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Synthesis, Characterization, Biological evaluation, Antioxidant and Molecular docking analysis of Aroylhydrazide Derivatives

S. Veeramanikandan* and H. Benita Sherine

PG and Research Dept. of Chemistry, Periyar E. V. R. College (Autonomous), Trichy-620 023

ABSTRACT

Using 4-chlorobenzohydrazide as a source of amine and substituted aromatic aldehydes as a carbonyl source, concentrated hydrochloric acid as catalyst at room temperature 4-chlorobenzohydrazide derivatives was synthesized. The compounds (S5-S6) were characterized by FT-IR, ^{1}H NMR and ^{13}C NMR. The compounds were screened for antimicrobial activity against S. aureus, E. coli and A. niger. The antioxidant activities of synthesized Schiff bases were investigated using the DPPH radical scavenging method. The designed compounds were further subjected for molecular docking study by in silico method to analyse anti-tuberculosis against InhA, the enoyl acyl carrier protein reductase (ENR) from Mycobacterium tuberculosis by Discover studio 2.1version software.

Keywords: 4-chlorobenzohydrazide, Antimicrobial activity, *Mycobacterium tuberculosis*.

INTRODUCTION

Schiff bases are considered as very important scaffolds of organic compounds having wide application in many biological aspects such as protein, enzyme aldolization and decarboxylation reaction [1-3]. The aroylhydrazone group in the organic molecule brings out several physical and chemical properties. The hydrazones bearing the –CH=N- group leads the molecule towards nucleophilic and electrophilic in nature. In the hydrazine moiety, the nitrogen atom behaves as nucleophilic and carbon atom behaves as nucleophilic as well as electrophilic in nature [4-6]. A long term interest in synthesis and characterization of benzohydrazide derivatives are due to its wide range of pharmacological applications [7]. A number of benzohydrazones were reported to possess antibacterial, antifungal, anti-HIV, anti-tumour, anti-inflammatory and herbicidal activities [8-12]. 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 [13]. In the present study, 4-chlorobenzohydrazide with different aldehydes are key building blocks for the synthesis of Schiff base. Further the synthesized compounds were evaluated for antimicrobial activity, antioxidant activity and were also docked to study the important binding orientations.

EXPERIMENTAL SECTION

2.1 Materials

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).

2.2 Synthesis of (E)-N′-((1H-indol-3-yl)methylene)-4-chlorobenzohydrazide(**S5**).
10 mL aqueous solution of 4-chlorobenzohydrazide (1a, 0.085g, 0.001 mol) is added to 5 mL ethanolic solution of Indole-3-carbaldehyde (1b, 0.0725g, 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 (50-60%) and dried over in a vacuum. The dried solid was recrystallised from ethanol. The same procedure is followed for the synthesis of **S6**.

2.3 Antimicrobial activity
The synthesized 4-chlorobenzohydrazide derivatives(**S5-S6**) were biologically evaluated for antibacterial and antifungal activities using 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) [14]. 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 DMSO was dissolved in the tested compounds. The synthesized compounds were tested for their *in vitro* growth inhibitory activity against *S. aureus* as gram positive, *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.

2.4 Screening for Antioxidant assays
The radical scavenging activities were determined using the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The radical scavenging effects of the synthesized compounds (**S5-S6**) were measured according to the method of Shimada et al., [15] 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:

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

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

2. 5 Molecular docking Analysis
Interaction studies were performed for the synthesized compounds (**S5-S6**) 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 [16]. The 2D structures of 4-chlorobenzohydrazide 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

4-chlorobenzohydrazide derivatives were synthesized through condensation reaction by Schiff base route. The yield was 96% and 92 % for **S5** and **S6** in the model reaction in which Con. HCl served as catalyst and water as solvent. The products S5 and S6 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 analysis.

