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Preliminary communication

3-(1,3,4-Thiadiazole-2-yl)quinoline derivatives: Synthesis, characterization and anti-microbial activity

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ABSTRACT

A new series of thiadiazoles and intermediate thiosemicarbazones were synthesized from the chloroquinone molecule, with an aim to explore their effect on *in vitro* growth of microorganisms causing microbial infection. The chemical structures of the compound were elucidated by elemental analysis, FTIR, 1H and 13C NMR and ESI-MS spectral data. *In vitro* anti-microbial activity was performed against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhimurium*, and *Escherichia coli*. The MIC was detected using the double dilution method. The results were compared by calculating percent inhibit area/μg of the compounds and the standard "amoxicillin". The selected compounds were tested for cytotoxic results using MTT assay H9c2 cardiac myoblasts cell line and the results showed that all the compounds offered remarkable >80% viability to a concentration of 200 μg/mL.

1. Introduction

The innovative research for antibiotics has improved mankind's health status by confining life threatening infections. However, the emergence and spread of bacterial resistance represent a severe global problem [1]. Multidrug-resistant gram-positive pathogens, such as methicillin-resistant *Staphylococci aureus* (MRSA), penicillin resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant *enterrococci* (VRE), compounded problems in the therapeutics [2–4]. Heterocyclic thiadiazoles are widely exposed to therapeutic world, because of their known anti-HIV [5], anti-inflammatory [6–8], anti-cancer [9], anti-tuberculosis [10,11], anti-convulsant [12] and anti-hypertensive [13,14] activities. Nitroheteroaryl-1,3,4-thiadiazole derivatives have shown impressive anti-microbial and antiparasitic activity particularly against *Trypanosomatid protozoa* [15]. Thiadiazole derivative, 2-(4-chloropheny1amino)-5-(4-aminophenyl)-1,3,4-thiadiazole showed 57% inhibition against *Mycobacterium tuberculosis* [16].

Quinolines have been of great interest as antibacterial and antiviral compounds for several decades, starting from the introduction of nalidixic acid in 1962 for the urinary tract infections. Various successful attempts have been made to produce the potent therapeutic agents such as torvafloxin [17], moxifloxacin [18], gemifloxacin [19] and gatifloxacin [20] etc. from quinoline. The incidence of gram-positive bacterial resistance to these antibacterial agents is growing and will be of major concern in near future [21,22]. Therefore, there is an urgent need for novel chemical entities that are particularly effective against gram-positive pathogens. In continuation of our efforts in developing heterocycles of biological interest [23–25] and considering the significant role of thiadiazoles [26–28] and quinolines in biological applications, we wish to report here the synthesis of a new series of quinoline-thiadiazole derivatives and their anti-microbial activities.

2. Result and discussion

2.1. Chemistry

The synthetic route of compounds (**1B**–**24B**) is shown in Fig. 1. Compounds were obtained from a starting material of 2,8-substituted-quinoline-3-carbaldehyde. The synthesized thiosemicarbazones (Fig. 1, Table 1) of substituted-quinoline were cyclized in presence of acetic anhydride to get the final thiadiazole compounds. All the reactions were monitored by TLC (aluminum sheet, silica gel 60 F_{254}, Merck). The precursor, thiosemicarbazone (**1A**–**24A**) and the final compounds (**1B**–**24B**) were purified by column chromatography using silica gel (pore size 60 Å, 200–400

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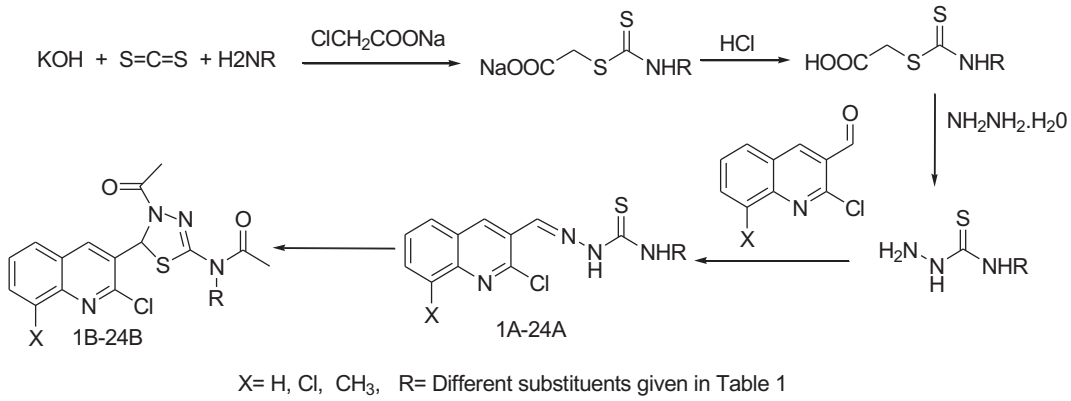
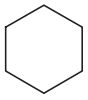
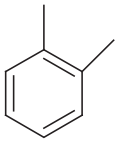
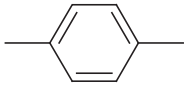
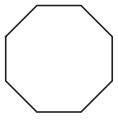
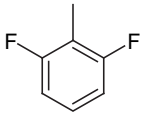
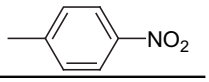


Fig. 1. Schematic Representation of the Synthetic route adopted for the synthesis N-4-acetyl-5-(2,8-substitutedquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)acetamide derivatives.

mesh) and eluted with proper solvent ($CHCl_3$:CH_3OH) system. The elutants were concentrated *in vacuo* and crystallized at 4 °C using chloroform hexane mixture to obtain a pure compound. The purity of the compounds was decided on the basis of melting points and elemental data analysis.

All the compounds were characterized using the ^{1}H NMR, IR and mass spectrophotometry. Some of the compounds were analyzed by using ^{13}C NMR. Almost all the compounds showed the molecular ion signal at M + 1, however in some cases [M + Na] signal was found. In ^{1}H NMR, the characterize signal around 7.28–6.72 ppm (CH=N) shows the condensation of substituted-quinoline-3-carbaldehyde and the different thiosemicarbazides. Other signals were found in accordance to the established structures. When the thiosemicarbazides were condensed in excess of acetic anhydride, the corresponding singlet shows an up-field shift from 7.28–6.72 ppm to 5.69–4.93 ppm range. The proton shift showed the change in status of carbon from unsaturated to saturated one. The cyclization was confirmed by the disappearance of NH signals and the appearance of a new singlet signal due to the six proton ($-CH_3$) around 2.13–3.10 ppm. To confirm the results, some molecules were analyzed by ^{13}C NMR. The ^{13}C NMR spectra of thiadiazoles showed some prominent signals, such as signals in 50–60 ppm, and around 22–26 ppm range, which were absent in corresponding thiosemicarbazones. These signals represent the change in carbon environment and were attributed to CH(NS) carbon and alkyl carbon, as the inclusion of two methyl groups was expected in the process of cyclization.

Table 1
Different substituent's "X" and "R" as indicated in Fig. 1.

Compound	X	R
1A, 1B	Cl	H
9A, 9B	H	
17A, 17B	CH_3	
3A, 3B	Cl	
11A, 11B	H	
19A, 19B	CH_3	
5A, 5B	Cl	
13A, 13B	H	
21A, 21B	CH_3	
7A, 7B	Cl	
15A, 15B	H	
23A, 23B	CH_3	
2A, 2B	Cl	
10A, 10B	H	
18A, 18B	CH_3	
4A, 4B	Cl	
12A, 12B	H	
20A, 20B	CH_3	
6A, 6B	Cl	
14A, 14B	H	
22A, 22B	CH_3	
8A, 8B	Cl	
16A, 16B	H	
24A, 24B	CH_3	

The structures were further confirmed by the appearance and disappearance of a band around 3400 cm^{-1} in the thiosemicarbazones and thiadiazoles, respectively. This suggests that the NH group had reacted in the process of cyclization. Besides the other characteristic signals for aromatic rings and C=N, a strong signal around 2930–2965 cm^{-1} indicates the presence of aliphatic methyl groups. The signal appeared as a new signal in the compound (**1B**–**24B**). The existence of a strong band in the region 1102–1133 cm^{-1} due to C=S and the absence of any band in the region 2500–2600 cm^{-1} due to C–SH suggest that all thiosemicarbazones retains the thione nature. In the compounds (**1B**–**24B**), two sharp carbonyl peaks were observed, backing inclusion of two acetyl groups to the newly cyclized thiadiazoles at the two different positions. Carbonyl group stretching frequency corresponded to esteric carbonyl stretching and amidic carbonyl stretching were observed at 1640–1678 cm^{-1}.

2.2. Pharmacology

2.2.1. In vitro evaluation of antibacterial activity against gram-positive and gram-negative bacteria

All the synthesized thiosemicarbazones (**1A**–**24A**) and their thiadiazole derivatives (**1B**–**24B**) were screened for their antimicrobial activity using the gram-positive and gram-negative bacteria. Four different cultures, two each of gram-negative (*Escherichia coli* and *Salmonella typhimurium*) and gram-positive (*Staphylococcus aureus* and *Staphylococcus pyogenes*) were treated

Table 2
Antibacterial activity of 1-((2,8-dichloroquinolin-3-yl)methylene)thiosemicarbazide derivatives (**1A**–**24A**), positive control (Amoxicillin), and negative control (DMSO) measured by the Halo Zone Test (Unit, mm).

