Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

One-pot Synthesis, Spectral Studies and Organic Molecules from 4-chlorobenzohydrazide derivatives

Veeramanikandan S. and SherineH. Benita*

PG and Research Dept. of Chemistry, Periyar E.V.R. College (A), Tiruchirappalli-620023
Tamil Nadu, India.Email. Id: veera3114chem@gmail.com (+91-9600302919).

ABSTRACT

The new 4-chlorobenzohydrazide derivatives have been synthesized. The synthesized compounds were confirmed by various analytical and spectral techniques such as melting points, elemental analysis, UV-Visible, FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy. The synthesized analogs were tested for antimicrobial activity at Minimum Inhibition Concentration (MIC) level. Molecular docking study revealed that thecompounds (S1-S4) utilized mycobacterium tuberculosis protein to identify important binding modes responsible for inhibition activity.

KEYWORDS: Synthesis, Spectral technique, Antibacterial activity, Dockingstudies.

*Corresponding author

Sherine H. Benita *

PG and Research Dept. of Chemistry,

Periyar E.V.R. College (A), Tiruchirappalli-620 023

Tamil Nadu, India.

Email. Id: veera3114chem@gmail.com Mob. +91-9600302919).

1. INTRODUCTION

Hydrazone constitute a vital role of reagents in organic synthesis of their easy accessibility, stability and diverse reactivity depending on their structure, the property of the reaction partner and reaction condition[1]. They are widely applied for the construction of different heterocycles skeletons and serve as precursors of functionalized carbonyl compounds and hydrazines. The multifaceted biological reactivity of many natural and synthetic hydrazones such as anticonvulsant, antidepressant, analgesic, antimalarial, antimycobacterial and anticancer activities among others, has made them interesting target compounds for developing new drugs. Hydrazones have also found wide applications in materials science[2]. For all these reasons, continuous research effort has been made for the development of useful synthetic methods of functionalized hydrazone. Recently, Schiff bases containing substituted benzohydrazide derivatives have been synthesized by various methods with modified procedures[3-6]. The compounds containing azomethine group haveinteresting biological and pharmaceutical activities[7-10].

A thorough literature survey reveals that substituted benzohydrazide derivatives from aromatic and heterocyclic compounds possess cytotoxic, anticonvulsant and antiproliferativeproperties[11-15]. In the present study, S1-S4 compounds were synthesized, characterized and screened for antimicrobial and antioxidant activities. Molecular docking was carried out to study the important binding orientations.

2. MATERIALS AND METHODS

All chemical reagents were purchased from Sigma-Aldrich Chemicals co., and used as received. Uncorrected melting points were determined by digital melting point apparatus. Elemental analysis (C, H and N) was carried out by an Element analyzer model Vario EL II instrument. The UV-Visible spectra were recorded on a Perkin Lambda-35 spectrophotometer. The FT-IR spectra were recorded in KBr pellets on a 400-4000 cm^{-1} Shimadzu-2 spectrophotometer. The ^{1}H NMR spectra were recorded on Bruker-400 MHz spectrometer, using deutrated DMSO-d_6 as a solvent with TMS as an internal standard. ^{13}C NMR spectra were recorded on a Bruker DPX-100 MHz.

2.0. General procedure for the synthesis of Schiff base compounds

2.1. Synthesis of (E)-4-chloro-N'-(3,4,5-trimethoxybenzylidene)benzohydrazide(S1)

To the ethanolic solution of 4-chlorobenzohydrazide (0.176 g, 0.001 mol), taken in a round bottom flask, 3,4,5-trimethoxybenzaldehyde (0.145 g,0.001 mol) and few drops of concentrated hydrochloric acid were added as catalyst. The reaction mixture was kept over a magnetic stirrer and stirred well at room temperature. The precipitate was filtered, then washed with petroleum ether (40-

60%) and dried in a desiccator. The obtained product was recrystallized from ethanol. The same procedure has been followed for rest of compounds.

Scheme: 1 Synthesis of 4-chlorobenzohydrazide derivatives

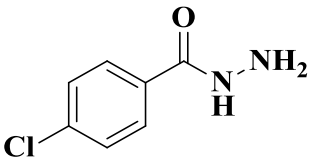
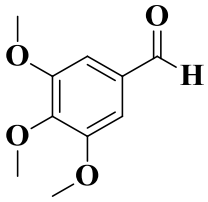
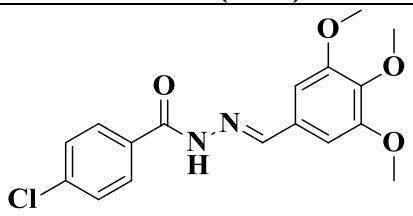
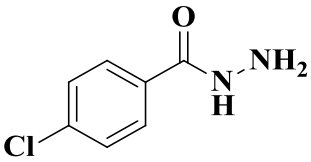
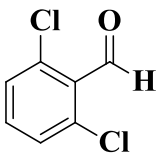
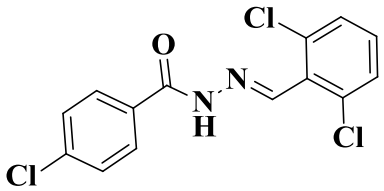
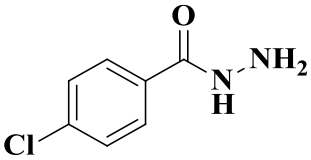
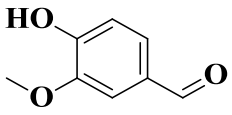
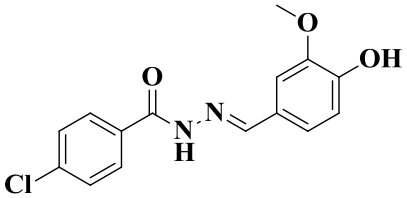
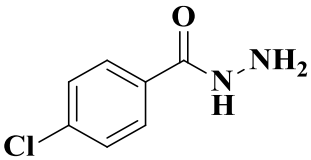
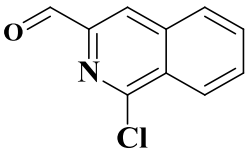
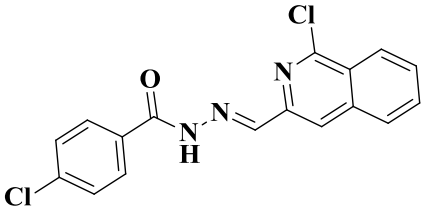
S.NO	4-chlorobenzohydrazide	Aldehydes	Products (S1-S4)
1.			 S1
2.			 S2
3.			 S3
4.			 S4

