Thieme

Graph Theoretical Analysis, In Silico Modeling, Synthesis, Anti-Microbial and Anti-TB Evaluation of Novel Quinoxaline Derivatives

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Key words
Graph theoretical analysis, Quinoxaline, Schiff base, Anti-bacterial, Anti-fungal and Anti-tubercular

received 04.08.2017
accepted 17.09.2017

Bibliography
DOI https://doi.org/10.1055/s-0043-120198
Published online: 2017
Drug Res

ISSN 2194-9379

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ABSTRACT

Background We designed to synthesize a number of 2-(2-(substituted benzylidene) hydrazinyl)-N-(4-((3-(phenyl imino)-3,4-dihydro quinoxalin-2(1 H)-ylidene)amino) phenyl) acetamide **S1-S13** with the hope to obtain more active and less toxic anti-microbial and anti-TB agents.

Methods A series of novel quinoxaline Schiff bases **S1-S13** were synthesized from o-phenylenediamine and oxalic acid by a multistep synthesis. In present work, we are introducing graph theoretical analysis to identify drug target. In the connection of graph theoretical analysis, we utilised KEGG database and Cytoscape software. All the title compounds were evaluated for their in-vitro anti-microbial activity by using agar well diffusion method at three different concentration levels (50, 100 and 150 µg/ml). The MIC of the compounds was also determined by agar streak dilution method.

Results The identified study report through graph theoretical analysis were highlights that the key virulence factor for pathogenic mycobacteria is a eukaryotic-like serine/threonine protein kinase, termed PknG. All compounds were found to display significant activity against entire tested bacteria and fungi. In addition the synthesized scaffolds were screened for their in vitro antituberculosis (anti-TB) activity against Mycobacterium tuberculosis (Mtb) strain $H_{37}Ra$ using standard drug Rifampicin.

Conclusion A number of analogs found markedly potent anti-microbial and anti-TB activity. The relationship between the functional group variation and the biological activity of the evaluated compounds were well discussed. The observed study report was showing that the compound **S6** (4-nitro substitution) exhibited most potent effective anti-microbial and anti-TB activity out of various tested compounds.

Introduction

The diverse parasitic bacteria such as Staphylococcus aureus, Staphylococcus pyogenes, Salmonella typhimurium, and Escherichia coli have significant impact on the mucosal health of humans. Infection with S. aureus, S. pyogenes, S. typhimurium, and E. coli may have resulted in massive destruction of host tissue and life threatening diseases. These bacterial parasites cause food poisoning, rheumatic fever and diarrhoea that affect millions of individuals in developing countries. More than 50 million people worldwide are infected and up to 1,10,000 of these die every year. Amoxicillin, Norfloxacin, Ciprofloxacin are the most common drugs used for this bacterial infection but are associated with severe side effects.

Toxicity and resistance to the drugs also play an important role in the failure of treatment [1]. The rapid development of bacterial strains resistant to anti-bacterial agents poses a significant threat to global health [2]. In particular, attention has focused on the Gram positive organism Staphylococcus aureus because many strains of this organism are now resistant against clinically useful antibiotics like Methicillin and Vancomycin [3]. The problem is further compounded by the rapid emergence of multidrug resistant organisms. For example, it look only a few years after the introduction of Linezolid [4], an oxazolidinone derivative, for clinical use before reports of resistant organisms and clinical failures emerged [5, 6]. In addition the statistics was indicates that millions of people worldwide die annually from difficulty of TB. In coincidence with the advent of the AIDS plague and the improved mobility of human populations, has led to the appearance of frequent multidrug- resistant Mycobacterium tuberculosis (Mtb). Given this situation, there is an urgent need to discover and develop new anti-bacterial agents. Fortunately, several promising heterocyclic compounds have been identified. It was found that quinoxaline and its analogs was one such important heterocyclic compound. A variety of chemical modifications were carried out so far around the quinoxaline nucleus. The quinoxalines have emerged as anti-microbial agents of an immense interest because of their broad spectrum of in-vitro activity and their in-vivo chemotherapeutic activity [7–14]. Schiff bases have gained importance because of physiological and pharmacological activities associated with them. Compounds containing azomethine group (–C = N–) in the structure are known as Schiff bases, which are usually synthesized by the condensation of primary amines and active carbonyl groups. Schiff bases are well known for their pharmacological properties as anti-bacterial, antifungal, anti-cancer and anti-viral agents [15]. On the other hand, diverse chemotherapeutic agents contain pharmacophore like phenolic hydroxy, nitro, and chlorine substitutions, are reported to possess anti-TB and anti-microbial activities [16]. Based on these findings, and in continuation of our drug research program concerning synthesis of new safer and more biologically active heterocyclic compounds, it was of interest to synthesize a new series of quinoxaline Schiff base derivatives with the hope to obtain more active and less toxic anti-TB and anti-microbial agents.

Experimental

General

The chemicals and reagents used were obtained from various chemical units Qualigens, E. Merck India Ltd., CDH, and SD Fine Chem. These solvents used were of LR grade and purified before their use. The Graph theoretical analysis was performed by using KEGG database and Cytoscape software. The docking study was performed using Sybyl-x 2.0, Tripos International, St. Louis, MO, USA, 2012." The silica gel G used for analytical chromatography (TLC) was obtained from E. Merck India Ltd. All the melting points were taken in open glass capillary and are uncorrected. ^{1}H-NMR spectra were recorded at 500 MHz on Bruker Avance-500 NMR spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. The chemical shifts are reported in ppm scale. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. All the IR spectra were recorded in KBr pellets on a Jasco FT-IR 410 spectrometer. Elemental analyses were performed on a Perkine Elmer model 2400C analyzer and were within ± 0.4 % of the theoretical values.

Network topology analysis

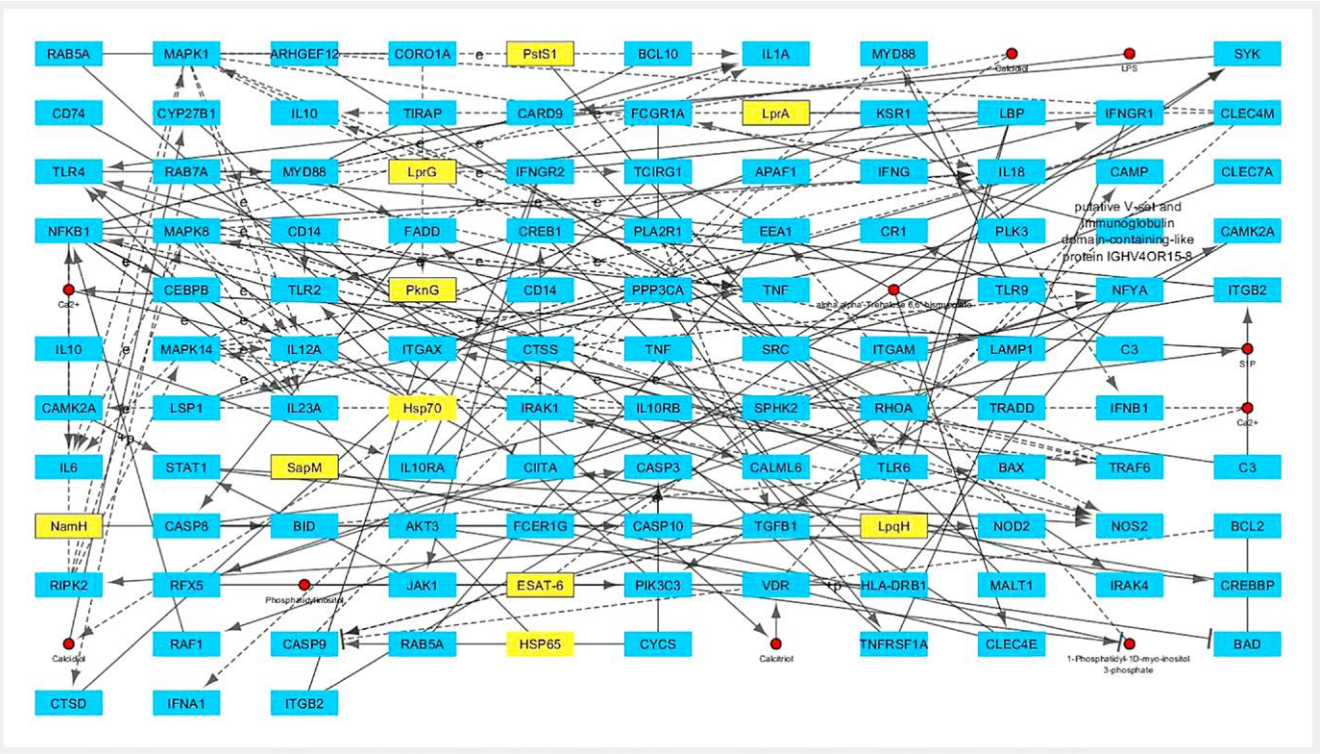
Graph theoretical analysis [17–19] was performed by using Kyoto Encyclopedia of Genes and Genomes database and network of protein interaction (hsa: 05152) in homo sapiens was in use to identify the influential proteins. The pathway was converted to a graph with proteins as nodes and interactions as edges and it was represented in ▶ **Figs. 1** and ▶ **2**. The network was carries 124 nodes and 167 edges, moreover the computed Degree Centrality, Betweenness Centrality, Eigenvector Centrality, Radiality Centrality, Stress Centrality and Closeness Centrality was used to identify the significant proteins from the network. Based on these measures with its threshold values, the target PknG was identified and Mtb drug target, PknG has more attention than ESAT-6 because of its interaction between 27 tuberculosis proteins and may help in the spread of disease. Based on the report of graph theoretical analysis, it was very clear proven that the PknG is a significant target to identify active quinoxiline motifs.

