Design, Synthesis, Characterization and Antimicrobial Evaluation of Novel 2,4-Disubstituted Quinazoline Derivatives

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ABSTRACT

Resistance to bacterial and fungal strains towards existing antimicrobial agents has become an increasing incietment for research and development of new molecules to guard them. Quinazoline ring system could be clubbed into many ring systems which lead to potent and highly active compounds. In this work, series of eighteen compounds were docked into the ecKAS III binding site (1HNJ, PDB ID) by using schrodinger software. Out of which, six derivatives showed highest glide score. A simple method has been developed for synthesis of six N'-(2-{[2-substituted phenyl)-2-oxoethyl]sulfanyl }quinazolin-4-yl) pyridine-4-carbohydrazide derivatives by condensing N'-(2-sulfanylquinazolin-4-yl)pyridine-4-carbohydrazide with substituted phenacyl bromides. The structures of these synthesized compounds of 5A series are confirmed by elemental analysis and spectral data. Screening of some selected compounds was carried out for antibacterial and antifungal activities by serial dilution method. The result of title compounds revealed that most of the compounds possess high to moderate activity against tested bacterial and fungal strains. Morever it indicates that, among the prepared compounds, 5A2 and 5A3 have higher inhibitory effect at MIC ranging from 0.4 to 12.5 µg/ml, while compounds 5A1 and 5A6 have moderate inhibitory effect with MIC between 12.5-50 µg/ml on the growth of gram positive bacterial strains. Especially, compounds 5A2 and 5A7 were found to be highly active against fungi at MIC 0.8 µg/ml each comparable with ciprofloxacin and fluconazole respectively. Molecular modeling studies have suggested probable inhibition mechanism making it the most potent antimicrobial agents in the series.

Keywords: Quinazoline; Phenacyl bromide; Molecular docking; Antimicrobial activity

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INTRODUCTION

Infections triggered by some pathogenic microorganisms can bring illnesses even a fatal one [1]. The WHO report of 2014 about antimicrobial resistance notify that resistance to common bacteria, viruses and fungi has reached startle levels in many parts of the world, and a post-antibiotic era, in which common infections and minor injuries can kill, is a very real possibility for the 21st century [2]. For instance, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are important pathogens causing invasive diseases such as bacteremia, meningitis, necrotizing fasciitis, pneumonia, nosocomial pneumonia, cystic fibrosis, acute leukemia, organ transplants, and intravenous-drug addiction. Some of these pathogens have been reported to develop resistance to the well-known commercially available drugs [3]. Among various heterocyclic compounds, Quinazoline motifs have extensively gained considerable importance owing to their wide gamut of biological activities including antibacterial antiviral , antifungal, antimalarial, antihypertensive, anticancer, diuretic, COX-2 inhibitory analgesic and anti-

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inflammatory activities [4-7]. Previous reports demonstrated that some alkylaminoquinazoline derivatives are observed to restore antibiotic activity in Gram-negative resistant isolates [8]. Moreover, fused quinazoline derivatives such as 1,2,4-triazino [3,4-c] quinazoline, benzimidazo[1,2-c] quinazoline [9], 6-fluoro-4-alkyl(aryl)thioquinazoline [10], 2-(4-chloro styryl) quinazoline and 2-Imidazolyl-*N*-(4-oxo-quinazolin-3(4*H*)-yl)-acetamides [11], N-phenyl-2-substituted phenyl amino quinazoline and many others[12] have also interesting antimicrobial properties. Recently some oxadiazolyl methyloxy quinazolines, pyrazolyl acetoxy methyl quinazolines are known to inhibit DNA gyrase [13]. 6-Aryl-2-Styrylquinazolin-4(3H)-ones bind within the active site of the dihydrofolate reductase and thymidylate synthase enzymes with better chemotherapeutic activity [14]. It was also reported that 1-methyl-3-substituted quinazoline-2,4-dione derivatives inhibit chitin synthase (CHS) and had strong inhibitory potential [15]. Structure activity relationship studies of quinazoline ring system revealed in aforementioned literature suggests position 3, 4 and 6 are very much important for structure activity studies and position-2 should be attached to different heterocyclic rings for better chemotherapeutic activity. In a similar study isoniazid incorporated styryl quinazolinones [16] , Azetidinyl-3-(isonicotinamide-yl)-6-iodo-quinazolin-4-ones were also well documented as antimicrobial as well as antimycobacterial agents [17].

During recent years, different kinds of targets in key areas of the bacterial cell cycle have been studied that would be a new approach against the problem of drug resistance. In bacteria, there seem to be three KAS enzymes, which are denoted as KAS I, KAS II, and KAS III (the *Escherichia coli* equivalents are FabB, FabF, and FabH, respectively). Among the related FAS II enzymes, the condensing protein, β-ketoacyl-acyl carrier protein synthase (KAS), is an intrinsic target for potential antibacterial drug design [18]. Reports regarding salicylanides, 1,3,4 oxadiazole and S-substituted phenacyl-1,3,4-thiadiazole-thiol derivatives as excellent inhibitors of β-ketoacyl-acyl carrier protein synthase III with antimicrobial activity against various strains are available in literature [19-21].This possible beneficial range of biological properties of quinazoline derivatives made them efficient synthetic molecular targets for many researchers. In view of this no reports were found in which quinazoline is clubbed with isoniazid and substituted at C-2 position through various phenacyl bromides as ecKAS III synthase inhibitors.

Inspired from these observations, in present study, firstly we designed top six *N'*-(2-{[2-(substituted phenyl)-2-oxoethyl]sulfanyl }quinazolin-4-yl) pyridine-4-carbohydrazide compounds which possess well binding affinities (Glide score, binding free energies, and hydrogen bonding) by docking into the active site of ecKAS III synthase (ecKAS III pdb id: 1HNJ) as a receptor highly active against growth of bacteria [22]. These 2,4 -disubstituted quinazoline derivatives are further synthesized and were picked out to test their antibacterial and antifungal activities against gram-negative and gram positive bacterial strains as well as two fungal strains.

MATERIALS AND METHODS

All chemicals used (analytical grade) procured from Merck and Sigma Aldrich. Melting points were determined by the open tube capillary method and are uncorrected. The reaction was monitored by thin layer chromatography (TLC) on pre-coated silica gel 60 F_{254} plates (Merck silica 100-200 mesh, Germany) using ethyl acetate: n-hexane (8:2) as eluent. The IR spectra were recorded on a Shimadzu 8400 FT-IR spectrometer (KBr pellets). The ^{1}H NMR and ^{13}C NMR spectra were obtained from a Bruker Ascend[TM] 500 FT-NMR spectrometer using TMS as the internal standard in DMSO-d_6. The mass spectra were recorded on Bruker Daltonik GmbH, Mass spectrometer ESI. Satisfactory analysis for C, H, and N on EuroVector E 3000 elemental analyser was obtained for the compounds within ± 0.4 % of the theoretical values. The following organisms were used in the antibacterial and antifungal screening, *Bacillus subtilis* ATCC-60511, *Staphylococcus aureus* ATCC 11632, *Staphylococcus epidermidis* ATCC 155, *Escherichia coli* ATCC 10536, *Klebsiella pneumoniae* ATCC 11298, *Pseudomonas aeruginosa* ATCC 10145, and *Candida albicans* ATCC 2091, *Aspergillus niger* ATCC 6275 respectively. The molecular docking study was carried out by using the software Schrodinger Maestro, version 9.5, LLC, New York, NY, 2013.