Table 1 Analytical and physical data of 4-chlorobenzohydrazide derivatives

Compound	Melting point(°C)	Yield(%)	MolecularFormula	Molecular Weight (g/mol)
S1	149	91	$C_{17}H_{17}ClN_2O_4$	348
S2	192	88	$C_{14}H_9Cl_3N_2O$	327
S3	169	81	$C_{15}H_{13}ClN_2O_3$	304
S4	185	78	$C_{17}H_{11}Cl_2N_3O$	344

Table 2 Elemental analysis of 4-chlorobenzohydrazide derivatives

Compound	CHN analysis Found (%)		
	C	H	N
S1	58.54 (58.50)	4.91 (4.93)	8.03 (8.11)
S2	51.33 (51.30)	2.77 (2.70)	8.55 (8.61)
S3	59.12 (59.09)	4.30 (4.33)	9.19 (9.29)
S4	59.32 (59.26)	3.22 (3.24)	12.21 (12.10)

3. RESULTS AND DISCUSSION

(E)-4-chloro-N'-(3,4,5-trimethoxybenzylidene)benzohydrazide (S1): was derived from 4-chlorobenzohydrazide and 3,4,5-dimethoxybenzaldehyde.

FT-IR: (ν in cm^{-1}) 3422(NH), 2923 (Ar-CH), 2856 (Ali-CH), 1656 (C=O), 1539 (C=N), 1255(C-Cl), 845(OCH_3). UV-Vis (λ in cm^{-1}): 267, λ_{max}: 37, 453 (Transition: π → π*), 317, λ_{max}:31,545 (Transition: n→π*). ^{1}H NMR (δ in ppm, 400 MHz, DMSO-d_6): 9.3(s, 1H, enolic NH proton), 8.2 (s, 1H, CH=N, azomethine group), 7.8(d, 2H, m-Ar-CH benzene ring), 7.4 (t, 2H, o-Ar-CH) 7.2 (s, 1H, o-Ar-CH, methoxy benzene ring), 3.8 (s, 9H, OCH_3). ^{13}C NMR δ in ppm (100 MHz DMSO-d_6): 163(C=O), 153 (m-Ar-C, benzene ring), 149 (CH=N), 140 (p-Ar-C, OCH_3 ring), 137 (C-Cl) 131 (Ar-CH), 129, 128, 104 (Ar-C, benzene ring) 56, 40 (OCH_3).

(E)-4-chloro-N'-(2,6-dichlorobenzylidene)benzohydrazide (S2): was derived from 4-chlorobenzohydrazide and 2,6-dichlorobenzaldehyde.

FT-IR: (ν in cm^{-1}) 3251 (NH), 2931 (Ar-CH), 2793 (Ali-CH), 1619 (C=O), 1487 (C=N), 1292 (C-O-C), 1090 (C-N). UV-Vis (λ in cm^{-1}): 264, λ_{max}: 37,878 (Transition: π → π*), 279, λ_{max}:35,842 (Transition: π → π*). ^{1}H NMR (δ in ppm, 400 MHz, DMSO-d_6): 12.2(d, 1H, enolic N-H proton), 8.6 (d, 1H, CH=N, azomethine group), 7.9 (d, 2H, o-Ar-CH benzene ring), 7.8-7.6 (m, 4H, m-Ar-CH, chlorine ring), 7.5-7.4 (m, 1H, Ar-CH, benzene ring). ^{13}C NMR δ in ppm (100 ppm DMSO-d_6): 162 (C=O), 143 (CH=N), 136, 135, 131, 130 129, 128 (Ar-CH, chlorine ring).

(E)-4-chloro-N'-(4-hydroxy-3-methoxybenzylidene)benzohydrazide (S3): was derived from 4-chlorobenzohydrazide and 4-hydroxy-3-methoxybenzaldehyde.