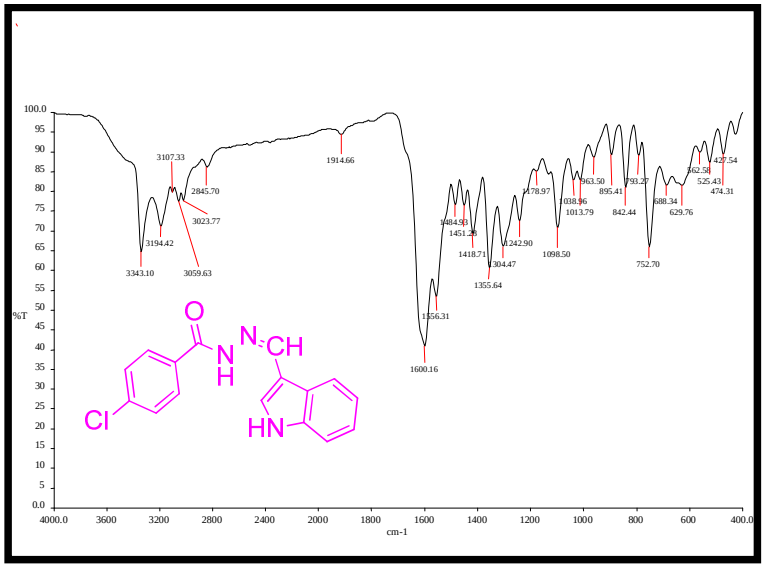


Fig. 1(a) FT-IR spectrum of compound (S5)

Fig. 1(b) FT-IR spectrum of compound (S6)

3.1 FT-IR spectral studies of compounds (S5-S6)

In order to confirm the functional groups present in the synthesized compounds (**S5-S6**) FT-IR spectra were recorded and shown in **Fig 1(a-b).** The bands observed in the range of 3343-3431 cm^{-1} are due to N-H stretching frequency of azomethine analogues, while the absorption band in the region 2958-3023 cm^{-1} and 2830-2845 cm^{-1} are ascribed to aromatic and aliphatic C-H stretching frequencies [17]. The band observed in the range of 1600-1610

cm^{-1} are due to C=O stretching frequency of carbonyl group. The presence of C=N stretching frequency around 1361-1484 cm^{-1} confirm the 4-chlorobenzohydrazide formation.

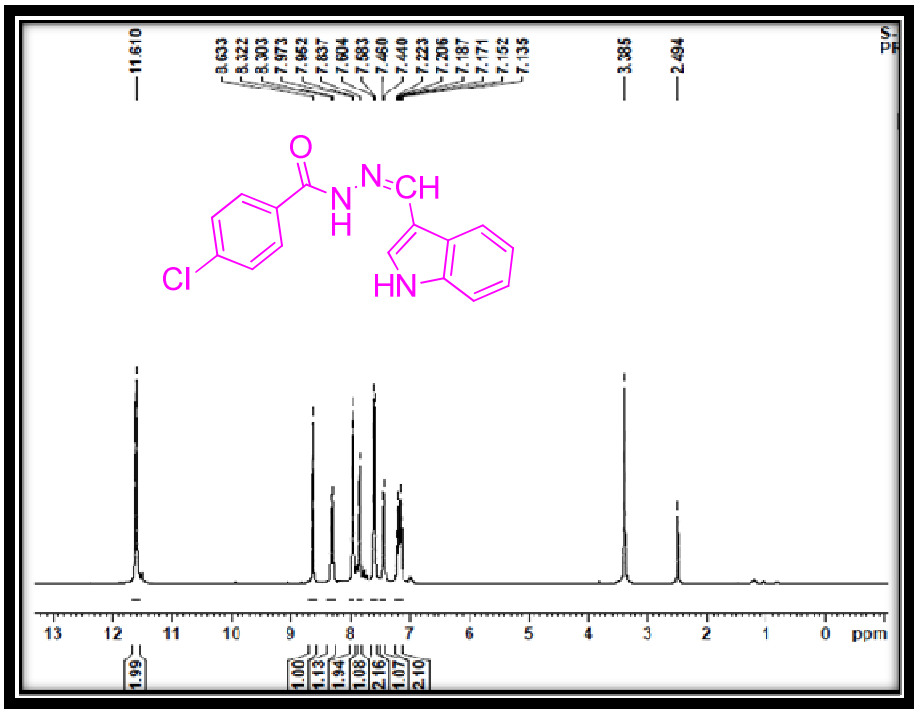


Fig. 2(a) ^{1}H NMR spectrum of compound (S5)

