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Synthesis, characterization and biological applications of substituted benzohydrazide derivatives

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ABSTRACT

*In the present article, a series of Schiff bases have been derived from aromatic aldehydes with acid hydrazide and using concentrated hydrochloric acid as catalyst. The synthesized compounds are (E)-4-Chloro-N'-(3,4-dimethoxybenzylidene)benzohydrazide(**S1**), (E)-4-Chloro-N'-(2,5-dimethoxybenzylidene)benzohydrazide(**S2**), (E)-4-Chloro-N'-(thiophene-2-ylmethylene) benzohydrazide(**S3**) and (E)-4-Chloro-N'-(4-chlorobenzylidene) benzohydrazide (**S4**) were characterized through FT-IR, ^{1}H NMR and ^{13}C NMR. These synthesized compounds have been screened for antimicrobial activity against S. aureus, E. coli and A. niger. The antioxidant activity of Schiff bases were investigated by DPPH radical scavenging method using UV spectrum. The designed compounds were further subjected for molecular docking studies for 2NSD protein in Mycobacterium tuberculosis by Discover studio 2.1version software.*

Keywords: 4-Chlorobenzohydrazide, Antimicrobial activity, DPPH, *Mycobacterium tuberculosis*.

INTRODUCTION

The chemistry of substituted benzohydrazide derivatives is one of the important subject in organic synthesis in recent years. The major problem in the effective antibacterial and antifungal treatment is increasing resistance of microorganisms to currently available antimicrobial drugs [1-4]. Therefore, the development of novel antimicrobial drugs is an active area of research. Most of the compounds bearing an azomethine group exhibit antimicrobial, antioxidant and antiproliferative properties [5-8]. Schiff bases such as nitrofurantoin of nifuroxazide are commonly applied in medicine as antibacterial agents [9]. Benzohydrazide have been reported to possess various biological activities such as antileishmanial [10], anti-inflammatory [11], anticancer [12], antimycobacterial [13], anti-tumoral studies was reviewed by Rollas et. al [14]. Benzohydrazides are easily converted into hydrazones by treating with aldehydes or ketones [15-17]. Applications of benzohydrazides are reported in medicinal and analytical chemistry [18]. The main mycobacterial infection in human is tuberculosis caused by Mycobacterium tuberculosis. Tuberculosis is the leading infectious cause of death in the world. Therefore, there is continuing and compelling need for new and improved treatment for tuberculosis [19-22]. In the present study, a series of Schiff bases have been synthesized. Further the synthesized compounds were evaluated for antimicrobial activity, antioxidant activity and molecular docking studies.

MATERIALS AND METHODS

All the reagents were obtained from commercial supplies and used without any further purification. Melting points were determined on an EZ-melt automated melting point apparatus without corrections. The reactions were carried out under the open atmosphere of oxygen. FT-IR spectra were recorded in KBr pellets on a perkin Elmer Spectrum-1 FT-IR spectrometer. ^{1}H-NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on Bruker spectrometer in DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal reference. The chemical shifts are mentioned in parts per million (δ in ppm), and The signals are described as s(singlet), d(doublet), t(triplet), q(quartet) and m(multiplet). The electronic spectra were recorded on a perkin Elmer UV-Vis 1100-100 nm Spectrophotometer.

Synthesis of (E)-4-chloro-N′-(3,4-dimethoxybenzylidene)benzohydrazide (S1).
10 mL aqueous solution of 4-chlorobenzohydrazide (1a, 0.176 g, 0.001 mol) is added 5 mL ethanolic solution of 3,4-dimethoxybenzaldehyde(1b, 0.145 g, 0.001 mol). The reaction mixture was kept in a magnetic stirrer, maintained at room temperature and stirred well for 5 min, followed by adding con. HCl. The obtained product was filtered, then washed with petroleum ether (40-60%) and dried over in a vacuum. The dried solid was recrystallised from ethanol. The same procedure is followed by the rest of compounds (S2-S4).

Antimicrobial activity
The synthesized compounds (**S1-S4**) were biologically evaluated for antibacterial and antifungal activities by the following method. The antimicrobial activities of these compounds were determined by nutrient agar well diffusion method as recommended by National Committee for Clinical Laboratory Standards (NCCLS) (Furtado and mederiros, 1980) [23]. The nutrient agar medium was prepared and sterilized by autoclaving at 121 °C and 15 lbs pressure for 15 minutes. The petri plates were allowed to solidify. The bacterial broth culture was swabbed on this petri plates using a sterile buds. The organic solvent dimethyl sulfoxide, was dissolved in the tested compounds. The substituted benzohydrazide derivatives were tested for their *in vitro* growth inhibitory activity against *S. aureus* as gram positive and *E. coli* as gram negative bacterial strains and *in vitro* antifungal potential against *A. niger* strain. The petri plates were incubated at 37 °C for 24 hrs for gram-positive, gram-negative bacteria and 48 hrs for fungi. After incubation, the plates were observed for the zone of inhibition. The antimicrobial activities of synthesized compounds were compared with Erythromycin and Gentamycin as standard.

Screening for Antioxidant assays
The radical scavenging activities were determined using the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The radical scavenging effects of all the synthesized compounds (**S1-S4**) were measured according to the method of Shimada et. al., [24-26] with a characteristic absorption using UV-Vis spectrophotometer. A fixed concentration of the experimental compounds were added to solution of DPPH in methanol (20 µM, 40 µM, 60 µM, 80 µM, 4 mL) and the final volume was made up to 4 mL with doubly distilled water. The solution was incubated at room temperature for 30 min in the dark. The decrease in absorbance of DPPH was measured at 517 nm. Here ascorbic acid is used as standard antioxidant. The percentage of activity was calculated using the following formula:

$$\text{Radical scavenging activity } (\%) = \frac{A_C - A_S}{A_C} \times 100$$

Where,
A_c- absorbance value of blank,
A_s -absorbance value of sample.

