Synthesis and biological evaluation of some novel schiff base hydrazide derivatives

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ABSTRACT: *Two novel Schiff base hydrazides with notable antibacterial and anticonvulsant properties have been synthesized. The present study illustrates that hydrazones possess wide applications in biological and therapeutic field. The synthesized compounds were characterized by analytical method and the docking studies were carried out by computational method. The compounds were tested for antituberculosis activity by in vitro methods. The results show that the compounds are stable and exhibit antituberculosis activity as evidenced by in silico and in vitro studies.*

Key Words: *in-vitro, in-silico studies, antimycobacterial studies, hydrazides, docking studies, anti tuberculosic activity.*

Introduction

Synthesis of organic molecule with good therapeutic effect is one of the main objectives in the field of pharmaceutical chemistry. This is because the society is always in need for development of novel and potent antimicrobial agents for the treatment of infectious diseases. The aromatic hydrazides and aroyl hydrazones exhibit antimicrobial activity towards *S. aureus*, *E. coli* and *P. aeruginosa*. The antibacterial activity experiments indicated that aroyl hydrazones possess more activity than the hydrazides. Also the compounds like hydrazide containing 2-aminobenzothiazoles, 2-(2-(benzofuran-2-ylsulfonylcarbamoyl)-5-methoxy-1H-indol-1-yl) acetic acid and 5-*tert*-butyl-*N*-pyrazol-4-yl-4,5,6,7-tetrahydrobenzo[*d*]isoxazole-3-carboxamide were found to possess antitubercular activity(1-3). The presence of –OH and –SH functional groups at ortho and para position increases the therapeutic properties than the other groups (4-5). Among amido substituted hydrazides namely N'-(substituted)-4-(butan-2ylideneamino)benzohydrazides were found to be potent against the fungi *C albicans* (6). Similarly Diaryl hydrazones show specific antioxidant activity similar to that of ascorbic acid and resveratrol (7).

Tuberculosis has become a primary health threat to the mankind. A series of hydrazide-hydrazones were found to possess antituberculosis activity against *M. tuberculosis* H37Rv (8). The synthesized trifluoromethylated homoallylic N-acylhydrazines or α-methylene-γ-lactams possess the pharmaceutical property in the field of pharmaceuticals (9). N'-(benzofuroxan-5-yl)methylene benzohydrazide derivatives were investigated for the properties with the potential anti- *Trypanosoma cruzi* agents (10). The phthalimido aromatic hydrazide derivatives are potent stabilizers for rigid PVC against thermal degradation and also potent antimicrobial agents against gram positive *B. subtilius* and *S. pneumonia,* gram-negative *E.coli, A. fumigates, S. racemosum* and *G.candidum* fungi (11). Similarly 3,4,5-trimethoxy benzyl moiety was screened for antioxidant activity and heterocyclic scaffolds are potent against *M. tuberculosis* H_{37}Rv as compared with that of the standard drug rifampicin. Many compounds were found to possess excellent activity against *P. falciparum* strain as compared to the standard drug quinine IC_{50} is 0.826 μM. Different compounds acquire antimicrobial, antituberculosis and antimalarial activities (12-13). Many novel hydrazones were screened for antimicrobial activity and they were found to be potent pharmaceuticals because of the presence of electron withdrawing groups which act as gateways for the design and development of new antimicrobial agents with potent activity and minimal toxicity (14).

The free ligand 1-(2-[(5-methylfuran-2yl)methylene)] hydrazono) phthalazine and nickel complex of the ligand were screened against *A. niger*, *A. flavus* and *C. albicans.* The results put forth modest antifungal activity of the complex compared to the parent ligand (15). Some of the synthesized compounds like phenoxy/naphthalene-1-yl/naphthalen-2-yloxy methyl-1H-benzimidazol-1-yl)acetohydrazide showed good antibacterial activity with MIC 12.5 and 25 μg/ml when compared with the series (16). All the compounds of the series were found to possess antimicrobial activity and 4-hydroxy-7-(trifluoromethyl)quinoline-3-carbohydrazide derivatives were having excellent anti tuberculosis activity and further studies can be

continued for the development of drug for tuberculosis disease (17**).** The different heterocyclic compounds like (*E*)-2-hydroxy-N'-[(2-hydroxynaphthalen-1-yl)methylene]benzohydrazide and (*E*)-4-hydroxy-N'-[(2-hydroxynaphthalen-1-yl)methylene]benzohydrazide found to exhibit potent antioxidant properties and variety of applications (18).