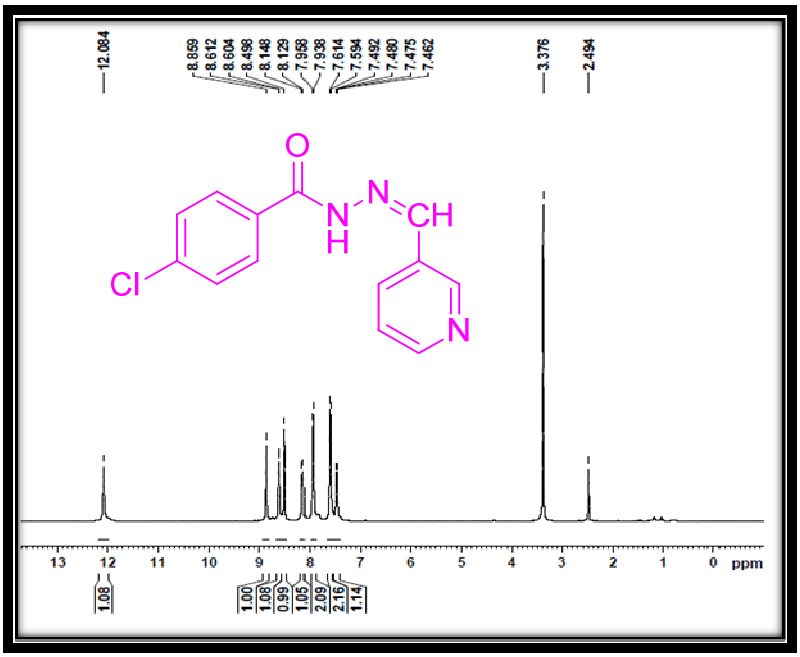


Fig. 2(b) ^{1}H NMR spectrum of compound (S6)

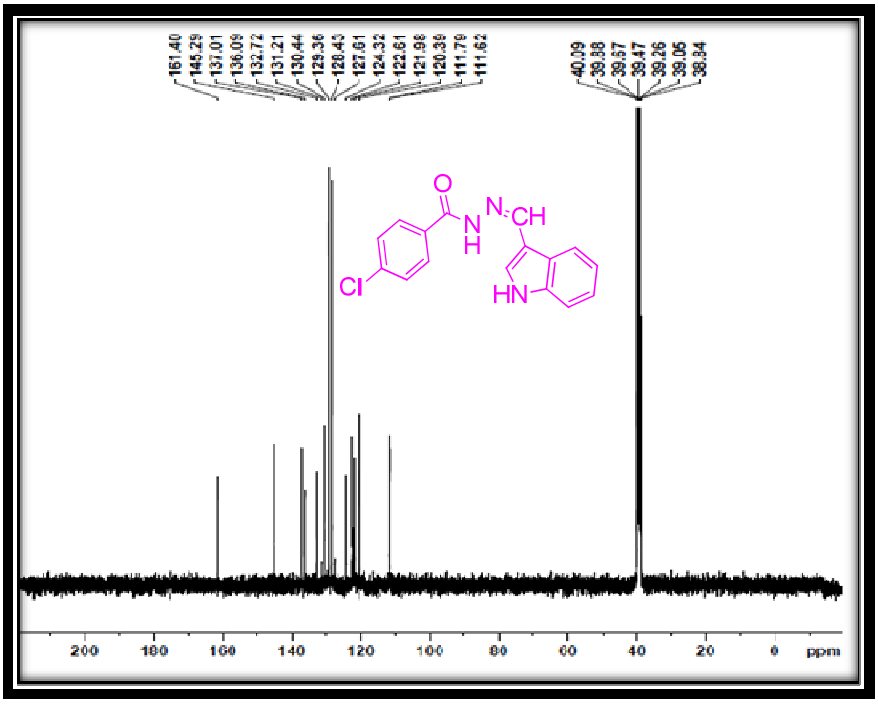


Fig. 3(a) ^{13}C NMR spectrum of compound (S5)