FT-IR: (ν in cm^{-1}) 3226 (N-H), 3074 (Ar-CH), 2845 (Ali-CH), 1644 (C=O), 1416 (C=N), 1221 (C-O-C), 1128 (C-C).UV-Vis (λ in cm^{-1}): 268 nm, λ_{max}: 37, 313 (Transition: π → π*), 330 nm, λ_{max}:30, 303 (Transition:n→π*). ^{1}H NMR (δ in ppm, 400 MHz, DMSO-d_6): 11.7(s, 1H, enolic NH proton), 9.5(s, 1H, Ar-OH) 8.3 (s, 1H, CH=N, azomethine group), 7.9 (d, 2H, o-Ar-CH chlorine ring), 7.5 (d, 2H, m-Ar-CH, Chlorine ring), 7.3 (s, 1H, o-Ar-CH, benzene ring), 7.0 (d, o-Ar-CH benzene ring), 6.8 (d, m-Ar-CH, benzene ring), 3.8 (s, 3H, m-OCH_3). ^{13}C NMR δ in ppm (100 ppm DMSO-d_6): 161.8 (C=O), 149 (Ar-OH) 148.7(CH=N), 136 (Ar-C-Cl), 132 (=CH-C), 129, 125, 122, 115, 108 (Ar-C benzene ring) 55 (OCH_3).

(E)-4-chloro-N'-((1-chloroisoquinolin-3-yl)methylene)benzohydrazide (S4): was derived from 4-chlorobenzohydrazide and 1-chloroisoquinoline-3-carbaldehyde.

FT-IR: (ν in cm^{-1}): 3356 (NH), 3062 (Ar-CH), 2819 (Ali-CH), 1682 (C=O), 1467 (C=N), 1245 (C-O-C), 745 (C-Cl). UV-Vis (λ in cm^{-1}): 232, λ_{max}: 43,103 (Transition: π → π*). ^{1}H NMR (δ in ppm, 400 MHz, DMSO-d_6): 12.3 (s, 1H, enolic N-H proton), 8.9 (d, 2H, Ar-p-CH, quinoline ring), 8.2 (d, 1H, Ar-o-CH, quinoline ring), 8.0 (s, 3H, Ar-o-CH, chlorine ring), 7.9 (t, 1H, Ar-o-CH, benzene ring), 7.8 (d, 1H, Ar-m-CH, Cl ring), 7.7 (d, 1H, Ar-o-CH, quinoline ring), 7.6 (s, 1H,

CH=N, azomethine group). ^{13}C NMR δ in ppm (100 ppm DMSO-d_6): 164 (C=O), 152(CH-Cl, quinoline ring), 147(CH=N, azomethine group), 145 (Ar-CH-Cl, benzene ring), 134 (Ar-o-CH, Cl benzene ring), 118 (Ar-p-CH, Cl benzene ring), 115 (Ar-m-CH, Cl benzene ring), 110 (Ar-m-CH, quinoline ring), 102 (Ar-m-CH, quinoline ring).

4. BIOLOGICAL STUDIES OF 4-CHLOROBENZOHYDRAZONE COMPOUNDS (S1-S4)

4.1. Antimicrobial Activity

The synthesized compounds (S1-S4) were screened for their antimicrobial activity against gram positive bacteria *B. polymyxa*, *B. megaterium*and gram negative bacteria *Vibrio cholera*, *F. antarctium.* The samples for antifungal as well as antibacterial evaluation were tested using standard protocols like Micro dilution/ Broth disc diffusion method. The stock solution (100 µg/mL)for each compound was prepared and screenedfor antibacterial activity and antifungal activity. The tubes along with the control were then kept for incubation at 37°C for 24 h. Suspensions were further inoculated on an appropriate media and growth was noted after 48 hrs. The obtained results (MIC) in µg/mL by observing the highest dilution were recorded and compared with MIC value of standard drugs Chloramphenicol and Fluconazole.

The antibacterial activities of 4-chlorobenzohydrazide derivatives (S1-S4) were tested against different microorganisms by disc diffusion method[16]. The microorganisms used in the present investigations included bacteria: *Bacillus polymyxa (B. polymyxa), Bacillus megaterium (B. megaterium), Vibrio cholera (V. cholera)* and *Flavobacterium antarctium (F. antarctium)*. The antibacterial results are given in Table 3 and the zone of inhibition is shown in Fig 1. The graphical representation of antibacterial activity is shown in Fig 3.

The compounds S1, S2 and S4 have been found to be the zone of inhibitions 25, 28 and 24 mm/mL which has higher antibacterial activity when compared to (21 mm/mL) of standard drug (Chloramphenicol). Among these, compound S2 has excellent antibacterial activity against *B.megaterium* bacteria. Overall,the compounds (S1-S4) were found to exhibit a slightly greater zone of inhibition than the standard which is due to presence of methoxy and chlorine groups in Schiff base derivatives.

Antifungal activities of the synthesized compounds (S1-S4) were tested against two fungal strains such as *C. albicans* and *A. fumigatus.* The test compounds showed varying degree of inhibition on the growth of the fungal strains and are given in Table 4. The zone inhibition inpetriplatesareshown in Fig 2. The graphical representation ofthe antifungal activity is shown in Fig 4.The compound S1 exhibitsgoodantifungal activity (24 mm/mL) against *C. albicans*strain compared

to fluconazole(24 mm/mL) as a standard drug. The zone of inhibition for compound S1 is 24 mm/mL, which is equal to the standard drug. The zone of inhibition for fungal strain *A. fumigatus*is found to be 28 mm/mL.The zone of inhibition for synthesized compounds(S1-S4) possessmoderate activity compared to the standard drug.