Compounds	Effect of Compounds on Microorganism			
	S. aureus	*S. pyogenes*	*S. typhimurium*	*E. coli*
1A	21.13 ± 0.56	19.58 ± 0.79	16.66 ± 0.53	15.80 ± 0.58
2A	17.68 ± 0.73	13.15 ± 0.76	15.17 ± 0.87	17.18 ± 0.71
3A	17.23 ± 0.33	13.73 ± 0.51	15.72 ± 0.53	15.23 ± 0.94
4A	–	17.54 ± 0.36	17.54 ± 0.34	17.62 ± 0.39
5A	18.90 ± 0.40	10.26 ± 0.78	10.53 ± 1.04	13.47 ± 0.89
6A	12.15 ± 0.18	15.92 ± 0.73	14.92 ± 0.71	13.75 ± 0.15
7A	15.01 ± 0.18	15.76 ± 0.47	17.55 ± 0.59	15.88 ± 0.18
8A	19.70 ± 0.60	15.26 ± 0.62	15.99 ± 0.68	–
9A	11.67 ± 0.59	16.97 ± 0.45	12.42 ± 0.70	17.76 ± 0.38
10A	19.52 ± 0.81	21.07 ± 0.64	22.58 ± 0.54	21.12 ± 0.57
11A	12.37 ± 0.50	12.38 ± 0.50	15.93 ± 0.60	17.68 ± 0.78
12A	09.90 ± 0.65	13.67 ± 0.74	19.73 ± 0.96	13.23 ± 0.34
13A	–	16.48 ± 0.75	14.73 ± 0.49	18.84 ± 0.41
14A	16.75 ± 0.37	14.07 ± 0.93	11.56 ± 0.57	10.13 ± 0.26
15A	17.93 ± 0.38	17.43 ± 0.59	18.02 ± 0.50	16.65 ± 1.19
16A	13.46 ± 0.42	12.25 ± 1.11	–	13.27 ± 0.79
17A	12.76 ± 0.51	17.10 ± 0.86	18.02 ± 0.88	–
18A	20.04 ± 0.42	18.71 ± 0.73	17.44 ± 0.73	16.97 ± 0.65
19A	12.75 ± 0.14	11.08 ± 0.50	15.66 ± 0.56	21.27 ± 0.64
20A	15.78 ± 0.16	14.45 ± 0.61	22.51 ± 0.53	16.61 ± 0.53
21A	14.80 ± 0.41	18.81 ± 0.41	13.94 ± 0.62	15.17 ± 0.47
22A	–	10.13 ± 0.36	15.88 ± 0.58	14.72 ± 0.53
23A	19.24 ± 0.95	17.65 ± 1.34	17.18 ± 0.91	18.67 ± 0.74
24A	17.31 ± 0.97	13.27 ± 0.89	14.23 ± 0.94	17.48 ± 0.75
+ive C	20.85 ± 0.64	19.57 ± 0.58	22.37 ± 0.27	22.05 ± 0.14
−ive C	–	–	–	–

with synthesized compounds using disk diffusion method [29]. The minimum inhibitory concentration (MIC), concentration at which the halo zone formation starts, was determined by using different dilutions of the treatment compounds. The results were compared with positive control, the standard drug amoxicillin and negative control, the DMSO poured disk. The MIC was evaluated by the macro-dilution test using standard inoculums of 10^{-5} CFL mL^{-1}. Serial dilutions of the test compounds, previously dissolved in dimethyl sulfoxide (DMSO), were prepared to final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 μg mL^{-1}. The dilutions were added to 24 h old inoculums. The susceptibility of the bacteria to the test compounds was determined by the formation of an inhibitory zone after 18 h of incubation at 37 °C. Tables 2 and 3 report the

Table 3
Antibacterial activity of 1,3,4-thiadiazole derivatives (**1B**–**24B**), positive control (Amoxicillin), and negative control (DMSO) measured by the Halo Zone Test (Unit, mm).

Compounds	Effect of Compounds on Microorganism			
	S. aureus	*S. pyogenes*	*S. typhimurium*	*E. coli*
1B	18.13 ± 0.57	19.91 ± 0.72	17.27 ± 0.42	16.72 ± 0.52
2B	18.03 ± 0.50	14.15 ± 0.27	15.17 ± 0.87	18.18 ± 0.95
3B	17.39 ± 0.62	17.73 ± 0.52	16.72 ± 0.61	15.23 ± 1.13
4B	17.62 ± 0.87	17.94 ± 0.41	18.54 ± 0.73	16.36 ± 0.90
5B	19.76 ± 0.64	13.84 ± 0.74	16.86 ± 0.51	15.59 ± 0.64
6B	12.99 ± 0.85	13.92 ± 0.38	17.59 ± 0.67	16.45 ± 0.62
7B	15.67 ± 0.72	17.09 ± 0.92	16.88 ± 0.49	18.11 ± 0.58
8B	19.81 ± 0.52	16.32 ± 0.32	17.25 ± 0.89	12.56 ± 0.61
9B	15.67 ± 0.60	16.97 ± 0.59	13.42 ± 0.63	17.76 ± 0.38
10B	20.63 ± 0.67	19.93 ± 0.62	18.60 ± 0.52	19.79 ± 0.62
11B	14.03 ± 0.82	17.38 ± 0.50	14.59 ± 0.55	15.63 ± 0.39
12B	14.57 ± 0.88	14.33 ± 0.36	18.82 ± 0.64	17.86 ± 0.72
13B	15.66 ± 0.33	17.14 ± 0.93	16.06 ± 0.72	15.84 ± 0.65
14B	17.41 ± 0.95	14.74 ± 0.51	16.67 ± 0.50	20.13 ± 0.36
15B	18.27 ± 0.90	17.00 ± 0.54	16.89 ± 0.50	16.40 ± 1.07
16B	14.29 ± 0.66	14.92 ± 0.49	17.03 ± 0.62	14.34 ± 0.91
17B	13.66 ± 0.68	16.77 ± 1.00	18.08 ± 0.82	18.34 ± 0.56
18B	19.00 ± 0.69	17.71 ± 0.44	15.13 ± 0.98	16.08 ± 0.55
19B	15.42 ± 0.43	13.09 ± 0.72	12.01 ± 0.78	16.75 ± 0.69
20B	18.77 ± 0.91	15.45 ± 0.72	17.84 ± 0.66	19.00 ± 0.85
21B	15.80 ± 0.73	19.14 ± 0.82	16.11 ± 0.84	17.90 ± 0.67
22B	21.99 ± 0.57	16.13 ± 0.71	16.60 ± 0.51	15.36 ± 0.52
23B	18.92 ± 0.53	18.32 ± 1.10	17.27 ± 0.16	18.16 ± 0.42
24B	16.54 ± 0.72	15.27 ± 1.12	19.16 ± 0.23	13.03 ± 0.30
+ive C	21.52 ± 0.53	20.90 ± 0.60	21.82 ± 0.42	23.39 ± 0.68
−ive C	–	–	–	–

Table 4
Minimum Inhibition Concentration (μg/mL) of 1-((2,8-dichloroquinolin-3-yl) methylene)thiosemicarbazide derivatives (**1A**–**24A**) and positive control.

Compound	Effect of Compounds on Microorganism			
	S. aureus	*S. pyogenes*	*S. typhimurium*	*E. coli*
1A	64	64	128	128
2A	128	256	128	256
3A	64	128	256	256
4A	256	256	256	128
5A	32	64	32	64
6A	64	32	64	64
7A	64	32	32	64
8A	32	64	64	256
9A	128	64	128	256
10A	64	128	128	128
11A	256	256	128	128
12A	256	128	128	256
13A	256	64	64	32
14A	32	64	32	64
15A	32	32	64	64
16A	64	64	256	32
17A	64	128	256	256
18A	256	256	256	256
19A	128	256	256	128
20A	256	128	128	256
21A	32	64	32	64
22A	256	32	64	64
23A	32	64	32	64
24A	64	32	64	32
Amoxicillin	32	32	32	32

Table 5
Minimum Inhibition Concentration (μg/mL) of 1,3,4-thiadiazole derivatives (**1B**–**24B**) and positive control.