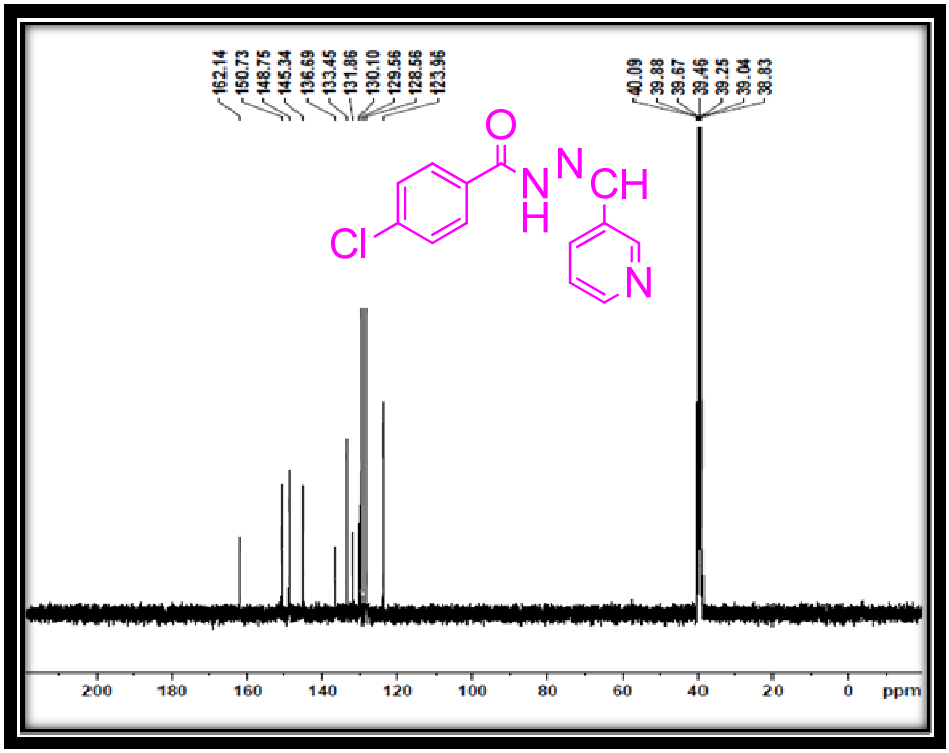


Fig. 3(b) ^{13}C NMR spectrum of compound (S6)

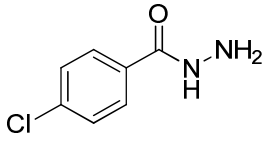
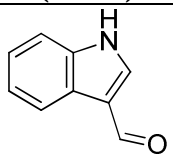
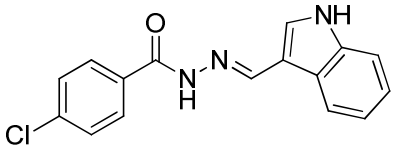
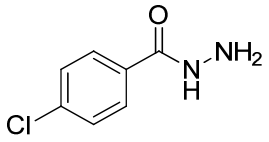
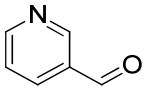
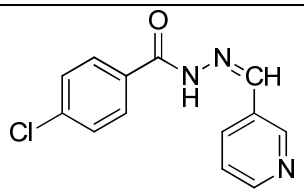
3.2 ^{1}H NMR spectra

In the present work, ^{1}H NMR Spectra were recorded for 4-chlorobenozhydrazide derivatives by Bruker-400 MHz Spectrometer. The ^{1}H NMR spectral data are shown in **Fig 2(a-b**). A singlet appears at δ 11.6-12.8 ppm is assigned to proton of NH adjacent to CO which is exhibited as enolic form and another broad singlet appears at δ 8.4-8.85 ppm is assigned to azomethine (CH=N) proton [18]. A multiplet shown in the range of δ 7.1-7.9 ppm is due to aromatic C-H protons.

3. 3 ^{13}C NMR Spectra

The structures of novel Schiff base compounds **S5-S6** was further supported by ^{13}C NMR (100 MHz) Spectrum and spectral data are shown in **Fig 3(a-b).** The carbonyl carbon signal appears at about δ 161-162 ppm respectively. The signals that appeared at values δ 148-150 ppm are due to the pyridine ring carbon. The signals that appeared at δ 145.29-145.34 ppm were assigned to the carbon of azomethine group [19]. A multiple signal for indole and pyridine ring in S5 and S6 appeared at δ 111-137 ppm.

Table 1 Products and their corresponding reactants

S. No	4-Chlorobenzohydrazide (1a&2a)	Aldehydes (1b&2b)	Products (S5-S6)	Yield (%)
S5	 **1a**	 **1b**	 **S5**	96
S6	 **1a**	 **2b**	 **S6**	92

(E)-N′-((1H-indol-3-yl)methylene)-4-chlorobenzohydrazide**(S5):** was derived from 4-chlorobenzohydrazide and Indole-3-carbaldehyde (1:1) Yield- 96%, m.p. 215-220°C; FT-IR (υ in cm^{-1}) : 3343 [NH], 3194 [NH], 3023 [Ar-CH], 2845 [Ali-CH], 1600 [C=O], 1556 [C=N], 1484 [C-N]; ^{1}H NMR δ in ppm (400 MHz, DMSO-d_6): 11.6 [s, 2H, enolic NH proton], 8.6 [s, 1H, CH=N, azomethine group], 8.3 [d, 1H, Ar-H, indole ring], 7.9 [d, 2H, Ar-o-H, ph], 7.8 [s, 1H, Ar-m-H, ph], 7.6 [d, 2H, Ar-H, indol], 7.4 [d, 1H, Ar-H, indol], 7.2-7.1 [m, 2H,indole ring]; ^{13}C NMR δ in ppm (100 ppm DMSO-d_6): 161 [C=O], 145.29 [CH=N], 137 [Ar-C-Cl], 136, 132, 124, 122, 121, 120, 111 [Ar-C, indol ring], 131, 130, 129, 128, 127 [Ar-C, chlorine ring].