General procedure for synthesis of *N'*-(2-{[2-(substituted phenyl)-2-oxoethyl] sulfanyl} quinazolin-4-yl) pyridine-4-carbohydrazide derivatives (5A1-5A3, 5A6-5A7 and 5A10)
To a mixture of intermediate 2-(2-sulfanylquinazolin-4-yl) pyridine-4-carbohydrazide (0.004 mol) compound (**4**) and anhydrous potassium carbonate (0.006 mol) in DMF (15ml), substituted phenacyl bromide (0.004 mol) was added portion wise with stirring. The stirring was continued for 5 hr at room temperature. The reaction mixture was further refluxed for 2 hr on oil bath. After completion of reaction the mixture was poured into ice cold water, neutralized with glacial acetic acid. The solid mass obtained was filtered, dried and purified by recrystallization with suitable solvent. All the titled compounds were prepared as per the literature procedures with slight modification [23-26].
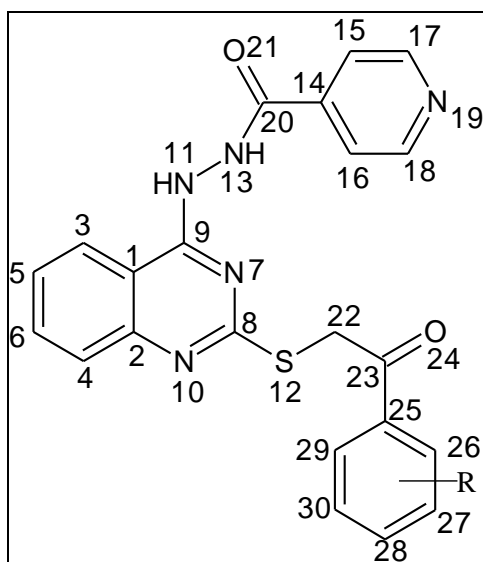
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Figure 1: Numbering system for ^{13}C NMR of compounds

The spectral characterizations of synthesized derivatives are given below

Compound 5A1: N'-(2-{[2-(4-fluorophenyl)-2-oxoethyl]sulfanyl}quinazolin-4-yl)pyridine-4-carbohydrazide
IR (KBr)υ: 3304.14 (N-H str), 3053.42 (C-H str aromatic), 2922.25, 2847.03 (C-H str aliphatic), 1699.34, 1672.42(C=O str), 1540.26(C=N str), 1604.83, 1488.95 (C=C str), 1500.67 (N-H bending), 1444.73 (C-H bending), 1294.28 (C-N str), 1232.55(N-N str), 1151.54(C-S str), 761.91 (C-F str), 565.16 (C-CO-C bend) cm^{-1}. ^{1}H NMR (500MHz, DMSO-d$_6$) δ ppm: 11.15 (s, 1H, NH-CO), 7.15-8.85 (m, 12H, Ar-H), 6.59 (s, 1H, NH), 4.42 (s, 2H, SCH$_2$); ^{13}C NMR (DMSO-d$_6$) δ ppm: 193.80 (C$_{23}$, C=O), 169.24(C, C$_8$), 163.30 (C$_{28}$, C-F), 156.76 (C$_{20}$, CONH), 150.76 (C, C$_9$, C$_{17-18}$) 147.62 (C, C$_2$), 141.33 (C, C$_{14}$), 135.42 (C, C$_{26}$, C$_{29}$), 114.79-127.41 (C, C$_1$,C$_{3-6}$, C$_{15-16}$, C$_{25}$), 39.48(C$_{22}$, CH$_2$); HRMS (ESI), Found: 434.1471(M+H). Anal calcd for C$_{22}$H$_{16}$FN$_5$O$_2$S: C, 60.96; H, 3.72; N, 16.16; Found: C, 60.88; H, 3.59; N, 16.11.

Compound 5A2: N'-(2-{[2-(4-bromophenyl)-2-oxoethyl]sulfanyl }quinazolin-4-yl)pyridine-4-carbohydrazide
IR (KBr) υ : 3314.53(N-H str), 3051.79(C-H str aromatic), 2918.40, 2847.03 (C-H str aliphatic), 1705.13, 1657. 87(C=O str), 1559.14(C=N str) 1618.33, 1498.74(C=C str), 1512.23 (N-H bending), 1444.73 (C-H bending), 1294.28 (C-N str), 1240.21(N-N str), 1182.40 (C-S str), 1041.60 (C-Br str), 565.16 (C-CO-C bend) cm^{-1}. ^{1}H NMR (500MHz, DMSO-d$_6$) δ ppm: 9.28 (s, 1H, NH-CO), 7.24-8.82 (m, 12H, Ar-H), 5.21 (s, 1H, NH), 4.15 (s, 2H, SCH$_2$); ^{13}C NMR (DMSO-d$_6$) δ ppm: 191.34 (C$_{23}$, C=O), 170.76 (C, C$_8$), 162.78 (C$_{20}$, CONH), 154.46 (C, C$_9$), 151.89 (C, C$_2$, C$_{17-18}$) 148.12 (C, C$_{25}$), 140.11 (C, C$_{14}$), 135.42 (C, C$_{27}$, C$_{30}$, C$_{28}$), 112.59-127.68(C, C$_1$,C$_{3-5}$, C$_{15-16}$, C$_{28-29}$), 39.21 (C$_{22}$, CH$_2$); HRMS (ESI), Found: 495.0919 (M+H). Anal calcd for C$_{22}$H$_{16}$BrN$_5$O$_2$S: C, 53.45; H, 3.26; N, 14.17; Found: C, 53.56; H, 3.19; N, 14.12.