In our research work we have synthesized 4-chloro-benzoic acid (2-chloro-quinolin-3-ylmethylene)-hydrazide (BQH) and 4-chloro-benzoic acid (2-chloro-quinolin-6-methyl-3-ylmethylene)-hydrazide (BQMH). These compounds were tested for antimicrobial properties.

Experimental

Materials and Physical Measurements

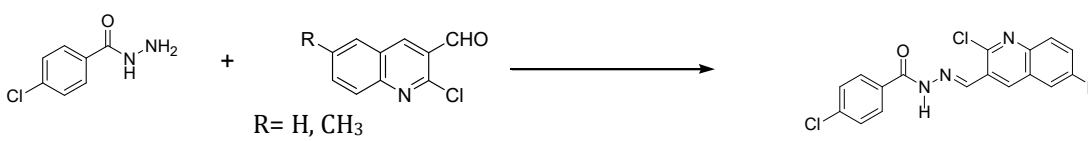
All the chemicals and solvents used are of analytical grade and were purchased from Royal scientific company and used without further purification. TLC was run on the silica coated aluminium sheets (TLC silica gel 60 F***254***, Analytical chromatography) and visualized in low UV light. IR spectra in KBr pellets were recorded on the FT-IR Perkin Elmer Spectrum spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 M Hz spectrometer with $CDCl_3$ as solvent and TMS as internal reference. Chemical shifts are quoted as ppm and the coupling constants *J* in *Hz* in signals are described as s (singlet), d (doublet), t (triplet), m (multiplet) and br (broad). Melting points were determined on a Sigma melting point apparatus without corrections. GC MS mass spectrum was taken in JEOLGCMATE II GC-MS with Data system in a high resolution, double focusing instrument.

General procedure for the synthesis of 2- chloro -3-formyl quinoline

2-Chloroquinoline-3-carbaldehyde was synthesized via Vilsmeier–Haack method using acetanilide(19).

Synthesis of N′ [(2-chloro-quinolin-3-yl)methylidene] benzohydrazide

10 mmol of ethanolic solution of 2- chloro-3-formyl quinoline was added to 10 mmol of ethanolic solution of benzohydrazides and the reaction mixture was stirred for 30 minutes at room temperature in the presence of glacial acetic acid as catalyst. After refluxing for 3-5 h the completion of the reaction was monitored by Thin Layer Chromatography (TLC), then the reaction mixture was cooled and placed in refrigerator overnight. The resulting solid was filtered and washed with petroleum ether then recrystallised with ethanol and characterized by spectral analysis.



Synthesis of N′ [(2-chloro-quinolin-3-yl)methylidene] benzohydrazide

In-silico studies

In silico studies were carried out by following the standard procedure as reported earlier (20)

Antimicrobial activity

Antibacterial screening of the synthesized compounds was evaluated against *Staphylococcus aureus* and *Escherichia coli,* and the zone of inhibition was measured in mm. The broth media was inoculated and grown at 37ºC for 18 h to revive the bacteria from the stock cultures. The agar plates with wells were inoculated by spreading evenly with 18h old bacterial cultures (100 µl, 10^{-4} cfu). After 20 min, various concentrations of the compound and the antibiotic were filled in the wells. Finally, the plates were incubated at 37ºC for 24 h and the inhibition zone was observed (21).

Luciferase reporter phage (LRP) assay (22)

The MTB H37Rv and clinical isolate of MTB were grown in Middle brook 7H9 complete medium 12 with and without test sample for 3 days at 37°C. LRP assay was done using concentrations of 200, 100 and 50 µg/mL of test samples. 50µLbacterial suspension equivalent to MacFarlands.No.2 standard was added to 400 µL of 7H9 with and without the test compound. For each sample, two drug-free controls and two drug concentrations were prepared and this set up was incubated for 72 h at 37°C. After incubation, 50 µLof the high titer luciferase reporter phage (phAETRC202) and 40 µLof 0.1 M $CaCl_2$ were added to all the vials and

this set up was incubated at 37°C for 4 h. After incubation, 100 µL of the mixture was taken from each tube into a Luminometer cuvette and an equal amount of working D-luciferin (0.3 mM in 0.05 M sodium citrate buffer, pH 4.5) solution was added. The Relative Light Units (RLU) was measured after 10s of integration in the Luminometer. Readings were recorded in duplicate for each sample and the mean was calculated. The percentage reduction in the RLU was calculated for each test sample and compared with that of control.