(E)-4-chloro-N′-(pyridine-3-ylmethylene)benzohydrazide**(S6):** was derived from 4-chlororbenzohydrazide and pyridine-3-carbaldehyde (1:1) Yield-92%, m.p. 230-235°C; FT-IR (υ in cm^{-1}) : 3431 [NH], 2958 [Ar-CH], 2830 [Ali-CH], 1610 [C=O], 1592 [C=N], 1361 [C-N]; ^{1}H NMR δ in ppm (400 MHz, DMSO-d_6): 12.08 [s, 1H, enolic NH proton], 8.85 [s,1H, o-pyridine ring], 8.6 [d, 1H, p-py ring], 8.4 [s, 1H, CH=N, azomethine group], 8.1 [d, 1H, o-Ar-H, py ring], 7.9 [d, 2H, o-Ar-H, benzene ring], 7.6 [d, 2H, m-Ar-H, benzene ring], 7.4 [m, 1H, m-Ar-H, py ring]; ^{13}C NMR δ in ppm (100 ppm DMSO-d_6): 162 [C=O], 150 [p-CH-py ring], 148 [Ar-o-CH, py ring], 145.34 [CH=N], 136, 133, 131, 130, 129, 128, 123 [Ar-benz ring & py ring].

3.4 Antimicrobial activity

The synthesized compounds (**S5-S6**) showed antimicrobial activity. One positive (Staphylococcus aureus) bacteria, gram negative (Escherichia coli) bacteria and (Aspergillus niger) fungi were selected for evaluation of antimicrobial activity of compounds under the study using Erythromycin and Gentamycin as standard drug. The compound (**S5**) showed good antimicrobial activity with amine group present in indole ring and found to be most effective against gram positive Staphylococcus aureus bacteria, gram negative Escherichia coli bacteria and Aspergillus niger fungi with high zone of inhibition. So the compound (**S5**) is most effective showing antimicrobial activity against Erythromycin and Gentamycin. The result is shown in **Fig. 4** and **Table 2.**

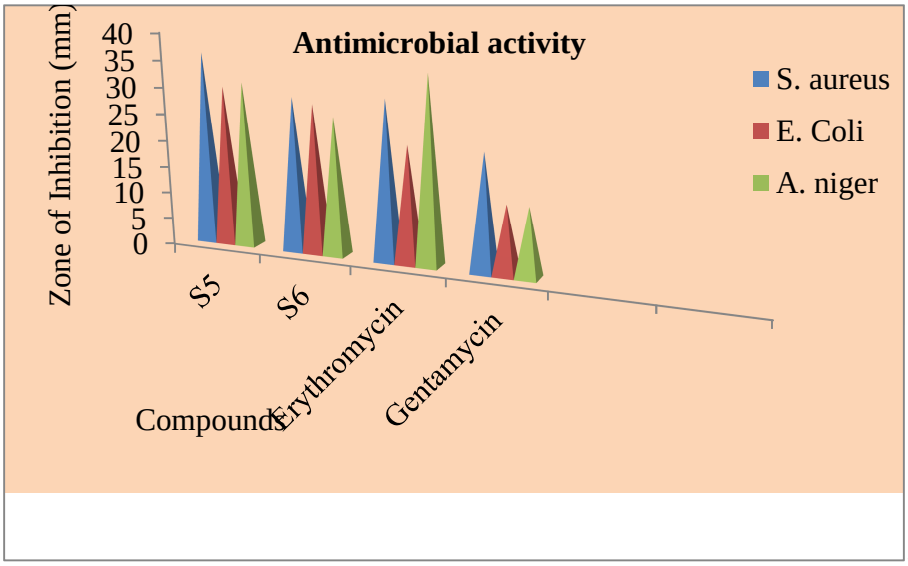


Fig. 4 Antimicrobial activity of 4-chlorobenzohydrazide derivatives(S5-S6)

Table 2 Antimicrobial activity of compounds (S5 & S6)

S. No	Organism	*S. aureus*	*E. Coli*	*A. niger*
1	S5	36	30	31
2.	S6	29	28	26
3.	Erythromycin	30	22	35
4.	Gentamycin	22	13	13

3.5 Antioxidant activity

The free radical-scavenging activity of the synthesized compounds (**S5-S6**) along with those of the standard ascorbic acid, was determined using a 1,1-Diphenyl-2-picrylhydrazyl assays [20]. DPPH radical scavenging activity evaluation is a rapid and convenient assay for screening the antioxidant activity of compound (S5-S6) can be observed from **Fig 5** and **Table 3**. The product (**S5**) showed excellent DPPH radical scavenging activity compared to synthetic commercial antioxidant ascorbic acid with IC_{50} values ranging from 45.76± 3.53µg/mL. The corresponding value for the standard antioxidant ascorbic acid, by contrast, was 36.37± 2.18 µg/mL. We also observed that the presence of an electron releasing amino group on the indole ring increased the scavenging ability.