Molecular docking Analysis
Interaction studies were performed for the synthesized compounds (**S1-S4**) with *Mycobacterium tuberculosis* (Protein id: 2NSD) protein using Discovery studio Accelrys software (version 2.1.) The X-ray crystallographic structure of *Mycobacterium tuberculosis* (2NSD) was downloaded from protein data bank. The protein was prepared for docking by the removal of water molecules and heteroatom from the downloaded protein structure. Crystallographic disorders and unfilled valence atoms were corrected using alternate conformations and valence monitor options were subjected to energy minimization by applying CHARMm (Chemistry at Harvard Macromolecular Mechanics) force fields. Active sites in the protein were explored using Discovery studio software [27]. The 2D structures of substituted benzohydrazide derivatives were retrieved from PubChem, a chemical database. The receptor cavities were explored and the active site residues selected were used for the interaction studies. Scoring functions implemented in docking programs make various assumptions and simplifications in the

evaluation of modeled ligands, which includes in terms of hydrogen bonds employed to rank the docked bases and to assess the binding site and the number of rotatable bonds present. Using these criteria (-CDocker interaction energy, vander waals energy) the best receptor-ligand was chosen and its stability was analyzed by the presence of hydrogen bond.

RESULTS AND DISCUSSION

Substituted benzohydrazide derivatives were synthesized through condensation reaction by Schiff base route. The yield was 97% for synthesized compound (**S1)** in the model reaction in which Con. HCl served as catalyst and water as solvent. All the products (**S1-S4**) were immiscible with polar solvents and soluble with DMSO and DMF. The postulated structures of the newly synthesized compounds were in good agreement with their FT-IR, ^{1}H NMR and ^{13}C NMR spectral data.

FT-IR Spectral studies

In order to confirm the functional groups present in the synthesized products (**S1-S4**) FT-IR spectra were recorded and shown in **Fig 1(a-d).** The bands observed in the range of 3347-3434 cm^{-1} are due to N-H stretching frequency of azomethine analogues, while the absorption band in the region 2829-3079 cm^{-1} and 2364-2723 cm^{-1} are ascribed to aromatic and aliphatic C-H stretching frequencies [28-30]. The band observed in the range of 1595-1654 cm^{-1} are due to C=O stretching frequency of carbonyl group. The presence of C=N stretching frequency around 1511-1598 cm^{-1}confirm the substituted benzohydrazide formation.

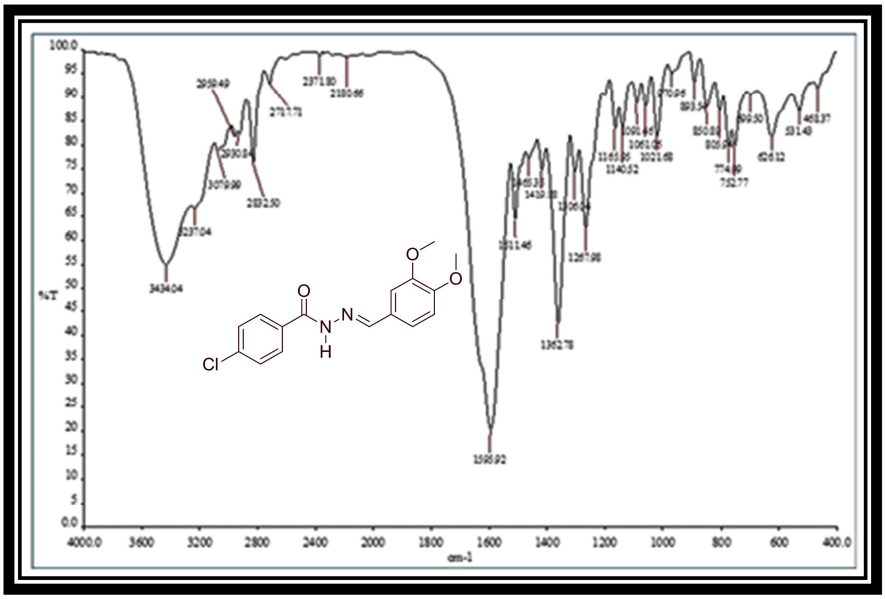

Figure 1(a). FT-IR Spectrum of Compound (S1)

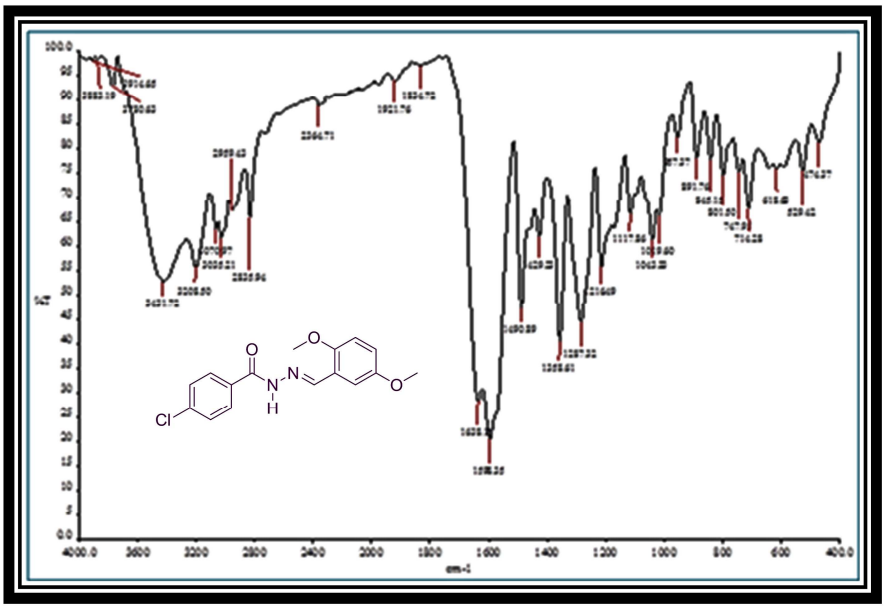

Figure 1(b). FT-IR Spectrum of Compound (S2)