Broth microdilution method

The MIC for MTB H37Rv and isoniazid (INH) resistant clinical isolate of MTB was determined using a broth microdilution method (23) in Middle brook 7H9 medium supplemented with OADC, with a final inoculum of 1x107cfu/mL. The compounds were dissolved in DMF (1.25 mg/ mL) and used as stock solution. Concentrations ranging from 1250 to 1 µg/mL were used to assess the effectiveness of the compounds. After inoculation the microtiter tubes were incubated at 37°C for 72 h, and the growth inhibition was recorded for 14 and 21 days, respectively. The MIC value represents the lowest dilution of the compound at which no bacterial growth was detected.

Results and discussion

2- chloro-3-formyl quinoline was prepared by refluxing acetanilide 5g (0.0337 mol) dimethylformamide 7.1mL (0.0925mol) phosphorous oxy chloride 23.8 mL (0.2352 mol), yellow solid, yield: 83%. m.p. 140˚C.

2-chloro-3-formyl-6-methyl quinoline was prepared by refluxing 4-methyl acetanilide 5g (0.0336 mol) dimethylformamide 7.1mL (0.0925mol) phosphorous oxy chloride 23.8 mL (0.2352 mol), yellow solid, yield: 80%. m.p. 124˚C.

4-chloro-benzoic acid (2-chloro-quinolin-3-ylmethylene)-hydrazide was prepared by the above mentioned method. 4-chlorobenzohydrazide (0.171g 0.001mol 1 equivalent) 2-chloro-3-formyl quinoline (0.096g 0.001mol 1 equivalent). FT-IR (υ_{max} KBr pellets, cm^{-1}) Ar (C-H) str- 3047, C=C str- 1552, (C=O) str- 1590, (C=N) str- 1548, (N-N)- 926, (C-Cl)- 744, (C-O-C)- 1285. ^{1}H NMR (DMSO, 400 MHz) δH: 12.3 (s, 1H), 8.9 (d, 2H, 23.2 Hz), 8.24 (d, 1H, 8Hz), 8 (m, 3H, 8.01-7.98Hz), 7.9 (m, 1H, 7.9-7.8 Hz), 7.7 (t, 1H, 7.73-7.69 Hz), 7.64 (d, 2H, 8.4Hz). ^{13}C NMR (DMSO 300 MHz) 162, 148, 147, 143, 136, 135, 131, 131, 129, 129, 128, 127, 127, 126 and 126. Pale white amorphous solid, yield: 76%. m.pt. 258-260˚C. m/z = 344 (M+1). Molecular Formula $C_{17}H_{11}Cl_2N_3O$.

4-chloro-benzoic acid (2-chloro-6-methyl quinolin-3-ylmethylene)-hydrazide was prepared by the above mentioned method. 4-chlorobenzohydrazide (0.171g 0.001mol 1 equivalent) 2-chloro-3-formyl-6-methyl quinoline (0.096g 0.001mol 1 equivalent), FT-IR (υ_{max} KBr pellets, cm^{-1}) Ar (C-H) str- 3048, (C-C) – 2821, C=C str-1551, (C=O) str- 1590, (C=N) str- 1547, (C-Cl)- 821, (C-O-C) -1285, (N-N) – 954. ^{1}H NMR (DMSO, 400 MHZ) δH: 12.3 (s, 1H), 8.8 (d, 2H, 16.8Hz), 7.9 (d, 3H, 8.4 Hz), 7.8 (d, 1H, 8.4 Hz), 7.7 (d, d, 1H, 10.4Hz), 7.6 (d, 2H, 8.4Hz), 2.9 (s, 3H). ^{13}C NMR (DMSO 300 MHz): 145, 143, 137, 135, 134, 131, 129, 128, 127, 127, 125 and 21. Yellowish white amorphous solid, yield: 80%. m.pt. 258˚C. m/z = 358 (M+1). Molecular Formula $C_{18}H_{13}Cl_2N_3O$

Table1: Antituberculosic activity prediction for the synthesized compounds

Compound code	Pa	Pi
BQH	0.566	0.007
BQMH	0.487	0.014

Pa – probability of activation; Pi – probability of inactivation

Table 2a: Docking results of 4-chloro-benzoic acid (2-chloro quinolin-3-ylmethylene)-hydrazide with the receptor