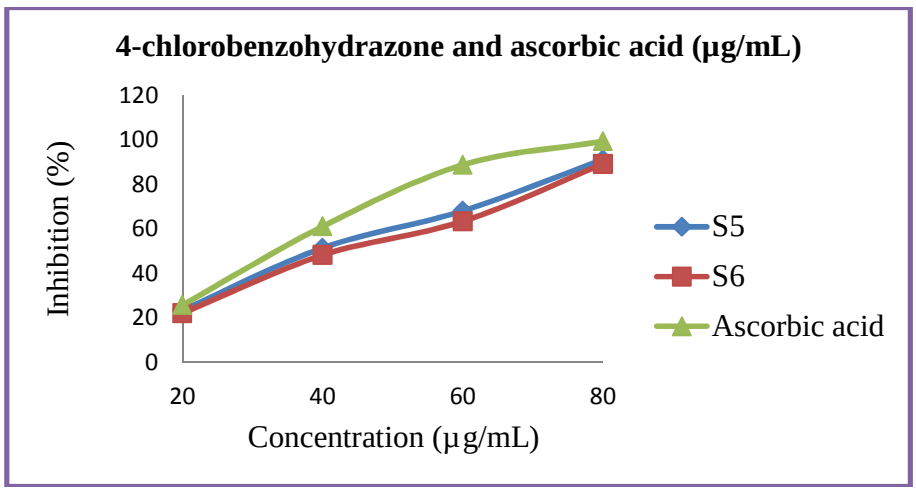


Fig. 5 Antioxidant activity of 4-chlorobenzohydrazide derivatives (S5-S6)

Table 3 Antioxidant activity of compounds (S5-S6)

DPPH	(S5)	(S6)	Ascorbic acid
20 (µl/ml)	23.4 ±0.43	22.1±1.03	25.65±1.78
40 (µl/ml)	51.4 ±2.37	48.2 ±2.51	61.12±4.23
60 (µl/ml)	68.1±4.41	63.4±3.43	88.75±6.19
80 (µl/ml)	91.2±5.08	89.2±1.01	99.38±6.89
IC_{50} µg/mL	45.76± 3.53	41.52± 2.03	36.37± 2.18

3.6 Molecular docking Analysis

The Molecular docking analysis of 4-chlorobenzohydrazide derivatives (**S5 &S6**) with a long chain trans-2-enoyl-ACP carried out for protein reductase (InhA) [21]. 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-c**) and **Table 4**. It was found that, in both the compounds, the carbonyl group of 4-chlorobenzohydrazide derivatives makes the electrostatic interaction with InhA protein. Compound **S5** shows two numbers of electrostatic interactions with less docking interaction energy of 46.2831. Therefore the compound **S5** may be suitable to overcome the drug resistance of *Mycobacterium tuberculosis* InhA protein.

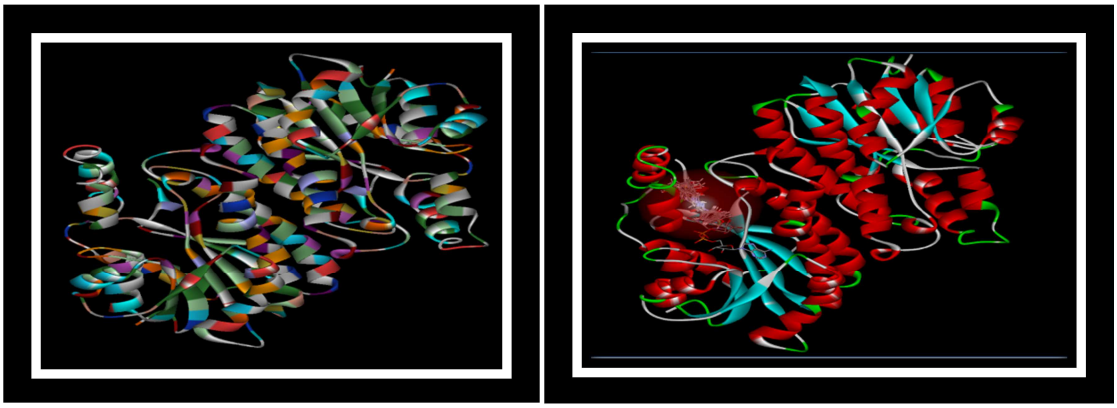


Fig. 6(a) Original image of 2NSD in running software and secondary structure of a protein with binding site in a chain