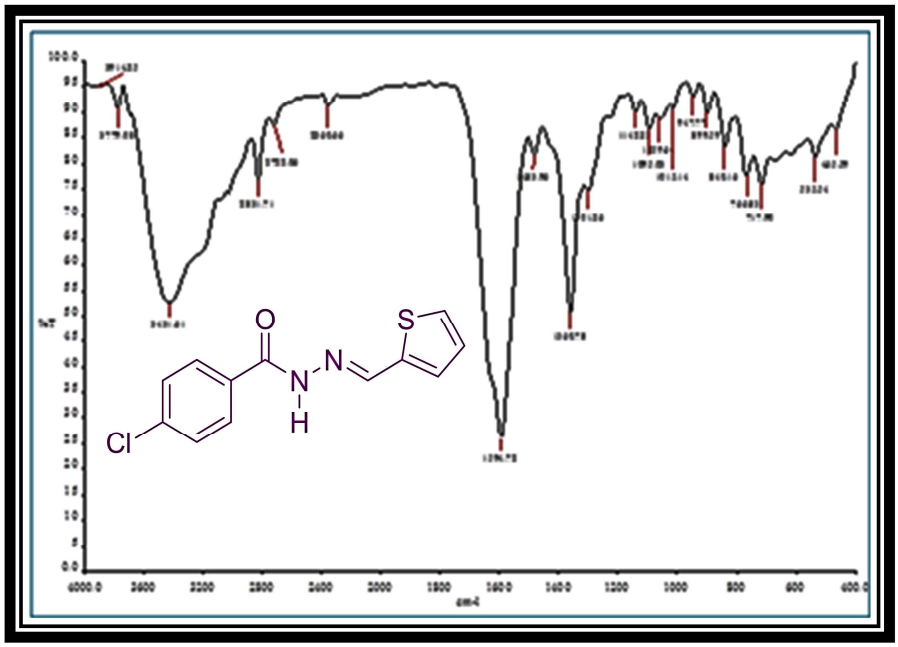

Figure 1(c). FT-IR Spectrum of Compound (S3)

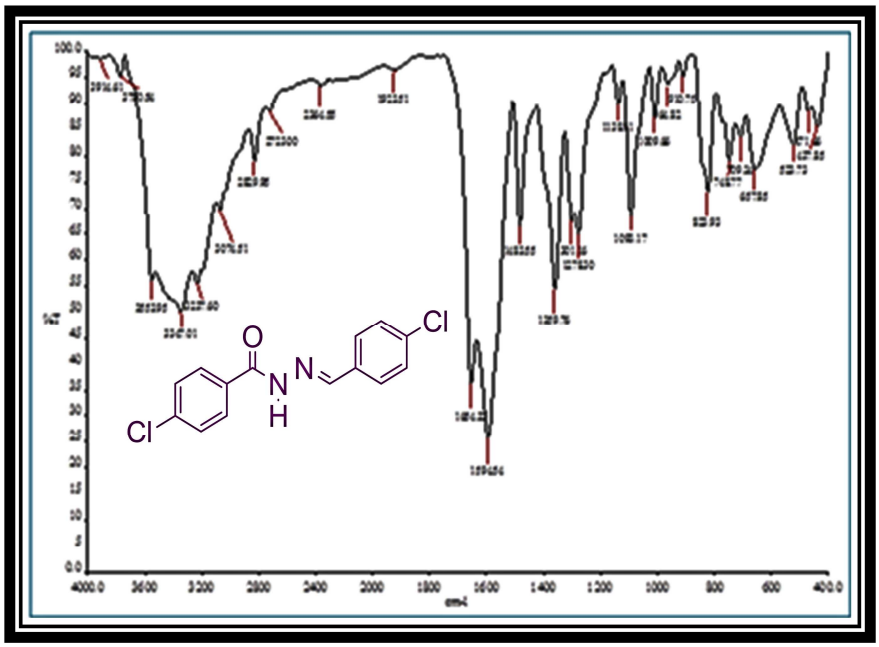

Figure 1(d). FT-IR Spectrum of Compound (S4)

^{1}H NMR Spectra

In the present study, ^{1}H NMR Spectra were recorded for substituted benzohydrazide derivatives using Bruker-400 MHz Spectrometer. The ^{1}H NMR spectral data are shown in **Fig 2 (a-d**). A singlet appears at δ 11.8-11.9 ppm is assigned to proton of NH adjacent to CO which is exhibited as enolic form and another broad singlet appears at δ 8.3-8.7 ppm is assigned to azomethine (CH=N) proton [31]. A multiplet shown in the range of δ 7.0-7.9 ppm, is due to aromatic C-H protons. A singlet appeared at δ 3.7 ppm and 3.8 ppm is assigned to methoxy proton present in **S1** and **S2** respectively.

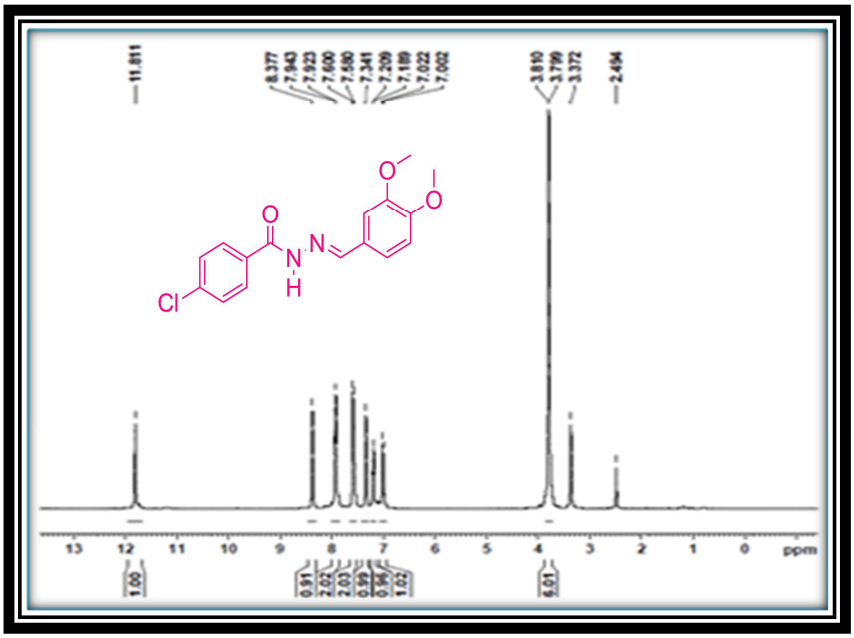

Figure 2 (a) ^{1}H NMR Spectrum of Compound (S1)