Insilico modeling

Molecular docking was used to clarify the binding mode of the compounds to provide straightforward information for further structural optimization. The "Sybyl-x 2.0, Tripos International, St. Louis, MO, USA, 2012.", was used to perform screening of docking based on earlier reported method [20]. The crystal structure of Protein kinase PknG from Mycobacterium tuberculosis in Complex with Tetrahydrobenzothiophene (PDB ID 2PZI) was extracted from the Brookhaven Protein Database (PDB http://www.rcsb.org/pdb). The proteins were prepared for docking by adding polar hydrogen atom with Gasteiger-Huckel charges and water molecules were removed. The 3D structure of the ligands was generated by the SKETCH module implemented in the SYBYL program (Tripos Inc., St. Louis, USA) and its energy-minimized conformation was obtained with the help of the Tripos force field using Gasteiger-Huckel charges and molecular docking was performed with Surflex-Dock program that is interfaced with Sybyl-X 2.0. and other miscellaneous parameters were assigned with the default values given by the software.

Biological Methods

All the synthesized compounds were screened for anti-microbial activities by agar well diffusion method. The anti-bacterial activity of the compounds was evaluated against one Gram-positive bacteria (Bacillus subtilis ATCC 6633) and two Gram-negative bacteria (Klebsiella pneumoniae ATCC 13883 & Pseudomonas aeruginosa ATCC 27853). The anti-fungal activities of the synthesized compounds were evaluated against three fungi (Trichoderma ATCC 26921, Aspergillus niger ATCC 9029 and Aspergillus flavus ATCC 10124). Bacterial strains were cultured over night at 37 °C in Muller-Hinton broth and the yeast was cultured over night at 30 °C in nutrient agar medium for anti-bacterial and anti-fungal activity tests. Ciprofloxacin and Ketoconozole were used as standard drugs for anti-bacterial and anti-fungal activities respectively. Nutrient agar medium and Sabouraud dextrose agar medium (Hi-Media Lab-



▶ **Fig. 1** The interaction of PknG pathway with nodes and edges.

oratories, India) was used as the medium for the study of anti-bacterial and anti-fungal activity respectively.

Paper disc diffusion technique

The sterilized (autoclaved at 120 °C for 30 min) medium (40–50 °C) was inoculated (1 ml/100 ml of medium) with the suspension (5×10^{-5} cfu/ml) of the microorganism (matched to McFarland barium sulphate standard) and poured into a petridish to give a depth of 3–4 mm. The paper impregnated with the test compounds (50, 100 and 150 µg/ml in dimethyl formamide) was placed on the solidified medium. The plates were pre-incubated for 1 h at room temperature and incubated at 37 °C for 24 and 48 h for antibacterial and antifungal activities, respectively. Ciprofloxacin (150 µg/ml/disc) and Ketoconazole (150 µg/ml/disc) were used as standard for antibacterial and antifungal activities respectively [21]. All the observed zone of inhibition is presented in ▶ **Table 1**.

Minimum inhibitory concentration (MIC)

MIC of the compound was determined by agar streak dilution method [22]. Using dimethyl formamide a stock solution of the synthesized compound were prepared in a concentration of 125 µg/ml. Graded quantities of the stock solution of test compounds were incorporated in a specified quantity of molten sterile agar (nutrient agar for antibacterial activity and Sabouraud's dextrose agar for antifungal activity) medium to get various concentrations of serially diluted test compounds. A specified quantity of the medium (40–50 °C) containing the compound was poured into a petridish to give a depth of 3–4 mm and allowed to solidify. Suspension of the microorganism were prepared to contain approximately 5×10^{-5}cfu/ml and applied to plates with serially diluted compounds in dimethyl formamide to be tested and incubated at 37 °C for 24 and 48 h for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate and the observed MIC data was represented in ▶ **Table 2**.

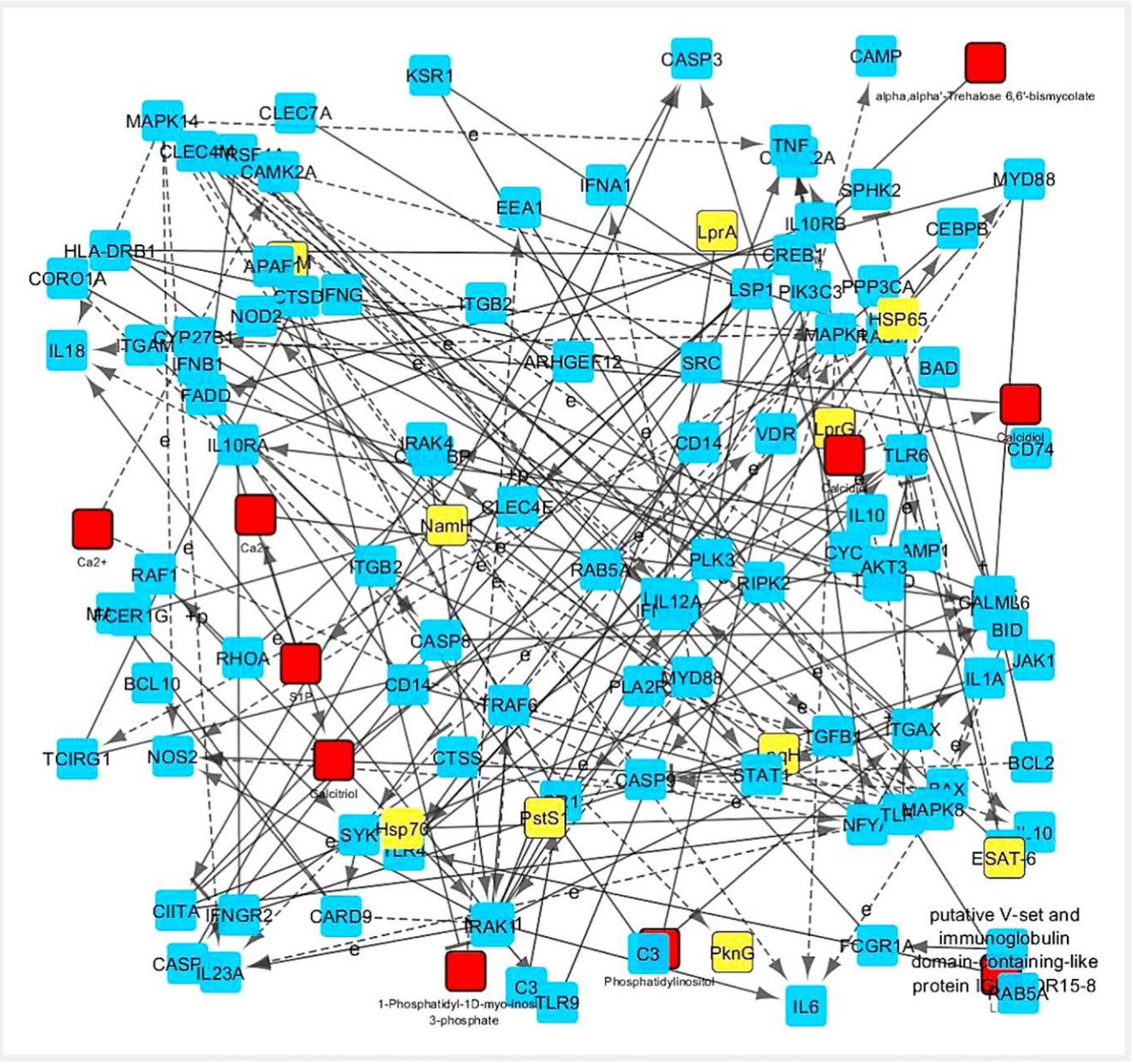
MABA Assay

The synthesized scaffolds were screened for their in vitro anti-TB activity against Mtb $H_{37}Ra$ and bioassay was performed using the microplate Alamar blue assay (MABA) [23] and INH was chosen as the reference compound. The indication of color change from blue to pink and minimum inhibitory concentration (MIC) was calculated based on earlier reported method [24] and the observed MIC data was represented in ▶ **Table 2**.

