0.0042 ^a	0.2±0.0024 ^{aa}	0.16±0.002 ^a	0.14±0.0019 ^a	60
IIe (100mg/kg)		O	0.17±0.004	0.19±0.002 ^a	0.22±0.0025 ^a	0.24±0.004 ^a	0.15±0.0029 ^a	0.14±0.0045 ^a	60
IIf (100mg/kg)		O	0.16±0.0057	0.17±0.0046 ^a	0.2±0.003 ^a	0.22±0.0024 ^a	0.18±0.006 ^a	0.15±0.0019 ^a	57.14
IIg (100mg/kg)		O	0.15±0.003	0.18±0.0017 ^a	0.21±0.0024 ^a	0.19±0.0017 ^a	0.16±0.0054 ^a	0.14±0.0038 ^a	60
IIh (100mg/kg)	NH ₂	O	0.14±0.0018	0.16±0.0055 ^a	0.18±0.002 ^a	0.2±0.003 ^a	0.17±0.004 ^a	0.11±0.0018 ^a	68.57
IIi (100mg/kg)		O	0.18±0.002	0.15±0.0028 ^a	0.22±0.0024 ^a	0.24±0.004 ^a	0.17±0.0038 ^a	0.12±0.005 ^a	62.85
IIj (100mg/kg)		O	0.17±0.0036	0.19±0.0018 ^a	0.21±0.0047 ^a	0.19±0.0016 ^a	0.15±0.0026 ^a	0.13±0.0048 ^a	65.71
IIIa (100mg/kg)		S	0.14±0.0017	0.16±0.002 ^a	0.19±0.0016 ^a	0.21±0.005 ^a	0.18±0.002 ^a	0.15±0.004 ^a	57.14
IIIb (100mg/kg)		S	0.15±0.003	0.18±0.0017 ^a	0.21±0.0024 ^a	0.19±0.0017 ^a	0.16±0.0054 ^a	0.15±0.0038 ^a	57.14

IIIc (100mg/kg)		S	0.16±0.0015	0.17±0.0036 ^a	0.2±0.003 ^a	0.21±0.0026 ^a	0.18±0.0017 ^a	0.14±0.0019 _a	60
III d (100mg/kg)		S	0.14±0.0018	0.16±0.0055 ^a	0.18±0.002 ^a	0.2±0.003 ^a	0.17±0.004 ^a	0.16±0.0018 _a	54.28
IIIe (100mg/kg)		S	0.15±0.0028	0.17±0.004 ^a	0.19±0.0015 ^a	0.21±0.003 ^a	0.19±0.002 ^a	0.14±0.002 ^a	60
III f (100mg/kg)		S	0.17±0.0042	0.19±0.002 ^a	0.2±0.0027 ^a	0.23±0.0048 ^a	0.18±0.0054 ^a	0.15±0.0019 _a	57.14
IIIg (100mg/kg)		S	0.15±0.003	0.17±0.0041 ^a	0.19±0.0014 ^a	0.22±0.0025 ^a	0.18±0.006 ^a	0.14±0.002 ^a	60
IIIh (100mg/kg)	NH ₂	S	0.16±0.006	0.18±0.0061 ^a	0.2±0.0024 ^a	0.23±0.0042 ^a	0.19±0.002 ^a	0.14±0.0018 _a	60
IIIi (100mg/kg)		S	0.17±0.0037	0.19±0.002 ^a	0.21±0.0045 ^a	0.2±0.0028 ^a	0.18±0.0016 ^a	0.14±0.005 ^a	60

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

Anti-inflammatory activity: (Carrageenan induced paw oedema method)

Anti-inflammatory activity was performed by carrageenan induced paw oedema method in rats (Bekhit and Fahmy, 2003). Oedema was induced by sub-plantar injection of 0.1 ml of freshly prepared 1% carrageenan into the right hind paw of the rats. The animals were divided into different groups of 6 each. Group-1 served as control and received carrageenan (1% w/v in saline), group-2 served as standard and received diclofenac sodium (20 mg/kg i.p), and group 3 to 22 received the test compound (100 mg/kg). The volume of paw oedema was measured at 0, 15, 30, 60, 120 and 180 min after injection of carrageenan using plethysmograph, the % of oedema inhibition was calculated for each animal group using the formula and the results were represented in the table 3.