Compound 5A3: N'-(2-{[2-(4-methylphenyl)-2-oxoethyl]sulfanyl }quinazolin-4-yl)pyridine-4-carbohydrazide
IR (KBr) υ: 3350.14 (N-H str), 3051.59 (C-H str Aromatic), 2918.40, 2847.03 (C-H str aliphatic), 1708.67, 1655.42 (C=O str for amide), 1567.16 (C=N str), 1618.33, 1478.74 (C=C str), 1498.74 (N-H bending), 1360.14 (C-CH$_3$ str. in aromatic ring), 1325.14 (C-N str), 1230.56(N-N str), 1141.90 (C-S str), 565.16 (C-CO-C bend) cm^{-1}. ^{1}H NMR (500MHz, DMSO-d$_6$) δ ppm: 9.15 (s, 1H, NH-CO), 7.17-8.81 (m, 12H, Ar-H), 4.07 (s, 1H, NH), 4.37 (s, 2H, SCH$_2$), 2.37 (s, 3H, CH$_3$); ^{13}C NMR (DMSO-d$_6$) δ ppm: 192.34 (C$_{23}$, C=O), 167.54(C, C$_8$), 163.30 (C$_{20}$, CONH), 154.47 (C, C$_9$), 150.76 (C, C$_{17-18}$), 146.82 (C, C$_2$), 141.32 (C$_{28}$, C-CH$_3$), 139.32 (C, C$_{14}$), 135.42 (C, C$_{25}$), 131.23 (C,C$_6$), 129.45 (C, C$_{27}$, C$_{30}$), 127.41 (C, C$_{26}$, C$_{29}$), 114.79-127.41(C, C$_{1-5}$, C$_{15-16}$), 39.99 (C$_{22}$, CH$_2$), 25.46 (C$_{31}$, CH$_3$). HRMS (ESI), Found: 430.1332 (M+H). Anal calcd for C$_{23}$H$_{19}$N$_5$O$_2$S: C, 64.32; H, 4.46; N, 16.31; Found: C, 64.19; H, 4.37; N,16.39 .

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Compound 5A6: N'-(2-{[2-(3-nitrophenyl)-2-oxoethyl]sulfanyl }quinazolin-4-yl)pyridine-4-carbohydrazide
IR (KBr) υ: 3339.19 (N-H str), 3053.42 (C-H str Aromatic), 2982.05, 2848.96 (C-H str aliphatic), 1711.34 , 1676.20 (C=O str), 1567.24, (C=N str), 1529.60 (-NO$_2$ aromatic), 1550.67 (N-H bending), 1608.69, 1488.74 (C=C str), 1429.30 (C-H bending), 1346.36 (C-N str), 1230.36 (N-N str), 1143.83 (C-S str), 636.53 (C-CO-C bend.) cm^{-1}. ^{1}H NMR (500MHz, DMSO-d$_6$) δ ppm: 11.28 (s, 1H, NH-CO), 7.17-8.83 (m, 12H, Ar-H), 6.70 (s, 1H, NH), 4.47 (s, 2H, SCH$_2$); ^{13}C NMR (DMSO-d$_6$) δ ppm: 187.12 (C$_{23}$, C=O), 171.34 (C, C$_8$), 163.30 (C$_{20}$, CONH), 156.78 (C, C$_9$), 150.76 (C, C$_{27}$, NO$_2$, C$_{17-18}$), 148.89 (C, C$_2$, C$_{25}$), 141.33 (C$_{14}$), 135.42 (C, C$_6$, C$_{29}$), 114.79-127.40 (C, C$_1$,C$_{3-5}$, C$_{15-16}$, C$_{26}$, C$_{28}$, C$_{30}$), 39.48 (C$_{22}$, CH$_2$); HRMS (ESI), Found: 461.1136 (M+H). Anal calcd for C$_{22}$H$_{16}$N$_6$O$_4$S: C, 57.38; H, 3.50; N,18.25; Found: C, 57.28; H, 3.56; N, 18.13.

Compound 5A7: N'-(2-{[2-(4-cyanophenyl)-2-oxoethyl] sulfanyl} quinazolin-4-yl) pyridine-4-carbohydrazide
IR (KBr) υ :3329.21 (N-H str), 3045.70 (C-H str Aromatic), 2978.19, 2848.96 (C-H str aliphatic), 2227.86 (C≡N str), 1710.45, 1674.27(C=O str), 1531.53 (C=N str), 1608.69, 1494.88 (C=C str), 1458.23, (C-H bending), 1292.35 (C-N str), 1228.70 (N-N str), 1143.83 (C-S str), 634.60 (C-CO-C bend) cm^{-1}. ^{1}H NMR (500MHz, DMSO-d6) δ ppm: 11.15 (s, 1H, NH-CO), 7.17-9.24 (m, 12H, Ar-H), 6.64 (s, 1H, NH), 4.46 (s, 2H, SCH$_2$); ^{13}C NMR (DMSO-d$_6$) δ ppm: 189.36 (C$_{23}$, C=O), 166.29 (C, C$_8$), 163.29 (C$_{20}$, CONH), 154.68 (C, C$_9$), 150.75(C, C$_{17-18}$), 149.36 (C, C$_2$), 141.32 (C, C$_{14}$), 135.42 (C, C$_{25}$), 133.15 (C, C$_{27}$, C$_{30}$), 127.40 (C, C$_6$,C$_{26}$, C$_{29}$), 114.79-125.31 (C, C$_1$,C$_{3-5}$, C$_{15-16}$, C$_{31}$),115.78 (C-28,CN), 39.81(C$_{22}$, CH$_2$); HRMS (ESI), found: 441.1563 (M+H). Anal calcd for C$_{23}$H$_{16}$N$_6$O$_2$S: C, 62.72; H, 3.66; N, 19.08; Found: C, 62.63; H, 3.56, N, 19.02.

Compound 5A10: N'-(2-{[2-(2-methoxyphenyl)-2-oxoethyl] sulfanyl} quinazoloin-4-yl) pyridine-4 Carbohydrazide
IR (KBr) υ : 3238.59 (N-H str), 3049.56 (C-H str aromatic), 2968.55, 2926.11(C-H str aliphatic), 2815.10 (CH$_3$-O-, C-H str), 1703.20, 1672.64 (C=O str), 1531.53 (C=N str), 1531.53(N-H bending), 1610.61, 1492.95 (C=C str), 1456.30, 1446.66 (C-H bending), 1330.93 (C-N str), 1292.35(ester C-O bend) 1246.06(N-N str), 1145.75(C-S str), 561.30 (C-CO-C bend) cm^{-1}. ^{1}H NMR (500MHz, DMSO-d$_6$) δ ppm: 11.161 (s, 1H, NH-CO), 7.11-9.26 (m, 12H, Ar-H), 6.34 (s, 1H, NH), 4.36 (s, 2H, SCH$_2$), 3.87 (s, 3H, OCH$_3$); ^{13}C NMR (DMSO-d$_6$) δ ppm: 192.26 (C$_{23}$, C=O), 167.57(C, C$_8$), 163.30 (C$_{20}$, CONH), 157.43 (C$_{26}$, OCH$_3$) 154. 68 (C, C$_9$), 150.75(C, C$_{17-18}$), 148.89 (C, C$_2$), 141.33(C, C$_{14}$), 135.42 (C, C$_{28}$), 131.39 (C, C$_6$, C$_{29}$), 111.50- 127.40 (C, C$_1$, C$_2$, C$_{4-5}$, C$_{15-16}$, C$_{25}$,C$_{27}$), 52.46 (C-32, OCH$_3$), 39.80 (C$_{22}$, CH$_2$); HRMS (ESI), found: 446.1804 (M+H). Anal calcd for C$_{23}$H$_{19}$N$_5$O$_3$S: C, 62.01; H, 4.30; N, 15.72; Found: C, 62.09; H, 4.21; N, 15. 64.