Synthetic methods

Synthesis of quinoxaline-2,3(1 H,4 H)-dione (1)

An equimolar solution of o-phenylenediamine (1.08 g, 0.01 mol) and oxalic acid (0.9 g, 0.01 mol) in 4 N hydrochloric acid (90 ml) was refluxed in water bath for 4 h and the reaction mixture was placed in refrigerator overnight to produce quinoxaline-2,3(1 H,4 H)-dione **1**. The needle shaped solid obtained was filtered, washed and re-crystallized by using ethanol and dried. Yield: 73 %, m.p.: 291–294 °C. IR (KBr, cm^{-1}): 3351 & 3279 (NH), 3084 (Ar-CH), 1698 (C = O), 1616 (C = C). ^1H NMR ($CDCl_3$, 500 MHz) δ ppm: 7.23–7.89 (m, 4 H, Ar-H), 8.34 (s, 1 H, CONH of quinoxaline), 8.51 (s, 1 H, CONH of quinoxaline). EI-MS m/z: 162 (M^+). Anal. Cald for $C_8H_6N_2O_2$: C, 59.26; H, 3.73; N, 17.28. Found: C, 59.43; H, 3.72; N, 17.22.



▶ **Fig. 2** The visualization of interaction of PknG using Cytoscape.

Synthesis of 3-(4-aminophenylimino)-3,4-dihydroquinoxalin-2(1 H)-one (2)

To a solution of quinoxaline-2,3(1 H,4 H)-dione **1** (1.62 g, 0.01 mol) in dimethyl formamide (30 ml), p-amino aniline (1.62 g, 0.015 mol) was added and the whole mixture was refluxed for 6 h at 150 °C and cooled overnight. The solid obtained was filtered, washed with water, dried and recrystallised using ethanol. Yield: 80 %, m.p.: 181–183 °C. IR (KBr, cm^{-1}): 3342 & 3256 (NH), 3053 (Ar-CH), 1678 (C = O), 1612 (C = N), 1591 (C = C). ^1H NMR ($CDCl_3$, 500 MHz) δ ppm: 4.13 (s, 2 H, NH_2), 4.45 (s, 1 H, NH of quinoxaline), 7.02–7.97 (m, 8 H, Ar-H), 8.26 (s, 1 H, CONH of quinoxaline). EI-MS m/z: 252 (M^+). Anal. Cald for $C_{14}H_{12}N_4O$: C, 66.65; H, 4.79; N, 22.21. Found: C, 66.82; H, 4.80; N, 22.14.

Synthesis of N-(3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene)benzene-1,4-diamine (3)

A mixture of 3-(4-aminophenylimino)-3,4-dihydroquinoxalin-2(1 H)-one **2** (2.52 g, 0.01 mol) and aniline (0.931 g; 0.01 mol) in ethanol (20 ml) was refluxed for 5 h in a water bath. Latter the mixture was cooled and poured in ice cold water. The precipitated product **3** was filtered, dried and recrystallised using alcohol. Yield: 70 %, m.p.: 248–250 °C. IR (KBr, cm^{-1}): 3367 & 3243 (NH), 3071 (Ar-CH), 1624 (C = N), 1609 (C = C). ^1H NMR ($CDCl_3$, 500 MHz) δ ppm: 4.05 (s, 2 H, NH_2), 4.28 (s, 1 H, NH of quinoxaline), 4.49 (s, 1 H, NH of quinoxaline), 6.84–7.80 (m, 13 H, Ar-H). EI-MS m/z: 327 (M^+). Anal. Cald for $C_{20}H_{17}N_5$: C, 73.37; H, 5.23; N, 21.39. Found: C, 73.12; H, 5.25; N, 21.45.

▶ **Table 1** Anti-microbial activity (zone of inhibition in mm) of title compounds S1-S13.

Micro-organism	Conc. (µg/ml)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	Con	Std
Bacillus subtilis	50	15	14	4	8	3	17	1	2	-	8	6	10	7	-	27
	100	19	19	8	15	8	20	3	4	4	14	9	16	10	-	
	150	27	25	13	22	12	28	7	7	6	20	14	23	15	-	
Klebsiella pneumoniae	50	14	14	4	10	4	15	1	-	-	8	5	11	6	-	29
	100	20	17	8	13	7	22	2	2	3	11	8	15	8	-	
	150	30	29	13	25	11	31	8	7	7	24	15	25	17	-	
Pseudomonas aeruginosa	50	15	13	3	7	2	18	-	-	-	6	5	9	5	-	30
	100	18	16	7	11	7	21	1	1	3	11	8	14	9	-	
	150	30	29	13	24	13	30	6	5	5	22	15	27	16	-	
Trichoderma	50	9	9	-	6	-	10	-	-	-	6	3	6	3	-	21
	100	14	13	5	10	5	15	2	2	3	9	5	11	5	-	
	150	21	19	16	16	14	23	5	4	4	14	10	18	10	-	
Aspergillus niger	50	11	10	-	7	-	13	-	-	-	7	3	8	5	-	18
	100	17	15	4	13	3	17	2	2	3	11	5	14	6	-	
	150	18	18	15	15	15	19	6	5	4	14	9	16	11	-	
Aspergillus flavus	50	12	11	2	8	2	14	-	-	-	8	4	9	5	-	22
	100	15	15	3	13	3	16	2	3	3	12	5	13	7	-	
	150	21	20	17	17	16	22	7	7	5	15	10	19	11	-	

Con: Control (DMF), Std: Standard drug in 150 µg/ml (Ciprofloxacin for bacteria & Ketoconazole for fungi), -: No zone of inhibition observed

▶ **Table 2** Minimum inhibitory concentration (MIC in µg/ml) of title compounds S1-S13.

Microorganism	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	Std
Bacillus subtilis	7.81	7.81	31.25	15.62	31.25	7.81	62.5	62.5	125	15.62	31.25	15.62	31.25	7.81
Klebsiella pneumoniae	7.81	7.81	62.5	15.62	62.5	7.81	62.5	125	125	15.62	31.25	7.81	31.25	3.9
Pseudomonas aeruginosa	15.62	31.25	62.5	31.25	62.5	15.62	125	125	125	31.25	62.5	31.25	62.5	7.81
Trichoderma	15.62	15.62	125	31.25	125	15.62	125	125	125	31.25	62.5	31.25	62.5	7.81
Aspergillus niger	31.25	31.25	125	62.5	125	15.62	125	125	125	62.5	62.5	62.5	62.5	15.62
Aspergillus flavus	15.62	15.62	62.5	15.62	62.5	7.81	125	125	125	31.25	62.5	15.62	31.25	7.81
Mycobecterium tuberculosis	**1.95**	**1.95**	-	**1.95**	-	**1.95**	15.62	15.62	31.25	3.90	7.81	**1.95**	7.81	0.0092

-: Inactive at tested concentrations

Synthesis of 2-chloro-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene) amino)phenyl)acetamide (4)

N^1-(3-(Phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene)benzene-1,4-diamine **3** (3.27 g; 0.01 mol) was dissolved in glacial acetic acid (50 ml) containing of saturated solution of sodium acetate (50 ml). The mixture was warmed to dissolve the substance completely. The solution was cooled in ice bath with stirring. A solution of chloroacetyl chloride (1.34 g; 0.012 mol) was added drop wise to the above stirred solution so that the vigorous reaction did not take place. After half an hour the product separated **4** is filtered, washed with water, dried, and recrystallised using alcohol. Yield: 74 %, m.p.: 186–189 °C. IR (KBr, cm^{-1}): 3364 & 3282 (NH), 3092 (Ar-CH), 2989 (CH_2-CH), 1682 (C = O), 1640 (C = N), 1613 (C = C), 768 (C-Cl). ^1H NMR ($CDCl_3$, 500 MHz) δ ppm: 4.09 (s, 2 H, CH_2), 4.21 (s, 1 H, NH of quinoxaline), 4.56 (s, 1 H, NH of quinoxaline), 7.18–8.25 (m, 13 H, Ar-H), 8.34 (s, 1 H, CONH). EI-MS m/z: 405 (M^{+2}), 403 (M^+). Anal. Cald for $C_{22}H_{18}ClN_5O$: C, 65.43; H, 4.49; N, 17.34. Found: C, 65.60; H, 4.47; N, 17.38.

Synthesis of 2-hydrazinyl-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene) amino)phenyl)acetamide (5)

Equimolar quantity of 2-chloro-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene)amino)phenyl)acetamide **4** (4.03 g; 0.01 mol) and hydrazine hydrate (0.05 g; 0.01 mol) in ethanol (30 ml) was refluxed for 10 h on a water bath**.** The resulting solution was cooled and poured in ice cold water with stirring. The product **5** obtained was filtered, dried and recrystallised from ethanol. Yield: 71 %, m.p.: 210–212 °C. IR (KBr, cm^{-1}): 3340 & 3268 (NH),

3040 (Ar-CH), 2975 (CH_2-CH), 1693 (C = O), 1632 (C = N), 1606 (C = C). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm: 2.03 (s, 2 H, NH_2), 2.66 (s, 1 H, NH of CH_2NH), 3.95 (s, 2 H, CH_2 of CH_2NH), 4.38 (s, 1 H, NH of quinoxaline), 4.50 (s, 1 H, NH of quinoxaline), 7.31–8.04 (m, 13 H, Ar-H), 8.12 (s, 1 H, CONH). EI-MS m/z: 399 (M^+). Anal. Cald for $C_{22}H_{21}N_7O$: C, 66.15; H, 5.30; N, 24.55. Found: C, 65.92; H, 5.32; N, 24.63.