CONF	B.E	L.E	IC (pM)	Int. E	Vdw	Elec. E	Totall E	Tor. E	Unb. E	No.of H bonds	Hydrogen bond
1	-14.17	-0.62	40.8 pM	-15.07	-15.05	-0.02	-0.62	0.89	-0.62	3	Lig1:N::Ala201:O Lig1:O::Ala201:O Lig1:N::Ala201:O
2	-13.99	-0.61	55.37pM	-14.89	-14.87	-0.02	-0.62	0.89	-0.62	2	Lig1:N::Ala201:O Lig1:O::Ala201:O
3	-13.86	-0.60	69.46pM	-14.75	-14.72	-0.03	-0.54	0.89	-0.54	1	Lig1:O::Ala201:O
4	-13.81	-0.60	74.97	-14.71	-14.70	-0.01	-0.62	0.89	-0.62	3	Lig1:N::Ala201:O Lig1:O::Ala201:O Lig1:N::Ala201:O
5	-13.56	-0.59	115.88	-14.45	-14.42	-0.03	-0.62	0.89	-0.62	1	Lig1:N::Ala201:O

6	-12.85	-0.56	379.8	-13.75	-13.74	-0.01	-0.62	0.89	-0.62	3	Lig1:N::Ala201:O Lig1:O::Ala201:O Lig1:N::Ala201:O
7	-12.19	-0.53	1.15nM	-13.09	-13.04	-0.04	-0.41	0.89	-0.41	2	Lig1:N::Ala201:O Lig1:O::Met98:O
8	-12.01	-0.52	1.57nM	-12.90	-12.84	-0.06	-0.63	0.89	-0.63	1	Lig1:N::Ala201:O
9	-11.22	-0.49	5.94nM	-12.12	-12.10	-0.01	-0.63	0.89	-0.63	1	Lig1:N::Gly96:O
10	-11.00	-0.48	8.62nM	-11.90	-11.82	-0.08	-0.51	0.89	-0.51	1	Gly:96:HN::Lig1:N,N

Table 2b: Docking results of 4-chloro-benzoic acid (2-chloro-6-methyl- quinolin-3-ylmethylene)-hydrazide with the receptor

CONF	B.E	L.E	IC (pM)	Int.E	Vdw	Elec. E	Total. IE	Tor. E	Unb. E	No. of H bonds	Hydrogen bond
1	-14.7	-0.61	16.8	-15.59	-15.57	-0.02	-0.63	0.89	-0.63	3	Lig1:N::Ala201:O Lig1:O::Ala201:O Lig1:N::Ala201:O
2	-14.6	-0.61	19.91	-15.49	-15.47	-0.02	-0.63	0.89	-0.63	3	Lig1:N::Ala201:O Lig1:O::Ala201:O Lig1:N::Ala201:O
3	-14.14	-0.59	42.99	-15.04	-15.03	-0.01	-0.63	0.89	-0.63	2	Lig1:N::Ala201:O Lig1:O::Ala201:O
4	-14.14	-0.59	42.95	-15.04	-15.03	-0.01	-0.63	0.89	-0.63	2	Lig1:N::Ala201:O Lig1:O::Ala201:O
5	-13.87	-0.58	68.04	-14.77	-14.75	-0.01	-0.63	0.89	-0.63	3	Lig1:N::Ala201:O Lig1:O::Ala201:O Lig1:N::Ala201:O
6	-11.84	-0.49	2.09nM	-12.74	-12.72	-0.01	-0.65	0.89	-0.65	2	Lig1:N::Asn106:OD1 Lig1:O::Met98:O
7	-11.71	-0.49	2.6nM	-12.61	-12.57	-0.04	-0.63	0.89	-0.63	0	-
8	-11.33	-0.47	4.97nM	-12.22	-12.21	-0.01	-0.63	0.89	-0.63	0	-
9	-10.83	-0.45	11.58nM	-11.72	-11.72	0.0	-0.62	0.89	-0.62	0	-
10	-9.74	-0.41	72.77nM	-10.63	-10.57	-0.07	-0.63	0.89	-0.63	0	-

The docking results of Enoyl-[acyl-carrier-protein] reductase [NADH] from *Mycobacterium tuberculosis* with the ligand 4-chloro-benzoic acid (2-chloro-quinolin-3-ylmethylene)-hydrazide (BQH) is presented in table 2a. The binding energies of the different binding conformations range between -14.17 and -11.0 kcal/mol. All the binding conformations had atleast 1 hydrogen bond formed between the ligand and the receptor. The conformations 1, 4 and 6 had formed the hydrogen bonds with the same 'Alanine 201' residue. The conformation 1 with three hydrogen bonds between the ligand BQH and 'O' of Alanine 201 is considered the best conformation because of the least binding energy than the other conformations and the orientation is displayed in Figure-1a.