Control - Test

$$\% \text{ Protection} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Acute toxicity study

Approximate 50% lethal dose (ALD₅₀) of the most lead compounds was determined using standard procedures (Bekhit and Fahmy, 2003). Albino mice of either sex weighing 25-35 g were divided in 21 groups of 6 mice each. The synthesized compounds were dissolved in DMSO in 500, 750 and 1000 mg/kg doses were injected. The toxic symptoms and mortality rates in each group were recorded in 24 hrs after drug administration.

RESULT AND DISCUSSION

Spectral characterisation

The proposed derivatives were synthesized as illustrated in the Figure 2. Physical properties and elemental analysis as well as all the spectral data are in accordance with the structure of the synthesized compounds.

The spectral data of I shows that Ar-CH_{stretch} (3072 cm⁻¹), C=O (1751 cm⁻¹), C=N (1592 cm⁻¹), cyclic C-O-C_{stretch} (1060 cm⁻¹), C-Cl (680 cm⁻¹). In addition, all the compounds displayed C₄-H deformation. The mass spectra of the compounds were studied and the molecular ion peaks (M⁺), which were found consistent for all the compounds.

The elemental analyses were within ± % of the theoretical values. ¹H NMR spectra of IIa – IIIj exhibited 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 6.50-8.725 ppm. The appearance of singlet protons around δ 3.46-4.50 ppm for single protons in the ¹H NMR spectra might be assigned to -NH- group.

The appearance of singlet proton δ 2.34-2.51 ppm for three protons in its ¹H NMR spectra which might be assigned to an aromatic methyl group confirms the formation of IIc/IIIc. The structures of the compounds are confirmed from the characteristics of the results obtained from analytical techniques.