Compound	Minimum Inhibition Concentration (μg/mL)			
	S. aureus	*S. pyogenes*	*S. typhimurium*	*E. coli*
1B	64	64	64	64
2B	64	64	128	128
3B	64	128	256	128
4B	64	128	64	64
5B	16	64	32	32
6B	64	64	64	64
7B	64	64	64	64
8B	32	64	32	32
9B	64	64	64	64
10B	32	32	32	64
11B	256	256	64	128
12B	128	128	64	256
13B	32	16	32	32
14B	16	64	32	32
15B	32	16	64	32
16B	64	64	64	64
17B	64	64	256	256
18B	128	64	128	128
19B	128	128	256	256
20B	256	256	128	256
21B	64	32	32	64
22B	64	16	32	64
23B	64	64	64	64
24B	64	64	64	64
Amoxicillin	32	32	32	32

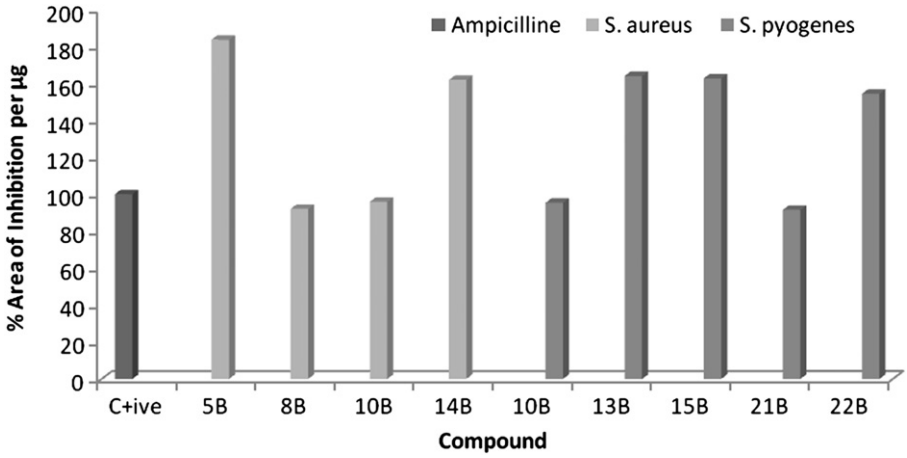


Fig. 2. The graph showing comparative percent area inhibition per µg of the compounds and the Amoxicillin, in case of gram-positive bacteria, *S. aureus and S. pyogenes*.

inhibition zones (mm) of each compound and the minimum concentration at which the inhibition zones appeared are presented in Tables 4 and 5.

The zone of inhibition was measured at the minimum inhibitory concentration. To compare the anti-microbial effect of the compounds with that of the ampicillin, the inhibition zone and MIC were considered. We calculated percent inhibition area per microgram of the compound and compared it with the positive control. All the thiosemicarbazones (**1A**–**24A**) were found less effective than amoxicillin when % inhibition area was compared. Although some of them showed 80% resemblance with the standard drug. From the initial screening of thiadiazoles (**1B**–**24B**) and comparing the % inhibition area per µg, it was found that compound **10B** and **21B** shows better inhibition in *S. pyogens* than amoxicillin, while **13B**, **15B** and **22B** were approximately similar to the amoxicillin inhibition. Similarly comparing the percent inhibition area in *S. aureus*, compound **5B** and **14B** showed better results than the control positive and compound **10B** and **8B** were found approximately similar to the control positive (Fig. 2). When the percent area inhibited ratio of gram-negative bacteria was compared, none of the compounds showed better result than the positive control.

The distinct difference in the antibacterial property of the thiosemicarbazones and the thiadiazoles justifies the purpose of this study. The importance of such work lies in the possibility that the new compound might be more effective against bacteria for which a thorough investigation like enzyme assay and other biological assays would be helpful in designing more potent antibacterial agents for therapeutic use.

2.2.2. Toxicity profile

To ensure the toxicity of the compounds **5B**, **8B**, **10B**, **13B**, **14B**, **15B**, **21B** and **22B** with better or nearer to, ratio of percent area inhibited per µg compared with positive control, were selected for cytotoxic studies. The compounds were tested against H9c2 cardiac myoblasts. The results were compared with a negative control (DMSO) and a positive control (amoxicillin). A subconfluent population of H9c2 cells was treated with increasing concentrations of test compounds and the positive control and the number of viable cells was measured after 48 h by MTT cell viability assay. Fig. 3 depicted that all these compounds exhibited >80% viability at the concentration range of 1.57–200 mg mL^{-1}. An ELISA plate reader (Labsystems Multiskan RC, Helsinki, Finland) at 570 nm with a reference wavelength of 655 nm was used.

2.2.3. Structure activity relationship

The new series of thiadiazoles were designed in a way to find-out the effect of substituted aliphatic and aromatic moieties on the biological activity. The thiadiazole molecule was substituted at C5 with three different 8-substitutedchloroquinoline (Cl, H, and CH_3) derivatives and at C2 with N-(substituted)acetamide (aromatic and

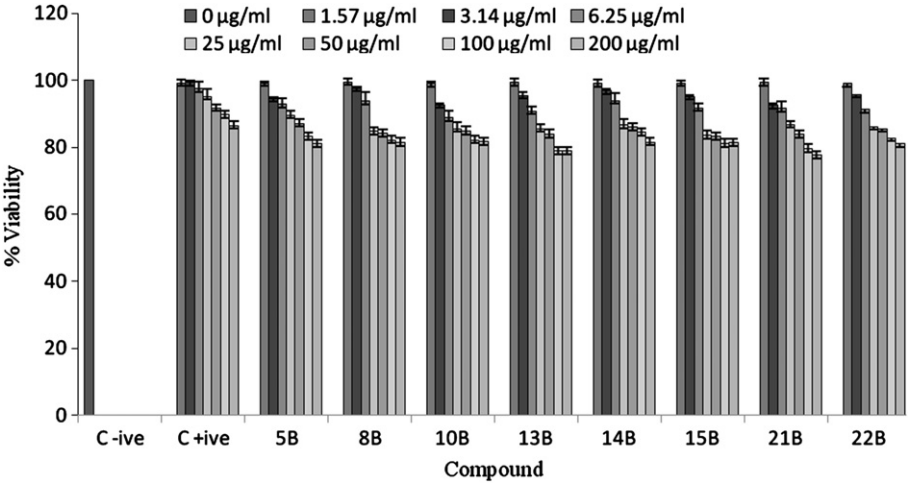


Fig. 3. The graph showing effect of different concentrations of thiadiazoles on percent viability of H9c2 cardiac myoblast cell line.

cyclic) derivatives. From the results it was found that thiadiazole derivatives substituted with N-(aromatic)acetamide group at C2 shows better inhibitory effects. Among the aromatic compounds the N-(o-tolyl)acetamide and N-(p-tolyl)acetamide showed better activity. However N-(o-tolyl)acetamide in combination with the 2,8-dichloroquinoline substituted at C5 of the thiadiazole ring showed much better inhibitory effect in *S. aureus,* compared on the basis of percent area of inhibition per microgram. This was followed by N-(o-tolyl)acetamide in combination with 2-chloroquinoline and N-(p-tolyl)acetamide in combination with chloroquinoline. When o-tolyl group was used in combination with 2-chloro-8-methylquinoline, the effect was less compared to 2-chloro and 2,8-dichloroquinoline. The trend was found opposite in case of N-(2,5-difluorophenyl) acetamide substituted at C2 of thiadiazole molecule. When difluorophenyl molecule was used in combination with 2-chloro,8-methyl quinolone attached at C5 of the thiadiazole ring, the effect was higher compared to 2-chloroquinoline. N-(p-nitropheyl)acetamide when used with 2-chloroquinoline also showed effect on the inhibition of growth of *S. pyogenes.* But the effect was not as prominent with other aromatic substituted groups. This shows that the substituent group play a definite role on the biological activity of these molecules.

3. Conclusion

A new library of thiosemicarbazones and thidiazoles were synthesized with an aim to target the microorganisms that cause microbial infection. The compounds were synthesized keeping in mind the molecular weight, antibiotics literally mean low molecular weight chemical compounds. Considering the individual drug activity of quinolines, thiosemicarbazones and thiadiazoles, we designed the synthesis in such a way that products will possess a quinoline moiety and the corresponding thiosemicarbazones and thiadizoles and will posses low molecular weight. In all the thiosemicarbazones (**1A**–**24A**) and thiadiazoles (**1B**–**24B**) synthesized and screened for anti-microbial activity compounds **5B** and **14B** showed better inhibitory effects for *S. aureus* and **13B** and **15B** showed better results for *S. pyogenes.*

4. Experimental

Reactions were conducted in oven dried glassware. Solvents were purified prior to use. Anhydrous sodium sulfate was used as the drying agent. All the melting points were recorded on a KSW apparatus and were uncorrected. Elemental analyses were performed by Regional Sophisticated Instrumentation Centre, Central drug Research Institute, Lucknow, India. IR spectra were recorded on Perkin–Elmer model 1600 FI-IR (KBr Pellet in the 4000–400 cm^{-1} range). The ^1H NMR spectra were obtained at 300 MHz in $CDCl_3$, DMSO-d_6 by using Bruker spectrospin and Varian instrument using TMS as internal standard and chemical shift values are given in ppm downfield to TMS (tetramethylsilane). ESI-MS was recorded on a Micromass Quattro II triple quadrupole mass spectrometer. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Chemical shift values are given in parts per million. The reactions were monitored by precoated aluminum-sheet TLC using chloroform:methanol (5:1) as eluant and column chromatography was accomplished using Silica gel, 60 Å (200–400 mesh).

4.1. Preparation of thiosemicarbazides

Substituted thiosemicarbazides were prepared by a two-step synthetic route as reported in our previous paper [30]. The data was compared with our previous work.

4.2. Preparation of 4-Substituted1-((2,8-dichloroquinolin-3-yl) methylene)thiosemicarbazide

Synthesized thiosemicarbazides (1 equiv.) were dissolved in 10 mL of absolute EtOH, followed by addition of a solution of 2,8-disubstituted quinoline-3-carboxaldehyde (1 equiv.) in EtOH. After stirring for an initial period of 15–30 min, AcOH was added in catalytic amount to increase the yield of the reaction. The mixture was heated to reflux at 60 °C for 12 h. The resultant mixture was evaporated *in vacuo* to give crude solid products, which were further purified by column chromatography (CH_2Cl_2/MeOH) to yield compounds **1A**–**24A**.