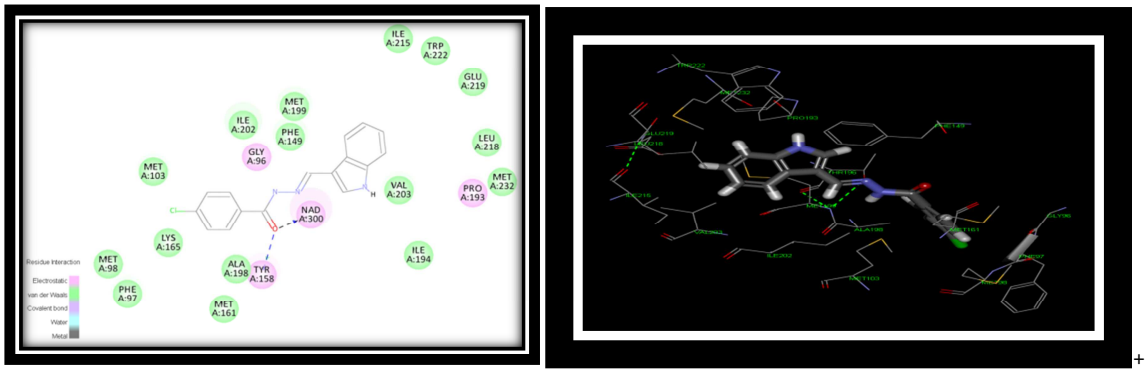


Fig. 6(b) Molecular docking analysis of *Mycobacterium tuberculosis* InhA protein with Compound (S5)

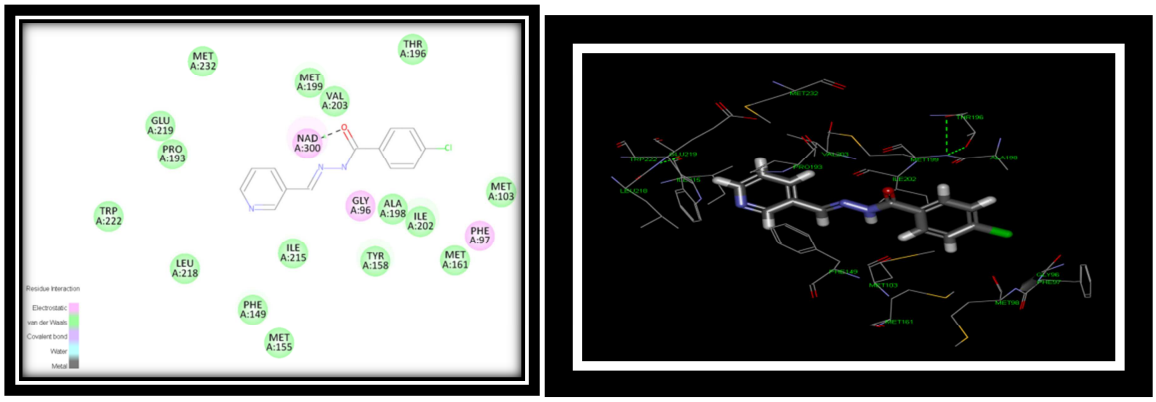


Fig. 6(c) Molecular docking analysis of *Mycobacterium tuberculosis* InhA protein with compound (S6)

Table 4 Molecular docking analysis of 4-chlorobenzohydrazide derivatives (S5-S6)

Com pound Code	RMS Gradient	Electrostatic Energy	Initial RMS gradient	Initial potential energy	CHARm Energy	van der Waals Energy	-CDocking energy	-CDocking interaction energy
S5	0.0958	20.2799	954,803	67.1244	29.6388	3.47329	30.7664	46.2831
S6	0.04457	24.0512	41.3638	335,487	33.2819	3.61255	27.6184	38.6609

