Organic Chemistry
Current Research

Prabhakar et al., Organic Chem Curr Res 2016, 5:4
DOI: 10.4172/2161-0401.1000174

Research Article Open Access

Synthesis, Characterisation and Biological Evaluation of Quinazoline Derivatives as Novel Anti-Microbial Agents

Prabhakar V[1]*, Sudhakar BK[2], Ravindranath LK[2], Latha J[3] and Venkateswarlu B[4]

[1]*Faculty of Engineering Chemistry, SVR Engineering College, Jawaharlal Nehru Technological University - Ananthapuramu (JNTU-A), Nandyal, Kurnool District, Andhra Pradesh, India*
[2]*Department of Chemistry, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India*
[3]*Department of Environmental Science, Sri Krishnadevaraya University College of Engineering and Technology, Ananthapuramu, Andhra Pradesh, India*
[4]*Jawaharlal Nehru Technological University (JNTU-A), Kavali, Nellore District, Andhra Pradesh, India*

Abstract

A novel series of Quinazolines were synthesised by cyclisation reaction of Anthranilic acid with urea to get 2,4 di hydroxy quinazoline (2) intermediate, which were further treated with $POCl_3$ to get 2,4 di chloro quinazoline (3), which was treated with Thio-morpholine (4) for 3 hrs to get compounds (5), which are reacted with aqueous ammonia to get compound(6), which was further reacts with different boronic acids 7(a-j) by using Chan-Lam coupling reaction conditions to get Target Novel Quinazoline derivatives (8a-8j). The structures of new compounds were confirmed by IR and ^{1}H NMR and ^{13}C NMR spectral data. Anti-bacterial and anti-fungal activities were evaluated and compared with the standard drugs, compounds 8i, 8d, 8h, 8g exhibited promising anti-microbial and anti-fungal activity compared to standard drugs.

Keywords: Quinazoline; Chan-Lam coupling; Anti-microbial activity; 2,4-di chloro quinazoline; Synthesis

Introduction

Quinazoline (1) is a fused six-member aromatic ring (a benzene ring and a pyrimidine ring are fused). Quinazoline is a fused bicyclic compound earlier known as benzo-1, 3-diazine. It was first prepared in the laboratory in 1903 by Gabriel [1]. Although its derivative were known much earlier. The name quinazoline (German: Chinazolin) was first proposed for this compound by weddige on observing that this was isomeric with the compounds cinnoline and quinoxaline. Paal and Bush suggested the numbering of quinazoline ring system, which is currently used [2-4]. The other less commonly used names for this ring system are 'phenmiazine' and 5, 6-benzopyrimidine. However, the name quinazoline is now universally accepted (Figure 1).

Quinazoline derivatives, which belong to the N-containing heterocyclic compounds, have caused universal concerns due to their widely and distinct biopharmaceutical activities. Researchers have already determined many therapeutic activities of quinazoline derivatives, including anti-cancer [5-8], anti-inflammation [9,10], anti-bacterial [11-14], analgesia [9,13], anti-virus [15], anti-cytotoxin [16], anti-spasm [13,17], anti-tuberculosis [18], anti-oxidation [19], anti malarial [20], anti-hypertension [21], anti-obesity [22], anti-psychotic [23], anti-diabetes [24], etc. Medicinal chemists synthesized a variety of quinazoline compounds with different biological activities by installing various active groups to the quinazoline moiety using developing synthetic methods. And the potential applications of the quinazoline derivatives in fields of biology, pesticides and medicine have also been explored.

Quinazoline derivatives have attracted much attention for their various biological and medicinal properties. For example, they act as the potent tyrosine kinase and cellular phosphorylation inhibitors [25], and they are also used as ligands for benzodiazepine and GABA receptors in the central nervous system (CNS) [26] or as DNA binders [27-29] Some of them show remarkable activity as anticancer [30], antiviral [31] and antitubercular agents [32,33]. Molecules containing the Quinazoline unit have been popular drugs. For example, Erlotinib is used in the treatment of several types of tumors [34] Prazosin acts as an R-adrenergic blocker [35] and Iressa as an epidermal growth factor receptor inhibitor was approved by the Food and Drug Administration in USA for the treatment of lung cancer [36].

The Quinazoline skeleton is present in a variety of biologically active compounds, among these are several marketed drugs such as prazosin (1), Gefitinib (2), Erlotinib (3), Vandetanib (4) (Figure 2).

Encouraged by the diverse biological activities of Quinazoline Heterocyclic compounds, it was decided to prepare a new series of Quinazoline derivatives. Literature survey revealed that incorporation of different groups in Quinazoline Heterocyclic ring enhanced antibacterial and antifungal activity. In the present communication 2,4 Di Chloro Quinazoline (3) was reacted with meta-Amine Sulphonamide (4) in tert-butanol at 90°C to form Compound (5), which was further reacted with aqueous ammonia in THF(Tetra Hydro Furan) at 90°C to get target compound (6), Which was further reacted with different boronic acids under Chan-Lam reaction conditions to form Target compounds (8a-8j). The synthesis of the compounds as per the following Figure 3 given below.

The structures of all synthesized compounds were assigned on the basis of IR, Mass, ^{1}H and ^{13}C NMR spectral data analysis. Further these compounds were subjected for antifungal and antibacterial activity.

Materials and Methods

In this Investigation chemicals were purchased from local dealer

***Corresponding author:** Prabhakar V, Faculty of Engineering Chemistry, SVR Engineering College, Jawaharlal Nehru Technological University - Ananthapuramu (JNTU-A), Nandyal-518 502, Kurnool District, Andhra Pradesh, India, Tel: +918297140295; E-mail: Virupakshi.prabhakar@gmail.com

Received: October 15, 2016; **Accepted:** October 30, 2016; **Published:** November 20, 2016

Citation: Prabhakar V, Sudhakar BK, Ravindranath LK, Latha J, Venkateswarlu B (2016) Synthesis, Characterisation and Biological Evaluation of Quinazoline Derivatives as Novel Anti-Microbial Agents. Organic Chem Curr Res 5: 174. doi: 10.4172/2161-0401.1000174

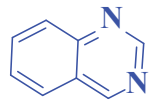


Figure 1: Quinazoline.

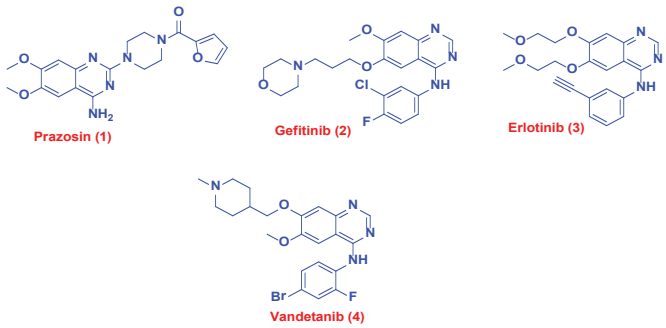


Figure 2: Quanazoline skeleton is present in a biologically active compounds.

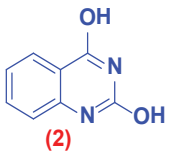


Figure 3: Structure of 2,4 di hydroxy Quinazoline (compound 2).

with S.D fine make was used. Chemicals were 99% pure; purity has been checked by Thin layer chromatography and melting point. Conventional method has been used for synthesis of Quinazoline derivatives. Stirring and reflux method were used for synthesis of Quinazoline derivatives 8(a-j) respectively. The Nomenclature was given for Synthetic compounds from compound (1) to Compounds 8a-8j by using Chem Bio draw Ultra -12 version.

The title compounds 8 (a-j) were synthesized in Seven steps using different reagents and reaction conditions, the 8 (a-j) were obtained in moderate yields. The structure were established by spectral (IR, ^{1}H-NMR, ^{13}C-NMR and mass) and analytical data (Scheme 1).

General procedure for synthesis of 2,4 di hydroxy Quinazoline [compound (2)]

A mixture of 2-aminobenzoic acid (1) (**50 g, 0.364 mole**) and urea (**109.2 gms**, **1.82 mol**) was heated at 180°C for 3 h. upon cooling to 120°C, The reaction mixture was poured into sodium hydroxide (1000 mL, 1N) solution and any insoluble material removed by filtration. The mixture was then acidified with HCl (2N), to yield 2,4 di hydroxy Quinazoline (compound 2) F as a white precipitate, which was collected by filtration and dried. (**95% yield, 56 gms**).

Yield: 90%; M.p. above 300°C (Figures 4-8);

^{1}H NMR (DMSO-d_6) δ ppm 7.15 (t, 2H, Ar-H), 7.6 (t, 1H, Ar-H), 7.85 (d, 1H, Ar-H), 11.05(1H,S), 11.1 (1H,S).

^{13}C NMR (DMSO-d_6) (δ/ppm): 120 (Ar C-H), 125.6 (Ar C-H),133.5(Ar C-H), 126.6(Ar C-H), 185(Ar C-OH),187(Ar C-OH), 110(Ar C), 151 (Ar C).

IR (KBr, *ν*/cm^{-1}): 3428 (OH, broad), 3079 (Ar C-H), 1604 (C=N);

LCMS Data shows That 2, 4 di hydroxy Quinazoline [Compound 2] Purity of 99.63%, RT 1.924, Mass 161.1 [M^{+}, **100%**].

General procedure for synthesis of 2, 4 di chloro Quinazoline [compound (3)]

A mixture of quinazoline-2,4-diol(compound 2) (**55 gms, 0.339 mol**), Phosphorous oxy chloride (550 mL), and catalytic amount of DMF (2 ml) was heated at reflux for 8 hrs, and the reaction was monitored by TLC. The reaction mixture was concentrated under reduced pressure and the residue was poured onto ice water with vigorous stirring yielding a precipitate. The mixture was then filtered to yield 2, 4 di chloro Quinazoline (compound 3) (Figure 9) as a white solid.

Yield: 75% (**51 gms**).

M.p. 116-118°C;

^{1}H NMR ($CDCl_3$-d_1) δ ppm 8.30-8.20 (d, IH), 8.10-8.00 (m, 2H), 7.80-7.70 (d, I H) (Figures 10 and 11).

^{3}C NMR ($CDCl_3$-d_1) (δ/ppm): 120(Ar C), 125 (Ar C), 128.6 (Ar C),136.5(Ar C), 151 (Ar C), 157(Ar C), 161.3(Ar C-) (Figures 12 and 13).