In vitro antimicrobial activity

The synthesized compounds of 5A series were tested for their in-vitro antibacterial activity against three gram-positive (*Bacillus subtilis, Staphylococcus aureus and Staphylococcus epidermidis*) and three gram-negative (*Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa*) bacterial strains and anti-fungal activity of the compounds was assayed against two fungal strains viz. (*Candida albicans and Aspergillus niger*) by serial plate dilution method [27-28]. The minimum inhibitory concentration (MIC, µg/ml) of the test compounds were visually determined as the lowest concentration of drug at which there was no visible growth of each strain. The synthesized compounds (test) compounds (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1ml), then diluted in culture medium (Mueller-Hinton Broth for bacteria and Sabouraud Liquid Medium for fungi), further increasing dilutions were done to obtain final concentrations of 0.2, 0.4, 0.8, 1.6, 3.12, 6.25, 12.5, 25, 50 and 100 µg/ml. 20 ml of agar media was poured into each petri dish. Excess suspension was decanted and plates were dried by placing in an incubator at 37°C for an hour. Using an agar punch, wells were made on these seeded agar plates and minimum inhibitory concentration of the test compounds in dimethylsulfoxide were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The petri dishes were prepared in triplicate and maintained at 37°C for 3-4 days. Ciprofloxacin and fluconazole were used as the standard drugs for antibacterial and antifungal activity respectively, while keeping DMSO as control which did not show inhibition against the tested organisms. Minimum inhibitory concentration (µg/ml) and diameter of the zone of inhibition (mm) was determined for all the synthesized compounds.

Molecular docking

With the aim to investigate the binding mode of newly synthesized compounds, molecular modeling study was performed by the means of Glide 6 (Schrodinger Maestro 9.5). The crystal structure of Escherichia coli β -Ketoacyl-acyl carrier protein synthase III having resolution of 1.46 Å was retrieved from the protein data bank (ecKAS III pdb id: 1HNJ) used as target for docking of synthesized compounds [29]. All molecules were effectively drawn in

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Maestro and converted into 3D conformations. The reported crystal structure is a monomer, having only one chain A with the inhibitor bound to it. The protein was prepared using protein preparation wizard and glide energy grids were generated for prepared protein complex. The binding site was defined by a grid box for the receptor was generated with a default inner box, which was centered on the corresponding ligand. The alignment was performed using the protein alignment module (Prime, Schrödinger). Bond orders and formal charges for hetero groups and hydrogens were added to all atoms in the system. Water molecules of crystallization beyond 5A° were removed from the complex except in the active site. In the protocol, The prepared ligands were docked against β-ketoacyl-acyl carrier protein synthase III the for the refinement and docking calculations, the default settings as available in the software package were used. Final scoring is then carried out on the energy-minimized poses. The minimized poses are rescored using Schrödingers proprietary Glide Score (g score) scoring function [30].

RESULT AND DISCUSSION

Chemistry

The synthetic approach leading to the target compound is illustrated in Scheme 1. 2-sulfanylquinazolin-4(3*H*)-one (2) was prepared from anthranilic acid (1) in thionyl chloride when heated at reflux for 2 hrs, dissolved in acetone and added to a suspension of NH_4SCN with stirring at room temperature. Upon stirring compound (2) in phosphorous oxychloride and N,N'-dimethylaniline under reflux at 108 ^oC for 3-4 hrs, residue was obtained. It was neutralized to pH 4-5 and further extracted with chloroform, it was removed under reduced pressure to yield 4-chloroquinazoline- 2-thiol (3). Refluxing mixture of (3) and pyridine-4-carbohydrazide in glacial acetic acid at 120^oC for 12-14 hrs, gave the corresponding *N'*-(2-sulfanylquinazolin-4-yl) pyridine-4-carbohydrazide (4) in 78-82% yield, reaction was monitored by TLC. Treatment of Intermediate (4) with appropriate phenacyl bromides in presence of K_2CO_3 and DMF heated under reflux for 2 hr in oil bath afforded a series of *N'*-(2-{[2-(substituted phenyl)-2-oxoethyl] sulfanyl} quinazolin-4-yl) pyridine-4-carbohydrazide derivatives (5A1-5A10), in quantitative yield. However treatment of intermediate *N'*-(2-sulfanylquinazolin-4-yl) pyridine-4-carbohydrazide (4) with four different phenacyl bromides yielded only a few mg of product in purified form, other derivatives which could not be purified are not reported. All the physicochemical data of the titled compounds (5A1-5A3, 5A6, 5A7 and 5A10) are presented in (Table-1). All the synthesized compounds were characterized by spectral data elemental analysis. The anthranilic acid to quinazolinone ring conversion in compound 2 (2-sulfanylquinazolin-4(3*H*)-one) was confirmed by C=O stretching observed at 1707.06 cm^{-1} and N-H, S-H and C=N stretching at 3373.81, 2573.13 and 1568.18 cm^{-1} respectively confirms that cyclization has been carried out successfully. Morever the ^{1}H NMR showed singlet at δ 12.69 for thiol proton. The formation of 4-chloroquinazoline-2-thiol (3) was confirmed by C-Cl str. observed at 759.96 cm^{-1} and disappearance of C=O stretching frequency in spectrum. Further, absorption bands at 3354.62 (N-H str.), 2580.84 (S-H str.), 1664.37(C=O str.) and 1562.39 (C=N str) cm^{-1} confirmed the formation of 2-(2-sulfanylquinazolin-4-yl) pyridine-4-carbohydrazide (4). Its ^{1}H NMR spectrum displayed singlet at δ 11.15 due to NH proton of sec. amide and at δ 3.85 due to aromatic C-NH proton.