Table 3 Antibacterial activity of synthesized compounds

Bacterial strain	Zone of inhibition (mm/mL)				
	Standard	S1	S2	S3	S4
B. polymyxa	24	12	21	21	18
B. megaterium	21	25	28	20	24
V. Cholera	27	23	20	17	15
F. antarctium	28	12	19	15	20

Table 4 Antifungal activity of synthesized compounds

Bacterial strains	Zone of inhibition (mm/mL)				
	Standard	S1	S2	S3	S4
C. albicans	24	24	14	16	18
A. fumigatus	28	11	17	19	20

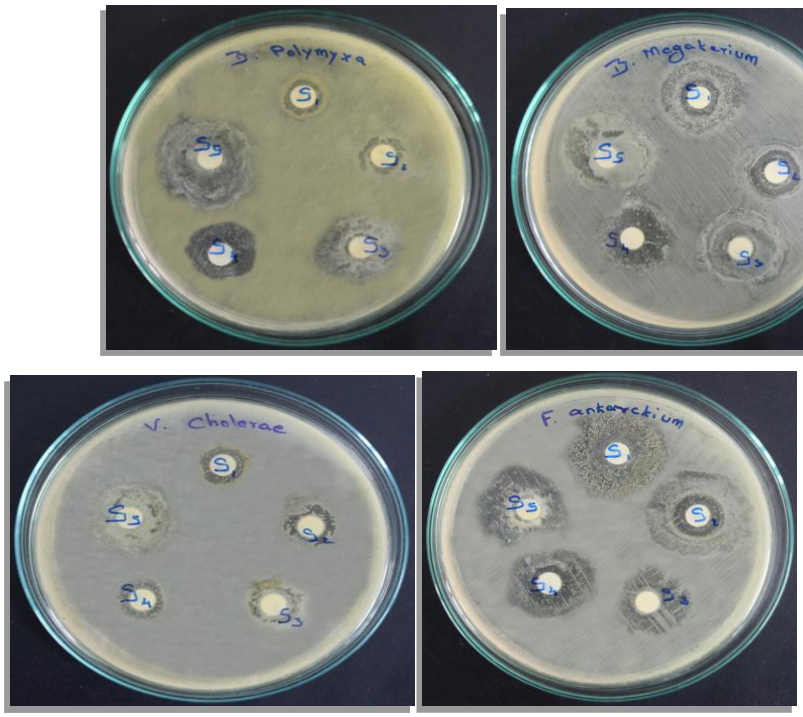


Figure 1 Antibacterial activity of 4-chlorobenzohydrazide derivatives

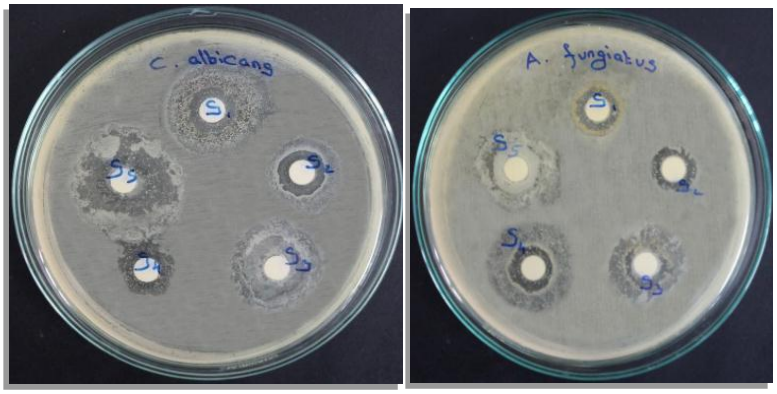


Figure 2 Antifungal activity 4-chlorobenzohydrazide derivatives

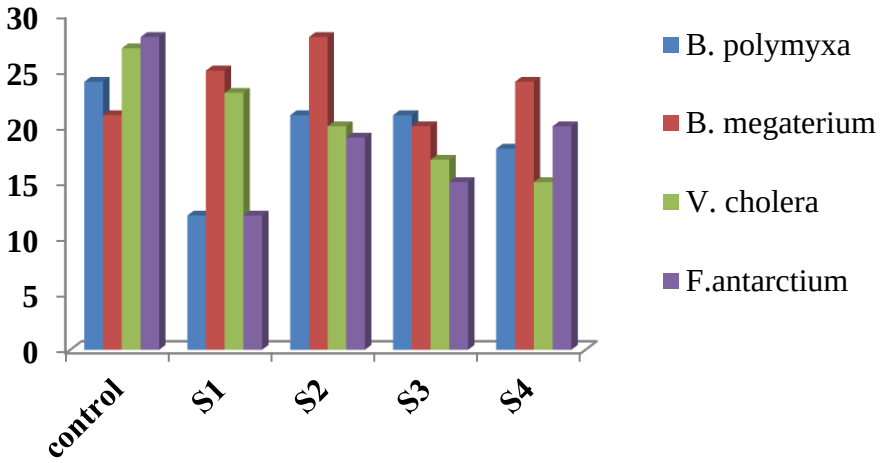


Figure 3 The plots of antibacterial activity using 4-chlorobenzohydrazide derivatives