IR (KBr, *ν*/cm^{-1}): 755 (C-Cl), 3040 (Ar C-H), 1619 (C=N);

GCMS Purity 89.75%, RT 8.738, Mass 198 [M^{+}], 200 [M+2], 202 [M+4], 9:6:1 It indicates molecule contains Two chlorine atoms and Even no. of Nitrogen atoms According to Nitrogen rule.

General procedure for synthesis of 4-(2-chloroquinazolin-4-yl) thiomorpholine [Compound 5]

To the mixture of 2,4 di chloro Quinazoline (Compound 3) (10 gms, 0.0505 mol), Na_2CO_3 (0.1262 mol,13.5 gms) and MeOH (100 mL), Thiomorpholine (2.1 eq, 0.106 mol, 10.93 gms), was added drop wise at 0°C. Then the reaction mixture was Stirred at Room Temperature for 3 hrs. After completion of reaction as indicated by TLC, the reaction mixture was concentrated under reduced pressure and the residue was poured onto ice water with vigorous stirring yielding a precipitate. The precipitate was then filtered to yield the title compound (Compound 5) (Figure 14) as a white solid (12.85 g, 96% yield).

M.p. 226-227°C.

ESI-MS m/z 266 [M+H]$^{+}$, 288[M+Na]$^{+}$

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.7 (4H, t, 2 × CH_2–**S**), 3.5 (4H, t, 2 × CH_2–**N**), 7.6 (1H,t, Ar-H), 7.85 (2H,m, Ar-H), 8.2 (1H,d, Ar-H).

^{13}C NMR (100 MHz, DMSO-d_6) (δ/ppm): 28(2C, C-S), 53(2C, C-O), 120, 123, 128, 132, 135, 153, 158, 181.

IR (KBr, *ν*/cm^{-1}): 748 (C-Cl), 3040 (Ar C-H), 1629 (C=N);

General procedure for synthesis of 4-thiomorpholinoquinazolin-2-amine [Compound 6]

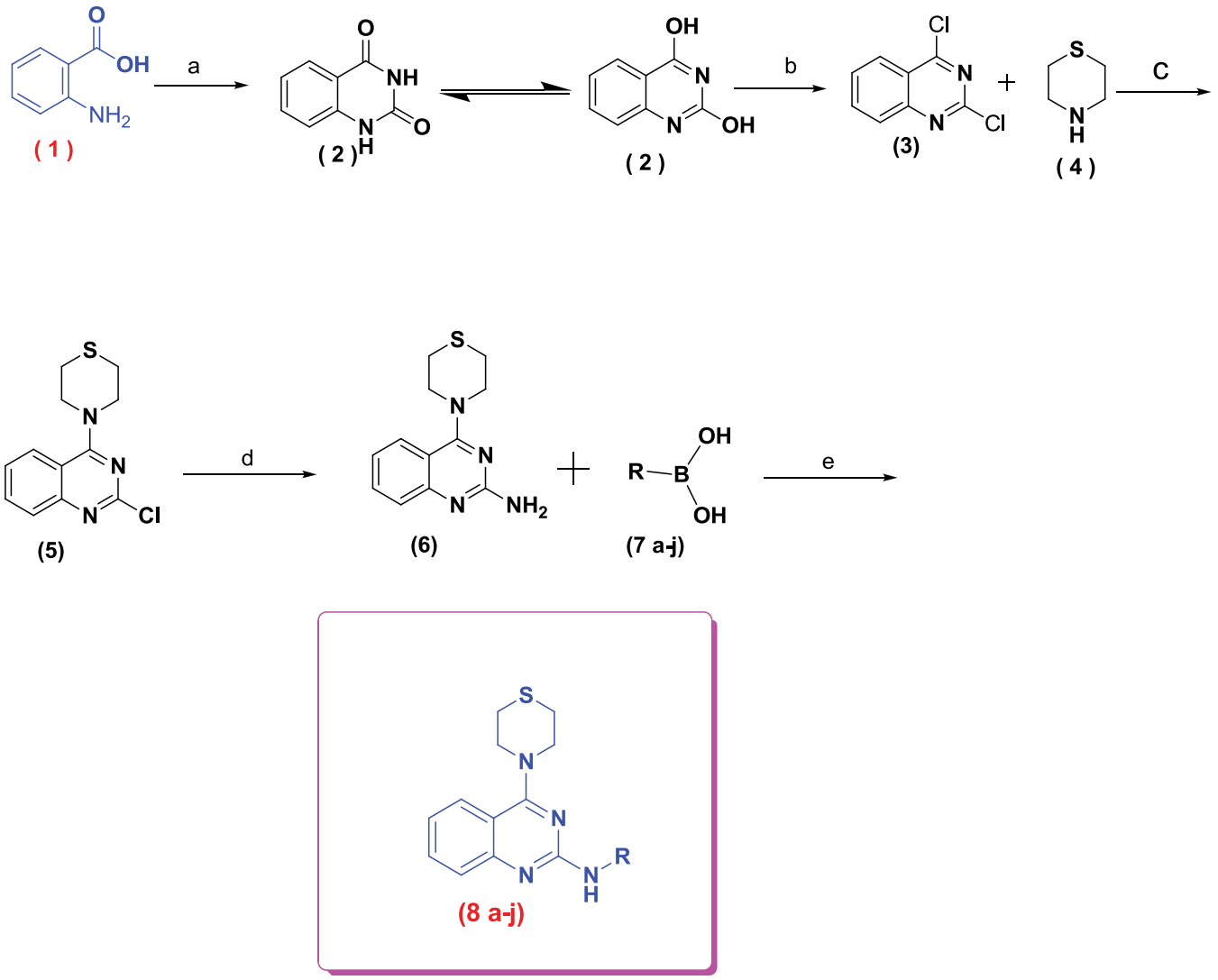
A solution of aqueous ammonia (NH_3.H_2O, 2.5 mol, 88 mL.), and compound (5) (0.039 mol, 10.335 g.) was stirred at 90°C for 6 hrs. The precipitate was collected by filtration and washed with water and dried to give compound (6) as a light white solid. (68%, 6.236 g) (Figure 15).

M.p.180-181°C.

ESI-MS m/z 247[M+H]$^{+}$.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.67(4H, t, 2 × CH_2 –**S**),

Synthetic Scheme



Scheme 1: Synthetic path way for compounds **8a-8j**.

R=-Phenyl, -4 Methyl phenyl, -4 Methoxy phenyl, -4 Tri fluoro phenyl, -4 Chloro phenyl, -4 Bromo phenyl, -4 Nitro phenyl, - pyridin-4-ylboronic acid, thiophen-2-ylboronic acid, furan-2-ylboronic acid

Reagents and reaction conditions: (a) Urea, 180°C, 3 hrs (d) $POCl_3$, DMF (catalytic), Reflux, 6 hrs (c). 2.1 eq. Thio morpholine, Na_2CO_3, MeOH, 0°C, 30 min, RT, 3 hrs (d) Aqueous Ammonia, 90°C, 6 hrs (e) $CuCl_2.2H_2O$/4A (10 mol%), CH_2Cl_2, 4 Å MS, 40°C, air, 24 hrs.

3.45(4H, t, 2 × CH_2 –**N**), 6.89(2H, bs) 7.6(1H,t, Ar-H),7.85(2H,m, Ar-H), 8.15(1H,d, Ar-H).

^{13}C NMR (100 MHz, DMSO-d_6) (δ/ppm): 28(2C, C-S), 53(2C, C-O), 115,123, 128, 130, 132, 153, 160, 180

IR (KBr, ν/cm^{-1}): 3340 and 3400(N-H Stretching, two bands), 3040 (Ar C-H), 1629 (C=N);

General procedure for synthesis of N-phenyl-4-thiomorpholinoquinazolin-2-amine (8a), 4-thiomorpholino-N-p-tolylquinazolin-2-amine (8b), N-(4-methoxyphenyl)-4-thiomorpholinoquinazolin-2-amine (8c), 4-thiomorpholino-N-(4-(tri fluoro methyl)phenyl)quinazolin-2-amine (8d), N-(4-chlorophenyl)-4-thiomorpholinoquinazolin-2-amine (8e), N-(4-bromophenyl)-4-thiomorpholinoquinazolin-2-amine (8f), N-(4-nitrophenyl)-4-thiomorpholinoquinazolin-2-amine (8g), 4-thiomorpholino-N-(thiophen-2-yl)quinazolin-2-amine (8i), N-(furan-2-yl)-4-thiomorpholinoquinazolin-2-amine (8j)

Amixtureofboronicacid7(a)(**1.5eq.**),4-thiomorpholinoquinazolin-2-amine (6) (**1 eq.**), pyridine (**2 m.mol**) and 0.5 g Cuo/4A in boiling dichloromethane (10 ml) was stirred under air atmosphere for 20 h. then the mixture was cooled, the solid was filtered, washed with dichloromethane, the filtrate was evaporated. The residue was purified by column chromatography (hexane: Ethyl acetate 4:1 eluent).