General procedure for the synthesis of 2-(2-(substituted-benzylidene)hydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene)amino)phenyl) acetamide (S1-S13)

Title compounds was synthesized by adding appropriate aromatic/heterocyclic aldehydes (0.01 mol) slowly with the well stirred mixture of 2-hydrazinyl-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene)amino)phenyl)acetamide **5** (3.99 g; 0.01 mol) in ethanol (50 ml), and glacial acetic acid (2–3 drops). The pH was maintained to 5–6. The reaction mixture was then refluxed for a period of 4–6 h, and kept aside for about 2 h. The products were separated by filtration, washed, and vacuum dried. Finally the products were recrystallised using ethanol to get pure form.

2-(2-(3-Nitrobenzylidene)hydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene) amino)phenyl)acetamide (S1)

Yield: 72 %, m.p.: 277–279 °C. IR (KBr, cm^{-1}): 3353 & 3289 (NH), 3095 (Ar-CH), 2953 (CH_2-CH), 1673 (C = O), 1621 (C = N), 1591 (C = C), 1534 & 1328 (NO_2). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm: 2.26 (s, 1 H, NH of CH_2NH), 3.91 (s, 2 H, CH_2 of CH_2NH), 4.20 (s, 1 H, NH of quinoxaline), 4.45 (s, 1 H, NH of quinoxaline), 6.90–7.72 (m, 17 H, Ar-H), 8.49 (s, 1 H, CONH), 8.65 (s, 1 H, CH of CH = N). EI-MS m/z: 532 (M^+). Anal. Cald for $C_{29}H_{24}N_8O_3$: C, 65.40; H, 4.54; N, 21.04. Found: C, 65.61; H, 4.53; N, 20.98.

2-(2-(2-Nitrobenzylidene)hydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene) amino)phenyl)acetamide (S2)

Yield: 75 %, m.p.: 244–246 °C. IR (KBr, cm^{-1}): 3348 & 3271 (NH), 3069 (Ar-CH), 2983 (CH_2-CH), 1697 (C = O), 1645 (C = N), 1620 (C = C), 1546 & 1312 (NO_2). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm: 2.43 (s, 1 H, NH of CH_2NH), 3.56 (s, 2 H, CH_2 of CH_2NH), 4.42 (s, 1 H, NH of quinoxaline), 4.58 (s, 1 H, NH of quinoxaline), 7.24–8.19 (m, 17 H, Ar-H), 8.32 (s, 1 H, CONH), 8.50 (s, 1 H, CH of CH = N). EI-MS m/z: 532 (M^+). Anal. Cald for $C_{29}H_{24}N_8O_3$: C, 65.40; H, 4.54; N, 21.04. Found: C, 65.19; H, 4.57; N, 21.09.

2-(2-(2-Hydroxybenzylidene)hydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene) amino)phenyl)acetamide (S3)

Yield: 70 %, m.p.: 230–234 °C. IR (KBr, cm^{-1}): 3531 (OH), 3335 & 3242 (NH), 3088 (Ar-CH), 2960 (CH_2-CH), 1674 (C = O), 1616 (C = N), 1589 (C = C). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm: 2.07 (s, 1 H, NH of CH_2NH), 3.70 (s, 2 H, CH_2 of CH_2NH), 4.17 (s, 1 H, NH of quinoxaline), 4.49 (s, 1 H, NH of quinoxaline), 5.13 (s, 1 H, OH), 7.06–8.38 (m, 17 H, Ar-H), 8.52 (s, 1 H, CONH), 8.64 (s, 1 H, CH of CH = N). EI-MS m/z: 503 (M^+). Anal. Cald for $C_{29}H_{25}N_7O_2$: C, 69.17; H, 5.00; N, 19.47. Found: C, 68.93; H, 5.02; N, 19.54.

2-(2-(2-Chlorobenzylidene)hydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene) amino)phenyl)acetamide (S4)

Yield: 77 %, m.p.: 226–228 °C. IR (KBr, cm^{-1}): 3364 & 3267 (NH), 3054 (Ar-CH), 2976 (CH_2-CH), 1679 (C = O), 1639 (C = N), 1590 (C = C), 785 (C-Cl). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm: 2.31 (s, 1 H, NH of CH_2NH), 3.97 (s, 2 H, CH_2 of CH_2NH), 4.45 (s, 1 H, NH of quinoxaline), 4.64 (s, 1 H, NH of quinoxaline), 6.82–7.95 (m, 17 H, Ar-H), 8.28 (s, 1 H, CONH), 8.39 (s, 1 H, CH of CH = N). EI-MS m/z: 523 (M^{+2}), 521 (M^+). Anal. Cald for $C_{29}H_{24}ClN_7O$: C, 66.73; H, 4.63; N, 18.78. Found: C, 66.95; H, 4.61; N, 18.72.

2-(2-(4-(Dimethylamino)benzylidene)hydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene) amino)phenyl)acetamide (S5)

Yield: 74 %, m.p.: 257–260 °C. IR (KBr, cm^{-1}): 3345 & 3254 (NH), 3079 (Ar-CH), 2958 (CH_2-CH), 1681 (C = O), 1643 (C = N), 1629 (C = C). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm: 2.19 (s, 1 H, NH of CH_2NH), 3.15 (s, 6 H, $N(CH_3)_2$), 3.62 (s, 2 H, CH_2 of CH_2NH), 4.09 (s, 1 H, NH of quinoxaline), 4.36 (s, 1 H, NH of quinoxaline), 7.03–7.86 (m, 17 H, Ar-H), 8.04 (s, 1 H, CONH), 8.31 (s, 1 H, CH of CH = N). EI-MS m/z: 530 (M^+). Anal. Cald for $C_{31}H_{30}N_8O$: C, 70.17; H, 5.70; N, 21.12. Found: C, 69.94; H, 5.72; N, 21.18.

2-(2-(4-Nitrobenzylidene)hydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1H)-ylidene) amino)phenyl)acetamide (S6)

Yield: 73 %, m.p.: 221–224 °C. IR (KBr, cm^{-1}): 3336 & 3274 (NH), 3034 (Ar-CH), 2941 (CH_2-CH), 1675 (C = O), 1573 (C = N), 1549 (C = C), 1529 & 1310 (NO_2). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm: 3.02 (s, 1 H, NH of CH_2NH), 3.69 (s, 2 H, CH_2 of CH_2NH), 4.25 (s, 1 H, NH of quinoxaline), 4.41 (s, 1 H, NH of quinoxaline), 7.16–8.24 (m, 17 H, Ar-H), 8.43 (s, 1 H, CONH), 8.78 (s, 1 H, CH of CH = N). EI-MS m/z: 532 (M^+). Anal. Cald for $C_{29}H_{24}N_8O_3$: C, 65.40; H, 4.54; N, 21.04. Found: C, 65.62; H, 4.55; N, 21.11.

2-(2-(Furan-2-ylmethylene)hydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene) amino)phenyl)acetamide (S7)

Yield: 70 %, m.p.: 190–194 °C. IR (KBr, cm^{-1}): 3336 & 3284 (NH), 3062 (Ar-CH), 2985 (CH_2-CH), 1690 (C = O), 1620 (C = N), 1598 (C = C), 1086 (C-O-C). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm: 2.04 (s, 1 H, NH of CH_2NH), 3.83 (s, 2 H, CH_2 of CH_2NH), 3.98 (s, 1 H, NH of quinoxaline), 4.31 (s, 1 H, NH of quinoxaline), 7.29–8.21 (m, 16 H, Ar-H), 8.36 (s, 1 H, CONH), 8.57 (s, 1 H, CH of CH = N). EI-MS m/z: 477 (M^+). Anal. Cald for $C_{27}H_{23}N_7O_2$: C, 67.91; H, 4.85; N, 20.53. Found: C, 68.10; H, 4.83; N, 20.48.

2-(2-(3-Phenylallylidene)hydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene) amino)phenyl)acetamide (S8)

Yield: 73 %, m.p.: 285–287 °C. IR (KBr, cm^{-1}): 3351 & 3247 (NH), 3074 (Ar-CH), 2963 (CH_2-CH), 1679 (C = O), 1648 (C = N), 1612 (C = C). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm: 2.35 (s, 1 H, NH of CH_2NH), 3.58 (s, 2 H, CH_2 of CH_2NH), 4.16 (s, 1 H, NH of quinoxaline), 4.42 (s, 1 H, NH of quinoxaline), 5.43 (d, 1 H, CH = CH), 6.29 (d, 1 H, CH = CH), 6.97–7.83 (m, 18 H, Ar-H), 8.13 (s, 1 H, CONH),

8.40 (s, 1 H, CH of CH = N). EI-MS m/z: 513 (M^+). Anal. Cald for $C_{31}H_{27}N_7O$: C, 72.50; H, 5.30; N, 19.09. Found: C, 72.27; H, 5.31; N, 19.01.