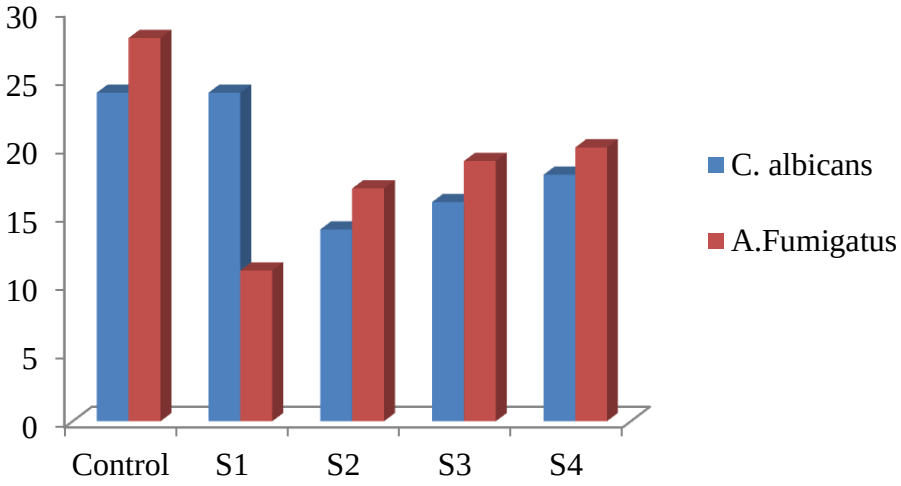


Figure4 The plots of antifungal activity using 4-chlorobenzohydrazide derivatives (S1-S4)

4.2. Antioxidant Activity of 4-chlorobenzohydrazide derivatives

The synthesized compounds (S1-S4) were subjected to screening for antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging technique. The major activities

of all the compounds were carried out based on the scavenging activity ofstable free radical with a characteristic absorption at 517 nm. The results are given in Table 5 and the percentage of antioxidant activity is shown in Fig 5. Different concentrations of compounds were determined by comparing with a standarddrug (ascorbic acid).

Since the compounds (S1-S4) exhibited good antioxidant activity, it is considered worthwhile to investigate their biological activities. The antioxidant activity of 4-chlorobenzohydrazide derivatives have attracted increasing interests and been extensively investigated, mainly *invitro* system[17]. It has been reported that overproduction of free radicals may induce some oxidative damages to Bio-molecules such as carbohydrates, proteins, lipids and DNA, thus accelerating ageing, cancer, cardiovascular diseases, inflammation and so on[18]. Generally, the compounds having lower absorbance value possess higher free radical scavenging activity.

The results showed that the synthesized Schiff bases S1, S2, S3 and S4 displayed IC_{50} values44.5µL/mL, 68.3µL/mL, 82.4µL/mL, 63.8µL/mL and compared to standard (ascorbic acid) value 41.5µL/mL.Among these, Compound S1has exhibits lower absorbance IC_{50} value (44.5µL/mL)and stronger antioxidant activity compared to rest of the compounds. Hence ascorbic acid used as a positive controlshowed the IC_{50} value 41.5 µL/mL. The IC_{50} value of ascorbic acid has lower absorbance than the compound S1 hasnearest IC_{50} value to the standard. Other compounds S2, S3 and S4 are showed moderate antioxidant activity compared to standard value.

Table 5DPPH radical scavenging activity of 4-chlorobenzohydrazide derivatives

Compound	DPPH 20 (µL/mL)	DPPH 40 (µL/mL)	DPPH 60 (µL/mL)	DPPH 80 (µL/mL)	IC_{50} Values
S1	24.1±0.42	45.5±2.42	54.2±5.22	83.2±2.23	44.5±2.81
S2	20.4±3.40	40.1±2.86	64.5±2.76	86.7±1.78	68.3±4.22
S3	24.1±2.44	44.3±0.59	66.2±1.50	88.2±3.54	82.4±5.13
S4	22.7±0.10	42.8±4.63	62.7±4.13	83.5±2.16	63.8±4.63
Ascorbic Acid	26.72±3.42	58.6±3.54	84.1±2.13	99.1±4.10	41.5±4.38

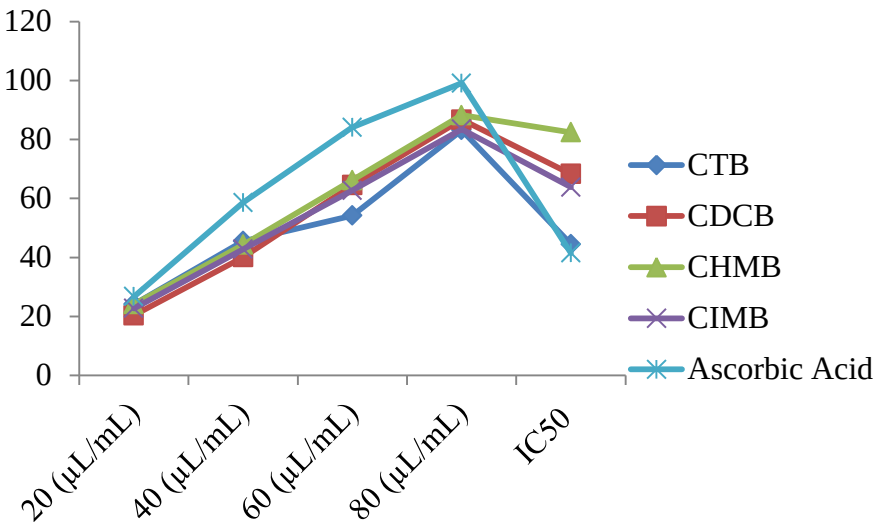


Figure 5 The plots of radical scavenging effects (%) of 4-chlorobenzohydrazide derivatives at different concentration