R= 4-F, 4-Br, 4-CH$_3$, 3-NO$_2$, 4-CN, 2-OCH$_3$
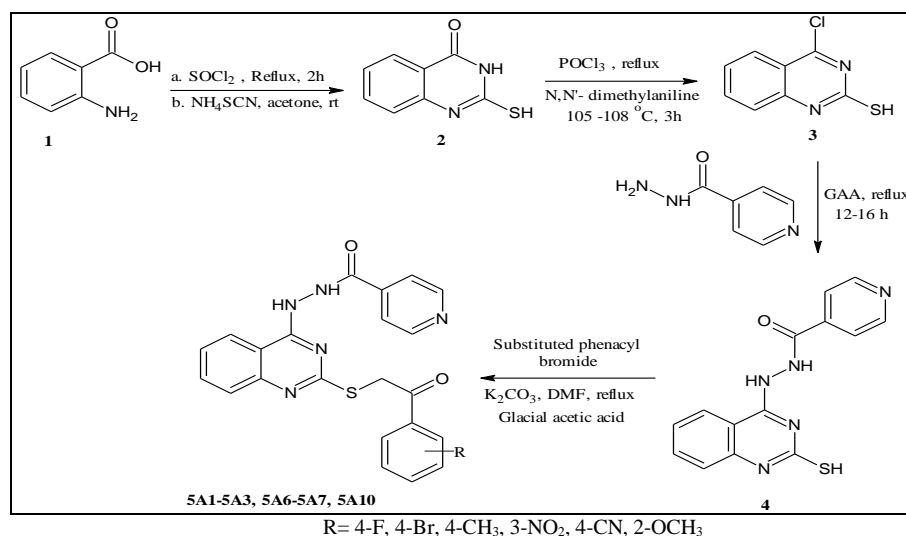
Scheme 1: Synthesis of *N'*- (2- {[2-(substituted phenyl) -2-oxo ethyl] sulfanyl} quinazolin-4-yl) pyridine-4-carbohydrazide derivatives (5A Series)

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The appearance of δ 12.70 peak of SH proton, a singlet at δ 8.71 corresponding to two protons adjacent to N in pyridine ring and aromatic protons were observed as multiplet at δ 8.30-7.15 ppm, further confirmed the successful formation of intermediate (4). These facts were further evidenced by ^{13}C NMR data which displayed signals of C-SH and C=N of aromatic ring around 174.75, 150.76-150.67 ppm respectively. Condensation of **4** with various substituted phenacyl bromides to give final compounds was confirmed by absence of SH peak. IR spectrum of 5A1 showed single band at 3304.14 cm^{-1} (N-H str.), C=O stretching of sec. amide and thioether at 1699.34 and 1672.42 cm^{-1} respectively. Morever the C=N and C-F stretching appeared at 1540.26 and 761.91 cm^{-1} Its ^{1}H NMR spectrum displayed a singlet at δ 11.15 for (NH-CO exchangeable) and 6.59 for NH proton. It showed δ 4.42 peak due to SCH$_2$ protons which confirms the formation of thioether linkage, while 12 aromatic protons resonated at δ 8.85-7.15 ppm. A molecular ion peak at m/z 433.1471(M-H) in HRMS spectrum has further confirmed the structure. IR spectrum of 5A3 showed C=O stretching of and sec. amide and thioether at 1708.67 and 1655.42 cm^{-1} respectively and C-CH$_3$ str. in aromatic ring at 1360.14 cm^{-1}. ^{1}H NMR spectrum of 5A3 indicate a singlet at δ 4.37 due to SCH$_2$ proton and a singlet at δ 4.37 for SCH$_2$ and at δ 2.37 for CH$_3$ protons. Further its aromatic protons are observed at δ 8.81 -7.17 ppm . Similarly the structures of other compounds were confirmed by the absence of SH peak, a singlet at δ 9.15- 11.28 for sec. amide proton and singlet at δ 6.70-4.07 for aromatic C-NH proton, while the signal of methylene proton SCH$_2$ was appeared at δ 4.47-4.36 ppm. The above facts were further evidenced by ^{13}C NMR data which displayed C=O signal at 193.80-187.12 and C of NHCO at 163.30-156.76 ppm, the heterocyclic carbons resonated at 170.76 to 112.59 ppm and CH$_2$ group resonated at 39.99-39.21 ppm respectively. The mass spectrum of compounds showed molecular ion peaks HRMS (ESI) at *m/z* 495.0919 to 430.1332 corresponding to molecular formula and elemental analysis of these compounds further confirmed the assigned structures.

Table1: Physical characterization data of *N'*- (2- {[2-(substituted phenyl) -2-oxo ethyl] sulfanyl} quinazolin-4-yl) pyridine-4-carbohydrazide derivatives (5A Series)

(5A Series)
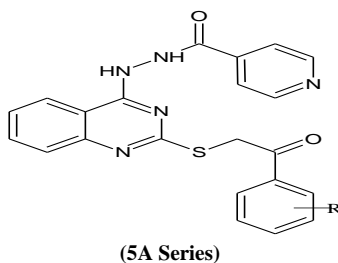
Compd. Code	R	Mol. Formula	Mol. Wt.	m. p. ºC	% Yield	R$_f$ value
5A1	4-F	C$_{22}$H$_{16}$FN$_5$O$_2$S	433.45	167-168	60	0.75
5A2	4-Br	C$_{22}$H$_{16}$BrN$_5$O$_2$S	494.36	188-190	68	0.36
5A3	4-CH$_3$	C$_{23}$H$_{19}$N$_5$O$_2$S	429.49	224-226	72	0.64
5A6	3-NO$_2$	C$_{22}$H$_{16}$N$_6$O$_4$S	460.46	210-212	62	0.56
5A7	3-CN	C$_{23}$H$_{16}$N$_6$O$_2$S	440.47	204-206	58	0.33
5A10	2-OCH$_3$	C$_{23}$H$_{19}$N$_5$O$_3$S	445.49	199-201	64	0.64

In vitro **antimicrobial activity**

The results of antibacterial and antifungal activity are summarized in Table 2. As per the in vitro antibacterial data summarized in (Table 2). It was confirmed that some of the compounds attached to the aryl ring at C$_2$ of quinazoline have exhibited moderate to most promising bacterial and fungal inhibition at all the tested concentration. Particularly compounds 5A2, 5A3 and 5A6 showed more promising activity against all tested gram positive bacterial strains with MIC values between 0.4-25 µg/ml while reduced activity against all tested gram negative bacterial strains. Compound 5A3 possessing substituent (4-CH$_3$) exhibited highest activity with MIC values between from 0.4-0.8 µg/ml against gram positive and moderate activity against gram negative bacterial strains at MIC ranging from 12.5-25 µg/ml as compared to the standard drug ciprofloxacin. Morever compound 5A1(4-F) and 5A2 (4-Br) showed promising activity with MIC 0.4-25 µg/ml against gram positive bacteria and moderate activity at MIC values between 12.5-50 µg/ml by 5A2 and less activity by 5A1 at MIC (>50 µg/ml) against gram negative bacteria. The presence of electron withdrawing group (3-NO$_2$) in compound 5A6 displayed moderate activity at MIC 12.5-25µg/ml against bacterial strains and reduced inhibition against gram negative bacteria. Whereas remaining compounds 5A7(3-CN) and 5A10 (2-OCH$_3$) showed reduced activity against gram positive bacteria and least activity towards gram negative bacteria. From the activity result it could be stated that introduction of halogen