2-(2-Benzylidenehydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1H)-ylidene)amino)phenyl)acetamide (S9)

Yield: 71 %, m.p.: 249–251 °C. IR (KBr, cm^{-1}): 3345 & 3239 (NH), 3090 (Ar-CH), 2957 (CH_2-CH), 1684 (C = O), 1618 (C = N), 1581 (C = C). ^1H NMR ($CDCl_3$, 500 MHz) δ ppm: 2.18 (s, 1 H, NH of CH_2NH), 3.95 (s, 2 H, CH_2 of CH_2NH), 4.33 (s, 1 H, NH of quinoxaline), 4.56 (s, 1 H, NH of quinoxaline), 7.34–8.22 (m, 18 H, Ar-H), 8.40 (s, 1 H, CONH), 8.52 (s, 1 H, CH of CH = N). EI-MS m/z: 487 (M^+). Anal. Cald for $C_{29}H_{25}N_7O$: C, 71.44; H, 5.17; N, 20.11. Found: C, 71.63; H, 5.16; N, 20.04.

2-(2-(4-Methylbenzylidene)hydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene) amino)phenyl)acetamide (S10)

Yield: 75 %, m.p.: 203–206 °C. IR (KBr, cm^{-1}): 3360 & 3257 (NH), 3056 (Ar-CH), 2972 (CH_2-CH), 1672 (C = O), 1638 (C = N), 1604 (C = C). ^1H NMR ($CDCl_3$, 500 MHz) δ ppm: 2.21 (s, 1 H, NH of CH_2NH), 2.78 (s, 3 H, CH_3), 3.79 (s, 2 H, CH_2 of CH_2NH), 4.14 (s, 1 H, NH of quinoxaline), 4.36 (s, 1 H, NH of quinoxaline), 7.19–8.07 (m, 17 H, Ar-H), 8.25 (s, 1 H, CONH), 8.37 (s, 1 H, CH of CH = N). EI-MS m/z: 501 (M^+). Anal. Cald for $C_{30}H_{27}N_7O$: C, 71.84; H, 5.43; N, 19.55. Found: C, 71.60; H, 5.45; N, 19.61.

2-(2-(4-Hydroxybenzylidene)hydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydro quinoxalin-2(1 H)-ylidene) amino)phenyl)acetamide (S11)

Yield: 79 %, m.p.: 198–201 °C. IR (KBr, cm^{-1}): 3590 (OH), 3357 & 3280 (NH), 3076 (Ar-CH), 2973 (CH_2-CH), 1674 (C = O), 1585 (C = N), 1550 (C = C). ^1H NMR ($CDCl_3$, 500 MHz) δ ppm: 2.29 (s, 1 H, NH of CH_2NH), 3.16 (s, 2 H, CH_2 of CH_2NH), 4.02 (s, 1 H, NH of quinoxaline), 4.38 (s, 1 H, NH of quinoxaline), 5.34 (s, 1 H, OH), 7.01–8.25 (m, 17 H, Ar-H), 8.85 (s, 1 H, CONH), 9.03 (s, 1 H, CH of CH = N). EI-MS m/z: 503 (M^+). Anal. Cald for $C_{29}H_{25}N_7O_2$: C, 69.17; H, 5.00; N, 19.47. Found: C, 69.39; H, 5.02; N, 19.50.

2-(2-(4-Chlorobenzylidene)hydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene) amino)phenyl)acetamide (S12)

Yield: 74 %, m.p.: 265–268 °C. IR (KBr, cm^{-1}): 3330 & 3265 (NH), 3076 (Ar-CH), 2969 (CH_2-CH), 1692 (C = O), 1621 (C = N), 1586 (C = C), 764 (C-Cl). ^1H NMR ($CDCl_3$, 500 MHz) δ ppm: 2.09 (s, 1 H, NH of CH_2NH), 3.84 (s, 2 H, CH_2 of CH_2NH), 4.37 (s, 1 H, NH of quinoxaline), 4.43 (s, 1 H, NH of quinoxaline), 6.95–8.01 (m, 17 H, Ar-H), 8.34 (s, 1 H, CONH), 8.66 (s, 1 H, CH of CH = N). EI-MS m/z: 523 (M^{+2}), 521 (M^+). Anal. Cald for $C_{29}H_{24}ClN_7O$: C, 66.73; H, 4.63; N, 18.78. Found: C, 66.50; H, 4.64; N, 18.82.

2-(2-(4-Methoxybenzylidene)hydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene) amino)phenyl)acetamide (S13)

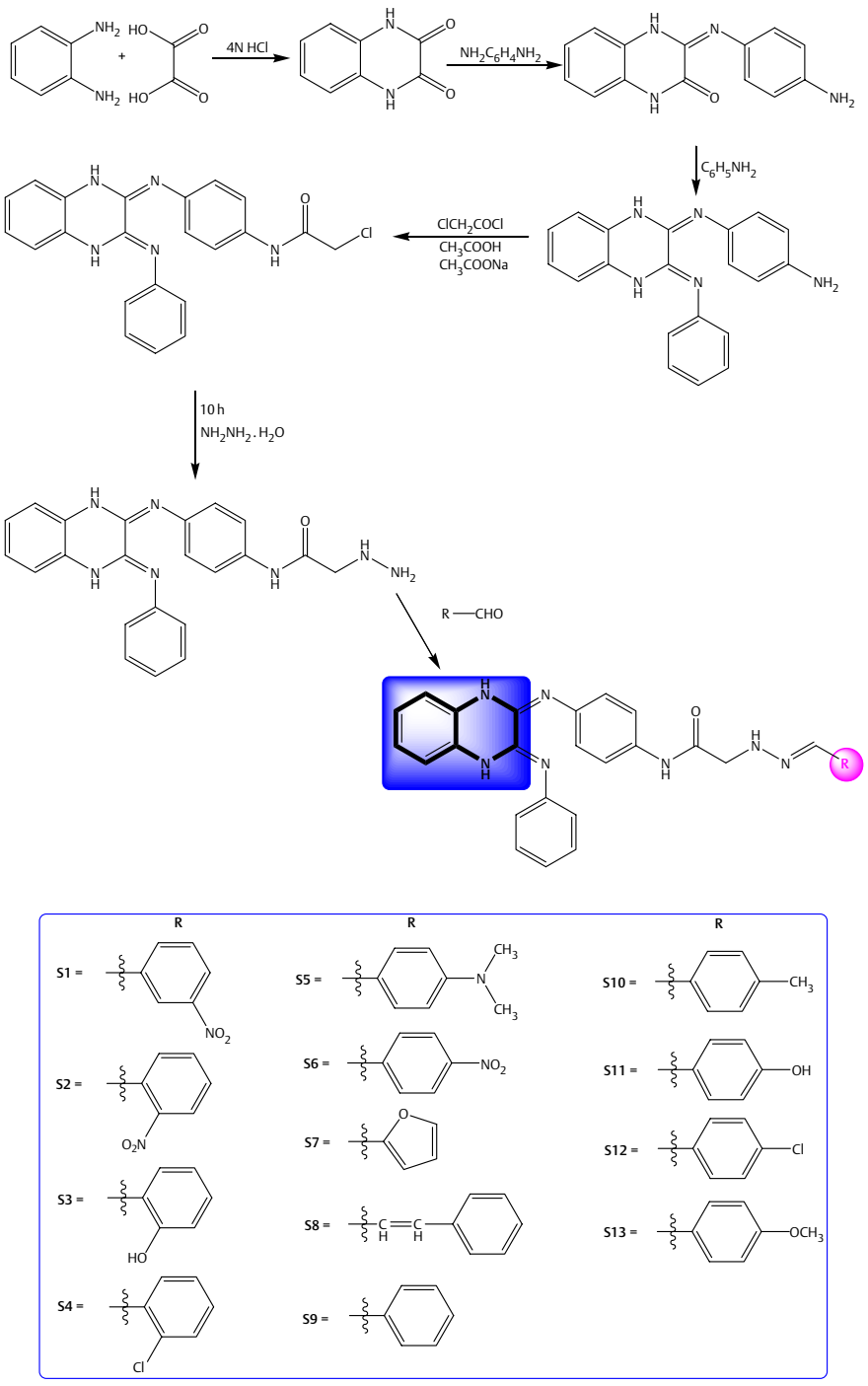
Yield: 76 %, m.p.: 293–295 °C. IR (KBr, cm^{-1}): 3356 & 3272 (NH), 3097 (Ar-CH), 2980 (CH_2-CH), 1685 (C = O), 1640 (C = N), 1619 (C = C), 1068 (C-O-C). ^1H NMR ($CDCl_3$, 500 MHz) δ ppm: 2.33 (s, 1 H, NH of CH_2NH), 3.65 (s, 2 H, CH_2 of CH_2NH), 3.82 (s, 3 H, OCH_3), 4.20 (s, 1 H, NH of quinoxaline), 4.57 (s, 1 H, NH of quinoxaline), 7.26–8.39 (m, 17 H, Ar-H), 8.51 (s, 1 H, CONH), 8.78 (s, 1 H, CH of CH = N). EI-MS m/z: 517 (M^+). Anal. Cald for $C_{30}H_{27}N_7O_2$: C, 69.62; H, 5.26; N, 18.94. Found: C, 69.83; H, 5.24; N, 18.89.