4.3. Molecular docking studies of compound (S1-S4)

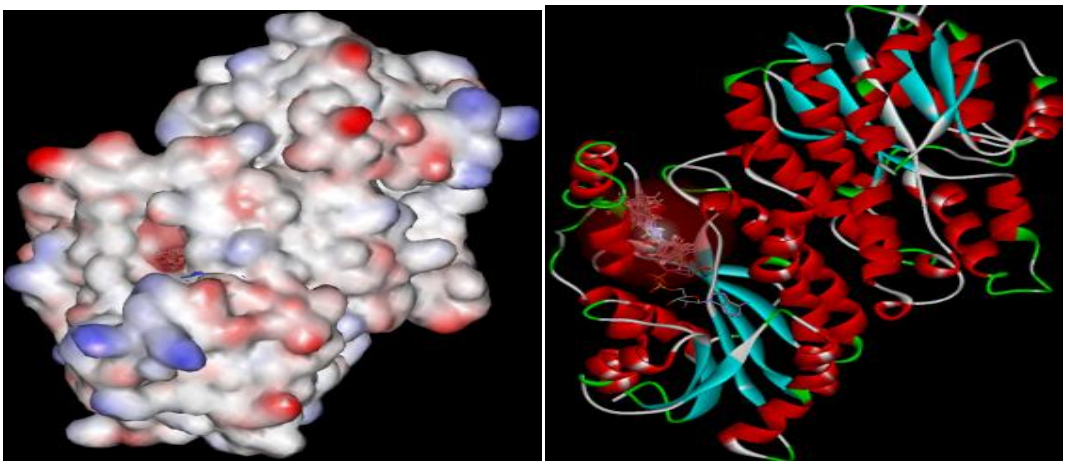
Molecular docking as one of the approaches to computer-based drug design play avital role in the design of potentialligands that are both sterically and chemically compatible withthe binding site of a target bio-macromolecule [19-20]. When performed with defaultsettings,docking reveals a number of possible conformations and orientations for the inhibitors at the binding site. Understanding thebinding site conformations helps to show the important interactions that stabilize the synthesized ligand-receptor. The 3Dstructures of the4-chlorobenzohydrazide derivatives were constructed usingstandard geometric parameters of the molecular docking software package Discovery studio 2.1 version.

The current docking studies clearly present in the binding modes of 4-chlorobenzohydrazide derivatives, active sites are azomethine, carbonyl, phenyl ring and substituent groups such as methoxy, chlorine and hydroxyl groups[21-22]. The molecular docking studies were further performed to verify the basic interactions of ligands (S1-S4)with the target protein inInhA [Enoy-acyl-carrier-protein]reductase. The potential binding modes of compounds S1, S2, S3 and S4 revealed using docking study are shown in Fig6-10 respectively. The active side of InhAwas carried out and the results of the analysis are summarized in Table 6. The compounds S2 and S4 found to be most active were also the best docked ligands having high Libdock score values 122.86 and less bond length 2.3285 indicating highest binding interaction towards the enzyme. The chlorine atom at 2 positions in S2 made one hydrogen bond and interaction residue with hydrazone moiety of LYS: 165. Similarly, compound S4 has interacted with a different set of residues GLY 96 and ALA A22 amino groups. Compound (E)-4-chloro-N-((1-chloroisoquinolin-3-yl)methylene)benzohydrazide (S4)

attached chlorineatomat 1 position of S4 made a double hydrogen bond with chlorine and hydrogen moiety. The two conventional hydrogen bond interaction of ligand with receptor binding interaction GLY A: 96 and ALA A: 22 amino groups. Hence the compounds S1 and S4 we expect may be anti-tuberculosis activity against *M. tuberculosis* in the *in vitro*level.

Table 6 Molecular docking results for 4-chlorobenzohydrazide derivatives

Ligand	H-bond	No of poses	Absolute energy	Libdock Score	H-bond length(Å)	Interacting residues	Interacting atom
S1	1	73	-77.595	121.891	2.4740	LYS165	O19
S2	1	43	-44.454	122.865	2.3285	LYS165	Cl20
S3	1	54	-46.898	121.844	2.4315	ALA157	H34
S4	2	47	-49.704	97.111	2.4417	GLY96	N11
					2.2838	ALA22	Cl23



Ligand with protein **Ligand with secondary protein**

Figure 6 The active site of mycobacterium tuberculosis protein

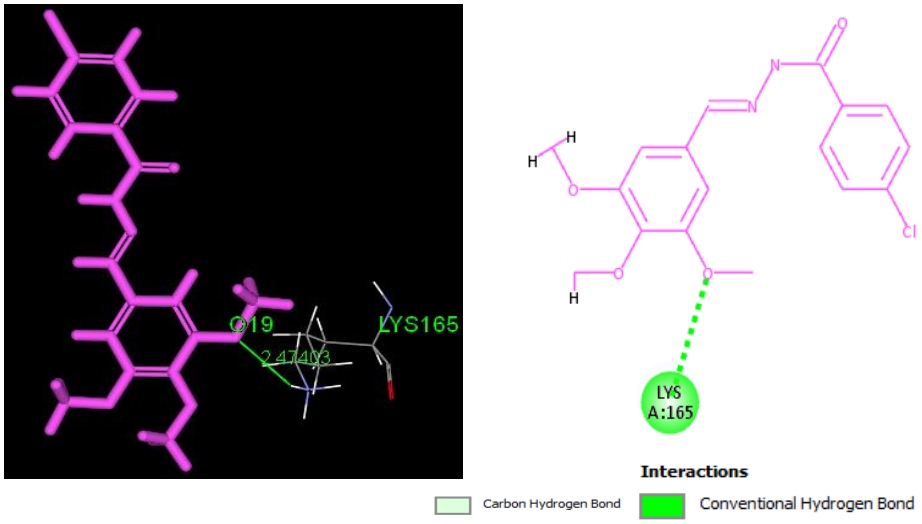


Figure 7 Binding analysis of compound S1 with *Mycobacterium tuberculosis* protein