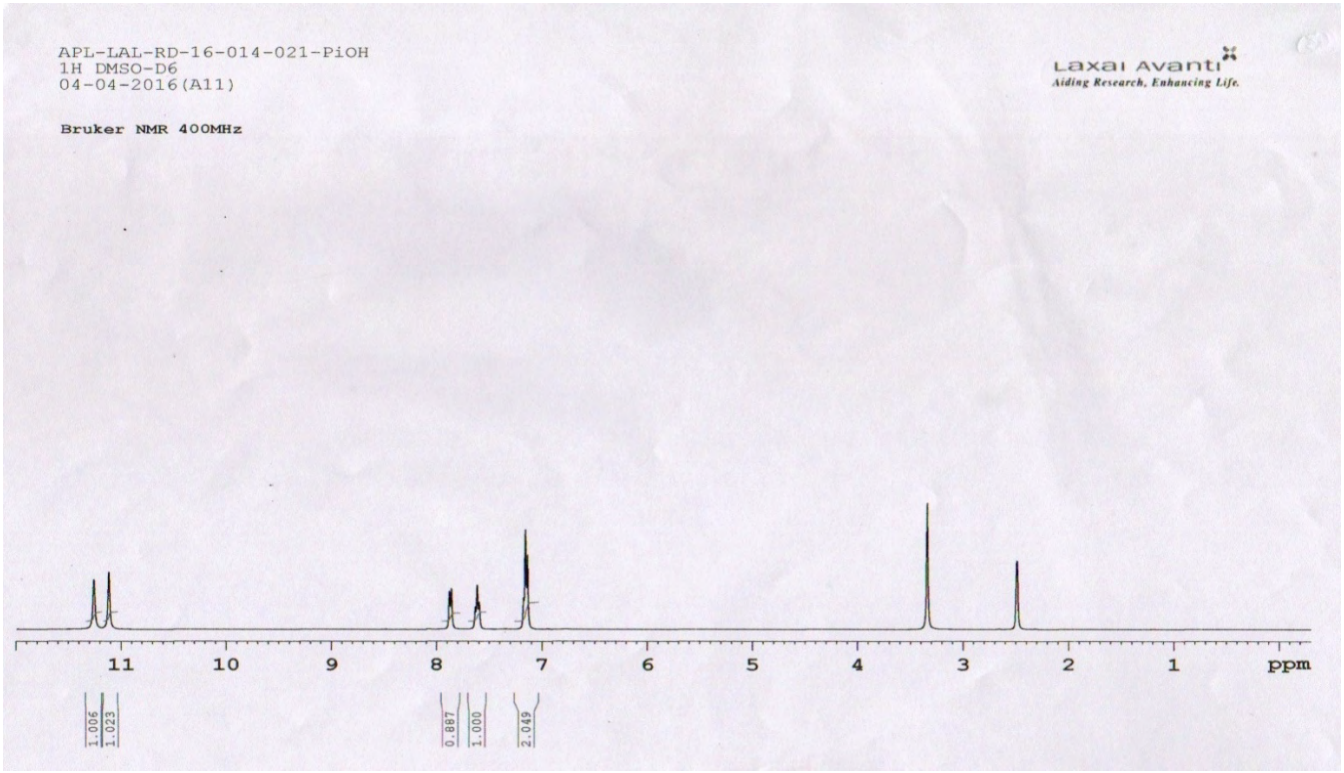


Figure 4: ^{1}H NMR Spectra of 2,4 di hydroxy Quinazoline (Compound 2).

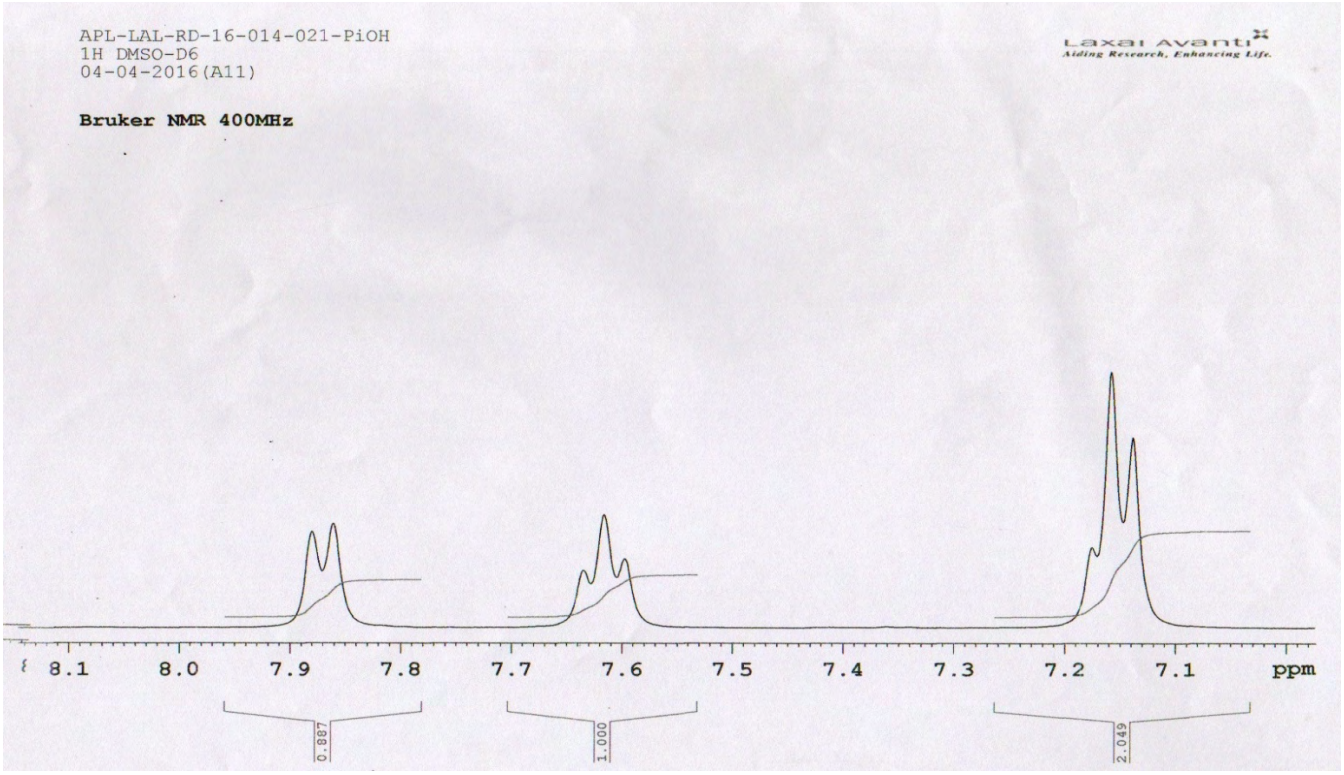


Figure 5: ^{1}H NMR Spectra Splitting pattern of 2,4 di hydroxy Quinazoline (compound 2).

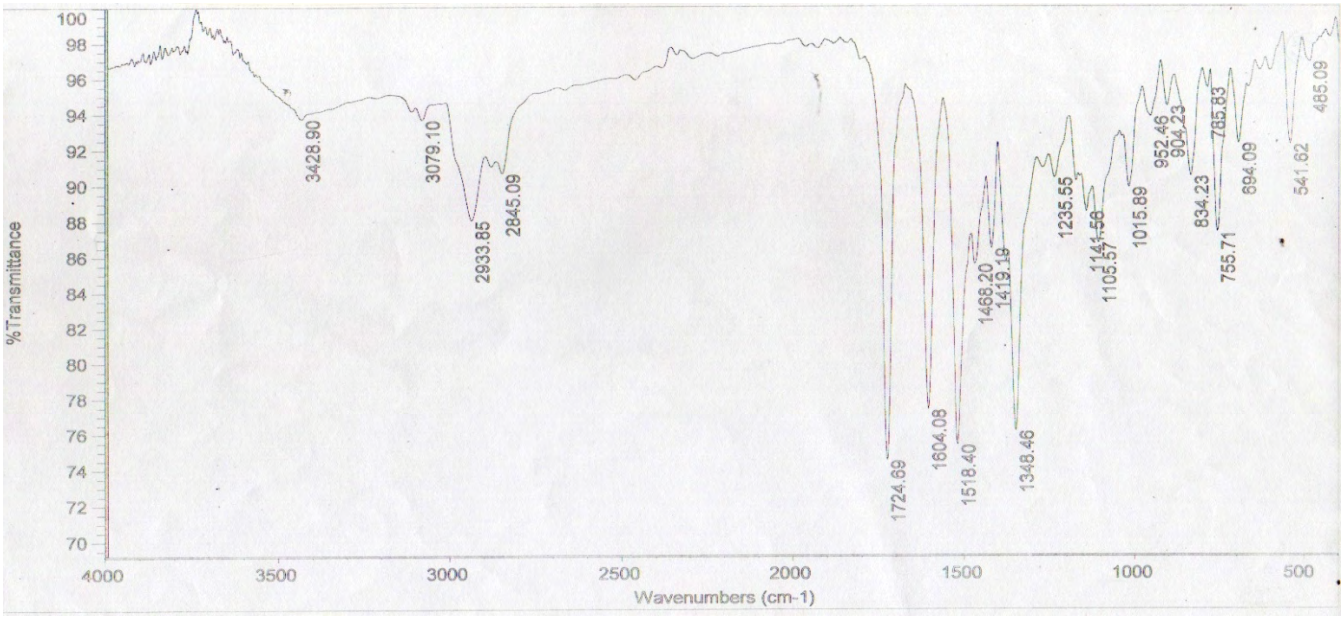


Figure 6: IR Spectra of 2,4 di hydroxy Quinazoline (compound 2).