Results and discussion

Chemistry

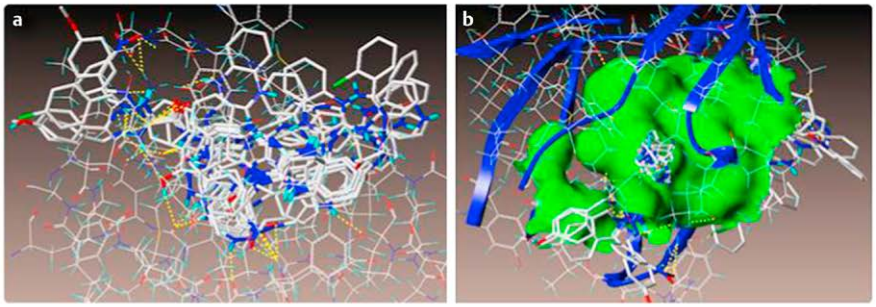
A series of novel 2-(2-(substitutedbenzylidene)hydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydro quinoxalin-2(1 H)-ylidene)amino) phenyl)acetamide **S1-S13** were synthesized from o-phenylenediamine and oxalic acid by a multistep synthesis. The synthetic pathway by which compounds synthesized were illustrated in ▶ **Fig. 3**. Initially o-phenylenediamine was treated with oxalic acid to obtain quinoxaline-2,3(1 H,4 H)-dione **1** by a ring closure reaction with removal of two water molecule. Latter, obtained quinoxaline-2,3(1 H,4 H)-dione **1** undergone Schiff base reaction with p-phenylenediamine and produced 3-(4-aminophenylimino)-3,4-dihydroquinoxalin-2(1 H)-one **2**. In the succeeding step, compound **2** was treated with aniline to produce N^1-(3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene)benzene-1,4-diamine **3** by Schiff base reaction. In the next step, compound **3** was treated with chloro acetyl chloride to get a corresponding chloro acetyl derivative **4** [2-chloro-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene)amino)phenyl) acetamide] by chloro acetylation in presence of acetic acid and sodium acetate. In the next step compound **4** was treated with hydrazine hydrate to produce a corresponding 2-hydrazinyl acetamide derivative **5** [2-hydrazinyl-N-(4-((3-(phenyl imino)-3,4-dihydro quinoxalin-2(1 H)-ylidene)amino)phenyl)acetamide]. Finally, title compounds 2-(2-(substituted benzylidene) hydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydro quinoxalin-2(1 H)-ylidene) amino)phenyl) acetamide **S1-S13** were synthesized by treating compound **5** with different aromatic/heterocyclic aldehydes by a Schiff base reaction according to the ▶ **Fig. 3**. TLC was performed throughout the reactions to optimize the reactions for purity and completion.

IR, ^1H NMR, mass spectra, and elemental analyses of the synthesized compounds are in accordance with the assigned structures. The IR spectra of all synthesized compounds showed some characteristic peaks indicating the presence of particular groups. Formation of the quinoxaline-2,3(1 H,4 H)-dione ring in compound 1 was confirmed by the presence of absorption peak at 3351 &3279 and 1698 cm^{-1} in IR due to presence of NH and C = O stretching, respectively. This is further supported by the appearance of two singlet in NMR at δ 8.34 & 8.51 ppm corresponds to CONH of quinoxaline ring. The formation of compounds 2 was confirmed by the appearance of singlet at δ 4.13 ppm for two protons in its ^1H NMR spectra which might be assigned to NH_2 group. Disappearance of carbonyl group absorbance in IR spectrum around 1700 cm^{-1} and appearance of two singlet in ^1H NMR spectra at δ 4.05 and 4.49 ppm for one proton each corresponds to NH of quinoxaline ring confirms the formation of compound 3. Appearance of sharp absorbance peak in IR spectra at 1682 and 768 cm^{-1} due to presence of C = O and C-Cl stretching, respectively confirms the formation of com-



▶ **Fig. 3** Synthetic scheme.

pound 4. This is further confirmed by appearance of singlet at δ 8.34 ppm for one proton in its ^{1}H NMR spectra which might be assigned to CONH group. Further presence of a two protons in NH_2 of compound 5 was confirmed by the appearance of singlet at δ 2.03 ppm. This is further supported by appearance of singlet in ^{1}H NMR spectra at δ 2.66 and 3.95 ppm for one and two protons, respectively which might be assigned to NH and CH_2 of CH_2NH. The IR spectrum of title compound shows absorption bands at 3330–3364 & 3239–3289 cm^{-1}, 3034–3097 cm^{-1}, 2941–2985 cm^{-1}, 1672–1697 cm^{-1}, 1573–1648 cm^{-1}, and weak band at 1549–1629 cm^{-1}, which can be assignable to NH, Ar-H, CH_2-H, C = O, C = C, and C = N vibrations, respectively. IR spectrum of compounds S1, S2 and S6 showed absorption band in the region of 1529–1546 cm^{-1} and 1310–1328 cm^{-1} which may be assigned to NO_2



▶ **Fig. 4** Docked view of all the compounds at the active site of the enzyme PDB ID: 2PZI.

▶ **Table 3** Surflex Docking score (kcal/mol) of the derivatives.

Compound Code	C Score[a]	Crash Score[b]	Polar Score[c]	D Score[d]	PMF Score[e]	G Score[f]	Chem Score[g]
S6	6.20	−2.04	2.15	−145.421	−11.794	−251.859	−33.618
S4	5.92	−5.39	2.69	−194.872	−33.140	−332.882	46.500
S12	5.91	−3.13	0.51	−195.043	−63.590	−340.197	−40.375
S3	5.81	−1.56	3.46	−129.754	−35.726	−185.072	−34.638
S11	5.73	−3.82	1.03	−204.548	−36.900	−309.049	−41.291
S5	5.71	−1.16	1.22	−138.943	−29.481	−225.376	−33.436
S10	5.49	−3.52	0.71	−202.452	−46.508	−316.260	−41.800
S1	5.23	−2.83	1.25	−194.606	−64.174	−309.830	−36.356
S7	4.91	−1.84	0.83	−165.176	−28.976	−252.999	−33.282
S13	4.70	−2.12	0.71	−197.668	−43.669	−300.765	37.517
S8	4.68	−3.82	0.91	−164.413	−11.803	−266.513	−35.859
S9	3.47	−2.50	2.65	−137.175	−46.216	−214.545	−35.192
S2	3.36	−0.84	1.35	−104.621	−15.626	−149.543	−26.310

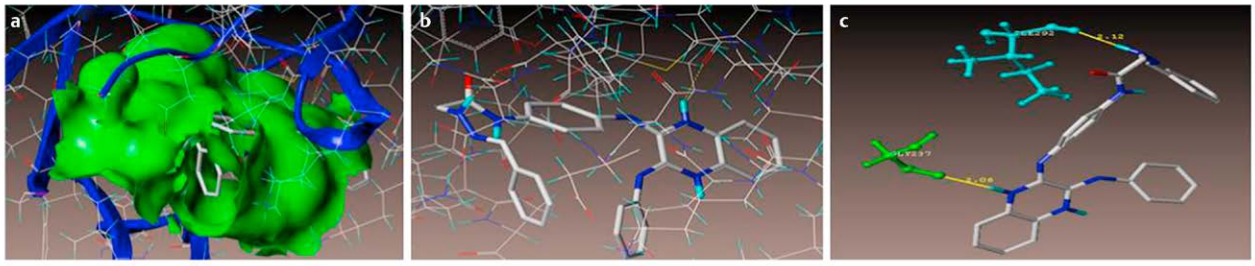
[a]CScore (Consensus Score) integrates a number of popular scoring functions for ranking the affinity of ligands bound to the active site of a receptor and reports the output of total score; [b] Crash-score revealing the inappropriate penetration into the binding site. Crash scores close to 0 are favorable. Negative numbers indicate penetration; [c] Polar indicating the contribution of the polar interactions to the total score. The polar score may be useful for excluding docking results that make no hydrogen bonds; [d] D-score for charge and van der Waals interactions between the protein and the ligand; [e] PMF-score indicating the Helmholtz free energies of interactions for protein-ligand atom pairs (Potential of Mean Force, PMF); [f] G-score showing hydrogen bonding, complex (ligand-protein), and internal (ligand-ligand) energies; [g] Chem-score points for H-bonding, lipophilic contact, and rotational entropy, along with an intercept term.