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(bromo) substitutent 5A2 displayed highest activity, the reason behind it may be due to optimal lipophilicity, while the presence of methoxy substitutent has reduced the activity that may be due to the presence of oxygen atom i.e higher hydrogen bonding capacity together with higher lipophilicity [31]. The order of antibacterial activity of the substitutent at the aryl ring is 4-CH3 > 4-Br > 4-F > 3-NO$_2$ > 4-CN > 2-OCH$_3$. Here the presence and position of the functional groups has a great impact on the activity as observed in structure activity relationship. The relative zone of inhibition of compounds 5A1-5A10 shown graphically in (Figure 2). Particularly single compound 5A10 having substituent methoxy, displayed least activity against gram positive and gram negative bacteria.

Table 2: In vitro anti-microbial activity of synthesized compounds (5A Series)

Compd Code	R	In vitro activity-zone of inhibition in mm (MIC in µg/ml)							
		Gram positive bacteria			Gram negative bacteria			Fungi	
		B.s. ATCC 60511	*S.a.* ATCC 11632	*S.e.* ATCC 155	*E.c.* ATCC 10536	*K.p.* ATCC 11298	*P.a.* ATCC 10145	*C.a.* ATCC 2091	*A.n.* ATCC 6275
5A1	4-Fluoro	20 (0.4)	12 (50)	18 (0.8)	13 (50)	11 (100)	15 (25)	13 (50)	22 (0.4)
5A2	4-Bromo	18 (0.8)	17 (12.5)	22 (0.4)	15 (25)	13 (50)	17 (12.5)	19 (0.8)	20 (0.8)
5A3	4-methyl	21 (0.4)	22 (0.4)	20 (0.8)	18 (12.5)	17 (12.5)	14 (25)	16 (16)	17 (3.12)
5A6	3-Nitro	17 (12.5)	15 (25)	16 (12.5)	14 (25)	13 (50)	15 (25)	22 (0.4)	17 (3.12)
5A7	4-Cyano	14 (25)	21 (0.4)	15 (25)	13 (50)	12 (50)	13 (50)	18 (0.8)	20 (0.8)
5A10	2-methoxy	16 (12.5)	14 (25)	13 (50)	13 (50)	11 (100)	15 (25)	12 (50)	14 (50)
CPR		24 (2)	25 (1)	25 (2)	21 (2)	22 (< 4)	21 (4)	-	-
FA		-	-	-	-	-	-	25 (16)	26 (8)
DMSO	-	-	-	-	-	-	-	-	-

B.c.-Bacillus subtilis, S.a.-Staphylococcus aureus, S.e.-Staphylococcus epidermidis, E.c.-Escherichia coli, K.p.-Klebsiella pneumoniae, P.a.-Pseudomonas aeruginosa, C.a.-Candida albicans, A. n.- Aspergillus niger. (-) indicates no inhibition zone, CPR- Ciprofloxacin, FA-Fluconazole.

The evaluation of in vitro antifungal data demonstrated the compounds 5A1, 5A2, 5A3 and 5A6, 5A7 and 5A10 showed moderate to highest activity with MIC values between 0.4 to 50 µg/ml . The compounds 5A2 (4-bromo) and 5A7 (4-cyano) displayed the highest activity at MIC 0.8 µg/ml against the fungal strains *A. Niger and C. Albicans* compared to the standard drug fluconazole. The remaining compounds 5A1, 5A3, 5A6 showed moderate to good activity with MIC values in between 0.4 -50 µg/ml, The least activity compound was found to be 5A10 with MIC of 50 µg/ml. Thus the MIC values of tested compounds are given in (Table 2) and the relative zone of inhibition of compounds 5A1-5A10 shown graphically in (Figure 3).
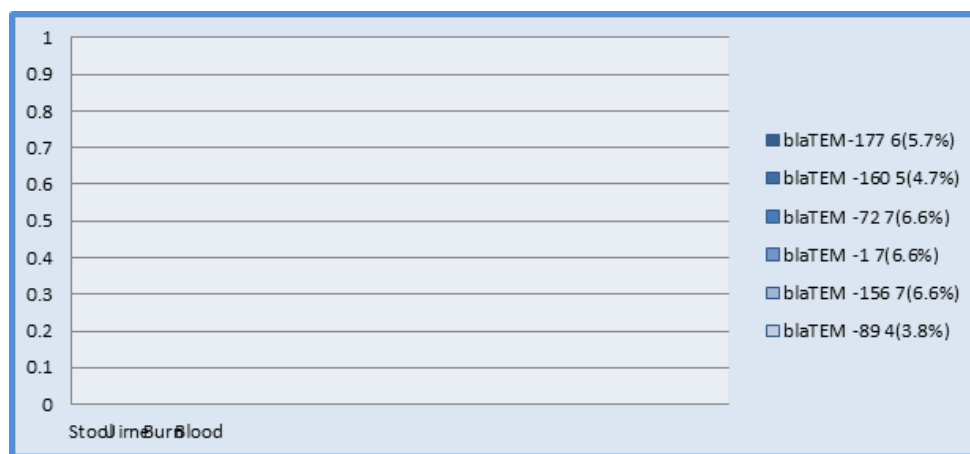

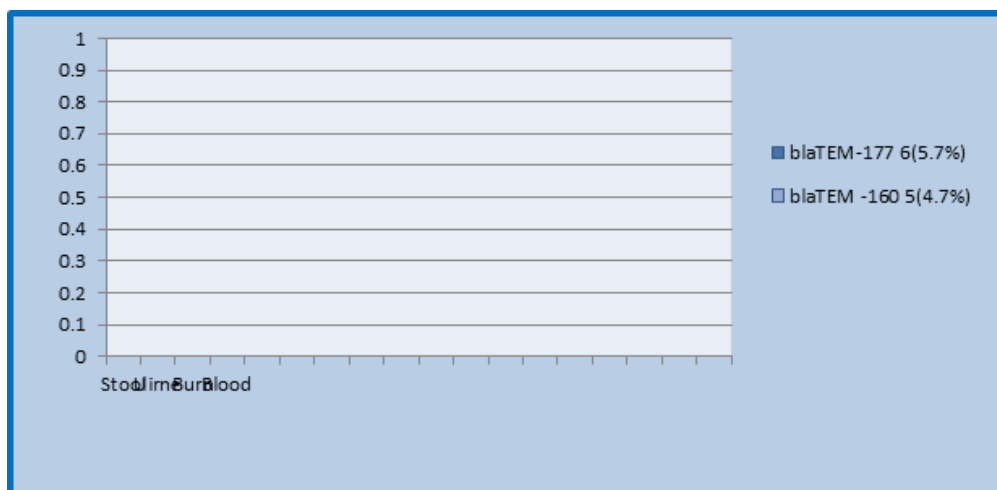**Figure 2: Zone of inhibition of synthesized derivatives and ciprofloxacin**

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Figure 3: Zone of inhibition of synthesized derivatives and Fluconazole

Molecular docking

The docking approach used in this study was aimed at identification of compounds that selectively bind to the active site of ecKAS III. The active site of ecKAS III (Figure 4) was defined based on the center and radius of the binding substrate in X-ray structure of enzyme complexed with CoA or inhibitor [32] towards optimization of the aforementioned compounds of the promising antimicrobial activities. The binding affinity was evaluated by glide gscore, glide energy and hydrogen bonding.