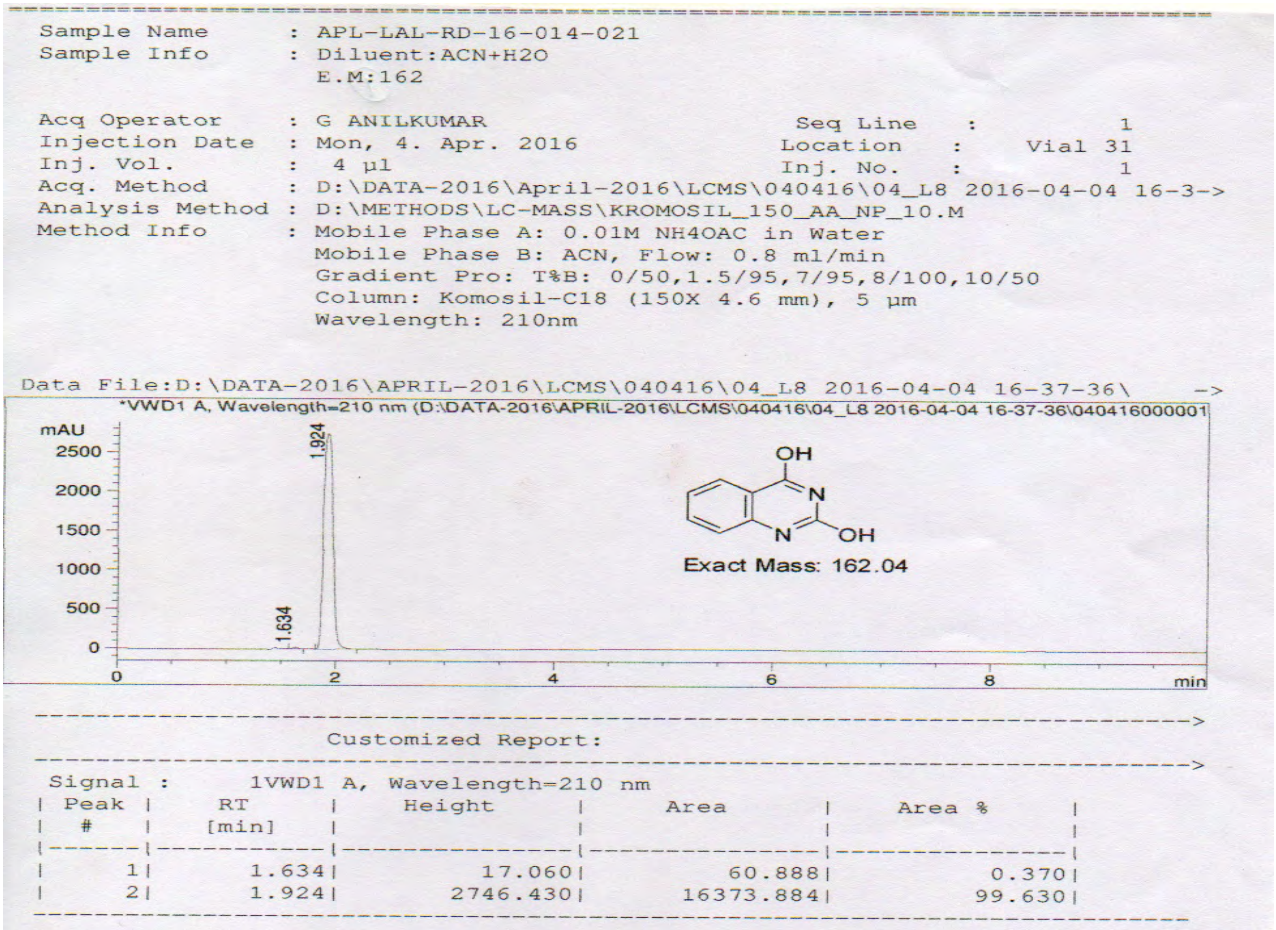


Figure 7: LCMS Data of 2,4 di hydroxy Quinazoline (compound 2).

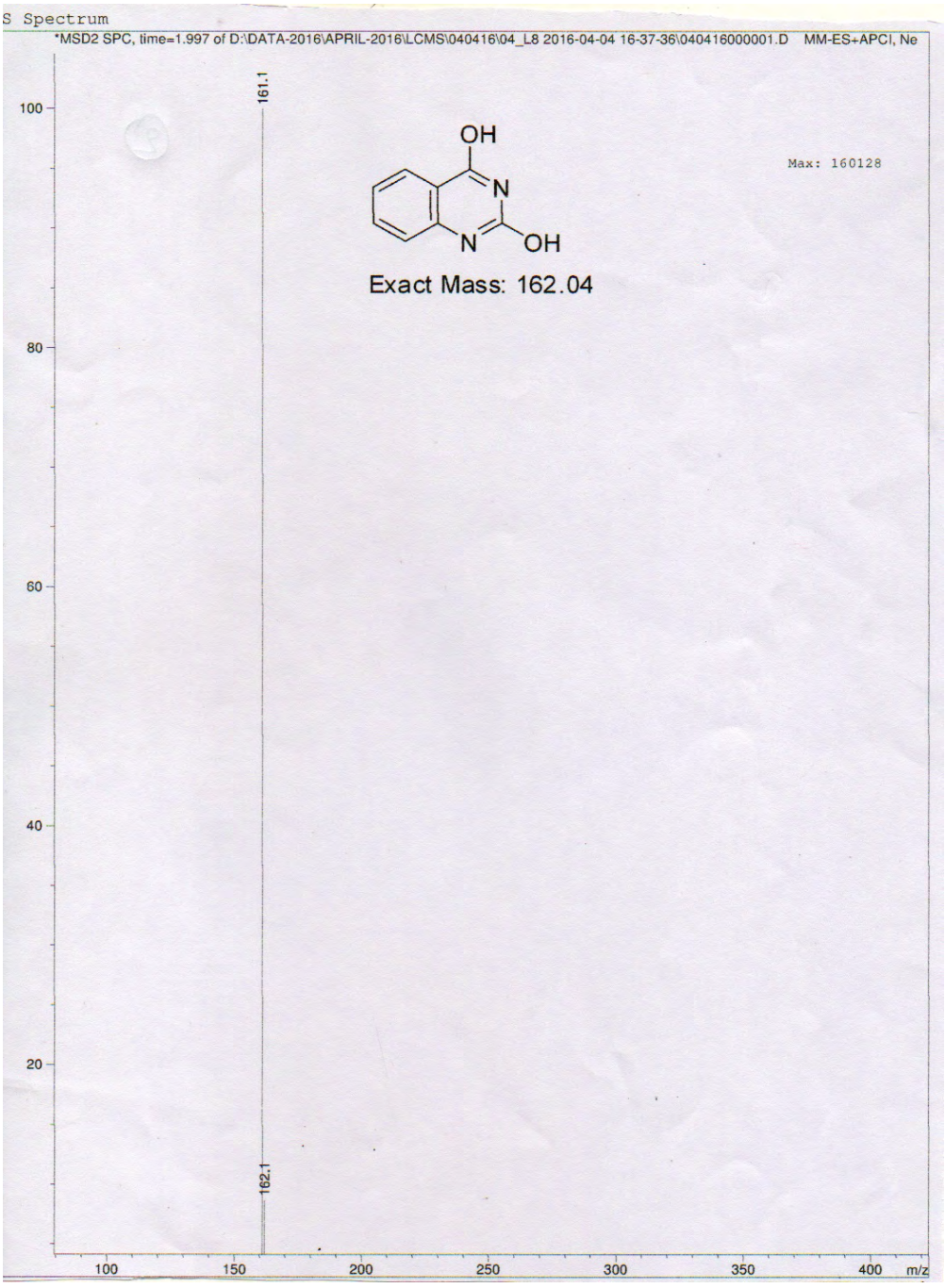


Figure 8: LCMS-Mass Spectra Data of 2,4 di hydroxy Quinazoline (compound 2).

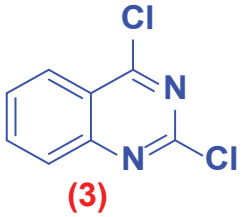


Figure 9: Structure of 2,4 di chloro Quinazoline (Compound 3).

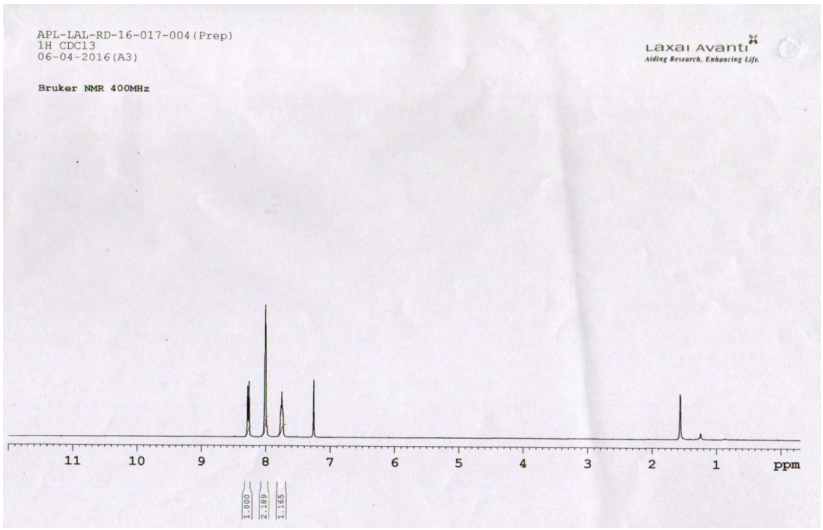


Figure 10: ^{1}H NMR Spectra of 2,4 di chloro Quinazoline (Compound 3).

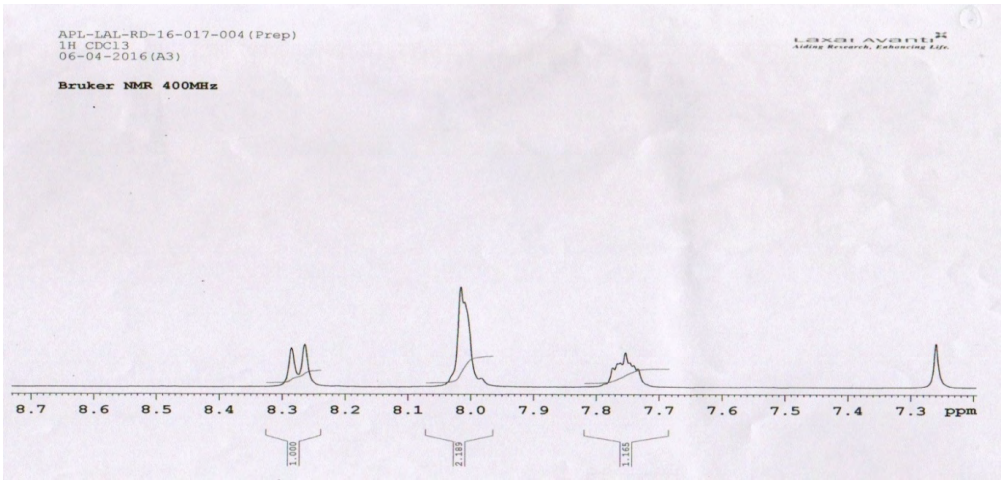


Figure 11: ^{1}H NMR Spectra Splitting pattern of 2,4 di chloro Quinazoline (Compound 3).

N-phenyl-4-thiomorpholinoquinazolin-2-amine (8a): From 4-thio morpholine quinazolin-2-amine (6) (**4.065 m.mol, 1 g**) and phenyl boronic acid (7a) (**6.097 m.mol, 745 mg**). The compound was obtained as off-white solid, 60% (**785 mg**) yield (Figure 16).

m.p. 256-258°C;

IR (KBr) cm^{-1}: 3360 (NH Stretching), 3060 (Ar CH Stretching), 1620 (C=N).

ESI-MS m/z 323[M+H]$^{+}$.