group. Compound S3 and S11 showed absorption bands at 3531–3590 cm^{-1} due to presence of OH stretching. In addition presence of chlorine in compounds S4 and S12 were confirmed by the appearance of absorption bands at 764–785 cm^{-1} in IR spectrum and appearance of M^{+2} peaks in mass spectrum. Compound S7 and S13 showed absorption bands at 1068–1086 cm^{-1} due to presence of C-O-C vibrations. The proton magnetic resonance spectrums of synthesized compounds were recorded in $CDCl_3$. The following conclusions can be derived by comparing the ^1H NMR spectra of title compounds:

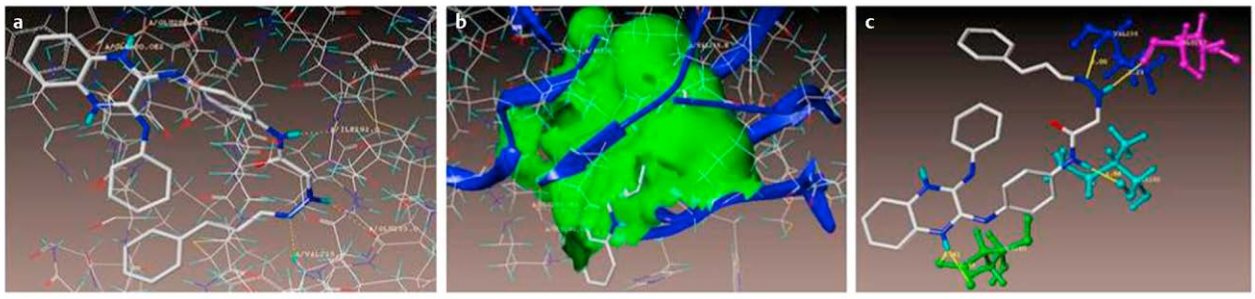
- A singlet at δ 2.04–3.02 ppm for NH of CH_2NH,
- A singlet at δ 3.16–3.97 ppm for CH_2 of CH_2NH,
- A singlet at δ 3.98–4.45 ppm for NH of quinoxaline,
- A singlet at δ 4.31–4.64 ppm for NH of quinoxaline,
- A multiplet at δ 6.82–8.39 ppm for Ar-CH,
- A singlet at δ 8.04–8.85 ppm for CONH,
- A singlet at δ 8.31–9.03 ppm for N = CH.

In silico screening

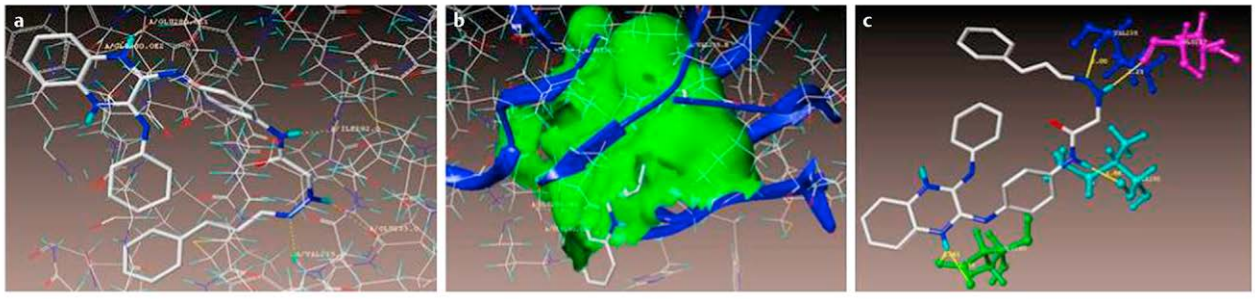
The PknG inhibition activity of 2-(2-(substituted benzylidene) hydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene)amino)phenyl) acetamide **S1-S13** compounds docked with active site of crystal structure of PknG. The molecular docking studies were performed on the crystal structure of Protein kinase PknG from Mtb in Complex with Tetrahydrobenzothiophene using the surflex-dock programme of sybyl-X 2.0 software. All the 13 inhibitors were docked into the active site of enzyme as shown in ▶ **Fig. 4**a and b. The predicted binding energies of the compounds are listed in ▶ **Table 3**. The docking study revealed that all the compounds have showed very good docking score against Mtb. As depicted in the ▶ **Fig. 5a-c**, compound **S6** makes two hydrogen bonding interactions at the active site of the enzyme (PDB ID: 2PZI), hydrogen atom present at hydrazineyl group makes an interaction with carbonyl group of ILE292 (-NH ------ O = C-ILE292, 2.12 Å), hydrogen atom present at the quinoxaline ring makes hydrogen



▶ **Fig. 5** Docked view of compound **S6** at the active site of the enzyme PDB: 2PZI.



▶ **Fig. 6** Interaction of compound **S4** at the binding site of the enzyme (PDB ID: 2PZI).



▶ **Fig. 7** Interaction of compound **S12** at the binding site of the enzyme (PDB ID: 2PZI).

bonding interaction with carbonyl group of GLY237 (NH-----O = C-GLY237, 2.06 Å).

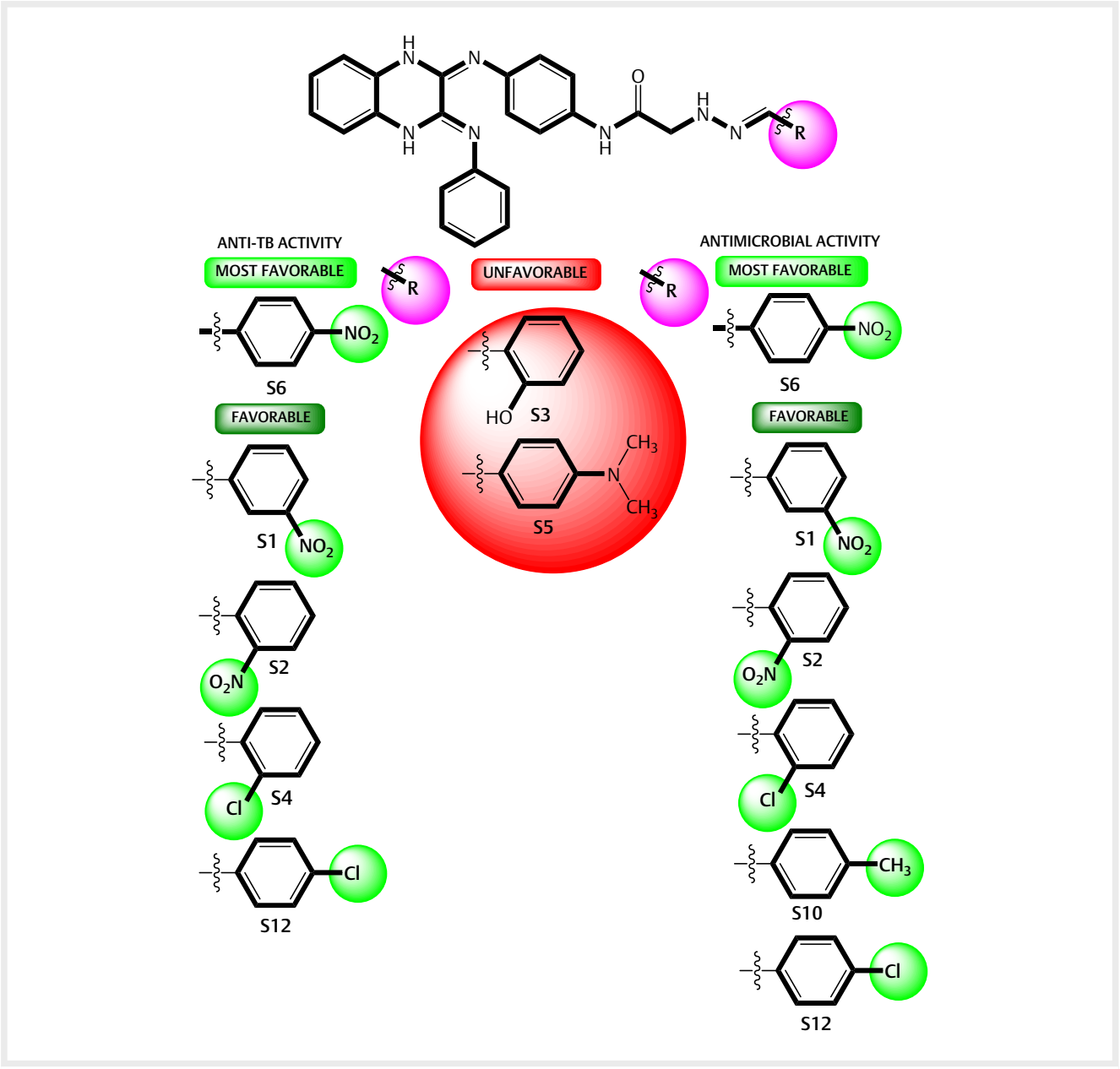
As depicted in ▶ **Fig. 6a-c**, compound **S4,** makes five hydrogen bonding interactions at the active site of the enzyme (PDB ID: 2PZI), among those, two interactions were of hydrogen atom of quinoxaline ring with oxygen of GLU280 (-NH ------ O-GLU280, 2.41 Å, 2.50 Å),

hydrogen atom of acetamide group makes an interaction with carbonyl group of ILE292 (-NH ------ O = C-ILE292, 1.88 Å), hydrogen atom of hydrazineyl group makes hydrogen bonding interaction with carbonyl group of GLU233 (NH ------ O = C-GLU233, 2.21 Å) and remaining another hydrogen bonding interaction raised from the nitrogen atom of hydrazineyl group with hydrogen of VAL235 (N ----- H-VAL235).