The docking results of Enoyl-[acyl-carrier-protein] reductase [NADH] from *Mycobacterium tuberculosis* with the ligand 4-chloro-benzoic acid (2-chloro-6-methyl- quinolin-3-ylmethylene)-hydrazide (BQMH) is presented in table 2b. The binding energies of the different binding conformations range between -14.7 and -9.74 kcal/mol. Among the 10 different binding conformations, 6 conformations had 2 or 3 hydrogen bonds. The best 5 docked conformations had hydrogen bonds formed between the ligand BQMH and 'Ala 201' of the receptor. The 6th best docked conformation had two hydrogen bonds formed between the Ligand BQMH and 'Asn 106' and 'Met 98' of the receptor. All the other four conformations had no hydrogen bonds formed between the receptor and the ligand. Hence, the best docked conformation is conformation 1 with 3 hydrogen bonds between the ligand BQMH and 'Ala 201' of the receptor which is displayed in figure-2b.

Table2c: Docking results of isoniazid with the receptor

CONF	B.E	L.E	IC (uM)	Int.E	Vdw	Elec.E	Total. IE	Tor. E	Unb. E	No. of H bonds	Hydrogen bond
1	-6.73	-0.67	11.61	-7.03	-7.0	-0.04	-0.09	0.3	-0.09	1	Iso:O::Ile 202:O
2	-6.73	-0.67	11.69	-7.03	-6.99	-0.04	-0.09	0.3	-0.09	1	Iso:O::Ile 202:O
3	-6.71	-0.67	12.11	-7.01	-6.97	-0.03	-0.09	0.3	-0.09	1	Iso:O::Ile 202:O

4	-6.67	-0.67	12.94	-6.97	-6.98	0.01	-0.09	0.3	-0.09	1	Iso:O::Ala 201:O
5	-6.67	-0.67	13.01	-6.96	-6.98	0.02	-0.09	0.3	-0.09	1	Iso:O::Ala 201:O
6	-6.54	-0.65	16.15	-6.84	-6.73	-0.11	-0.09	0.3	-0.09	0	-
7	-6.04	-0.60	37.35	-6.34	-6.26	-0.08	-0.09	0.3	-0.09	2	Tyr158:HH::Iso:O Ile194:HN::Iso:N
8	-6.04	-0.60	37.62	-6.33	-6.25	-0.08	-0.09	0.3	-0.09	2	Tyr158:HH::Iso:O Ile194:HN::Iso:N
9	-6.00	-0.60	39.78	-6.30	-6.21	-0.09	-0.09	0.3	-0.09	1	Ile194:HN::Iso:N
10	-5.83	-0.58	53.14	-6.13	-6.06	-0.07	-0.09	0.3	-0.09	1	Ile194:HN::Iso:O

The docking results of Enoyl-[acyl-carrier-protein] reductase [NADH] from *Mycobacterium tuberculosis* with the standard drug Isoniazid is presented in table 2c**.** The binding energies of the different binding conformations range between -6.73 and -5.83 kcal/mol. Among the ten different docked conformations, conformation 7 and 8 show 2 hydrogen bonds between the drug and Tyr158 and Ile194. The conformation 1, Conformation 2, Conformation 3, Conformation 4 and Conformation 5 had only one hydrogen bond formed with binding energies -11.81, -10.68, -10.33, -9.82 and -9.56 kcal/mol respectively. Hence, conformation 8 with 2 hydrogen bonds and binding energy of -9.84 kcal/mol is considered the best docked conformation and represented in figure-3.

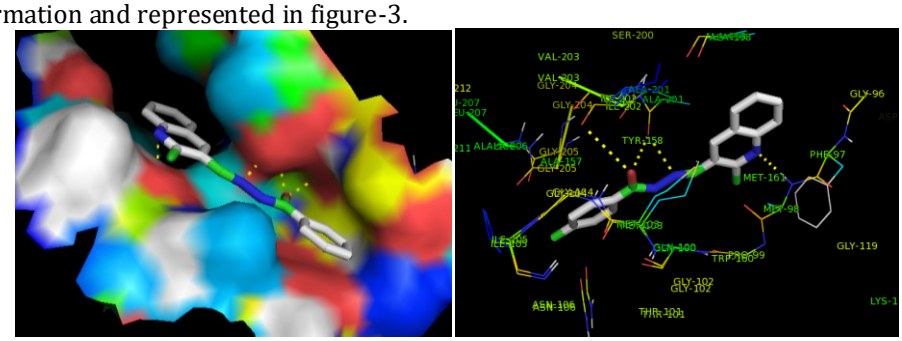


Fig 1a Surface representation of binding interactions between BQH with the receptor