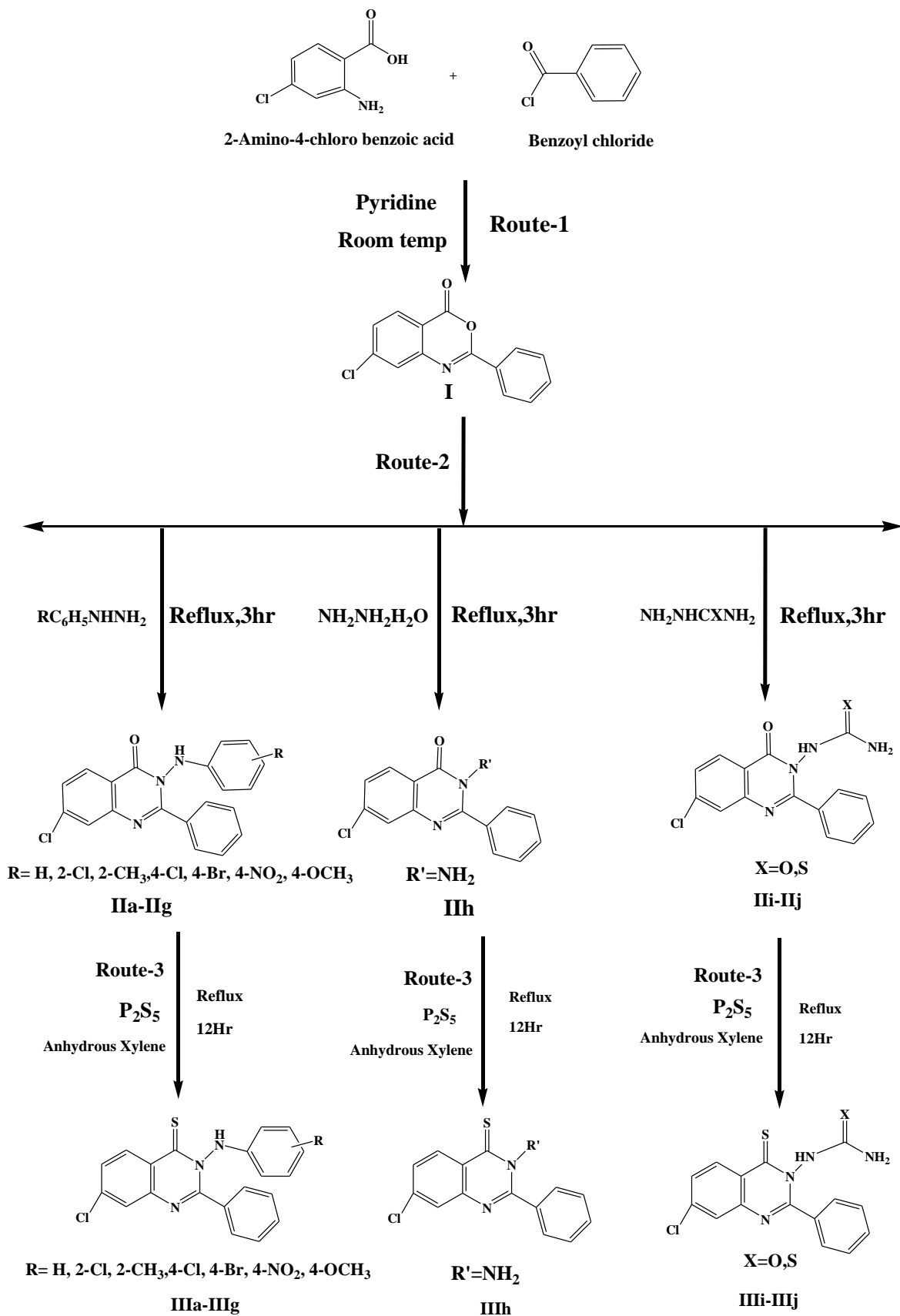


Fig. 2: Scheme for the synthesis of 7-chloro-3-[substituted (amino/ phenyl amino)]-2-phenyl quinazolin-4 (3H)-one/thione derivatives and 1- (7-chloro-4-oxo/-2-phenylquinazolin-3 (4H-yl)) substituted urea

Pharmacological activity

Anti-microbial activity

All the synthesized compounds were evaluated for their anti-bacterial and anti-fungal activities. A comparative anti-microbial study of the synthesized compounds with that of the standard drugs is effectively presented in table 1. The present study showed that some of the synthesized quinazoline derivatives performed significant anti-microbial potency. By correlating the structure of the sample compounds with their pharmacological activity, it has been observed that the compounds **IIg** and **IIIh** showed potent activity against bacteria *S. aureus*, and *B. subtilis*. This may be due to the presence of electron donating substituents i.e NH₂ and p-methoxy phenyl hydrazine group substitution at the 3rd position of quinazoline ring. Similarly compounds like **IIc**, **IIIi**, **IIj** also exhibited good activity against *A.niger* and *C.albicans* respectively due to the aforesaid reason.

The influence of these electron donating groups in improving the antimicrobial activity was revealed through literature (Fu *et al.*, 2010). Compounds containing electron withdrawing groups such as 2-chloro phenyl hydrazine (**IIb**), 4-chloro phenyl hydrazine (**IIId**), p-bromo phenyl hydrazine (**IIe**), p-nitro phenyl hydrazine (**IIIf**) did not exhibit significant anti-microbial activity. Compounds **IIIc**, **IIIg**, **IIIh**, **IIIi**, **IIIj** showed mild to moderate anti-microbial activity.