4.2.1. 1-((2,8-Dichloroquinolin-3-yl) methylene)thiosemicarbazide (*1A*)

Light Yellowish; yield 79%; mp 165 °C; Anal. Calc. for $C_{11}H_8Cl_2N_4S$: C 44.16, H 2.70, N 18.73, found C 44.13, H 2.71, N 18.71; IR ν(cm^{-1}): 3466 (NH_2),3163 (NH), 1465 (C=N), 1109 (C=S); ^1H NMR ($CDCl_3$) δ(ppm): 7.39–7.69 (m, 4H, Ar-H), 7.1 (s, 1H, CH=N), 5.96 (s, 1H, NH_2), 2.4 (s, 2H, NH_2); ESI-MS *m*/*z*: [M^+ + Na] 322.1

4.2.2. 1-((2,8-Dichloroquinolin-3-yl)methylene)-4-cyclopentylthiosemicarbazide (*2A*)

Dark Brown; yield 76%; mp 158 °C; Anal. Calc. for $C_{16}H_{16}Cl_2N_4S$: C 52.32, H 4.39, N 15.25, found C 52.29, H 4.41, N 15.24; IR ν(cm^{-1}): 3197 (NH), 2829 (CH_2), 1447 (C=N), 1110 (C=S); ^1H NMR ($CDCl_3$) δ/ppm: 11.74 (s, 1H, NNH), 8.6 (s, 1H, Ar-H), 7.43–7.56 (m, 3H, Ar-H), 6.91(s, 1H, N=CH), 5.99 (s, 1H, CNH), 2.82–2.93 (m, 1H, NCH), 1.93–2.3 (m, 8H, CH_2); ESI-MS *m*/*z*: [M^+ + H] 368.3

4.2.3. 1-((2,8-Dichloroquinolin-3-yl)methylene)-4-cyclohexylthiosemicarbazide (*3A*)

Light Yellowish; yield 81%; mp 160 °C; Anal. Calc. for $C_{17}H_{18}Cl_2N_4S$: C 60.92, H 4.26, N 15.79, found C 62.94, H 4.24, N 15.77; IR ν(cm^{-1}): 3259 (NH), 2863 (CH_2), 1475 (C=N), 1117 (C=S); ^1H NMR (DMSO) δ(ppm): 11.17 (s, 1H, NNH), 8.19 (s, 1H, Ar-H), 7.39–7.53 (m, 3H, Ar-H), 7.28 (s, 1H, CH=N), 6.13 (s, 1H, CNH), 2.93 (m, 1H, NCH), 2.29–2.50 (m, 10H, CH_2); ESI-MS *m*/*z*: [M^+ + H] 382.2

4.2.4. 1-((2,8-Dichloroquinolin-3-yl)methylene)-4-cyclooctylthiosemicarbazide (*4A*)

Blackish; yield 62%; mp 161 °C; Anal. Calc. for $C_{19}H_{22}Cl_2N_4S$: C 55.74, H 5.42, N 13.69, found C 55.76, H 5.44, N 13.71; IR ν(cm^{-1}): 3250 (NH), 2822 (CH_2), 1777 (C=C), 1115 (C=S); ^1H NMR ($CDCl_3$) δ(ppm): 11.53 (s, 1H, NNH), 8.68 (s, 1H, Ar-H), 7.45–7.69 (m, 3H, Ar-H), 7.13 (s, 1H, CH=N), 5.67 (s, 1H, CNH), 2.42–2.53 (m, 1H, NCH), 2.07–2.31 (m, 4H, CH_2), 1.73–1.92 (m, 10H, CH_2); ESI-MS *m*/*z*: [M^+ + Na] 432.3

4.2.5. 1-((2,8-Dichloroquinolin-3-yl)methylene)-4-o-tolylthiosemicarbazide *5A*

White; yield 69%; mp 163 °C; Anal. Calc. for $C_{18}H_{14}Cl_2N_4S$: C 55.53, H 3.62, N 14.39, found C 55.55, H 3.59, N 14.37; IR ν(cm^{-1}): 3260 (NH), 3020 (Ar,C–H), 2928 (CH_3), 1446 (C=N), 1113 (C=S); ^1H NMR ($CDCl_3$) δ(ppm): 11.93 (s, 1H, NNH), 9.06 (s, 1H, CNH), 7.34–8.69 (m, 8H, Ar-H), 7.12 (s, 1H, CH=N), 2.31 (s, 3H, CH_3); ESI-MS *m*/*z*: [M^+ + H] 390.4

4.2.6. 1-((2,8-Dichloroquinolin-3-yl)methylene)-4-(2,5-difluorophenyl)thiosemicarbazide (*6A*)

White; yield 73%; mp 165 °C; Anal. Calc. for $C_{17}H_{10}Cl_2F_2N_4S$: C 49.65, H 2.45, N 13.62, found C 49.69, H 2.50, N 13.58; IR ν(cm^{-1}): 3199 (NH), 3092 (Ar, C–H),1476 (C=N), 1125 (C=S); ^1H NMR (DMSO) δ(ppm): 11.23 (s, 1H, NNH), 7.9–8.65 (m, 7H, Ar-H), 7.21 (s, 1H, CH=N); ESI-MS *m*/*z*: [M^+ + H] 412.26.

*4.2.7. 1-((2,8-Dichloroquinolin-3-yl)methylene)-4-p-tolylthiosemicarbazide (**7A**)*

Brownish; yield 76%; mp 164 °C; Anal. Calc. for $C_{18}H_{14}Cl_2N_4S$: C 55.53, H 3.62, N 14.39, found C 55.51, H 3.60, N 16.43; IR ν(cm^{-1}): 3255 (NH), 3062 (Ar,C–H), 2914 (CH_3),1486 (C=N), 1127 (C=S); ^{1}H NMR ($CDCl_3$) δ(ppm): 12.13 (s, 1H, NNH), 9.01(s, 1H, Ar-H), 8.31 (s, 1H, NH), 7.38–7.86 (m, 7H, Ar-H.), 7.13 (s, 1H, CH=N), 2.12 (s, 3H, CH_3); ESI-MS *m*/*z*: [M^+] 389.1.

*4.2.8. 1-((2,8-Dichloroquinolin-3-yl)methylene)-4-p-nitrophenylthiosemicarbazide (**8A**)*

Light Pinkish; yield 67%; mp 171 °C; Anal. Calc. for $C_{17}H_{11}Cl_2N_5O_2S$: C 48.58, H 2.64, N 16.66, found C 48.62, H 2.61, N 16.68; IR ν(cm^{-1}): 3187 (NH), 3065 (Ar,C–H), 1463 (C=C), 1131 (C=S); ^{1}H NMR (DMSO) δ(ppm): 11.69 (s, 1H, NNH), 9.69 (s, 1H, NH), 7.51–8.09 (m, 8H, Ar-H), 6.72 (s,1H, CH=N); ESI-MS *m*/*z*: [M^+ + H] 421.3.

*4.2.9. 1-((2chloroquinolin-3-yl)methylene)thiosemicarbazide (**9A**)*

Brown; yield 73%; mp 160 °C; Anal. Calc. for $C_{11}H_9ClN_4S$: C 49.91, H 3.43, N 21.16, found C 49.89, H 3.47, N 21.14; IR ν(cm^{-1}): 3477 (NH_2), 3160 (NH), 1469 (C=N), 1108 (C=S); ^{1}H NMR ($CDCl_3$) δ(ppm): 10.96 (s, 1H, NNH), 9.3 (s, 1H, Ar-H), 7.13–7.26 (m, 4H, Ar-H), 7.11 (s, 1H, CH=N), 2.38 (s, 2H, NH_2); ESI-MS *m*/*z*: [M^+ + H] 266.6.