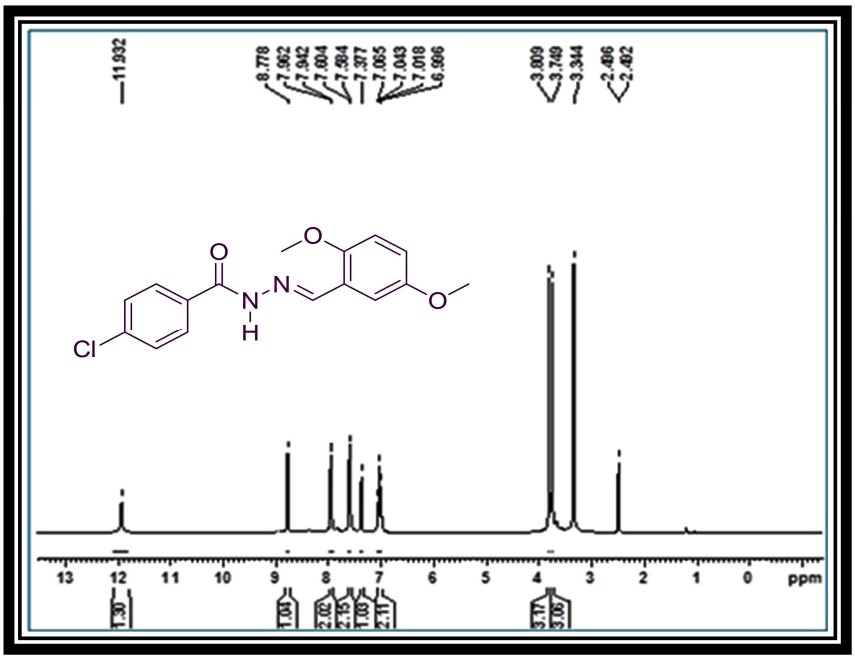

Figure 2(b). ^{1}H NMR Spectrum of Compound (S2)

Figure 2(c). ^{1}H NMR Spectrum of Compound (S3)

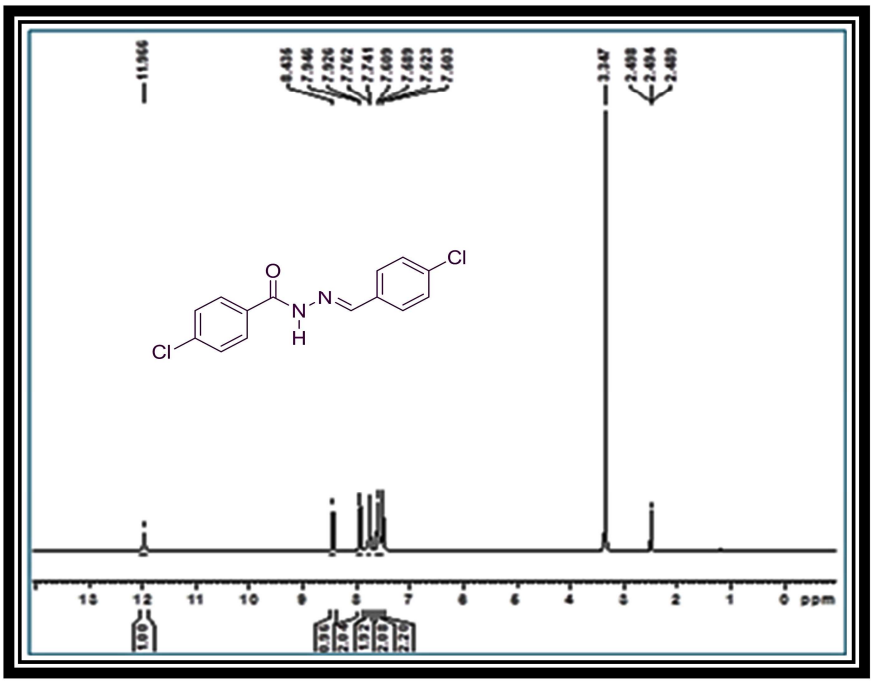

Figure 2(d). ^{1}H NMR Spectrum of Compound (S4)

Figure 2(a-d). ^{1}H NMR Spectra of substituted benzohydrazide derivatives (S1-S4)

^{13}C NMR Spectra of compounds (S1-S4)

The structures of novel Schiff base compounds **S1-S4** was further supported by ^{13}C NMR (100 MHz) Spectrum and spectral data are shown in **Fig 3(a-d).** The carbonyl carbon signal appears at about δ 161-162 ppm respectively. The five signals that appeared at values δ 153-149 ppm and 136-108 ppm are due to the substituted benzene ring carbon. The signals that appeared at δ 143-148 ppm were assigned to the carbon of azomethine group [32]. The signal for methoxy carbon (OCH_3) in S1and S2 appeared at δ 56-55 ppm [32].

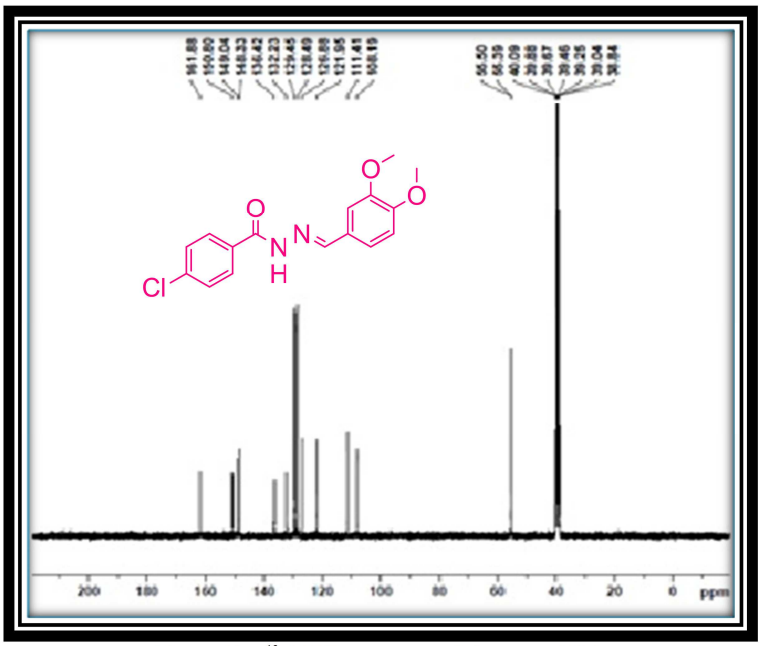

Figure 3(a). ^{13}C NMR spectrum of Compound (S1)