^{1}H NMR (400 MHz, DMSO-d$_6$) δ ppm 2.80(4H, t, 2 × CH_2 –**S**), 3.60(4H, t, 2 × CH_2 –**N**), 4.7(1H, bs),6.9(1H,t), 7.3(2H,m), 7.6(1H,t, Ar-H),7.7(2H,m),7.85(2H,m, Ar-H), 8.15(1H,d, Ar-H).

^{13}C NMR (100 MHz, DMSO-d$_6$) (δ/ppm): 28(2C, C-S), 53(2C, C-N), 115,123, 128, 130, 133,140, 153, 175, 180.

4-thiomorpholino-N-p-tolylquinazolin-2-amine (8b): From 4-thiomorpholinoquinazolin-2-amine (6) (**4.065 m.mol, 1 g**) and 4-methyl phenyl boronic acid (7b) (**6.097 m.mol, 830 mg**). The compound was obtained as light yellow powder, 62% (**846 mg**) yield (Figure 17).

m.p. 247-249°C;

IR (KBr) cm^{-1}: 3360 (NH Stretching), 3070 (CH aryl), 2960 (CH alkyl), 1610 (C=N).

ESI-MS m/z 337[M+H]$^{+}$, 359[M+Na]$^{+}$.

^{1}H NMR (400 MHz, DMSO-d$_6$) δ ppm 2.34(3H,S),2.80(4H, t, 2 × CH_2 –**S**), 3.55(4H, t, 2 × CH_2 –**N**), 4.4(1H, bs),6.9(2H,d), 7.3(2H,d), 7.6(1H,t, Ar-H), 7.80(2H,m, Ar-H), 8.15(1H,d, Ar-H).

^{13}C NMR (100 MHz, DMSO-d$_6$) (δ/ppm): 28(2C, C-S), 23(Aromatic methyl carbon),53(2C, C-N), 115,123, 126, 130, 133,138, 153, 175, 180.

N-(4-methoxyphenyl)-4-thiomorpholinoquinazolin-2-amine (8c): From 4-thiomorpholinoquinazolin-2-amine (6) (**4.065 m.mol, 1 g**) and 4-methoxy phenyl boronic acid (7c) (**6.097 m.mol, 926 mg**). The compound was obtained as off-white solid, 64% (**915 mg**) yield (Figure 18).

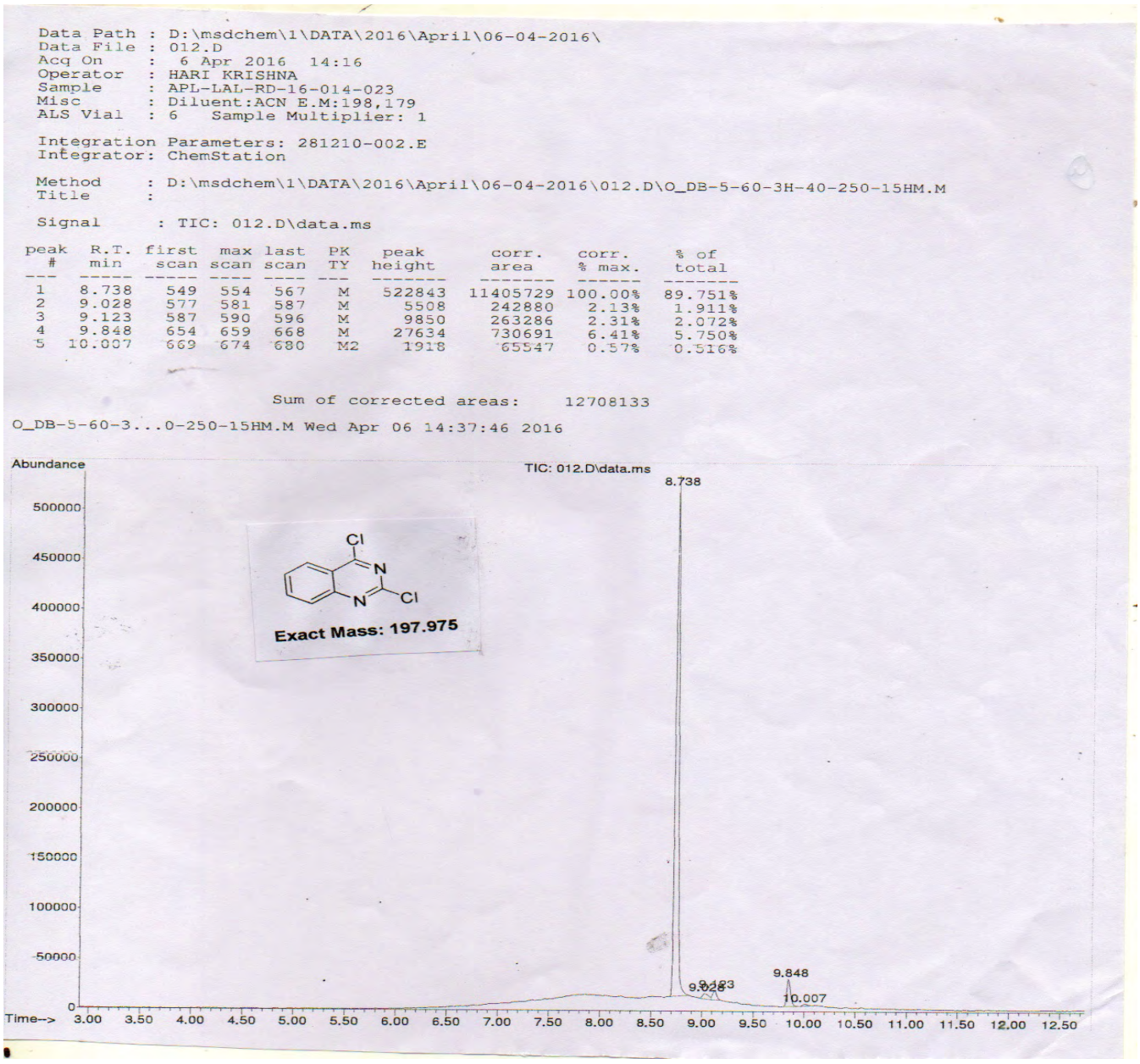


Figure 12: LCMS Data of 2,4 di chloro Quinazoline (Compound 3).

m.p. 238-240°C;

IR (KBr) cm^{-1}: 3340 (NH Stretching), 3090 (CH aryl), 2960 (CH alkyl), 1150(C-O-C), 1600 (C=N), 1590 (C=C);

ESI-MS m/z 353[M+H]$^+$, 375[M+Na]$^+$

^{1}H NMR (400 MHz, DMSO-d$_6$) δ ppm 2.80(4H, t, 2 × CH_2 –**S**), 3.55(4H, t, 2 × CH_2 –**N**), 3.9(3H,S,

-OCH_3),4.4(1H, bs),7(2H,d), 7.6(2H,d), 7.7(1H,t, Ar-H), 7.80(2H,m, Ar-H), 8.15(1H,d, Ar-H).

^{13}C NMR (100 MHz, DMSO-d$_6$) (δ/ppm): 29(2C, C-S), 53(2C, C-N), 56(Aromatic methyl carbon), 115,123, 126, 130, 133,138, 153, 175, 180.

4-thiomorpholino-N-(4-(tri fluoro methyl) phenyl) quinazolin-2-amine (8d): From 4-thiomorpholinoquinazolin-2-amine (6) (**4.065 m.mol, 1 g**) and 4-Trifluoro methyl phenyl boronic acid (7d) (**6.097 m.mol, 1158 mg**). The compound was obtained as light yellow solid, 63% (**998 mg**) yield (Figure 19).

M.p. 221-223°C;

IR (KBr) cm^{-1}: 3350 (NH Stretching), 3010 (CH aryl), 1340(C-F), 1640 (C=N), 1615 (C=C);

ESI-MS m/z 391[M+H]$^+$, 413[M+Na]$^+$.

^{1}H NMR (400 MHz, DMSO-d$_6$) δ ppm 2.80(4H, t, 2 × CH_2 –**S**), 3.55(4H, t, 2 × CH_2 –**N**), 4.6(1H, bs),7.4(2H,d), 7.55(2H,d), 7.7(1H,t, Ar-H), 7.80(2H,m, Ar-H), 8.15(1H,d, Ar-H).

