Synthesis, antibacterial activity and molecular docking studies of *N*'-benzylidene/*N*'-(1-phenylethylidene)hexa-2,4-dienehydrazide derivatives

Yusuf Adamu Muhammad, Rakesh Narang*, Surendra Kumar Nayak and Sachin Kumar Singh

Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara-144401, India

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ABSTRACT

*In present study a series of sorbic acid (2,4-hexadienoic acid) hydrazide-hydrazone derivatives was synthesized and screened for their in vitro antibacterial potential against Gram-negative Escherichia coli and Gram-positive Bacillus subtilis, Staphlococcus aureus and Staphlococcus epidermidis by tube dilution method. Molecular docking study was also performed as potential FabH inhibitor. The results of antibacterial screening showed that the compounds having electron withdrawing groups viz. NO_2 (**4** and **11**), Br (**10**) and electron donating groups viz. p-OCH_3, m-OC_2H_5 (**8**) are more active against tested bacterial strains as compared to other synthesized derivatives and may be further developed as antibacterial agent. Moreover, compounds **4** and **11** were pointed to be most active against all tested bacterial strains with MIC value ranging from 0.023 to 0.46 µM/ml. Docking studies revealed that dipolar interactions with amino acid residues (Cys112 and Ala246) and H_2O of FabH found to be crucial and results are comparable with in vitro antimicrobial activities of synthesized derivatives.*

Keywords: Sorbic acid, 2,4-Hexadienoic acid, Hydrazide, Antibacterial, Molecular docking

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INTRODUCTION

The rapid emergence and spreading of antimicrobial resistance is a serious health crisis, as resistant microbes cause difficult-to-treat infections [1]. Even though a large number of antibiotics are available against pathogenic microbes, the antimicrobial resistance problem against existing antibiotics produced a substantial need for new class of antimicrobial agents [2].

Sorbic acid (2,4-hexadienoic acid) is a natural straight chain unsaturated fatty acid with double bonds at α, β and γ, δ position. It is generally used as food preservative [3-4]. The antimicrobial action of sorbic acid is primarily against yeasts and molds [5-7]. Literature studies showed that derivatization of sorbic acid led to increase in antimicrobial spectrum and potency of sorbic acid [3]. Further, reported data have suggested that inhibition of bacterial growth by sorbic acid and their derivatives may result from disruption of cell membrane, creation of a proton flux into the cell, inhibition of key enzymes and transport systems. Sorbates are also reported to inhibit the amino acids uptake in microbial cells, resulting in either destruction or disruption of the cell membrane [8].

Chemical compounds having azomethine –NHN=CH moiety (hydrazide-hydrazone) represent an important class for the development of antimicrobial agents [9]. Literature study of compounds having hydrazide moities claimed to possess antibacterial, antifungal [10], antitubercular [11] trypnocidal [12], antimalarial [13], antiviral [11], anti-

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inflammatory [14] and anti-tumour [15] activities. Moreover, isoniazid [antitubercular, 16], nifuroxazide [antidiarrheal and antitumor, 17], nifurtimox [antiamoebic, 18], furacin [antibiotic, 19] and furazolidone [antibacterial, 20] are hydrazide containing important biologically active drug molecules. Structure activity relationship study of hydrazide compounds revealed that conversion hydrazide moiety to hydrazone based molecules and substituents attached to aromatic moiety at a particular position affect the antimicrobial potency to a great extent [21].

Molecular docking is a computer-assisted drug design (CADD) method used to predict the favourable orientation of a ligand (*viz.* drug) to a target (*viz.* receptor) when bound to each other to form a stable complex. By understanding of the favoured orientation in turn can be used to find out the strength of binding affinity between ligand and target site, *e.g.* by docking score [22]. Docking study can also be used to find out type of interactions between ligand and receptor *viz.* hydrogen bonding and hydrophobic interactions. Hence, molecular docking can be considered as first-line technique for a pharmaceutical lead discovery [23].

In silico studies on Schiff bases derived from N-(2/4-benzaldehyde-amino)phenyl-N' -phenyl-thiourea carried out by the research group of Zhang and co-workers [24] and found that Schiff based analogues *viz.* hydrazone have potential to bind with β-ketoacyl-acyl carrier protein synthase III (FabH) enzyme. FabH has an important role in catalysis of branched-chain fatty acid biosynthesis, both in Gram-positive and Gram-negative bacteria, while there are no significantly homologous proteins in humans [24-25]. Hence, in present study FabH target was selected for performing the *in silico* studies.

In view of above findings and continuation of our research programme to study antimicrobial activity of acid hydrazides [11, 21, 26-29] we synthesized sorbic acid hydrazide-hydrazone derivatives and screened their antimicrobial activity against selected pathogenic bacterial strains.