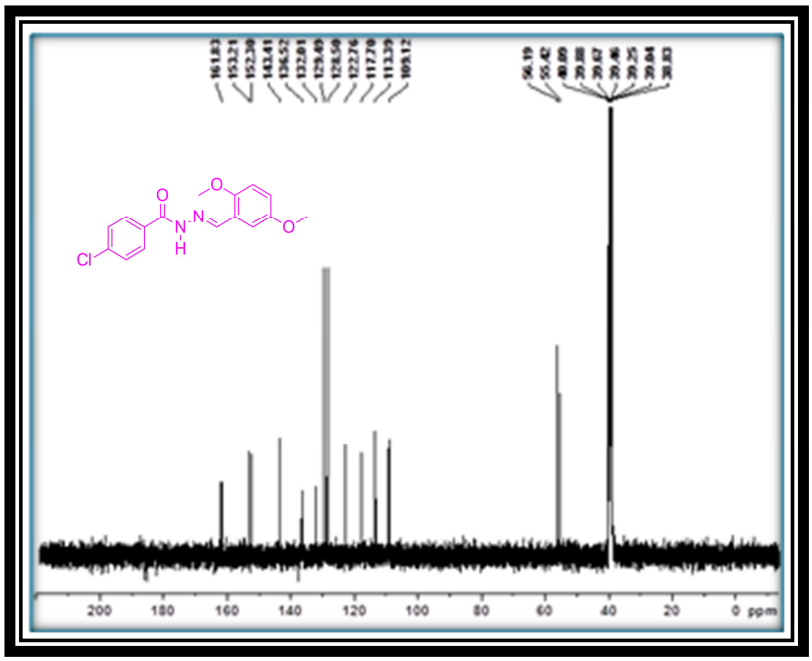

Figure 3(b). ^{13}C NMR spectrum of Compound (S2)

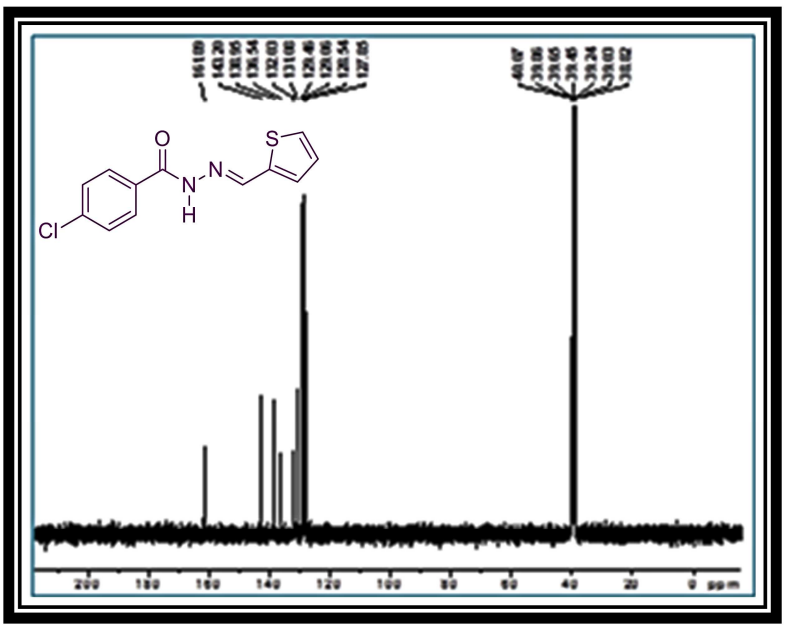

Figure 3(c). ^{13}C NMR spectrum of Compound (S3)

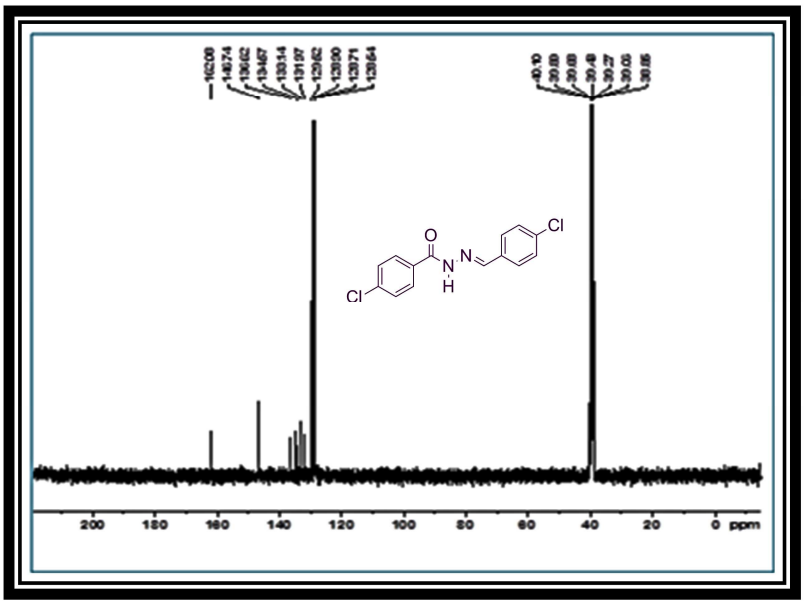

Figure 3(d). ^{13}C NMR spectrum of Compound (S4)

Figure 3(a-d).^{13}C NMR spectra of substituted benzohydrazide derivatives (S1-S4)

Table 1: List of Products and their corresponding reactants

S. No	Substituted benzohydrazide (1a-4a)	Aldehydes (1b-4b)	Products (S1-S4)	Yield (%) in HCl
S1	1a	1b	**S1**	97
S2	2a	2b	**S2**	94
S3	3a	3b	**S3**	86
S4	4a	4b	**S4**	91

(E)-4-chloro-N′-(3,4-dimethoxybenzylidene)benzohydrazide (S1): was derived from 4-chlorobenzohydrazide and 3,4-dimethoxybenzaldehyde (1:1) Yield : 97%. FT-IR: (υ in cm^{-1}) 3434 (NH), 3079 (Ar-CH), 2717 (Ali-CH), 1595 (C=O), 1511 (C=N), 1465 (C-N). ^{1}H NMR δ in ppm (400 MHz, DMSO-d_6): 11.8 (s, 1H, enolic NH proton), 8.3 (s, 1H, CH=N, azomethine group), 7.9 (d, 2H, 0-Ar-H, Ar-Cl), 7.6 (d, 2H, m-Ar-H, Ar-Cl), 7.3 (s, 1H, m-Ar-H, OCH_3), 7.2 (d, 1H, o-Ar-H, OCH_3), 7.0 (d, 1H, o-Ar-H, OCH_3), 3.8 (d, 6H, Ar-H, OCH_3). ^{13}C NMR δ in ppm (100 ppm DMSO-d_6): 161 (C=O), 150 (p-Ar-C, OCH_3), 149 (m, Ar-C, OCH_3), 148 (CH=N), 136,132, 128, 126 (Ar-C, Ar-Cl), 129, 121, 111, 108, (Ar-C, OCH_3), 55.5, 55.3 (Ar-OCH_3).