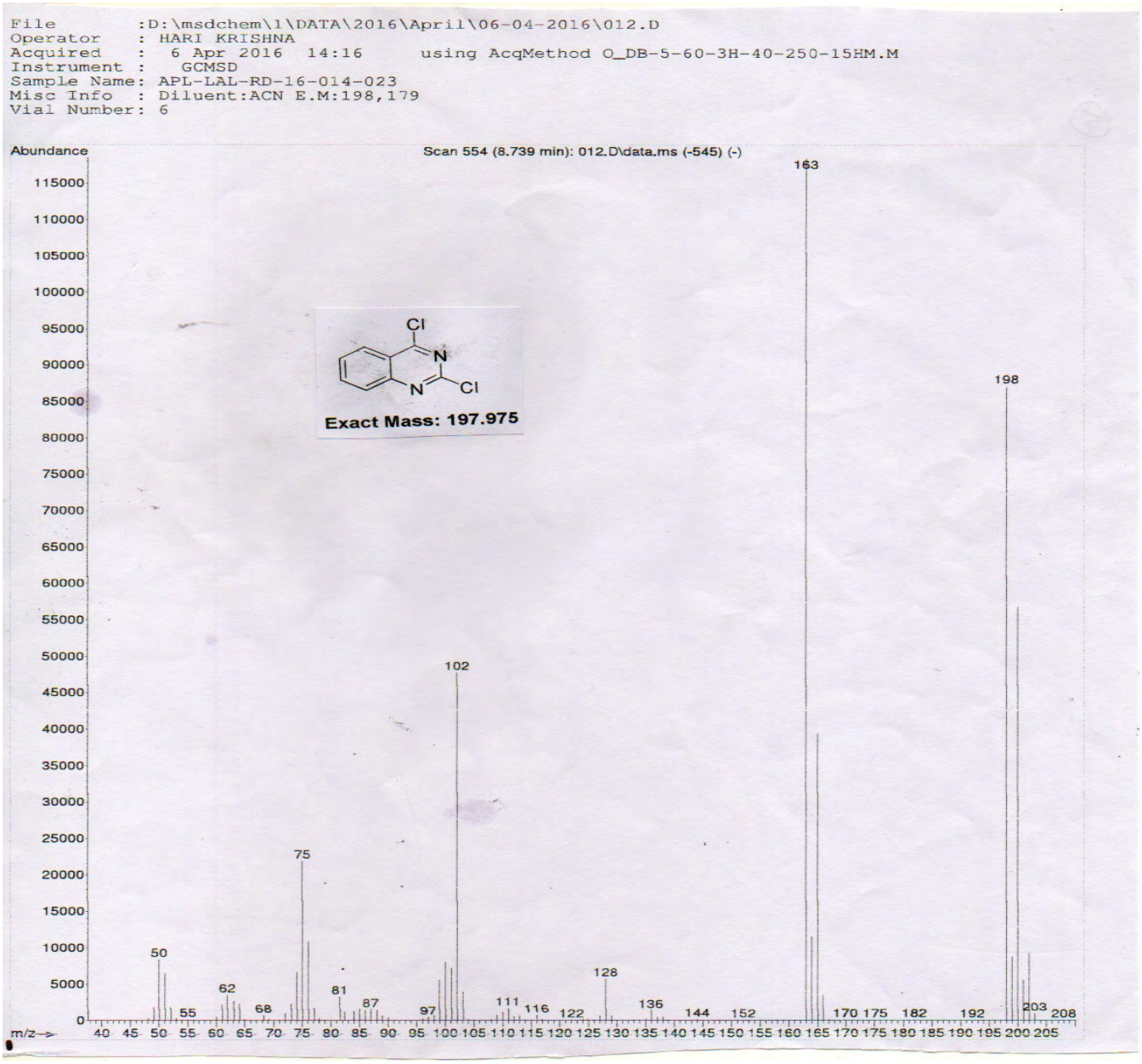


Figure 13: LCMS-Mass spectral Data of 2,4 di chloro Quinazoline (Compound 3).

^{13}C NMR (100 MHz, DMSO-d$_6$) (δ/ppm): 29(2C, C-S), 53(2C, C-N), 115,124, 126, 130, 133,138, 153, 175, 180.

N-(4-chloro phenyl)-4-thiomorpholinoquinazolin-2-amine (8e): From 4-thiomorpholinoquinazolin-2-amine (6) (**4.065 m.mol, 1 g**) and 4-chlorophenylboronic acid (7e) (**6.097 m.mol, 951 mg**). The compound was obtained as light yellow solid, 65% (**940 mg**) yield (Figure 20).

m.p. 177-179°C;

IR (KBr) cm^{-1}: 3360 (NH Stretching), 3010 (CH aryl), 1640 (C=N), 1615 (C=C); 746(C-Cl).

ESI-MS m/z 357[M+H]$^+$, 359[M+2]$^+$

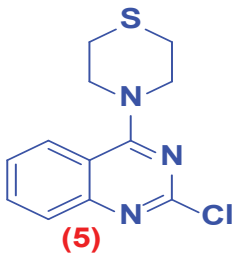
^{1}H NMR (400 MHz, DMSO-d$_6$) δ ppm 2.7(4H, t, 2 × CH_2 –**S**), 3.55(4H, t, 2 × CH_2 –**N**), 4.9(1H, bs),7.3(2H,d), 7.75(2H,d), 7.7(1H,t, Ar-H), 7.80(2H,m, Ar-H), 8.15(1H,d, Ar-H).

^{13}C NMR (100 MHz, DMSO-d$_6$) (δ/ppm): 30(2C, C-S), 53(2C, C-N), 115,124, 126, 130, 133,138, 153, 175, 180.

N-(4-bromophenyl)-4-thiomorpholinoquinazolin-2-amine (8f): From 4-thiomorpholinoquinazolin-2-amine (6) (**4.065 m.mol, 1 g**) and 4-bromophenylboronic acid (7f) (**6.097 m.mol, 1219 mg**). The compound was obtained as off-white solid, 64% (**1043 mg**) yield (Figure 21).

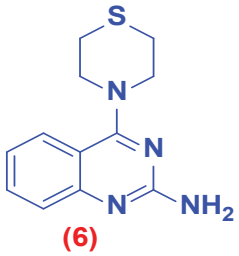
m.p. 248-250°C;

IR (KBr) cm^{-1}: 3360 (NH Stretching), 3010 (CH aryl), 1640 (C=N), 1615 (C=C); 546(C-Br).



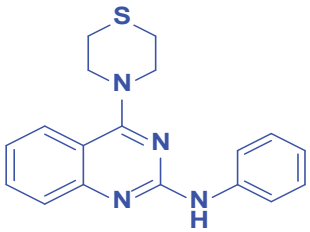
(5)

Figure 14: Structure of 4-(2-chloroquinazolin-4-yl) thiomorpholine (Compound 5).



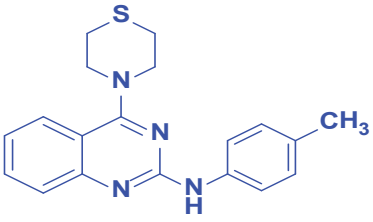
(6)

Figure 15: Structure of 4-thiomorpholinoquinazolin-2-amine [Compound 6].



***N*-phenyl-4-thiomorpholinoquinazolin-2-amine (8a)**

Figure 16: Structure of N-phenyl-4-thiomorpholinoquinazolin-2-amine (8a).



4-thiomorpholino-*N*-*p*-tolylquinazolin-2-amine (8b)

Figure 17: Structure of 4-thiomorpholino-N-p-tolylquinazolin-2-amine (8b).

ESI-MS m/z 401[M+H]$^+$, 403[M+2]$^+$

^{1}H NMR (400 MHz, DMSO-d$_6$) δ ppm 2.57(4H, t, 2 × CH_2 –**S**), 3.45(4H, t, 2 × CH_2 –**N**), 4.9(1H, bs),7.1(2H,d), 7.4(2H,d), 7.7(1H,t, Ar-H), 7.80(2H,m, Ar-H), 8.15(1H,d, Ar-H).

^{13}C NMR (100 MHz, DMSO-d$_6$) (δ/ppm): 28(2C, C-S), 53(2C, C-N), 115,124, 126, 130, 133,138, 153, 175, 180.

N-(4-nitrophenyl)-4-thiomorpholinoquinazolin-2-amine (8g): From 4-thiomorpholino quinazolin-2-amine (6) (**4.065 m.mol, 1 g**) and 4-nitrophenylboronic acid (7g) (**6.097 m.mol, 1018 mg**). The compound was obtained as pale yellow solid, 60% (**895 mg**) yield (Figure 22).

m.p. 234-236°C;

IR (KBr) cm^{-1}: 3360 (NH Stretching), 3010 (CH aryl), 1640 (C=N), 1615 (C=C); 1340 and 1560 (N-O Symmetric and Asymmetric stretching in Nitro Group).

ESI-MS m/z 368[M+H]$^+$, 390[M+Na]$^+$.

^{1}H NMR (400 MHz, DMSO-d$_6$) δ ppm 2.57(4H, t, 2 × CH_2 –**S**), 3.45(4H, t, 2 × CH_2 –**N**), 4.9(1H, bs),7.1(2H,d), 7.7(1H,t, Ar-H), 7.80(2H,m, Ar-H), 8.15(1H,d, Ar-H),8.2(2H,d).

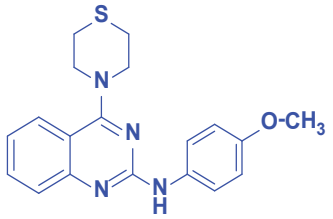
^{13}C NMR (100 MHz, DMSO-d$_6$) (δ/ppm): 30(2C, C-S), 55(2C, C-N), 115,124, 126, 130, 133,138, 153, 175, 180.

N-(pyridin-4-yl)-4-thiomorpholinoquinazolin-2-amine (8h): From 4-thiomorpholinoquinazolin-2-amine (6) (**4.065 m.mol, 1 g**) and pyridin-4-ylboronic acid (7h) (**6.097 m.mol, 750 mg**). The compound was obtained as pale yellow powder, 63% (**826 mg**) yield (Figure 23).

m.p. 237-239°C;

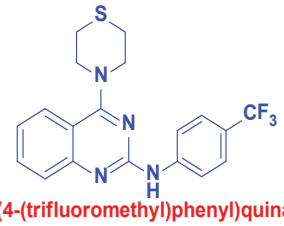
IR (KBr) cm^{-1}: 3370 (NH Stretching), 3019 (CH aryl), 1660 (C=N), 1645 (C=C);

ESI-MS m/z 324[M+H]$^+$, 346[M+Na]$^+$



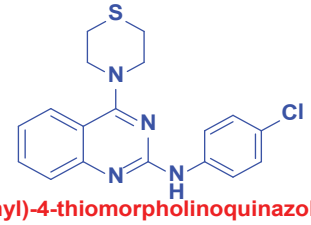
***N*-(4-methoxyphenyl)-4-thiomorpholinoquinazolin-2-amine (8c)**

Figure 18: Structure of N-(4-methoxy phenyl)-4-thiomorpholinoquinazolin-2-amine (8c).



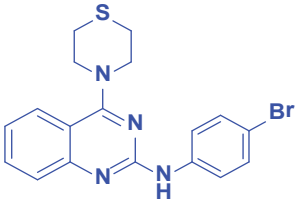
4-thiomorpholino-*N*-(4-(trifluoromethyl)phenyl)quinazolin-2-amine (8d)

Figure 19: Structure of 4-thiomorpholino-N-(4-(tri fluoro methyl)phenyl) quinazolin-2-amine (8d).



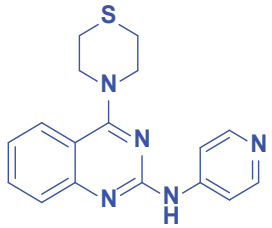
***N*-(4-chlorophenyl)-4-thiomorpholinoquinazolin-2-amine (8e)**

Figure 20: Structure of N-(4-chloro phenyl)-4-thiomorpholinoquinazolin-2-amine (8e).



N-(4-bromophenyl)-4-thiomorpholinoquinazolin-2-amine (8f)

Figure 21: Structure of N-(4-bromophenyl)-4-thiomorpholinoquinazolin-2-amine (8f).



N-(pyridin-4-yl)-4-thiomorpholinoquinazolin-2-amine (8h)

Figure 22: Structure of N-(4-nitrophenyl)-4-thiomorpholinoquinazolin-2-amine (8g).