*4.2.10. 1-((2-Chloroquinolin-3-yl)methylene)-4-cyclopentylthiosemicarbazide (**10A**)*

Reddish brown; yield 82%; mp 162 °C; Anal. Calc. for $C_{16}H_{17}ClN_4S$: C 57.73, H 5.15, N 16.83, found C 57.77, H 5.13, N 16.85; IR ν(cm^{-1}): 3179 (NH), 2886 (CH_2), 1431 (C=N), 1113 (C=S); 1H NMR ($CDCl_3$) δ(ppm): 11.14 (s,1H, NNH), 7.21–7.37 (m 5H, Ar-H), 7.03 (s, 1H, N=CH), 2.76–2.81 (m, 1H, N–CH), 1.53–1.69 (m, 8H, CH_2); ESI-MS *m*/*z*: [M^+] 332.5

*4.2.11. 1-((2-Chloroquinolin-3-yl)methylene)-4-cyclohexylthiosemicarbazide (**11A**)*

Creamy White; yield 73%; mp 162 °C; Anal. Calc. for $C_{17}H_{19}ClN_4S$: C 58.86, H 5.52, N 16.15, found C 58.89, H 5.54, N 16.14; IR ν(cm^{-1}): 3189 (NH), 2867 (CH_2),1461 (C=N), 1107 (C=S); ^{1}H NMR (DMSO) δ(ppm): 11.07 (s,1H, NNH), 7.49–7.61 (m. 4H, Ar-H), 6.88 (s, 1H, CH=N), 4.93 (s, 1H, CNH), 2.93–3.04 (m, 1H, NCH), 1.89–2.10 (m, 10H, CH_2); ESI-MS *m*/*z*: [M^+ + Na] 370.1

*4.2.12. 1-((-Hloroquinolin-3-yl)methylene)-4-cyclooctylthiosemicarbazide (**12A**)*

Light Brownish; yield 82%; mp 165 °C; Anal. Calc. for $C_{19}H_{23}ClN_4S$: C 60.87, H 6.18, N 14.94, found C 60.90, H 6.16, N 14.90; IR ν(cm^{-1}): 3250 (NH),2861 (CH_2), 1465 (C=N), 1130 (C=S); ^{1}H NMR (CDCl3) δ(ppm): 10.89 (s, 1H, NNH), 7.63–7.71 (m, 5H, Ar-H), 7.18 (s, 1H, CH=N), 5.78 (s, 1H, CNH), 2.34–2.52 (m, 1H, NCH), 1.97–2.13 (m, 4H, CH_2), 1.61–1.74 (m, 10H, CH_2); ESI-MS *m*/*z*: [M^+ + H] 375.8

*4.2.13. 1-((2-Chloroquinolin-3-yl)methylene)-4-o-tolylthiosemicarbazide (**13A**)*

Whitish; yield 79%; mp 171 °C; Anal. Calc. for $C_{18}H_{15}ClN_4S$: C 60.92, H 4.26, N 15.79, found C 60.92, H 4.25, N 18.81; IR ν(cm^{-1}): 3160 (NH), 3064 (Ar,C–H), 1472 (C=N), 1123 (C=S); ^{1}H NMR ($CDCl_3$) δ(ppm): 11.93 (s, 1H, NNH), 9.06 (s, 1H, CNH) 7.12–7.68 (m, 9H, Ar-H), 7.05 (s, 1H, CH=N), 2.56 (s, 3H, CH_3); ESI-MS *m*/*z*: [M^+ + H] 355.8

*4.2.14. 1-((2-Chloroquinolin-3-yl)methylene)-4-(2,5-difluorophenyl)thiosemicarbazide (**14A**)*

Bluish; yield 74%; mp 179 °C; Anal. Calc. for $C_{17}H_{11}ClF_2N_4S$: C 54.19, H 2.94, N 14.87, found C 54.15, H 2.89, N 14.90, IR: 3458 (NH), 1504 (C=C), 1035 (C=S). ^{1}H NMR (DMSO) δ(ppm): 10.89 (s, 1H, NNH), 7.63–7.85 (m, 8H, Ar-H), 6.93 (s, 1H, CH=N); ESI-MS *m*/*z*: [M^+ + Na] 399.6

*4.2.15. 1-((2-Chloroquinolin-3-yl)methylene)-4-p-tolylthiosemicarbazide (**15A**)*

Pinkish; yield 77%; mp 163 °C; Anal. Calc. for $C_{18}H_{15}ClN_4S$: C 60.92, H 4.26, N 15.79, found C 60.89, H 4.30, N 15.76; IR ν(cm^{-1}): 3155 (NH), 3064 (Ar,C–H),1506 (C=N), 1132 (C=S); ^{1}H NMR ($CDCl_3$) δ(ppm): 11.57 (s, 1H, NNH), 8.71 (s, 1H, NH), 8.21 (s, 1H, Ar-H), 7.29–7.64 (m, 8H, Ar-H), 6.97 (s, 1H, CH=N), 2.37 (s, 3H, CH_3); ESI-MS *m*/*z*: [M^+ + 1] 355.9

*4.2.16. 1-((2-Chloroquinolin-3-yl)methylene)-4-p-nitrophenylthiosemicarbazide (**16A**)*

Light Black; yield 87%; mp 162 °C; Anal. Calc. for $C_{17}H_{12}ClN_5O_2S$: C 52.92, H 3.13, N 18.15, found C 52.96, H 3.17, N 18.12; IR ν(cm^{-1}): 3187 (NH), 3057 (Ar,C–H), 1457 (C=N), 1131 (C=S); ^{1}H NMR (DMSO) δ(ppm): 11.13 (s, 1H, NNH), 9.54 (s, 1H, NH), 7.37–8.10 (m, 9H, Ar-H), 6.97 (s, 1H, CH=N); ESI-MS *m*/*z*: [M^+ + 1] 386.6

*4.2.17. 1-((2-Chloro-8-methylchloroquinolin-3-yl)methylene) thiosemicarbazide (**17A**)*

Yellowish; yield 74%; mp 162 °C; Anal. Calc. for $C_{12}H_{11}ClN_4S$: C 51.70, H 3.98, N 20.10, found C 51.66, H 4.02, N 20.13; IR ν(cm^{-1}): 3465 (NH_2), 3199 (NH), 2956 (CH_3), 1421 (C=N), 1121 (C=S); ^{1}H NMR ($CDCl_3$) δ(ppm): 11.74 (s, IH, NNH), 9.3 (s, 1H, Ar-H), 7.19–7.39 (m, 3H, Ar-H), 6.85 (s, 1H, N=CH), 2.51 (s, 3H, CH_3), 2.19 (s, 2H, NH_2); ESI-MS *m*/*z*: [M^+ + Na] 301.9

*4.2.18. 1-((2-Chloro-8-methylchloroquinolin-3-yl)methylene)-4-cyclopentylthiosemicarbazide (**18A**)*

Brownish; yield 78%; mp 154 °C; Anal. Calc. for $C_{17}H_{19}ClN_4S$: C 58.86, H 5.52, N 16.15, found C 58.82, H 5.55, N 16.19; IR ν(cm^{-1}): 3497 (NH), 2946 (CH_3), 2857 (CH_2),1477 (C=N), 1110 (C=S); ^{1}H NMR ($CDCl_3$) δ(ppm): 11.11 (s, 1H, NNH), 8.69 (s, 1H, Ar-H), 7.29–7.46 (m, 3H, Ar-H), 7.06 (s, 1H, N=CH), 2.81–2.94 (m, 1H, N–CH), 1.73–1.96 (m, 8H, CH_2); ESI-MS *m*/*z*: [M^+ + 1] 347.7

*4.2.19. 1-((2-Chloro-8-methylchloroquinolin-3-yl)methylene)-4-cyclohexylthiosemicarbazide (**19A**)*

Blackish; yield 75%; mp 155 °C; Anal. Calc. for $C_{18}H_{21}ClN_4S$: C 59.90, H 5.86, N 15.52, found C 59.93, H 5.89 N 15.449; IR ν(cm^{-1}): 3189 (NH), 2852 (CH_2),1411 (C=N), 1107 (C=S); ^{1}H NMR (DMSO) δ(ppm): 12.07 (s, 1H, NNH), 8.19 (s, 1H, Ar-H), 7.56–7.72 (m, 3H, Ar-H), 7.14 (s, 1H, CH=N), 6.23 (s, 1H, CNH), 2.89–3.01 (m, 1H, NCH), 2.45 (s, 3H, CH_3), 1.91–2.05 (m, 10H, CH_2); ESI-MS *m*/*z*: [M^+ + 1] 361.9

*4.2.20. 1-((2-Chloro-8-methylchloroquinolin-3-yl)methylene)-4-cyclooctylthiosemicarbazide (**20A**)*

Yellowish; yield 80%; mp 158 °C; Anal. Calc. for $C_{19}H_{23}ClN_4S$: C 60.87, H 6.18, 14.94, found C 60.91, H 6.21, N 14.91; IR ν(cm^{-1}): 3245 (NH), 2859 (CH_2), 1465 (C=N), 1125 (C=S); ^{1}H NMR ($CDCl_3$) δ(ppm): 11.12 (s, 1H, NNH), 8.68 (s, 1H, Ar-H), 7.48–7.65 (m, 3H, Ar-H), 7.09 (s, 1H, CH=N), 5.32 (s, 1H, CNH), 2.54–2.61 (m, 1H, NCH), 2.39 (s, 3H, CH_3), 2.05–2.25 (m, 4H, CH_2), 1.76–1.91 (m, 10H, CH_2); ESI-MS *m*/*z*: [M^+ + 1] 375.8

*4.2.21. 1-((2-Chloro-8-methylchloroquinolin-3-yl)methylene)-4-o-tolylthiosemicarbazide (**21A**)*

Creamy; yield 76%; mp 155 °C; Anal. Calc. for $C_{19}H_{17}ClN_4S$: C 61.86 H 4.65 N 15.19 found C 61.85, H 4.68, N 15.23; IR ν(cm^{-1}): 3160 (NH), 3095 (Ar,C–H), 2964 (CH_3), 1433 (C=N), 1113 (C=S); ^{1}H NMR ($CDCl_3$) δ(ppm): 11.53 (s, 1H, NNH), 9.72 (s, 1H, CNH), 7.85–8.10 (m, 8H, Ar-H), 6.85 (s, 1H CH=N), 2.46 (s, 6H, CH_3); ESI-MS *m*/*z*: [M^+ + 1] 369.6