Fig 1b: Binding interactions of BQH with protein Enoyl-[acyl-carrier-protein] reductase [NADH] showing hydrogen bonds

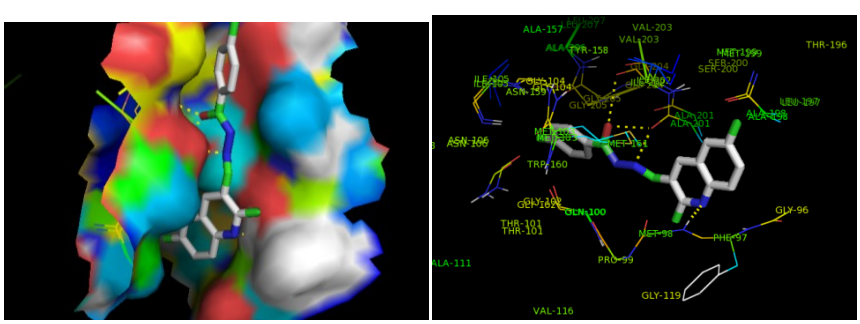


Fig 2a: Surface representation of binding interactions between BQMH with the receptor

Fig 2b: Binding interactions of BQMH with protein Enoyl-[acyl-carrier-protein] reductase [NADH]

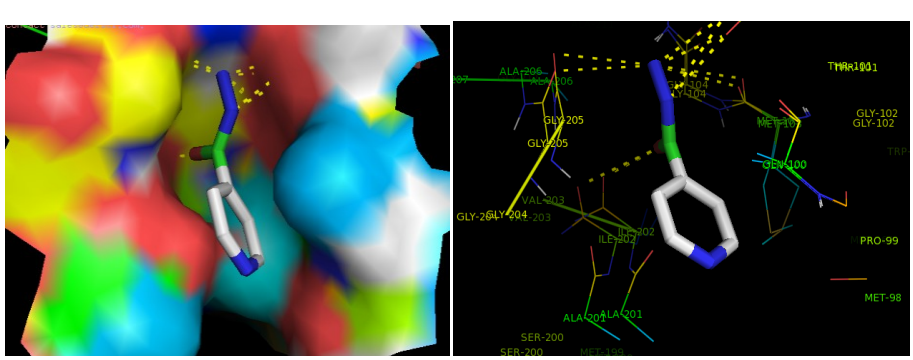


Fig 3a: Binding interactions between isoniazid with the receptor

Fig 3b:Binding interactions of isoniazid with protein Enoyl-[acyl-carrier-protein] reductase [NADH]

Bactericidal activity

Antimycobacterial tuberculosis activity of the compounds synthesized is portrayed in the table 3. The bactericidal activity of the compounds BQH and BQMH, depict that both the compounds very good anti bacterial agents. Further, maximum inhibition (27.95%) was observed at 50 µg/ml of BQMH. Similarly at 50 µg/ml concentration of BQH there is 40.97% inhibition in the growth of bacteria.

Table 3: Antimicrobial activity data of BQH and BQMH by LPR assay

Compound (µg/ml)	Cfu/ml	% reduction	% inhibition
DRUG FREE1 control	1.2×10^5	1.7×10^5	-
DRUG FREE2 Control	2.2×10^5		
BQMH 50ug	3×10^4	82	27.95498
BQMH 100ug	3.2×10^4	81	24.07529
BQMH 200ug	2.3×10^4	86	26.05151
BQH 50ug	2.6×10^4	84	40.97012
BQH 100ug	2.9×10^4	82	16.26944
BQH 200ug	2×10^4	88	27.02749

Conclusion

In the present study two compounds BQH and BQMH have synthesized in good yield adopting standard procedures and characterized by spectral and analytical techniques. Docking studies show that the compounds exhibit good antibacterial activity especially anti-tuberculosis activity. The compounds show better activity when compared to that of the existing drug isoniazid for Tuberculosis. It is also confirmed from the in vivo studies that the compound BQH (84%) and BQMH (82%) has maximum inhibition at 50µg/mL concentration. Hence both these compounds can be further evaluated for their efficiency in treating Tuberculosis.

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