^{1}H NMR (400 MHz, DMSO-d$_6$) δ ppm: 2.67(4H, t, 2 × CH_2 –**S**), 3.45(4H, t, 2 × CH_2 –**N**), 4.9(1H, bs),7.1(2H,d), 7.7(1H,t, Ar-H), 7.80(2H,m, Ar-H), 8.15(1H,d, Ar-H),8.5(2H,d).

^{13}C NMR (100 MHz, DMSO-d$_6$) (δ/ppm): 30(2C, C-S), 55(2C, C-N), 110,115,124, 126, 130, 133,138, 153, 175, 180.

4-thiomorpholino-N-(thiophen-2-yl)quinazolin-2-amine (8i): From 4-thio-morpholinoquinazolin-2-amine (6) (**4.065 m.mol, 1 g**) and thiophen-2-ylboronic acid (7i) (**6.097 m.mol, 780 mg**). The compound was obtained as light yellow solid, 60% (**800 mg**) yield (Figure 24).

m.p. 148-149°C;

IR (KBr) cm^{-1}: 3360 (NH Stretching), 3017 (CH aryl), 1640 (C=N), 1635 (C=C);

ESI-MS m/z 329[M+H]$^+$.

^{1}H NMR (400 MHz, DMSO-d$_6$) δ ppm : 2.7(4H, t, 2 × CH_2 –**S**), 3.45(4H, t, 2 × CH_2 –**N**), 4.9(1H, bs),6.1(1H,d),6.94(1H,d), 6.8(1H,t), 7.7(1H,t, Ar-H), 7.80(2H,m, Ar-H), 8.15(1H,d, Ar-H),8.5(2H,d).

^{13}C NMR (100 MHz, DMSO-d$_6$) (δ/ppm): 30(2C, C-S), 55(2C, C-N), 110,118,124, 126, 130, 133,138, 153, 175, 180.

N-(furan-2-yl)-4-thiomorpholinoquinazolin-2-amine (8j): From 4-thiomorpholinoquinazolin-2-amine (6) (**4.065 m.mol, 1 g**) and furan-2-ylboronic acid (7j) (**6.097 m.mol, 685 mg**). The compound was obtained as light yellow solid, 63% (**798 mg**) yield (Figure 25).

m.p. 138-140°C;

IR (KBr) cm^{-1}: 3380 (NH Stretching), 3017 (CH aryl), 1640 (C=N), 1055 (C-O-C);

ESI-MS m/z 313[M+H]$^+$.

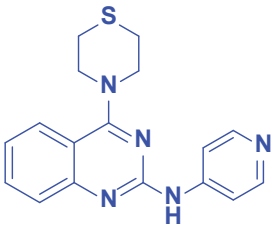
^{1}H NMR (400 MHz, DMSO-d$_6$) δ ppm: 2.7(4H, t, 2 × CH_2 –**S**), 3.45(4H, t, 2 × CH_2 –**N**), 4.9(1H, bs), 6.74(1H,d), 6.8(1H,t), 7.7(1H,t, Ar-H), 7.8(1H,d), 7.80(2H,m, Ar-H), 8.15(1H,d, Ar-H),8.5(2H,d).

^{13}C NMR (100 MHz, DMSO-d$_6$) (δ/ppm): 30(2C, C-S), 55(2C, C-N), 110,118,124, 126, 130, 133,138, 153, 175, 180.

Biological Activity

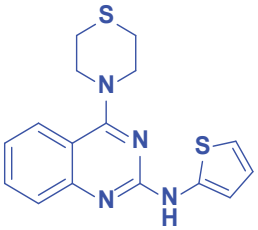
Antibacterial studies

The newly prepared compounds were screened for their antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli* (clinical isolate) bacterial strains by disc diffusion method. A standard inoculum (1-2 × 10^7 cfu/ml 0.5 McFarland standards) were introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums. The disks measuring 6 mm in diameters were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140°C for 1 h. The sterile disks previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. Amoxicillin (30 µg) was used as positive control and the disk poured in DMSO was used as negative control



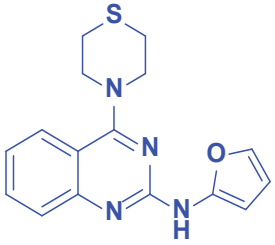
N-(pyridin-4-yl)-4-thiomorpholinoquinazolin-2-amine (8h)

Figure 23: Structure of N-(pyridin-4-yl)-4-thiomorpholinoquinazolin-2-amine (8h).



4-thiomorpholino-*N*-(thiophen-2-yl)quinazolin-2-amine (8i)

Figure 24: Structure of 4-thiomorpholino-N-(thiophen-2-yl)quinazolin-2-amine (8i).



N-(furan-2-yl)-4-thiomorpholinoquinazolin-2-amine (8j)

Figure 25: Structure of N-(furan-2-yl)-4-thiomorpholinoquinazolin-2-amine (8j).

and the test compounds were dissolved in DMSO at concentration of 100 and 50 µg/mL. The plates were inverted and incubated for 24 h at 37°C. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition of zone of measured and compared with controls. The bacterial zone of inhibition values are given in Table 1. The order of activity was 8i>8d>8h>8g>8j >8e >8f>8a>8b>8c (Table 1).

Antifungal studies

The newly prepared compounds were screened for their antifungal activity against *Candida albicans* and *Aspergillus flavus* in DMSO by agar diffusion method. Sabourauds agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting pH 5.7. Normal saline was used to make suspension of corresponding species. Twenty millilitres of agar media was poured into each Petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37°C for 1 h using an agar punch, wells were made and each well was labelled. A control was also prepared in triplicate and maintained at 37°C for 3-4 days. The fungal activity of each compound was compared with Fluconazole as a standard drug. Inhibition zone were measured and compared with the controls. The fungal zone of inhibition values are given in Table 2.

Results and Discussion

Chemistry

The reaction sequences Employed for synthesis of title compounds are shown. In the present work, the starting quinazoline-2,4-diol (2) was prepared from 2-aminobenzoic acid (1) and Urea according to synthetic procedure was prepared according to synthetic procedure [37]. 2,4-dichloroquinazoline (3) was prepared according to synthetic procedure [38]. The 4-(2-chloroquinazolin-4-yl)thiomorpholine (5) was prepared from thio-morpholine(4) and Compound (3) according to synthetic procedure [39], which on further treatment with aqueous ammonia to get 4-thiomorpholinoquinazolin-2-amine (6) according to synthetic procedure [40], which were treated with different substituted phenyl boronic acids and Heterocyclic boronic acids under Chan-lam coupling reaction conditions to get Target Novel Quinazoline derivatives (8a-j) according to synthetic procedure [41]. All compounds displayed IR, ^{1}H and ^{13}C NMR and mass spectra consistent with the assigned structures. ^{1}H NMR and IR spectrum of compounds (8 a-j) showed singlet at 2.3 ppm, 3.8 ppm are due to the aromatic methyl group protons and Aromatic methoxy group protons. The most characteristic IR absorption bands are at 1140 cm^{-1} (C-O-C), 740 cm^{-1} (C-Cl) and 1324 and 1552 cm^{-1} (N-O Stretching in Nitro group). The mass spectra of all the final derivatives showed comparable molecular ion peak with respect to molecular formula.

Anti-microbial studies

The newly synthesized compounds (8a-j) were screened for their *in vitro* anti-bacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli* using Amoxicillin as standard by disc diffusion method (zone of inhibition) [42,43]. The test compounds were dissolved in di methyl sulfoxide (DMSO) at concentrations of 50 and 100 µg/mL. The antibacterial screening revealed that all the tested compounds showed good inhibition against various tested microbial strains compared to the standard drug. Along with the synthesized compounds 8i, 8d, 8h, 8g were found to be more active against tested bacterial strains as compared to the standard. The *in vitro* antifungal activities for compounds 8a-8j were determined by agar diffusion method [43]. The results indicate that, among the tested compounds 8i, 8d, 8h, 8g were active against all tested fungal strains. The enhanced activities are due to electron withdrawing groups viz., -CF_3 and Thiophene ring attached to Quinazoline core ring. All other compounds such as phenyl ring with methyl and methoxy groups in Quinazoline core structure showed lesser antifungal activity as compared with standard Fluconazole. The Tables 1 and 2 depict the antimicrobial screening results of the final compounds.

Conclusion

The research study reports the successful synthesis and anti-microbial activity of novel Quinazoline as a core unit containing different Substituted Phenyl / Heterocyclic derivatives. The anti-microbial activity study revealed that all the tested compounds showed good antibacterial and antifungal activities against pathogenic strains. The structure and biological activity relationship of title compounds indicate that the presence of electron withdrawing groups like –CF_3 and Thiophene ring attached to the Quinazoline ring were responsible for good antimicrobial activity and hence compounds 8i, 8d, 8h and 8g exhibited more potent anti-microbial activity of all tested pathogenic strains.

Synthesised Compounds	Zone of inhibition measure in mm							
	Gram positive				Gram negative			
	Bacillus subtilis		*Staphylococcus aureus*		*Klebsiella pneumonia*		*Escherichia coli*	
	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL
8a	7.5	3.5	8	7	9.5	7	10.5	7.5
8b	7	4.5	7	4.5	8.5	6.5	9	7
8c	6	3	7.5	5	8	6	9.5	6
8d	13	10	14.5	10.5	15	13.5	16.5	12.5
8e	9.5	7	9.5	7.5	12	10	12.5	10.5
8f	8.5	6.5	9.0	6.5	10.15	8	11	8
8g	11	9.5	11.5	8.5	12.5	12	13	11.5
8h	11.5	9	12.5	11	14.5	11.5	15.5	12
8i	14	11	16	12.3	17	14.2	18	14
8j	10	8	11.1	9.5	12	11	13.5	11
Amoxicillin	16	13	18	14	18	14.8	20.3	15.5
Control **(DMSO)**	---	---	----	-----	----	-----	-----	-----

Table 1: Anti-bacterial activity of Novel Quinazoline Derivatives 8(a-j).