CONCLUSION

In the present study, We have synthesized (E)-N′-((1H-indol-3-yl)methylene)-4-chlorobenzohydrazide(**S5**) using 4-chlorobenzohydrazide (**1a**) with Indole-3-carbaldehyde (**1b**) and **1a** with **2b** characterized by FT-IR, ^{1}H NMR and ^{13}C NMR . The reaction occurred very fast, under mild condition using reasonable reagent and solvents and the yield is also higher for **S5**. Compound **S5** was effectively screened against gram positive *S. aureus*, gram negative *E. Coli* and *A. niger* bacterial and fungal strain. The antioxidant ability of the synthesized compound **S5** shows good activity which increases with increasing number of amine group. To expand the knowledge of anti-tuberculosis activities of 4-chlorobenzohydrazide derivatives against *Mycobacterium tuberculosis* InhA protein, molecular docking analysis were performed. It revealed that compound **S5** 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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REFERENCES

[1] Hina zafar, Anis ahmad, U. Asad khan, Tahir Ali Khan, *J. Mol. Stru*. 1097 (**2015**) 129-135.
[2] X. Li, Y. Bao, C. Bi, Y. Fan, *J. Pharm. Med. Res*. 1(1) (**2015**) 17-20.
[3] R. N. Singh, A. Kumar, P. Rawat, A. Srivastsva, *J. Mol. Stru*. 1049 (**2013**) 182- 184.
[4] A. Richard Friesner, B. Robert Murphy, P. Matthew Repasky, L. Leah Frye, R. Jeremy Greenwood, A. Thomas Halgren et al. *J. Med. Chem*.1 (10) (**2004**) 974-80.
[5] Natchimuthu Santhi, Sekar Aiswarya, *Bioinfor*. 7 (1) (**2011**) 38- 43.
[6] Liang Wang, Da-Gang Guo, Yan-Yan Wang, Chang-Zheng, *Rsc. Adv*. 4 (**2014**) 58895-58901.
[7] H. Bayrak, A. Demirbas, N. Demirbas, S. A. Karaoglu, *Eur. Med. Chem*. 44 (**2009**) 4362–4366.
[8] S. D. Joshi, H. M. Vagdevi, V. P. Vaidya, G. S. Gadaginamath, *Eur. J. Med. Chem* 43 (**2008**) 1989-1996.
[9] Rakesh Narang, Balasubramanian Narasimhan, sunil Sharma, Dharmarajan Sriram, Perumal Yogeeswari , Erik De Clercq, Christophe Pannecouque, Jan Balzarini. *Med Chem Res*. 21 (**2012**)1557-1576.
[10] J. Easmon, G. Purstinger, K. S. Thies, G. Heinisch, J. Hoffmnn, *J. Med. Chem*. 49 (**2006**) 6343-6350.
[11] V. Shashikant, Bhandari, G. Kailash Bothara, K. Mayuresh Raut, A. Ajit Patil, P. Aniket Sarkate, J. Vinod Mokale, *Bioorg. Med. Chem*. 4 (**2008**) 1822-1831.

[12] S. D. Joshi, H. M. Vagdevi, V. P. Vaidya, G. S. S. Gadaginamath, *Eur. J. Med. Chem*. 43 (9) (**2008**) 1989-1996.
[13] T. Aboul-Fadl, H. A. Abdel-Aziz, T. Elsaman, M. K. Abdel-Hamd, J. Thanassi, M. J. Pucci, *Molecules*. 16 (**2011**) 7864-7879.
[14] Rakesh Narang, Balasubramanian Narasimhan, sunil Sharma, Dharmarajan Sriram, Perumal Yogeeswari , Erik De Clercq, Christophe Pannecouque, Jan Balzarini. *Med. Chem. Res*. 21 (**2012**) 1557-1576.
[15] K. Shimada, K. Fujikawa, K. Yahara, T. Nakamura, *J. Agric. Food. Chem.* 40 (**1992**) 945-948.
[16] S. D. Joshi, H. M. Vagdevi, V. P. Vaidya, G. S. S. Gadaginamath, *Eur. J. Med. Chem*. 43 (9) (**2008**) 1989-1996.
[17] V. Balamurugan, S. Shankar, S. Chandramohan, *Inter. J. Bio. Chem*. 8 (**2014**) 85-90.
[18] M. M. Abd-Elzaher, W. H. Hegazy, A. M. Gaafar, *Appl. Organomet. Chem*. 19 (**2005**) 911-916.
[19] H. Bayrak, A. Demirbas, N. Demirbas, S. A. Karaoglu, *Eur. J. Med. Chem*. 44 (**2009**) 4362-4366.
[20] R. Maheswari, J. Manjula, *Inter. J. Appl. Res*. 1 (10) (**2015**) 587-592.
[21] A. S. Raja, A. K. Agarwal, N. Mahajan, S. N. Pandeya, A. Ananthan, *Ind. J. Chem*. 49B (**2010**) 1384-1388.