(E)-4-chloro-N′-(2,5-dimethoxybenzylidene)benzohydrazide (S2): FT-IR: (υ in cm^{-1}) 3431 (NH), 3035 (Ar-CH), 2364 (Ali-CH), 1638 (C=O), 1598 (C=N), 1490(C-N). ^{1}H NMR δ in ppm (400 MHz, DMSO-d_6): 11.9 (s, 1H, enolic NH proton), 8.7 (s, 1H, CH=N, azomethine group), 7.9 (d, 2H, Ar-OH, Benzene ring), 7.3 (s, 1H, Ar-o-H, OCH3), 7.0 (q, 2H, Ar-H, OCH3), 3.8 (s, 3H, Ar-H, OCH3), 3.7 (s, 3H, Ar-H, OCH_3). ^{13}C NMR δ in ppm (100 ppm DMSO-d_6): 161 (C=O), 153 (Ar-p-C, OCH_3), 143 (CH=N), 136, 132, 129, 128, 122, 117, 113, 109 (Ar-benzene ring), 56.1, 55 (OCH_3).

(E)-4-chloro-N′-(thiophene-2-ylmethylene)benzohydrazide (S3): FT-IR: (υ in cm^{-1}) 3431 (NH), 2831 (Ar-CH), 2722 (Ali-CH), 1620 (C= O), 1594 (C=N), 1483 (C-N). ^{1}H NMR δ in ppm (400 MHz, DMSO-d_6): 11.8 (s, 1H, enolic NH proton), 8.6 (s, 1H, CH=N, azomethine group), 7.9 (d, 2H, Ar-H, benzene ring), 7.6 (d, 1H, thio), 7.5 (d, 2H, Ar-Ph), 7.1 (s, 1H, thio ring). ^{13}C NMR δ in ppm (100 ppm DMSO-d_6): 161 (C=O), 143 (CH=N), 138 (Ar-C-Cl), 136 (Ar-C-Cl), 131 (Ar-C-thio), 129 (Ar-C-Cl), 128 (Ar-C-thio), 127 (Ar-C-thio).

(E)-4-chloro-N′-(4-chlorobenzylidene)benzohydrazide (S4): FT-IR: (υ in cm^{-1}) 3347 (NH), 2829(Ar-CH), 2723 (Ali-CH), 1654 (C=O), 1594 (C=N), 1483(C-N). ^{1}H NMR δ in ppm (400 MHz, DMSO-d_6): 11.9 (s, 1H, enolic NH proton), 8.4 (s, 1H, CH=N, azomethine group), 7.9 (d, 2H, Ar-H, benzene ring), 7.7 (d, 2H, Ar-H-Cl), 7.6 (d, 2H, Ar-H, benzene ring), 7.5 (d,2H, Ar-H, Cl ring). ^{13}C NMR δ in ppm (100 ppm DMSO-d_6): 162 (C=O), 146 (CH=N), 133 (Ar-C-benzene ring), 136,134, 131, 128 (Ar-Cl ring).

Antimicrobial activity

The synthesized substituted benzohydrazide derivatives were evaluated for their *in vitro* antibacterial activity against gram-positive *S. aureus*, and gram-negative *E. Coli* and antifungal activity against *A. niger* by agar well diffusion method. The results of antimicrobial activity are presented in **Fig 4** and **Table 2.**

In case of *E Coli,* (E)-4-chloro-N′-(thiophen-2-ylmethylene)benzohydrazide (**S3**) was found to be more active than the other synthesized derivatives with $pMIC_{ec}$ value of **15** which is comparable to the reference drug Gentamycin (pMCec=13). Among the tested bacterial strain, gram-negative bacteria *E. Coli* showed relatively high sensitivity towards the tested compound. On the other hand, investigation of antifungal activity revealed that the substituted benzohydrazide derivatives having sulfur group (**S3**) was able to produce good inhibitory activity against *A. niger* having a pMICan value 14. (E)-4-chloro-N′-(thiophen-2-ylmethylene)benzohydrazide (**S3**) have shown marked antifungal potential having pMICan value greater than **14** as comparable to the reference drug Gentamycin (pMICan=13). It can be seen from **Table 2** that synthesized substituted benzohydrazide (**S3**) have higher antifungal potential as well as antibacterial activity. Further, the results of antimicrobial activity revealed that compounds S1, S2 and S4 displayed minimum inhibitory effect on growth of tested bacterial and fungal strains of all the synthesized derivatives.

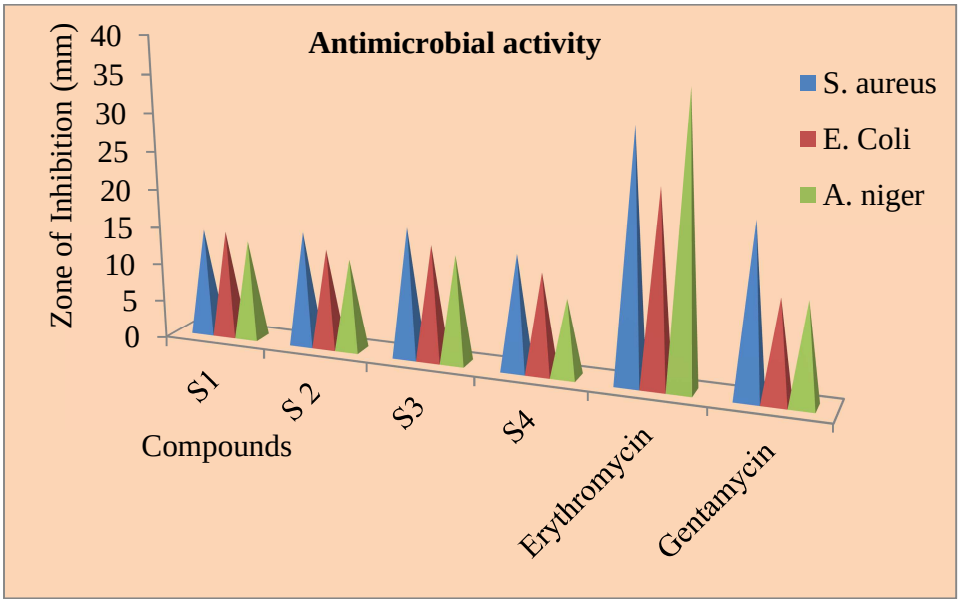

Figure 4. Zone of Inhibition of compound (S1-S4)