Individual binding poses of each compound was assessed and their interactions in the active site of the enzyme were analyzed. After analyzing the different docking interactions of ligands, the compounds namely 5A6 and 5A7 showed better interaction with ecKAS III with the lowest glide gscore value than the other molecules. Theoretically all other molecules showed very good glide score ranging from -7.432 to -8.600 and glide emodel -83.583 to -85.909 kJ/mol respectively. The compounds which revealed the highest binding affinities, lowest glide gscore within KAS III and the hydrogen bond interactions into the target macromolecule are represented in (Table 3).
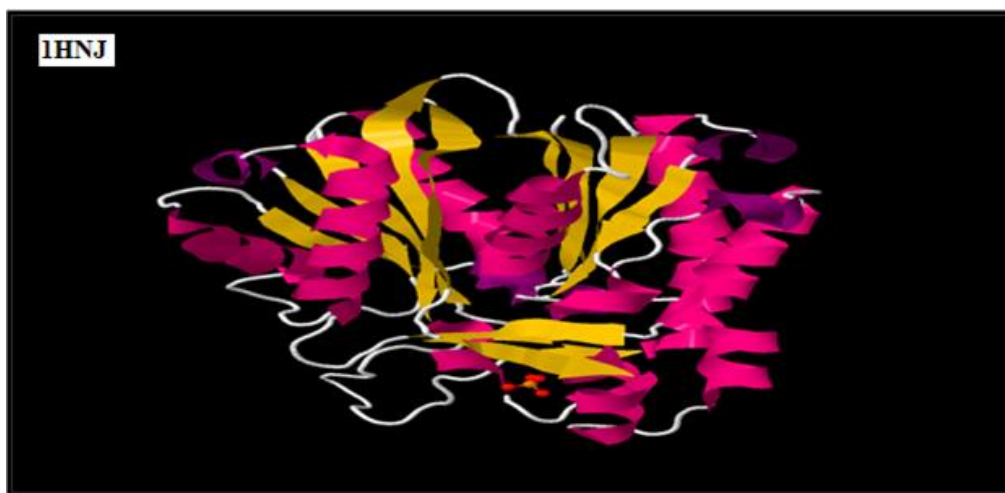

**Figure 4: Structure of β-ketoacyl-acyl carrier protein synthase III (PDB ID: 1HNJ)**

From the binding model, it was revealed that compound 5A2 is bound into ecKAS III receptor site via hydrophilic binding by hydrogen bond between oxygen atom of -NHCO of pyridine-4-carbohydrazide and H –N of Arg 151 and oxygen atom of 2-oxo ethyl linker of C-2 substitutent and H–N of Asn 247 and Nitrogen atom of pyridine ring of isoniazid and H–O of Thr 28 residue. The quinazolin-4(3*H*)-one ring was surrounded by hydrophobic amino acids such as Ala 246, Ile56 and Phe157, the phenacyl bromide substitutent was enclosed by Leu189, Phe304, Val 212,

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Phe213, Ile250, Met207 and pyridine-4-carbohydrazide surrounded by Ile155, Ile33 and Trp 32 hydrophobic amino acids. The ligand binding analysis of compound 5A3 showed hydrogen bonding interactions by oxygen atom of 2-oxoethyl of phenacyl linker with N-H of Asn 247 residue and by nitrogen atom of pyridine ring of isoniazid with N-H of Thr 28. The compound is buried into hydrophobic amino acid residues Met 207, Phe 304, Val 212, Phe 213, Ile 250, Ala 216, Ala 246, Ile156, Ile 33, Ile 155. The binding analysis of compound 5A6 its association with two hydrogen bonds, one between oxygen atom of pyridine-4-carbohydrazide and N-H of Arg 36, other with oxygen atom of nitro group as substitutent at C-2 of quinazoline. The compound 5A7 exhibited two hydrogen bonds, one between nitrogen atom of pyridine ring and N-H of Thr 28 and other between oxygen atom of linkage in pyridine-4-carbohydrazide and N-H of Arg151. All compounds showed pi-pi stacking interactions between pyridine ring of isoniazid clubbed with quinazoline and aromatic amino acid residues Arg 151 and Trp 32, but were weak as compared to observed hydrogen bonding interactions.

Table 3: Molecular docking results of 5A1, 5A3, 5A6 and 5A7 against β-Keto acyl carrier protein

Compound Code	Glide model	Glide score	No. of hydrogen bond and distance in A°
5A2	-80.271	-7.961	3
			O (5A2) and H–N (Arg 151) : 2.53
			O (5A2) and H–N (Asn 247): 1.80
			N (5A2) and H–O (Thr 28): 1.97
5A3	-79.077	-7.95	2
			O(5A3) and H–N (Asn 247): 2.15
			N (5A3) and H–O (Thr 28) : 1.84
5A6	-83.583	-7.432	2
			O (5A2) and H–N (Arg 151): 2.44
			N (5A2) and H–O (Thr 28): 1.91
5A7	-85.909	-8.6	2
			O (5A2) and H–N (Arg 151): 2.24
			N (5A2) and H–O (Thr 28): 1.81