*4.2.22. 1-((2-Chloro-8-methylchloroquinolin-3-yl)-4-(2,5-difluorophenyl)thiosemicarbazide (**22A**)*

Light black; yield 83%; mp 158 °C; Anal. Calc. for $C_{18}H_{13}ClF_2N_4S$: C 55.32, H 3.35, N 14.34, found C 55.35, H 3.51, N 14.36; IR ν(cm^{-1}):

3158 (NH), 3035 (Ar,C–H), 2934 (CH_3), 1474 (C=N), 1115 (C=S); ^{1}H NMR (DMSO) δ(ppm): 11.71(s,1H, NNH), 7.76–7.98 (m, 7H, Ar-H), 7.17 (s, 1H, CH=N), 2.64 (s, 3H, CH_3); ESI-MS *m/z*: [M^+ + H] 391.9

*4.2.23. 1-((2-Chloro-8-methylchloroquinolin-3-yl)-4-p-tolyl-thiosemicarbazide (**23A**)*

White; yield 76%; mp 160 °C; Anal. Calc. for $C_{19}H_{17}ClN_4S$: C 61.86, H 4.65, N 15.19, found C 61.82, H 4.69, N 15.16; IR ν(cm^{-1}): 3155 (NH), 3062 (Ar,C–H), 2912 (CH_3), 1486 (C=N), 1102 (C=S); ^{1}H NMR ($CDCl_3$) δ(ppm): 11.96 (s, 1H, NNH), 9.69 (s, 1H, NH), 7.28–7.56 (m, 8H, Ar-H), 6.94 (s, 1H, CH=N), 2.69 (s, 6H, CH_3); ESI-MS *m/z*: [M^+ + H] 369.7

*4.2.24. 1-((2-Chloro-8-methylchloroquinolin-3-yl)-4-p-nitrophenyl-thiosemicarbazide (**24A**)*

White; yield 80%; mp 165 °C; Anal. Calc. for $C_{18}H_{14}ClN_5O_2S$: C 54.07, H 3.53, N 17.51, found C 54.04, H 3.55, N 17.49; IR ν(cm^{-1}): 3187 (NH), 3021 (Ar,C–H), 2953 (CH_3), 1477 (C=N), 1131 (C=S); ^{1}H NMR (DMSO) δ(ppm): 11.43 (s, 1H, NNH), 9.34 (s, 1H, NH), 7.68–7.96 (m, 8H, Ar-H), 6.79 (s, 1H, CH=N); ESI-MS *m/z*: [M^+ + Na] 422.7.

*4.3. Synthesis of N-4-acetyl-5-(2,8-substitutedquinolin-3-yl)-4,5-dihydro-1,3,4-thiadi-azole-2-yl)N-substituted-acetamide derivatives (**1B**–**24B**)*

Compounds (**1A**–**24A**) were refluxed with excess of acetic aicd anhydride, with a constant stirring for 15–24 h. The reaction was monitored by TLC. The solution was poured into ice-water and stirred for 3 h. The solution was filtered and the solid obtained was washed with diethyl ether. The precipitated solid was recrystalized from appropriate solvents to get the desired products. Some compounds were subjected to column chromatography to get the desired compounds.

*4.3.1. N-4-acetyl-5-(2,8-dichloroquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)acetamide (**1B**)*

Brownish yellow; yield 66%; mp 193 °C; Anal. Calc .for $C_{15}H_{22}Cl_2N_4O_2S$: C 47.01, H 3.16, N 14.62 found: C 46.98, H 3.13, N 14.68; IR ν(cm^{-1}): 3211 (NH), 2953 (CH_3)1658 & 1641 (CO), 1479 (C=N); ^{1}H NMR ($CDCl_3$) δ(ppm): 7.13–7.26 (m, 4H, Ar-H), 5.31 (s, 1H, SCHN), 2.53 (s, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ/ppm: 169.24 (C=O), 163.36 (C=O), 158.61 (C=N), 149.32 (C=N), 139.16, 134.95, 129.24, 128.84, 126.26, 125.21 (Ar-C), 65.98 (SCN), 24.32, 26.10 (Aliphatic). ESI-MS *m/z*: [M^+ + H] 384.2

*4.3.2. N-4-acetyl-5-(2,8-dichloroquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-cyclopentylacetamide (**2B**)*

Brownish; yield 61%; mp 189 °C; Anal. Calc. for $C_{20}H_{20}Cl_2N_4O_2S$: C 53.22, H 4.47, N 12.41, found C 53.20, H 4.49, N 12.43; IR ν(cm^{-1}): 2911 (CH_3), 2851 (CH_2), 1671&1661 (CO), 1440 (C=N); 1H NMR ($CDCl_3$) δ(ppm): 8.51 (s, 1H, Ar-H), 7.21–7.37 (m, 3H, Ar-H), 5.25(s, 1H, SCHN), 2.65–2.2.78 (m, 1H, N–CH), 2.35 (s, 6H, CH_3), 1.99–2.28 (m, 8H, CH_2); ^{13}C NMR ($CDCl_3$) δ/ppm: 171.23 (C=O), 168.21 (C=O), 158.86 (C=N), 149.96 (C=N), 143.01, 137.25. 135.56, 129.24, 126.21 (Ar-C), 65.49 (SCN), 34.27, 27.26, 24.37, 23.73 21.98 (aliphatic, cyclic); ESI-MS *m/z*: [M^+ + 1] 452.2

*4.3.3. N-4-acetyl-5-(2,8-dichloroquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-cyclohexylacetamide (**3B**)*

Yellowish; yield 61%; mp 208 °C; Anal. Calc. for $C_{21}H_{22}Cl_2N_4O_2S$: C 54.20, H 4.76, N 12.04, found C 54.22, H 4.73, N 12.06; IR ν(cm^{-1}): 2952 (CH_3), 2859 (CH_2), 1651&1640 (CO), 1430 (C=N). ^{1}H NMR (DMSO) δ(ppm): 7.56–7.72 (m. 4H, Ar-H), 5.56 (s, 1H, SCHN), 2.68 (s, 6H, CH_3), 1.73–1.91 (m, 10H, CH_2); ESI-MS *m/z*: [M^+ + Na] 488.3

*4.3.4. N-4-acetyl-5-(2,8-dichloroquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-cyclooctylacetamide (**4B**)*

Light black; yield 57%; mp 210 °C; Anal. Calc. for $C_{23}H_{26}Cl_2N_4O_2S$: C 55.98, H 5.31, N 11.35, found: C 55.94, H 5.33, N 11.332; IR ν(cm^{-1}): 2933 (CH_3), 2872 (CH_2), 1674, 1641 (CO), 1492 (C=N); ^{1}H NMR ($CDCl_3$) δ(ppm): 8.61 (s, 1H, Ar-H), 7.48–7.69 (m, 3H, Ar-H), 5.36 (s, 1H, SCHN), 2.69 (s, 6H, CH_3), 2.32–2.41 (m, 1H, NCH), 1.99–2.11 (m, 4H, CH_2), 1.58–1.85 (m, 10H, CH_2); ESI-MS *m/z*: [M^+ + 1] 494.2

*4.3.5. N-4-acetyl-5-(2,8-dichloroquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-o-tolylacetamide (**5B**)*

White; yield 58%; mp 196 °C; Anal. Calc. for: $C_{22}H_{18}Cl_2N_4O_2S$: C 55.82, H 3.83, N 11.84, found: C 55.84, H 3.80, N 11.80; IR ν(cm^{-1}): 3135 (Ar,C–H), 2963 (CH_3), 1655&1643 (CO), 1435 (C=N); ^{1}H NMR ($CDCl_3$) δ(ppm): 8.53 (s,1H, Ar-H), 6.95–7.31 (m, 7H, Ar-H), 5.69 (s,1H, SCHN), 2.35 (s, 3H, CH_3), 2.13 (s, 6H, CH_3); ESI-MS *m/z*: [M^+ + H] 474.0

*4.3.6. N-4-acetyl-5-(2,8-dichloroquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-(2-5-difluorophenyl)acetamide (**6B**)*

White; yield 67%; mp 206 °C; Anal. Calc. for $C_{21}H_{14}Cl_2F_2N_4O_2S$: C 50.92, H 2.85, N 11.31, found C 50.88, H 2.82, N 11.35; IR ν(cm^{-1}): 3092 (Ar,C–H), 2952 (CH_3), 1664, 1642 (CO), 1435 (C=N); ^{1}H NMR (DMSO) δ(ppm): 7.78–8.62 (m, 7H, Ar-H), 5.69 (s, 1H, SCHN), 2.61 (s, 6H, CH_3); ESI-MS *m/z*: [M^+ + H] 496.1

*4.3.7. N-4-acetyl-5-(2,8-dichloroquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-p-tolylacetamide (**7B**)*

Brown; yield 53%; mp 201 °C; Anal. Calc. for $C_{22}H_{18}Cl_2N_4O_2S$: C 55.82, H 3.83, N 11.84, found C 55.84, H 3.79, N11.81; IR ν(cm^{-1}): 3049 (Ar,C–H), 2942 (CH_3), 1678, 1640 (CO), 1449 (C=N); 1H NMR ($CDCl_3$) δ(ppm): 7.38–7.86 (m, 8H, Ar-H), 4.98 (s, 1H, SCHN), 2.39 (s, 6H, CH_3), 2.13 (s, 3H, CH_3); ESI-MS *m/z*: [M^+ + Na] 518.2