Zone of inhibition measure in mm				
Synthesised Compounds	***Candida albicans***		***Aspergillus flavus***	
	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL
8a	8.5	5	7.5	5.5
8b	8	5.5	7	3.5
8c	6.5	4.5	7	4
8d	15	13	13.5	12.5
8e	11	9	10	9
8f	9.5	7.5	8	6.5
8g	12.5	16	12	11
8h	13	11.5	12.5	10
8i	18.5	16.5	16	14
8j	12	11.6	11.5	10
Flucanazole	20	17	17.5	15
Control (DMSO)	--	--	--	--

Table 2: Anti-fungal activity of Novel Quinazoline Derivatives 8a-j.

Acknowledgments

Authors are thankful to our Research Supervisor Prof. K. Sudhakar Babu (KSB) Sir and L. K. Ravindranath Sir for providing us required facilities and motivation for completion of the Research work. We also extend our gratitude towards Laxai Avanti Life Sciences Pvt Ltd., Hyderabad for providing us facilities of IR Spectra, ^{1}H NMR for characterization of Novel Synthesized compounds.

References

1. Gabriel P (1903) Ber Dtsch Chem Ges 36: 800.
2. Oriefs (1869) Ber Dtsch Chem Ges 2: 416.
3. Weddige J (1887) Prakt Chem 2: 141.
4. Paal B (1889) Ber Dtsch Chem Ges 22: 2683.
5. Chandregowda V, Kush AK, Chandrasekara RG (2009) Synthesis and in vitro antitumor activities of novel 4-anilinoquinazoline derivatives. Eur J Med Chem 44: 3046-3055.
6. Al-Rashood ST, Aboldahab IA, Nagi MN, Abouzeid LA, Abdel-Aziz AA, et al. (2006) Synthesis, dihydrofolate reductase inhibition, antitumor testing, and molecular modeling study of some new 4(3H)-quinazolinone analogs. Bioorg Med Chem 14: 8608-8621.
7. Vasdev N, Dorff PN, Gibbs AR, Nandanan E, Reid LM, et al. (2005) Synthesis of 6-acrylamido-4-(2-[18F] fluoroanilino) quinazoline: A prospective irreversible EGFR binding probe. J Lablelled Compd Rad 48: 109-115.
8. Wakeling AE, Guy SP, Woodburn JR, Ashton SE, Curry BJ, et al. (2002) ZD1839 (Iressa): an orally active inhibitor of epidermal growth factor signalling with potential for cancer therapy. Cancer Res 62: 5749-5754.
9. Alagarsamy V, Solomon VR, Dhanabal K (2007) Synthesis and pharmacological evaluation of some 3-phenyl-2-substituted-3H -quinazolin-4-one as analgesic, anti-inflammatory agents. Bioorg Med Chem 15: 235-241.
10. Baba A, Kawamura N, Makino H, Ohta Y, Taketomi S, et al. (1996) Studies on disease-modifying antirheumatic drugs: synthesis of novel quinoline and quinazoline derivatives and their anti-inflammatory effect. J Med Chem 39: 5176-5182.
11. Rohini R, Muralidhar RP, Shanker K, Hu A, Ravinder V (2010) Antimicrobial study of newly synthesized 6-substituted indolo[1,2-c]quinazolines. Eur J Med Chem 45: 1200-1205.
12. Antipenko L, Karpenko A, Kovalenko S, Katsev A, Komarovska-Porokhnyavets E, et al. (2009) Synthesis of new 2-thio-[1,2,4]triazolo[1,5-c] quinazoline derivatives and its antimicrobial activity. Chem Pharm Bull 57: 580-585.
13. Jatav V, Kashaw S, Mishra P, Gupta V (2008) Synthesis and antimicrobial activity of some new 3-[5-(4-substituted)phenyl-1,3,4-oxadiazole-2yl]-2-styrylquinazoline- 4(3H)-ones. Med Chem Res 17: 205-211.
14. Aly AA (2003) Synthesis of novel quinazoline derivatives as antimicrobial agents. Chin J Chem 21: 339-346.
15. Li H, Huang R, Qiu D, Yang Z, Liu X (1998) Synthesis and bioactivity of 4-quinazoline oxime ethers. Prog Nat Sci 8: 359-365.
16. Chandrika PM, Yakaiah T, Narsaiah B, Sridhar V, Venugopal G (2009) Synthesis leading to novel 2,4,6-trisubstituted quinazoline derivatives, their antibacterial and cytotoxic activity against THP-1, HL-60 and A375 cell lines. Indian J Chem 48B: 840-847.
17. Paneersalvam P, Raj T, Ishar PSM, Singh B, Sharma V (2010) Anticonvulsant activity of Schiff bases of 3-amino-6,8-dibromo-2-phenylquinazolin-4(3H)-ones. Indian J Pharm Sci 72: 375-378.
18. Nandy P, Vishalakshi MT, Bhat AR (2006) Synthesis and antitubercular activity of Mannich bases of 2-methyl-3H-quinazolin-4-ones. Indian J Heterocycl Chem 15: 293-294.
19. Saravanan G, Alagarsamy V, Prakash CR (2010) Synthesis and evaluation of antioxidant activities of novel quinazoline derivatives. Int J Pharm Pharm Sci 2: 83-86.
20. Lakhan R, Singh OP, Singh JRL (1987) Studies on 4 (3H)-quinazolinone derivatives as anti-malarials. J Indian Chem Soc 64: 316-318.
21. Hess HJ, Cronin TH, Scriabine A (1968) Antihypertensive 2-amino-4(3H)-quinazolinones. J Med Chem 11: 130-136.
22. Sasmal S, Balaji G, Kanna Reddy HR, Balasubrahmanyam D, Srinivas G, et al. (2012) Design and optimization of quinazoline derivatives as melanin concentrating hormone receptor 1 (MCHR1) antagonists. Bioorg Med Chem Lett 22: 3157-3162.
23. Alvarado M, Barceló M, Carro L, Masaguer CF, Raviña E (2006) Synthesis and biological evaluation of new quinazoline and cinnoline derivatives as potential atypical antipsychotics. Chem Biodivers 3: 106-117.
24. Malamas MS, Millen J (1991) Quinazoline acetic acids and related analogs as aldose reductase inhibitors. J Med Chem 34: 1492-1503.
25. Fry DW, Kraker AJ, McMichael A, Ambroso LA, Nelson JM, et al. (1994) A specific inhibitor of the epidermal growth factor receptor. A J Science 265: 1093.
26. Colotta V, Catarzi D, Varano F, Lenzi O, Filacchioni G (2006) Structural Investigation of the 7-Chloro-3-hydroxy-1H-quinazoline-2,4-dione Scaffold to Obtain AMPA and Kainate Receptor Selective Antagonists. Synthesis, Pharmacological, and Molecular Modeling Studies. J Med Chem 49: 6015-6026.
27. Doyle LA, Ross DD (2003) Multidrug resistance mediated by the breast cancer resistance protein BCRP (ABCG2) Oncogene 22: 7340-7358.
28. Henderson EA, Bavetsias V, Theti DS, Wilson SC, Clauss R, et al. (2006) Targeting the α-folate receptor with cyclopenta [g]quinazoline-based inhibitors of thymidylate synthase Bioorg Med Chem 14: 5020-5045.
29. Foster A, Coffrey HA, Morin MJ, Rastinejad F (1999) Pharmacological Rescue of Mutant p53 Conformation and Function. Science 286: 2507-2510.
30. Chien TC, Chen CS, Yu FH, Chern JW (2004) Nucleosides XI. Synthesis and Antiviral Evaluation of 5′-Alkylthio-5′-deoxy Quinazolinone Nucleoside Derivatives as S-Adenosyl-L-homocysteine Analogs. Chem Pharm Bull 52: 1422.
31. Herget T, Freitag M, Morbitzer M, Kupfer R, Stamminger T, et al. (2004) Novel Chemical Class of pUL97 Protein Kinase-Specific Inhibitors with Strong Anticytomegaloviral Activity. Antimicrob Agents Chemother 48: 4154-4162.

32. Waisser K, Gregor J, Dostal H, Kunes J, Kubicova L, et al. (2001) J Farmaco 56: 803

33. Kunes J, Bazant J, Pour M, Waisser K, Slosarek M, et al. (2000) Quinazoline derivatives with antitubercular activity. J Farmaco 55: 725-729.

34. Gundla R, Kazemi R, Sanam R, Muttineni R, Sarma JARP (2008) Discovery of Novel Small-Molecule Inhibitors of Human Epidermal Growth Factor Receptor-2: Combined Ligand and Target-Based Approach. N J Med Chem 51: 3367-3377.

35. Mendes da Silva JF, Walters M, Al-Damluji S, Ganellin CR 2008 Bioorg Med Chem 16: 7254.

36. Rewcastle GW, Palmer BD, Bridges AJ, Showalter HD, Sun L, et al. (1996) Tyrosine kinase inhibitors. 9. Synthesis and evaluation of fused tricyclic quinazoline analogues as ATP site inhibitors of the tyrosine kinase activity of the epidermal growth factor receptor. J Med Chem 39: 918-928.

37. Luth A, Lowe W (2008) Syntheses of 4-(indole-3-yl)quinazolines - A new class of epidermal growth factor receptor tyrosine kinase inhibitors. Eur J Med Chem 43: 1478-1488.

38. Zhu L, Jing J, Chang L, Zhang C, Sun Y, et al. (2011) Synthesis and biological evaluation of novel quinazoline-derived human Pin1 inhibitors. Bioorganic and Medicinal Chemistry, 19: 2797-2807.

39. Smits RA, Herman DL, Meer TVD, Sebastiaan K, Karin B, et al. (2012) Ligand based design of novel Histamine H_4 receptor antagonists; fragment optimization and analysis of binding kinetics Bioorganic & Medicinal Chemistry Letters 22: 461-467.

40. Zhu W, Yajing L, Xin Z, Xiao W, Yan Z, et al. (2012) Design,Synthesis and 3-D QSAR analysis of Novel 2-hydrazinyl -4-morpholino Thieno[3,2-d]pyrimidine derivatives as potential anti-tumor agents. European Journal of Medicinal Chemistry. 57: 162-175.

41. Nora D, Anna F, Zoltan H (2014) Coupling of Boronic Acids with Amines in the Presence of a Supported Copper Catalyst. Catal Lett 144: 1547-1551.

42. Collins CH (1976) Microbiological methods. Butterworth, London, UK.

43. Varma RS (1998) Anti-fungal agents: past, present and future prospects. National Academy of Chemistry & Biology, Lucknow, India.