Table 2: Antimicrobial activity of substituted benzohydrazide derivatives

S. No	Organism	*S. aureus*	*E. Coli*	*A. niger*
1	S1	14	14	13
2	S 2	15	13	12
3	S3	17	15	14
4	S4	15	13	10
7	Erythromycin	32	25	37
8	Gentamycin	22	13	13

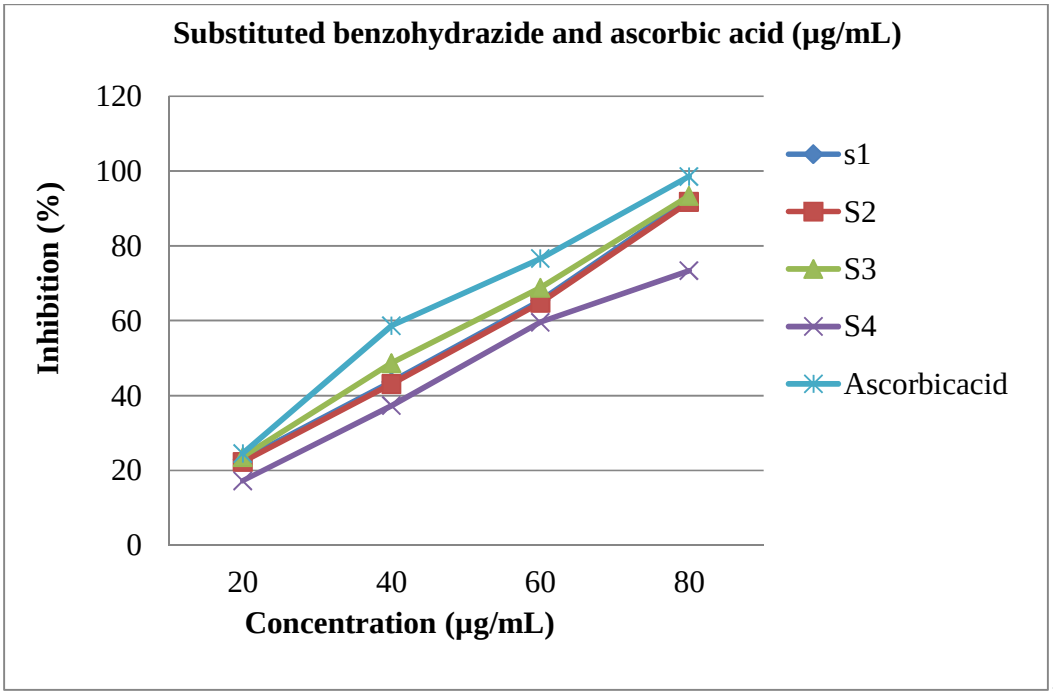

Figure 5. Antioxidant activity of substituted benzohydrazide derivatives (S1-S4)

Table 3: Radical scavenging activity of different concentrations of substituted benzohydrazide derivatives and DPPH (S1-S4)

DPPH	S1	S2	S3	S4	Ascorbic acid
20 (µL/mL)	22.9±0.51	22.3±0.24	23.6±0.37	17.2±0.28	24.53± 2.18
40 (µL/mL)	43.7± 2.87	43.1±3.31	48.7±2.16	37.3±2.91	58.7±4.26
60 (µL/mL)	65.4±4.83	64.8±4.56	68.8±4.51	59.6±4.30	76.64±6.53
80 (µL/mL)	92.5±6.51	91.8±6.16	93.4±6.34	73.4±6.52	98.6±6.71
IC_{50} value	45.2±4.5	42.1±3.4	64.7±3.4	34.1±3.6	35.8±6.71

Antioxidant activity

In the present study, all the synthesized compounds (**S1-S4**) were subjected to screening for their possible antioxidant activity using *in vitro* 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. The radical scavenging ability of synthesized compound is shown in **Fig 5** and **Table 3.** At different concentrations of synthesized compounds and DPPH, the antioxidant activity was measured by comparing with ascorbic acid as standard. It was observed from the results that the maximum antioxidant activity was exhibited by compound (**S3**) at concentration of 0.1 mg. The highest radical scavenging activity of 64.7±3.4% was due to the presence of sulfur group.

Molecular docking Analysis

The Molecular docking analysis of substituted benzohydrazide derivatives (**S1-S4**) with a long chain trans-2-enoyl-ACP carried out for protein reductase (InhA) [33-35]. Inhibition of InhA disrupts the biosynthesis of the mycolic acids that were central constituents of the mycobacterial cell wall. The results were shown in **Fig 6 (a-d**) and **Table 4**. Molecular docking analysis of compound **S1** and **S2** were best ligand which showed high score of Cdocker energy, vander waals interaction and interaction energy. These compounds showed hydrogen bond pi interaction with **LYS165**, good vander waals interaction ranging from **TYR A:158** and electrostatic interaction with **NAD A:300** is due to compounds **S1** and **S2** having electron releasing methoxy group which is directly attached to phenyl ring. Therefore these compounds **S1**and **S2** may be suitable to overcome the drug resistance of *Mycobacterium tuberculosis* InhA protein.