*4.3.8. N-4-acetyl-5-(2,8-dichloroquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-(4-nitrophenyl)acetamide (**8B**)*

Light pink; yield 56%; mp 194 °C; Anal. Calc. for $C_{21}H_{15}Cl_2N_5O_4S$: C 50.01, H 3.00, N 13.89, found C 49.99, H 3.03, N 13.87; IR ν(cm^{-1}): 3065 (Ar,Ctyl-5-(2,8-dichloroquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-(4-nitrophenyl)H), 2971 (CH_3), 2551 (CH_2) 1673, 1640 (CO), 1445 (C=N); ^{1}H NMR (DMSO) δ(ppm): 7.73–8.21 (m, 8H, Ar-H), 5.41 (s, 1H, SCHN), 2.13 (s, 6H, CH_3); ESI-MS *m/z*: [M^+ + 1] 505.7

*4.3.9. N-4-acetyl-5-(2-chloroquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)acetamide (**9B**)*

Reddish brown; yield 61%; mp 198 °C; Anal. Calc. for $C_{15}H_{13}ClN_4O_2S$: C 51.65, H 3.76, N 16.06, found C 51.62, H 3.79, N 16.11; IR ν(cm^{-1}): 3183 (NH), 2926 (CH_3), 1658, 1641 (CO), 1479 (C=N); ^{1}H NMR ($CDCl_3$) δ(ppm): 8.76 (s, 1H, Ar-H), 6.98–7.39 (m, 7H, Ar-H), 5.41(s, 1H, SCHN), 2.36 (s, 6H, CH_3); ESI-MS *m/z*: [M^+ + H] 349.8

*4.3.10. N-4-acetyl-5-(2-chloroquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-cyclopentylacetamide (**10B**)*

Brownish yellow; yield 66%; mp 205 °C; Anal. Calc. for $C_{20}H_{21}ClN_4O_2S$: C 57.62, H 5.08, N 13.44, found C 57.65, H 5.06, N 13.46, IR ν(cm^{-1}): 2928 (CH_3), 2896 (CH_2) 1661, 1652 (CO), 1440 (C=N); ^{1}H NMR ($CDCl_3$) δ(ppm): 7.29–7.46 (m 5H, Ar-H), 5.51(s, 1H, SCHN), 2.71–2.81 (m, 1H, N–CH), 2.35 (s, 6H, CH_3), 1.73–1.86 (m, 8H, CH_2); ESI-MS *m/z*: [M^+ + H] 417.8

*4.3.11. N-4-acetyl-5-(2-chloroquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-cyclohexylacetamide (**11B**)*

Creamy white; yield 53%; mp 221 °C; Anal. Calc. for $C_{21}H_{23}ClN_4O_2S$: C 58.53, H 5.38, N 13.00, found C58.49, H 5.34,

N 13.03; IR ν(cm^{-1}): 2918 (CH_3), 2876 (CH_2), 1651, 1640 (CO), 1430 (C=N); ^{1}H NMR (DMSO) δ(ppm): 7.39–7.353 (m, 5H, Ar-H), 5.37 (s, 1H, SCHN), 2.63 (s, 6H, CH_3), 2.32–2.41 (m, 1H, NCH), 1.99–2.13 (m, 10H, CH_2); ^{13}C NMR (DMSO) δ/ppm: 169.35 (C=O), 166.92 (C=O), 158.39 (C=N), 153.24 (C=N), 139.22, 136.95, 132.10,130.29, 129.13, 123.12 (Ar-C), 65.87 (SCN), 37.02, 29.95, 27.36, 26.95, 22.01, 21.23, 19.89 (aliphatic); ESI-MS *m*/*z*: [M^+ + H] 431.6

*4.3.12. N-4-acetyl-5-(2-chloroquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-cyclooctylacetamide (**12B**)*

Brownish; yield 69%; mp 208 °C; Anal. Calc. for $C_{23}H_{27}ClN_4O_2S$: C 60.20, H 4.36, N 12.76, found : C 60.24, H 4.33, N 12.79; IR ν(cm^{-1}): 2937 (CH_3), 2861 (CH_2), 1672, 1651 (CO), 1492 (C=N); ^{1}H NMR ($CDCl_3$) δ(ppm): 10.89 (s, 1H, NNH), 7.45–7.69 (m, 5H, A-H), 5.39 (s, 1H, SCHN), 2.56–2.61 (m, 1H, NCH), 2.43 (s, 6H, CH_3), 1.72–1.81 (m, 4H, CH_2), 1.49–1.65 (m, 10H, CH_2); ^{13}C NMR ($CDCl_3$) δ(ppm): 175.52 (C=O), 165.32 (C=O), 153.95 (C=N), 147.86 (C=N), 137.32, 135.20, 129.01, 128.24, 127.35 (Ar-C), 53.11 (SCN), 38.96, 33.25, 29.94, 27.27, 24.95, 21.43 (aliphatic); ESI-MS *m*/*z*: [M^+ + H] 460.2

*4.3.13. N-4-acetyl-5-(2-chloroquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-o-tolylacetamide (**13B**)*

Creamy white; yield 64%; mp 192 °C; Anal. Calc. for: $C_{22}H_{19}ClN_4O_2S$: C 60.20, H 4.36, N 12.78, found: C 60.23, H 4.34, N 12.75; IR ν(cm^{-1}): 3064 (Ar,C–H), 2926 (CH_3), 1655,1642 (CO), 1435 (C=N); ^{1}H NMR ($CDCl_3$) δ(ppm): 7.16–7.69 (m, 9H, Ar-H), 5.59 (s, 1H, SCHN), 2.51 (s, 3H, CH_3), 2.43 (s, 6H, CH_3); ESI-MS *m*/*z*: [M^+ + Na] 461.7

*4.3.14. N-4-acetyl-5-(2-chloroquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-(2,5-difluorophenyl)acetamide (**14B**)*

Blue black; yield 71%; mp 204 °C; Anal. Calc. for $C_{21}H_{15}ClN_4O_2S$: C 54.73, H 3.28, N 12.61, found C 54.69, H 3.31, N 12.58; IR ν(cm^{-1}): 3072 (Ar,C–H), 2909 (CH_3), 1678, 1664 (CO), 1435 (C=N); ^{1}H NMR (DMSO) δ(ppm): 2.58 (s, 6H, CH_3), 5.43 (s, 1H, SCHN), 7.89–8.61 (m, 8H, Ar-H); ESI-MS *m*/*z*: [M^+ + H] 461.5

*4.3.15. N-4-acetyl-5-(2-chloroquidnolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-p-tolylacetamide (**15B**)*

Light pink; yield 68%; mp 207 °C; Anal. Calc. for $C_{22}H_{19}ClN_4O_2S$: C 60.20, H 4.36 N 12.76, found C 60.23, H 4.33, N 12.79; IR ν(cm^{-1}): 3056 (Ar,C–H), 2926 (CH_3), 1678,1640 (CO), 1445 (C=N); ^{1}H NMR ($CDCl_3$) δ(ppm): 7.29–7.64 (m, 9H, Ar-H), 5.19 (s, 1H, SCHN), 2.36 (s, 3H, CH_3), 2.19 (s, 6H, CH_3),; ESI-MS *m*/*z*: [M^+ + H] 484.7

*4.3.16. N-4-acetyl-5-(2-chloroquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-(4-nitophenyl)aceamide (**16B**)*

Blackish; yield 65%; mp 204 °C.; Anal. Calc. for $C_{22}H_{16}ClN_5O_4S$: C 60.20, H 4.36, N 12.76, found C 60.16, H 4.39, N 12.71; IR ν (cm^{-1}): 3027 (Ar,C–H), 2952 (CH_3), 1671,1658 (CO), 1449 (C=N); ^{1}H NMR (DMSO) δ(ppm): 7.51–8.09 (m, 9H, Ar-H), 5.12 (s, 1H, SCHN), 2.49 (s, 6H, CH_3); ESI-MS *m*/*z*: [M^+ + Na] 492.5

*4.3.17. N-4-acetyl-5-(2-chloro-8-methylquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)acetamide (**17B**)*

Yellowish; yield 63%; mp 213 °C Anal. Calc. for $C_{16}H_{15}ClN_4O_2S$: C 52.96, H 4.17, N 15.44, found C 53.01, H 4.13, N 15.48; IR ν(cm^{-1}): 3194 (NH), 2961 (CH_3), 1658, 1645 (CO), 1479 (C=N); ^{1}H NMR ($CDCl_3$) δ(ppm): 8.13 (s, 1H, Ar-H), 7.39–7.69 (m, 3H, Ar-H), 5.28 (s, 1H, N=CH), 2.65 (s, 6H, CH_3), 2.48 (s, 3H, CH_3), 2.19 (s, 1H, NH_2); ESI-MS *m*/*z*: [M^+ + H] 363.5

*4.3.18. N-4-acetyl-5-(2-chloro-8-methylquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-cyclpentylacetamide (**18B**)*