Among the six molecules, compounds 5A2 and 5A3 have emerged as highly active against gram positive bacterial strains, whereas moderately active against gram negative bacteria. The antifungal evaluation revealed that compound 5A2 and 5A6 are most potent against fungi indicating that the docking method was most appropriate for clarifying the binding mode of this novel series of compounds as good inhibitor of ecKAS III synthase as illustrated in (Fig. 5) It can be predicted as the activity may be due to inhibition of enzyme ecKAS III synthase except in case of compound 5A10 having least activity against all tested microorganism though it has lowest glide score than others [33-34].
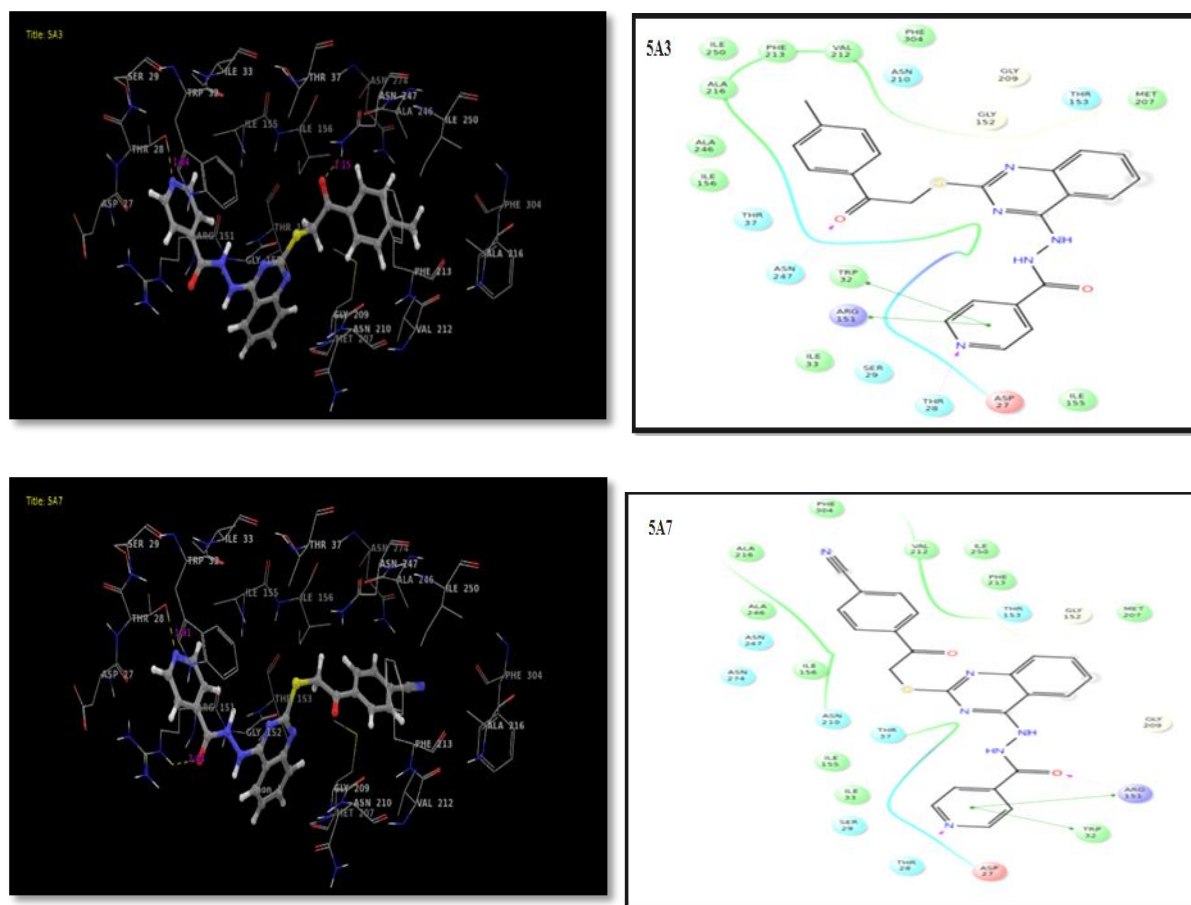
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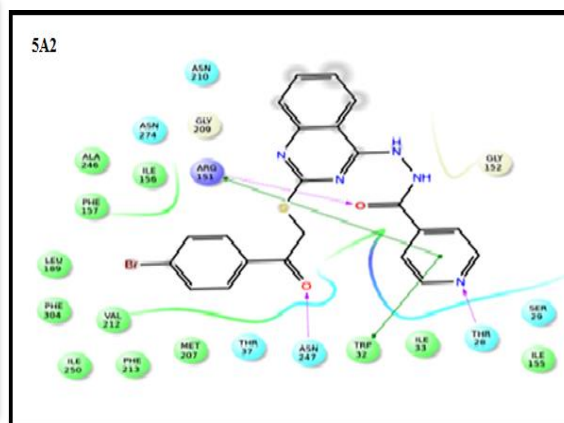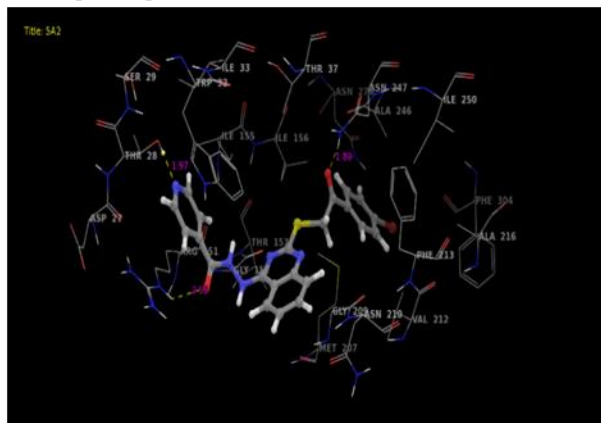
Figure 5: Compound 5A2, 5A3 and 5A7 bound into ecKAS III receptor site via hydrophobic interactions and hydrophilic binding by hydrogen bond between compound and amino acids

CONCLUSION

The antimicrobial study of newly synthesized N'-2-{[2-(4-substitutedphenyl)-2-oxoethyl]sulfanyl }quinazolin-4-yl)pyridine-4-carbohydrazide derivatives by the introduction of pyridine-4-hydrazide at position-4 of quinazoline nucleus with substituted phenacyl bromides at C-2 offers facile entry into synthesis with short reaction time and excellent yields to get more potent drugs for microbial infections. Also, it is evident that some of the compounds of 5A series are showing good potency against tested bacterial strains (except *Klebsiella pneumoniae*) and all fungal strains (except 5A10) compared to ciprofloxacin and fluconazole respectively. Especially it was observed that compounds 5A2 and 5A3 showed excellent potency against all bacterial and fungal strains when compared to respective standard drugs. Morever compounds 5A2 and 5A7 exhibited highest activity against all the tested fungal strains. It is important to point out that activity is affected by the introduction of substituents at different positions of phenyl ring but not affected by the electronic properties of the substituents. Molecular docking studies also revealed that 5A2, 5A3, 5A6 and 5A8 have minimum glide energy and glide score and may be considered as a good ecKAS III inhibitors. Hence we conclude that the analogs with electron donating substituent and analogs bearing bromo, nitro and cyano substituent showed better to moderate antimicrobial activity. Thus by analyzing these data, *N'*-[2-(substituted sulfanyl) quinazolin-4-yl] pyridine-4-carbohydrazide derivatives can be considered as potent inhibitor against the enzyme ecKAS III synthase.

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