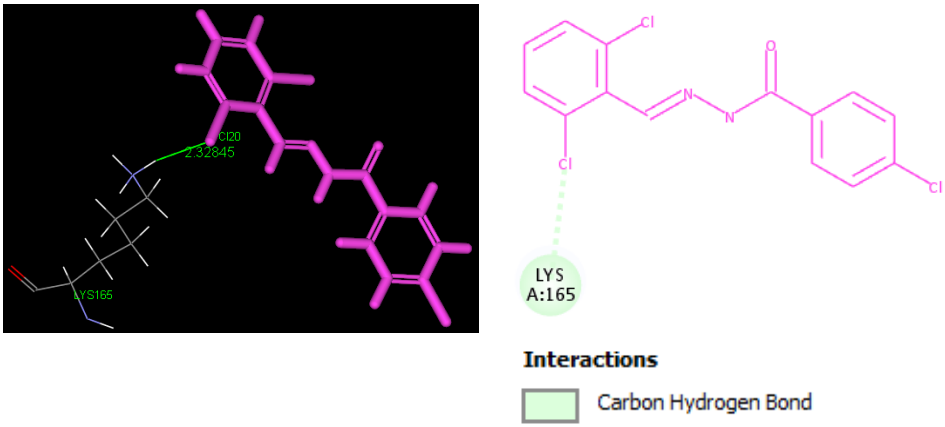


Figure 8 Binding analysis of compound S2 with *Mycobacterium tuberculosis*protein

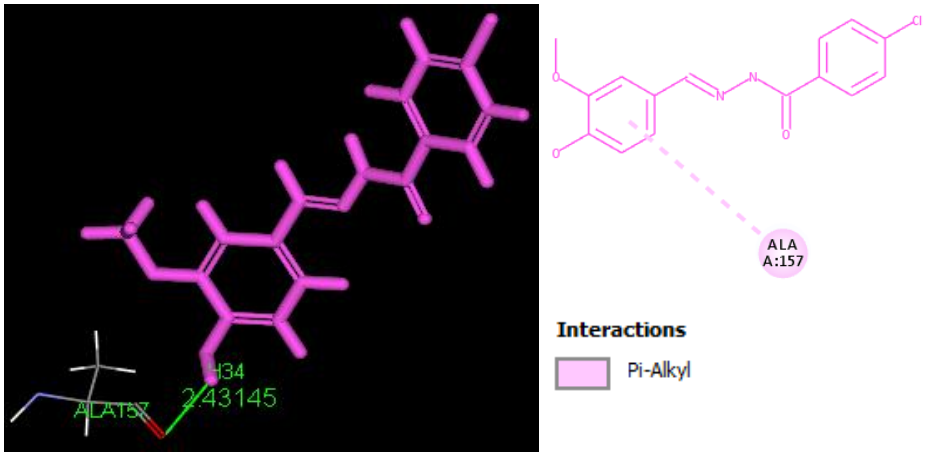


Figure9 Binding analysis of compound S3 with *Mycobacterium tuberculosis* protein

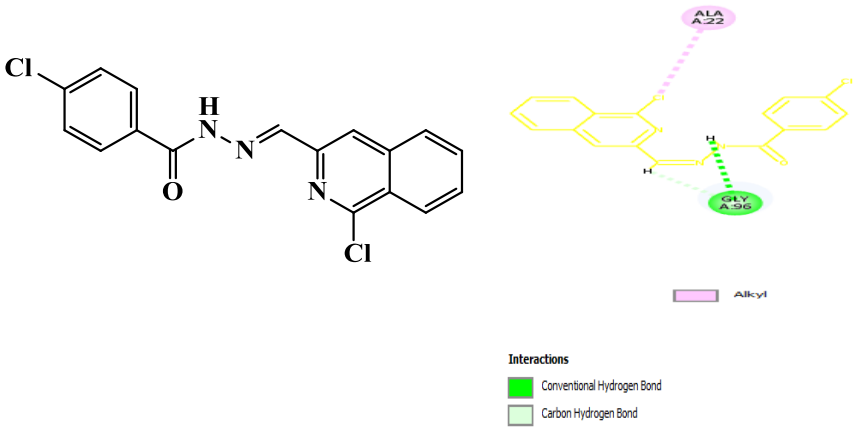


Figure 10 Binding analysis of compound S4 with *Mycobacterium tuberculosis* protein

5. CONCLUSION

In this study, the compounds were synthesized, analytical and spectral techniques were determined using melting point, Elemental analysis, UV-Visible, FT-IR,^{1}H NMR and ^{13}C NMR respectively.All synthesized product were screened for their in vitro antimicrobial and antioxidant

activities. Most of the compounds exhibited significant antimicrobial activity and some of them showed good DPPH scavenging activity. The compound S1 and S4 were found to be the most active, particularly for *Mycobacterium tuberculosis* protein and it may possess anti-tuberculosis activity.