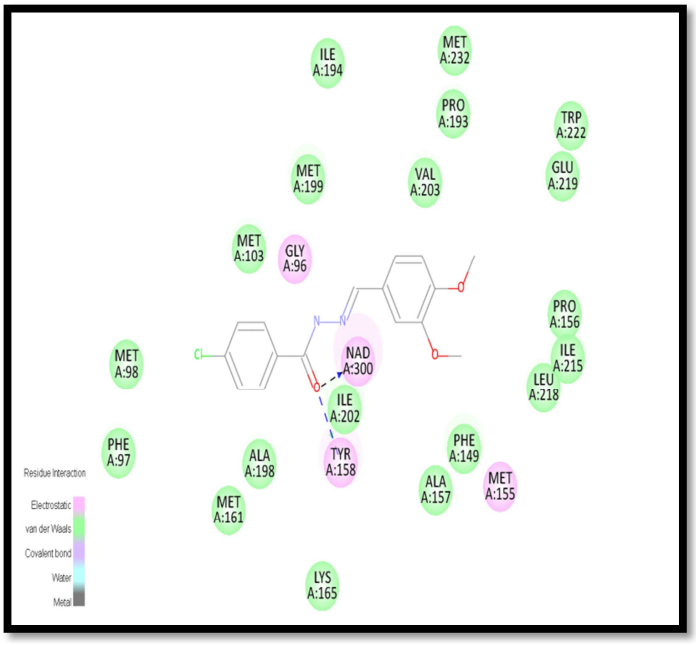

Figure 6(a). Docking interaction of compound S1 with (Mtb) InhA Protein

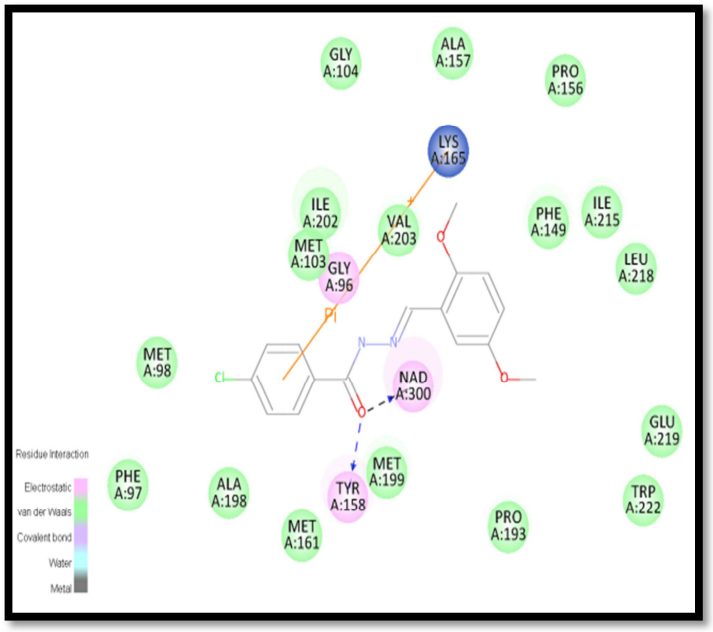

Figure 6(b). Docking interaction of compound S2 with (Mtb) InhA protein

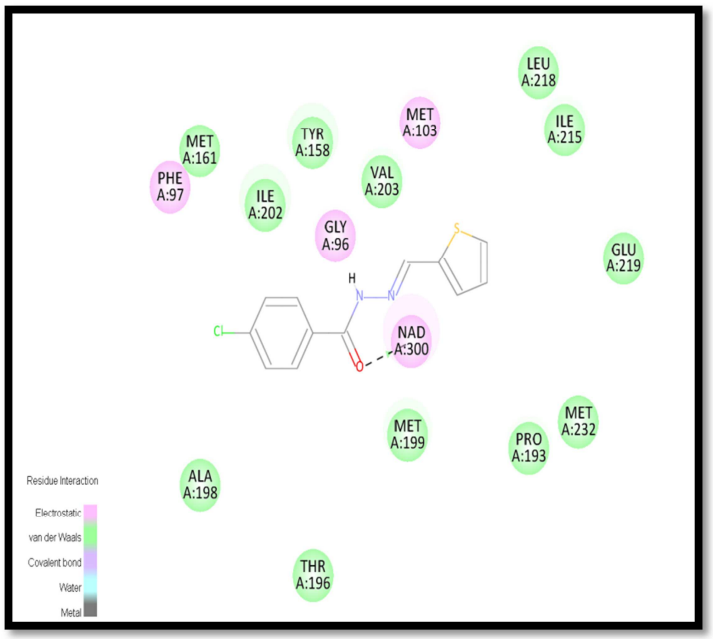

Figure 6(c). Docking interaction of compound S3 with (Mtb) InhA protein

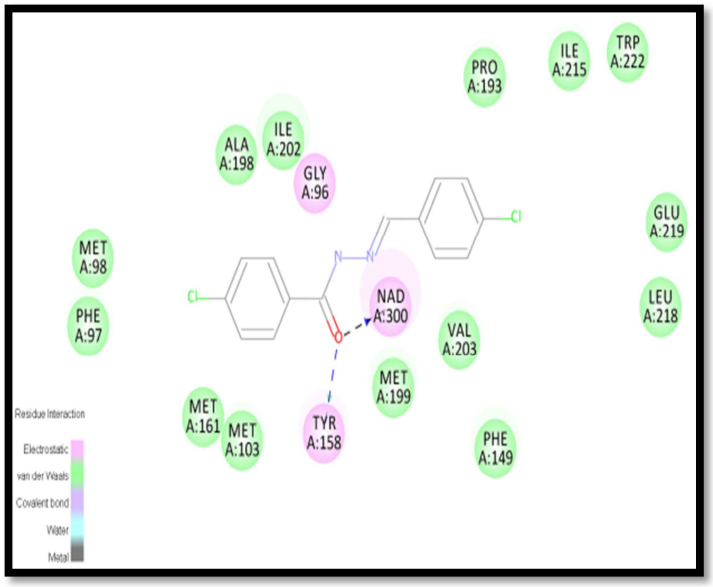

Figure 6(d). Docking interaction of compound S4 with (Mtb) InhA protein

Table 4: Molecular docking analysis of substituted benzohydrazide derivatives (S1-S4)

Compound Code	Force field	Electrostatic Energy	Initial RMS gradient	Initial potential energy	CHARmEnergy	van der Waals Energy	Docking energy	Docking interaction energy
S1	CHARMm	4.55746	38.676	85.7222	22.4755	2.20093	30.0622	51.3753
S2	CHARMm	23.4142	38.7473	84.2547	38.4945	4.25337	29.8528	50.1553
S3	CHARMm	20.0957	45.4544	65.1707	26.9095	0.34518	26.6283	35.199
S4	CHARMm	15.4312	42.7066	59.1679	24.3142	2.96761	30.3605	44.2796

CONCLUSION

In the present work, the synthesized compounds (**S1-S4**) along with spectral data as well as antimicrobial activity using simple and convenient method have been reported. The antimicrobial activity of the synthesized compounds were effectively screened against gram positive *S. aureus*, gram negative *E. Coli* bacterial and *A. Niger* fungi strains. Compound **S3** showed good antimicrobial activity. Further the supporting medicinal chemistry effects for the development, antioxidant activity of the synthesized compounds **S1-S4** using DPPH radical scavenging method using ethanol as solvent was carried out. The results obtained in the present study clearly demonstrate that the compound **S3** exhibited good antioxidant activity than compared to other compounds. To expand the knowledge of anti-tuberculosis activities of substituted benzohydrazide derivatives against *Mycobacterium tuberculosis* InhA protein, molecular docking studies were performed. It revealed that compound **S1** and **S2** which showed high score of -Cdocker interaction energy and it may be suitable to overcome the drug resistance of *Mycobacterium tuberculosis* InhA protein.

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