These substitutions are quite similar to the structure i.e. **IIc**, **IIg**, **IIIh**, **IIIi**, **IIj** respectively. But there is a substitution at 4th position with sulphur instead of oxygen in the quinazoline ring. So the alteration of activity may be due to the replacement in the quinazoline scaffold.

Analgesic and anti-inflammatory activity

In the present study, we have screened the analgesic activity by tail immersion method and anti-inflammatory activity by carrageenan induced paw edema method as shown in the table 2 and 3. Among the synthesized compounds, compounds **IIc** and **IIg** showed significant analgesic and anti-inflammatory activity as compared to the standard drug diclofenac sodium (20 mg/kg) respectively. This is assumed to be due to the presence of o-methyl phenyl hydrazine and p-methoxy phenyl hydrazine group at 3rd position of the quinazoline ring respectively. The literature survey showed that the therapeutic potency is due to the presence of alkyl or alkyloxy group (Alagarsamy *et al.*, 2005). Similarly compounds containing an amino group (**IIIh**), semicarbamoyl group (**IIIi**), thio-semicarbamoyl group (**IIIj**) showed good anti-inflammatory activity. The activity is probably due to the presence of electron donating substituents in the quinazoline scaffold as mentioned above (Alafeefy *et al.*, 2010). Compounds (**IIIc**, **IIIg**, **IIIh**, **IIIi** and **IIIj**) showed mild to moderate analgesic and anti-inflammatory activity.

The process of pain and inflammation includes sensitization of the nociceptors, transmission of signal of pain through impulses to the spinal cord and then the pain centre in the thalamus followed by integration of the sensation in the sensory

cortex as pain (Yokota *et al.*, 1989). Thus the anti-nociceptive action of the synthesized compounds may exert action either through peripherally or centrally at the site of the nociceptors. The potent inhibitory effects of the synthesized compounds of tail immersion method induced peripheral or cerebral mechanisms. This can only be pinpointed by investigating the effect of the compounds on the main sensitizers of the peripheral pain receptors mainly the prostaglandins.

The carrageenan induced edema is supposed to be mediated through the release of various pain mediators such as serotonin (5-Hydroxy typtamine), prostaglandin etc (Damas *et al.*, 1999). Hence the experimentally observed anti-inflammatory activities of the compounds were found to be due to the inhibition of these mediators.

Acute toxicity studies

Toxicological studies of the most promising anti-microbial (**IIc**, **IIg**, **IIIh**, **IIIi** and **IIj**) analgesic (**IIc** and **IIg**) and anti-inflammatory (**IIc**, **IIg**, **IIIh**, **IIIi** and **IIj**) active agents were performed using LD₅₀ standard method in mice in 500, 750 and 1000 mg/kg (body weight). However, no toxic symptoms or mortality rates were observed 24 hr post administration implying their good safety margin. But apart from these synthesized compounds rest of the synthesized quinazoline derivatives induced urination while compounds induced calmness, muscle relaxation and vasodilation. However, no toxic symptoms or mortality rates were observed after 24 hr.

CONCLUSION

A new series of quinazoline derivatives with a common skeleton were synthesised and evaluated for their anti-microbial, analgesic and anti-inflammatory principle. In general, electron donating group substituted derivatives exhibited better anti-microbial principle than electron withdrawing compounds. The quinazoline derivatives obtained from this research work indicates that the methyl/methoxy group in phenyl hydrazine ring at 3rd position, amine, urea and thiourea substitution at 3rd position of quinazoline derivatives are essential for anti-microbial, analgesic and anti-inflammatory principle.

Compounds **IIc**, **IIg**, **IIIh**, **IIIi** and **IIj** were found to be potent compound which may be effective as potential source for the development of anti-microbial, analgesic and anti-inflammatory compound having common quinazoline scaffold with lesser side effects. Therefore the study deserves further investigation with respect to *in vitro* activity of the above potent quinazoline derivatives.

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