REFERENCES

1. Lazny R and Nodzewska A, N,N-Dialkylhydrazones in Organic synthesis from simple N,N-Dimethylhydrazones to supported chiral Auxiliaries.Chemical Reviews, 2010; 110: 1386-1434.
2. RollasS and Küçükgüzel SG, Biological activities of Hydrazone Derivatives, Molecule.2007;12:1910.
3. Jumbad H. Tomma, Mustafa Khazaal S, Ammar H, Al-DujailiParikh and Vyas SP,Synthesis, Characterizationof novel Schiff bases containing pyridine Unit. Pharmaceutical Letters. 2012; 4 (2): 638-640.
4. Ray S, Jana S, Jana A, Konar S, Das K, Chatterjee S, Butcher RJ and Kar SK,Dicopper(II) complexes of a tridentate pyrimidine derived Schiff base ligand: Syntheses, crystal structures and catalytic reactions.Polyhedron.2012; 46 (1): 74-80.
5. Kandeel MM, Ali SM, Abed El Ali EKA, AbdelgawadMA andLamie PF, Synthesis and antitumor activity of novel pyrazolo[3,4-d]pyrimidin-4(5H)-one derivatives,OrganicChemistry: AnIndian Journal.2013; 9 (3): 81-91.
6. Petrie CR, Cottam HB, Mckernan PA, Robins RK and Revankar GR, Synthesis and biological activity of 6-azacadeguomycin and certain 3,4,6-trisubstituted pyrazolo[3,4-d] pyrimidine ribonucleosides, Journal of Medicinal Chemistry. 1985;28 (1): 010-1016.
7. Antre RV, Cendilkumar A, Goli D, AndhaleGS and Oswal RJ, Microwave assisted synthesis of novel pyrazolone derivatives attached to a pyrimidine moiety and evaluation of their anti-inflammatory, analgesic and antipyretic activities, Saudi Pharmaceutical Journal. 2011; 19(4): 233-243.
8. Sondhi SM, Johar M, Rajvanshi S, Dastidar S.G, Shukla R, RaghubirR and Lown JW, Anticancer, Anti-Inflammatory and Analgesic Activity Evaluation of Heterocyclic Compounds Synthesized by the Reaction of 4-Isothiocyanato-4-Methylpentan-2-One with Substituted o-Phenylenediamines, o-Diaminopyridine and (Un) Substituted o, Australian Journal of Chemistry. 2001;54: 69-74.
9. Taha M, Ismail NH, Imran S and Khan KM, 4-[5-(2-methoxyphenyl)-1,3,4- oxadiazol-2-yl]benzamide, Molbank. 2014;826:1-4.

10. Parikh KS and Vyas SP, synthesis and microbial studies of some novel schiff base containing pyrimidine**,**International Journal of Pharmaceutical Science & Research. 2012; 3(9): 3425-3427.
11. Singh NP and Srivastava AN, Synthesis, Characterization and Antimicrobial Studies of Novel Binuclear Transition Metal Complexes of Schiff Base Derived from 1-Amino-5-methyl-2,6-pyrimidine-dione and 2,3-Butanedione, Asian Journal Chemistry. 2013;25(1): 533-537.
12. Jumbad H.Tomma, Mustafa S, Khazaal and Al-Dujaili AH, Synthesis and Characterization of novel Schiff bases containing pyrimidine Unit, Arabian Journal of Chemistry.2014; 7: 157-163.
13. HassaninHM and El-Edfawy SM,Novel heterocyclic derivatives of 2-quinolinone associated with antibacterial and antitumor potencies, Heterocyclic.2012; 85 (10): 2421-2436.
14. S.J. Wadher, N.A. Karande, D.S. Borkar and P.G. Yeole, Synthesis and Biological evaluation of Schiff bases of cinchopen as antimicrobial agents, International Journal of ChemTech. 2009;1(4): 1297-1302.
15. Rajapakse HA, Zhu H, Young MB and Mott BT, A Mildand efficient one pot Synthesis of 1,3,4-oxadiazoles from carboxylic acids and acyl hydrazides,Journal of TetrahedranLetters, 2006; 47: 4827-4830.
16. Vicini P, Geronikaki A, Incerti M, Busonera B, Ooni G, KabrasCA and Colla PL,Synthesis and biological evaluation of benzo[d]isothiazole, benzothiazole and thiazoleSchiff bases, BiologicalMedicinal Chemistry, 2003;11: 4785-4791.
17. Wang Q, Yang YZ, Qi GF and Qin DD, Synthesis, crystal structure, antioxidant activities and DNA-binding studies of the Ln(III) complexes with 7-methoxychromone-3carbaldehyde-(4′-hydroxy) benzoyl hydrazone, European Journal of Medicinal Chemistry,2009; 44(6): 2425-2433.
18. Proctor PH, and Reynolds ES, Research Methodology in Food Sciences: Integrated Theory and Practice, Physiological Chemistry Physics Medicinal NMR,1984; 16(3): 175-195.
19. Cohen NC, Guidebook on Molecular Modeling in Drug Design, Academic Press, San Diego, 1996; 361.
20. Wermuth CG,The Practice of Medicinal Chemistry, Academic Press, San Diego,1996; 81-99.

21. RakeshNarang,BalasubramanianNarasimhan, Sunil Sharma, Dharmarajansriram, PerumalYogeeswari, Erik declercq Christophe Panneconque and Jan balzarini,MedicinalChemistryResearch, 2012;21: 1557-1576.
22. Ritesh. P. Bhole, Deepak. D. Borkar, Kishore. P. Bhusari and Prashant. A. Patil, Design and Synthesis of p-hydroxybenzohydrazide Derivatives for their Antimycobacterial Activity, Journal of Korean Chemical Society, 2012;56 (2): 236-245.