As depicted in ▶ **Fig. 7a-c**, compound **S12**, makes four hydrogen bonding interactions at the active site of the enzyme (PDB ID: 2PZI), among those, two interactions were of nitrogen atom of hydrazineyl group with hydrogen of LYS181 (-N ------ H-LYS181, 2.68 Å, 2.57 Å), hydrogen atom of carboxamide group with carbonyl group of ASN281 (NH ------ O = C-ASN281, 2.42 Å) and the carbonyl group of the carboxamide has one hydrogen bonding with hydrogen of LYS181 (C = O-----H-LYS181, 2.73 Å). ▶ **Fig. 8** represents the selected area of interest for comparative analysis.

Antimicrobial activity

All the synthesized compounds were evaluated for their in vitro antibacterial and antifungal activity. A comparison of antimicrobial activity of the synthesized compounds with that of standard drugs



▶ **Fig. 8** Selected area of interest for comparative analysis.

was effectively presented in ▶ **Table 2**. All compounds were found to exhibit significant activity against all the tested bacteria and fungi. Compounds S1, S2, S4, S6, S10 and S12 showed excellent antimicrobial properties against the entire microorganisms that were included in the present evaluation. When compared to standard drug, compound S6 showed remarkable activity against Bacillus subtilis ATCC 6633, Klebsiella pneumoniae ATCC 13883, Trichoderma ATCC 26921, and Aspergillus niger ATCC 9029. In addition compound S6 showed equipotent activity against Pseudomonas aeruginosa ATCC 27853, and Aspergillus flavus ATCC 10124. Moreover, compound S1 exhibited more activity against Klebsiella pneumoniae ATCC 13883 when compared with the reference drug Ciprofloxacin. Among the tested compounds against Bacillus subtilis ATCC 6633, Pseudomonas aeruginosa ATCC 27853, Trichoderma ATCC 26921, and Aspergillus niger ATCC 9029 compound S1 showed equipotent activity like standard drug; whereas compound S2 shown equipotent activity against Klebsiella pneumoniae ATCC 13883 and Aspergillus niger ATCC 9029. Compounds S3, S5, S11 and S13 showed moderate to good anti-microbial activity against tested micro organism. Rest of compounds S7, S8 and S9 showed weaker anti-microbial activity.

All the synthesized compounds were subjected to MIC (minimum inhibitory concentration) studies against all microorganisms. The MICs of Ciprofloxacin and Ketoconazole were determined in parallel experiments in order to control the sensitivity of the test organisms. MIC values of the compounds and the standards are presented in ▶ **Table 2**. As seen in ▶ **Table 2** While all compounds showed lower activities than the standard against B. subtilis, com-

pounds S1, S2 and S6 showed the same activity (MIC: 7.81 µg/ml). Against K. pneumoniae, all compounds exhibited lower activity (MIC: 7.81–125 µg/ml) than standard (MIC: 3.9 µg/ml). Similarly all test compounds exhibited lower activity (MIC: 15.62–125 µg/ml) against P. aeruginosa than standard Ciprofloxacin (MIC: 7.81 µg/ml). Compared to Ketoconazole (MIC: 7.81 µg/ml) entire test compounds exhibited lesser activity (MIC: 15.62–125 µg/ml) against Trichoderma. Compound S6 showed the same activity (MIC: 15.62 µg/ml) as Ketoconazole against A. niger, while others demonstrated lower activity (MIC: 31.25–125 µg/ml) than standard. Compound S6 exhibited the same activity (MIC: 7.81 µg/ml) against A. flavus, while other compounds exhibited lower activity (MIC: 15.62–125 µg/ml) than standard Ketoconazole.

In vitro anti TB screening

Herein we report anti TB screening of title compounds 2-(2-(substituted benzylidene) hydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)- ylidene) amino) phenyl) acetamide **S1-S13**. The anti-TB effect of these synthetic compounds on the growth of Mtb H37Ra was recorded after 7 days of incubation at 37 °C using by MABA method. There are six different concentrations 0.97, 1.95, 3.90, 7.81, 15.62 and 31.25 µg/ml were used. The data of the anti-TB activity screening reveals that the compound **S3, S5** was inactive in all concentrations against MTB H37Ra strain. In the MABA screening, compounds **S1, S2, S4, S6** and **S12** were sensitive at 1.95 µg/ml concentration; compound **S10** were sensitive at 3.90 µg/ml concentration; compounds **S11** and **S13** were sensitive at 7.81 µg/ml concentration; compounds **S7**, and **S8**, were sensitive at 15.62 µg/ml concentration and compound **S9** were sensitive at 31.25 µg/ml concentration. As like antimicrobial activity it is interesting to highlight that compounds **S1, S2, S4, S6** and **S12** showed enhanced activity and these compounds were well thought-out as a most effective scaffolds against microbial strains and was found to indicate alike anti-TB potency.

Structure activity relationships (SAR)

Structure activity relationship (SAR) studies indicated that nature of the substituent attached at 2-hydrazinyl acetamide is important for anti-microbial and anti-TB activity ▶ **Fig. 8**. In general it was found that most potent anti-microbial and anti-TB activities was exhibited compounds (**S1, S2, S4, S6** and **S12**) possessing electron withdrawing substituent like nitro, chlorine, and methyl group on phenyl ring attached to 2-hydrazinyl acetamide. While other compounds containing electron donating substituents such as methoxy, hydroxyl and dimethylamino groups (**S3** and **S5**) exhibited moderate in-vitro anti-microbial activity and poor anti-TB activity. The unsubstituted / heterocyclic derivative (**S7, S8** and **S9**) showed moderate to weaker activity. The chemical structure and anti-microbial and anti-TB activities are relationship of the synthesized compounds revealed that the compounds having electron withdrawing moiety exhibited better activity than compounds having electron releasing moieties; whereas the unsubstituted derivative exhibited weaker activity. On correlating the structures of the sample candidate within the same substituent, para substituted derivatives (**S6** and **S12**) showed potent activity than corresponding ortho/meta substituted analogs (**S1, S2** and **S4**).

Conclusions

In summary, a series of novel quinoxaline Schiff bases (**S1-S13**) were synthesized and evaluated for anti-microbial activities against various pathogenic microorganisms. IR, ^{1}H-NMR, Mass spectroscopy and elemental analyses data of the synthesized compounds are in accordance with the assigned structures. All compounds were found to display significant activity against entire tested bacteria, fungi and Mtb H37Ra. Towards entire tested bacteria, fungi & Mtb H37Ra compounds **S1, S2, S4, S6, S10** and **S12** showed excellent anti-microbial and anti-TB properties. SAR studies indicated that nature of the substituent attached at 2-hydrazinyl acetamide is important for anti-microbial and anti-TB activities. The chemical structure and anti-microbial and anti-TB activities are relationship of the synthesized compounds revealed that the compounds having electron withdrawing moiety exhibited better activity than compounds having electron releasing moieties; whereas the unsubstituted derivative exhibited weaker activity. On correlating the structures of the sample candidate within the same substituent, para substituted derivatives showed potent activity than corresponding ortho / meta substituted analogs. Out of thirteen tested compounds, 2-(2-(4-nitrobenzylidene)hydrazinyl)-N-(4-((3-(phenylimino)-3,4-dihydroquinoxalin-2(1 H)-ylidene) amino)phenyl)acetamide **S6** exhibited most potent anti-bacterial, anti-fungal and anti-TB activities compared to Ciprofloxacin and Ketoconazole. As a result the compound **S6** could therefore serve as a pilot molecule for further development as novel class of anti-microbial agent.

Acknowledgement

The authors are thankful to the management of MNR College of Pharmacy, Fasalwadi, Sangareddy, Telangana, India, for providing infrastructure facilities to carry out this research work. The authors gratefully acknowledge the Central Instrumentation Facility, IIT Chennai, India for the spectral analysis of the compounds used in this study. The authors also thank Kalasalingam University, Tamilnadu and VIT University, Vellore for providing insilico facilities to carry out this research work.

Conflict of Interest

The authors have declared no conflict of interest.

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