Dark brown; yield 58%; mp 209 °C; Anal. Calc. for $C_{21}H_{23}ClN_4O_2S$: C 58.53, H 5.38, N 13.00, found C 58.56, H 5.34, N 12.96; IR ν(cm^{-1}): 2918 (CH_3), 2851 (CH_2), 1661, 1649 (CO), 1440 (C=N); ^{1}H NMR ($CDCl_3$) δ(ppm): 1.92–2.16 (m, 8H, CH_2), 2.38 (s, 6H, CH_3), 2.64 (s, 3H, CH_3), 2.81–2.94 (m, 1H, N–CH), 5.34 (s, 1H, N=CH), 7.73–7.56 (m, 3H, Ar-H), 8.24 (s, 1H, Ar-H); ESI-MS *m*/*z*: [M^+ + H] 431.6

*4.3.19. N-4-acetyl-5-(2-chloro-8-methylquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-cyclohexylacetamide (**19B**)*

Light black; yield 63%; mp 188 °C; Anal. Calc. for $C_{22}H_{25}Cl\,N_4O_2S$: C 59.38, H 5.66, N 12.59 found C 59.42, H 5.63, N 12.56; IR ν (cm^{-1}): 2933 (CH_3), 2867 (CH_2), 1660, 1651 (CO), 1430 (C=N); ^{1}H NMR (DMSO) δ(ppm): 8.36 (s, 1H, Ar-H), 7.49–7.61 (m. 3H, Ar-H), 6.23 (s, 1H, CNH), 5.26 (s, 1H, SCHN), 2.92–3.01 (m, 1H, NCH), 2.59 (s, 6H, CH_3), 2.51 (s. 3H, CH_3), 1.73–1.93 (m, 10H, CH_2); ESI-MS *m*/*z*: [M^+ + H] 445.1

*4.3.20. N-4-acetyl-5-(2-chloro-8-methylquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-cyclooctylacetamide (**20B**)*

Brownish; yield 54%; mp 219 °C; Anal. Calc. for $C_{24}H_{29}ClN_4O_2S$: C 60.94, H 6.18, N 11.84 found : C 60.92, H 6.21, N 11.80; IR ν(cm^{-1}): 2937 (CH_3), 2891 (CH_2), 1670, 1640 (CO), 1492 (C=N); ^{1}H NMR ($CDCl_3$) δ(ppm): 5.12 (s, 1H, SCHN), 7.63–7.82 (m, 4H, Ar-H), 2.51–2.62 (m, 1H, NCH), 2.48 (s, 6H, CH_3), 2.37 (s, 3H, CH_3), 1.65–1.73 (m, 4H, CH_2), 1.31–1.56 (m, 10H, CH_2); ESI-MS *m*/*z*: [M^+ + H] 438.2

*4.3.21. N-4-acetyl-5-(2-chloro-8-methylquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-o-tolylacetamide (**21B**)*

Creamy white; yield 56%; mp 203 °C; Anal. Calc. for: $C_{23}H_{21}ClN_4O_2S$: C 60.99, H 4.67, N 12.37, found: C 60.95, H 4.71, N 12.34; IR ν(cm^{-1}): 3095 (Ar,C–H), 2964 (CH_3), 1655, 1642 (CO), 1435 (C=N); ^{1}H NMR ($CDCl_3$) δ(ppm): 2.68 (s, 6H, CH_3), 2.73 (s, 6H, CH_3), 5.37 (s, 1H, SCHN), 7.12–7.44 (m, 8H, Ar-H); ESI-MS *m*/*z*: [M^+ + Na] 475.7

*4.3.22. N-4-acetyl-5-(2-chloro-8-methylquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-(2,5-difluorophenyl)acetamide (**22B**)*

Blackish; yield 58%; mp 198 °C; Anal. Calc. for $C_{22}H_{17}ClF_2N_4O_2S$: C 55.64, H 3.61, N 11.80, found C 55.69, H 3.59, N 11.82; IR ν(cm^{-1}): 3075 (Ar,C–H), 2934 (CH_3), 1664, 1640 (CO), 1435 (C=N); ^{1}H NMR (DMSO) δ(ppm): 11.71(s, 1H, NNH), 7.76–7.98 (m, 7H, Ar-H), 7.17 (s, 1H, CH=N), 2.64 (s, 3H, CH_3); ESI-MS *m*/*z*: [M^+ + H] 475.8

*4.3.23. N-4-acetyl-5-(2-chloro-8-methylquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-p-phenylacetamide (**23B**)*

White; yield 64%; mp 201 °C; Anal. Calc. for $C_{23}H_{21}ClN_4O_2S$: C 60.99, H 4.67, N 12.37, found C 61.02, H 4.65 N 12.41; IR ν(cm^{-1}): 3062 (Ar,C–H), 2911 (CH_3), 1678, 1610 (CO), 1445 (C=N); ^{1}H NMR ($CDCl_3$) δ(ppm): 7.23–7.61 (m, 8H, Ar-H), 5.61 (s, 1H, SCHN), 2.69 (s, 6H, CH_3), 2.34 (s, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ/ppm: 174.36 (C=O), 168.95 (C=O), 153.48 (C=N), 149.95 (C=N), 141.49, 139.10,135.08, 130.65, 128.76, 125.91, 124.34, 122.11 (Ar-C), 49.02 (SCN), 24.03, 22.01, 21.24 (aliphatic); ESI-MS *m*/*z*: [M^+ + H] 453.8

*4.3.24. N-4-acetyl-5-(2-chloro-8-methylquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazole-2-yl)-N-(4-nitrophenyl)acetamide (**24B**)*

Creamy white; yield 55%; mp 207 °C; Anal. Calc. for $C_{22}H_{18}ClN_5O_2S$: C 54.80, H 3.75, N 14.47, found C 54.74, H 3.78, N 14.51; IR ν(cm^{-1}): 3054 (Ar,C–H), 2952 (CH_3), 1640, 1604 (CO), 1449 (C=N); ^{1}H NMR (DMSO) δ(ppm): 7.19–7.39 (m, 8H, Ar-H), 4.93 (s, 1H, SCHN), 2.51 (s, 3H, CH_3), 2.24 (s, 6H, CH_3); ^{13}C NMR (DMSO) δ/ppm: 172.36 (C=O), 175.39 (C=O), 156.64 (C=N), 151.37 (C=N), 140.19, 139.74, 136.38, 133.27, 131.24, 129.84, 128.68, 124.84,

(Ar-C), 47.84 (SCN), 26.94, 25.72, 23.19, 21.96 (aliphatic carbon); ESI-MS m/z: $[M^+ + H]$ 484.9

4.4. Biological activity

Organism culture and *in vitro* screening for antibacterial activity was done by the disk diffusion method with minor modifications. *S. aureus, S. pyogenes, S. typhimurium*, and *E. coli* were subcultured in BHI medium and incubated for 18 h at 37 °C. Following the incubation the bacterial cells were suspended, according to the McFarland protocol in saline solution to produce a suspension of about 10^5 CFU/mL. 10 mL of this suspension was mixed with 10 mL of sterile antibiotic agar at 40 °C and poured on to an agar plate in a laminar flow cabinet. Five paper disks (6.0 mm diameter) were fixed onto nutrient agar plate. One milligram of each test compound was dissolved in 100 mL DMSO to prepare stock solution. From the stock solution different dilutions of each test compound were prepared and poured over disk plate. Amoxicillin was used as a standard drug (positive control). DMSO poured disk was used as negative control. The susceptibility of the bacteria to the test compounds was determined by the formation of an inhibitory zone after 18 h of incubation at 36 °C. Tables 2 and 3 report the inhibition zones (mm) of thiosemicarbazones (**1A**–**24A**) and thiadiazoles (**1B**–**24B**) respectively. The results were compared with the negative and positive controls and the zone of inhibitions was measured at the minimum inhibitory concentration.

The minimum inhibitory concentration (MIC) was evaluated by the macro-dilution test using standard inoculums of 10^5 CFL/mL. Serial dilutions of the test compounds, previously dissolved in dimethyl sulfoxide (DMSO) were prepared to final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 μgm/L. To each tube was added 100 mL of a 24 h old inoculum. The MIC, defined as the lowest concentration of the test compound, which inhibits the visible growth after 18 h, was determined visually after incubation for 18 h, at 37 °C, and the results are presented in Tables 4 and 5. DMSO and amoxicillin were used as negative and positive controls.

4.5. Cytotoxicity profile

MTT Assay: H9c2 rat cardiac myoblasts were cultured and maintained as monolayer in Dulbecco's modified Eagle's medium (DMEM), high glucose, supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 mg/mL streptomycin, and 2.5 mg/mL amphotericin B, at 37 °C in humidified incubator with 5% CO_2 [31]. Cells were incubated with different concentrations of compounds **5B, 8B, 10B, 13B, 14B, 15B, 21B, 22B** and amoxicillin for 48 h at 37 °C in 5% CO_2 humidified incubator together with untreated control sample. At appropriate time points, cells were washed in PBS, treated with 600 mL MTT solution (5 mg/mL, tetrazolium salt) and incubated for 45 min at 37 °C. After 45 min of incubation at 37 °C, the cell supernatants were discarded, MTT crystals were dissolved with acid isopropanol and the absorbance measured at 570 nm. All assays were performed in triplicate. Percent viability was defined as the relative absorbance of treated versus untreated control cells.

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