EXPERIMENTAL SECTION

Thin layer chromatography (TLC) was used to check the progress and completion of the reaction using silica gel G as an adsorbent (stationary phase) and ethyl acetate and hexane as mobile phase. Open glass capillaries were used to determine the melting point on popular melting point apparatus and were uncorrected. H^1 nuclear magnetic resonance (H^1 NMR) spectra were recorded on Bruker Avance II 400 NMR spectrometer (400 MHz) at 298K, in appropriate deuterated solvent. Chemical shift were reported as δ (ppm) relative to tetramethyl silane (TMS) as internal standard. Infrared spectra (IR) were recorded as KBr pellet on Shimadzu FT-IR spectrometer. The unit of IR peaks is presented in cm^{-1}. Mass spectra were recorded on Waters Micromass Q-ToF Micro instrument.

General procedure for preparation of sorbic acid hydrazide/hydrazone derivatives (Scheme 1)
Synthesis of ethyl hexa-2,4-dienoate (2)
A mixture of sorbic acid (0.1mol), 20 ml ethanol and 2–3 drops of conc. H_2SO_4 was refluxed for 8 hrs in a RBF. Saturated solution of sodium carbonate was added to neutralize unreacted sorbic acid. Ester of sorbic acid was separated using dichromethane (DCM) as organic solvent and on evaporation of DCM yielded the crude ester. Synthesized ester was recrystallized from ethanol and TLC was carried out to ascertain the purity of the product.

Synthesis of hexa- 2,4-dienehydrazide (3)
Ethanolic solution of above synthesized ester (0.01 mole, **2**) and hydrazine-hydrate (0.03 mole) was refluxed for 10 hrs. The reaction mixture was then cooled and the precipitates of acid hydrazide (**3**) were filtered off, washed with water, dried and recrystallized from ethanol.

Synthesis of N'-benzylidene/N'-(1-phenylethylidene)hexa-2,4-dienehydrazide derivatives (4-11) Solution of acid hydrazide (0.08 mole) and appropriate benzaldehydes/ acetophenone (0.08 mole) was refluxed in ethanol for 5-6 hrs. The precipitated title compounds (**4-11**) were then filtered off, washed and recrystallized from ethanol.

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Scheme 1. Synthetic scheme for synthesis of hydrazide derivatives of sorbic acid

Comp.	R_1	R_2	R_3	R_4	R_5
4	H	H	NO_2	H	H
5	H	H	CI	H	H
6	H	OC_2H_5	OH	H	H
7	H	H	$N(CH_3)_2$	H	H
8	H	OC_2H_5	OCH_3	H	H
9	H	H	OH	H	H
10	H	H	Br	H	H
11	H	H	NO_2	H	H

Analytical data
Sorbic acid (1)
Mp (^{o}C) 135-137; IR (KBr pellets): cm^{-1} 2968.55 (C-H aliphatic str.), 1695.49 (C=O str.), 1637.63 (C=C str.), 1415.80 (C-H bending aliphatic), 997.23 (C-H bending, alkene); H^1 NMR (400 MHz, DMSO) δ: 11.2 (s, 1H, OH), 5.7 (m, 1H, CH of -CH-CH$_3$), 5.4 (s, 1H, CH of -CH-C=O), 6.1-7.3 (m, 3H, CH of =CH-CH=CH-), 2.04 (d, 3H, CH$_3$)

Hexa- 2,4-dienehydrazide (3)
Bp (^{o}C) 84-87, yield-65.4%, IR (KBr pellets): cm^{-1} 3513.26, 3408.33 (NH str.), 2881.75 (C-H str., aliphatic), 1666.56 (C=O str., amide), 1589.40 (NH bending), 1442.80 (C-H bending, aliphatic). H^1 NMR (400 MHz, DMSO) δ: 8.3 (s, 1H, NH), 5.6-7.3 (m, 3H, CH of –CH=CH-CH=), 5.1 (d, 1H, CH of CH-C=O), 2.0-2.2 (d, 3H, CH$_3$).

4-Nitrobenzylidenehexa-2,4-dienehydrazide (4)
Mp (^{o}C) 210-213, yield–73.6%, IR (KBr pellets): cm^{-1} 3193.72 (NH str.), 3033.20 (C-H str., aromatic), 2843.17 (C-H str., aliphatic), 1595.18 (C=O str. amide), 1521.89 (N-O str. asymmetric), 1346.36 (N-O str. symmetric). H^1 NMR (400 MHz, DMSO) δ: 8.5 (s, 1H, CH of N=CH), 8.1-8.4 (m, 4H, ArH), 8.04 (s, 1H, NH), 5.90-7.80 (m, 3H, CH of –CH=CH-CH=), 5.3 (d, CH of CH-C=O), 2.05 (d, 3H, CH$_3$)

4-Chlorobenzylidenehexa-2,4-dienehydrazide (5)
Mp (^{o}C) 214-217; yield– 56.2%, IR (KBr pellets): cm^{-1} 3030.34 (C-H str., aromatic), 2943.47, (C-H str., aliphatic), 1624.12 (C=O str. amide), 1593.25 (C=C str., aromatic), 702.11 (C-Cl str.). H^1 NMR (400 MHz, DMSO) δ: 8.6 (s, 1H, CH of N=CH), 8.1 (s, 1H, NH), 5.9-7.4 (m, 3H, CH of CH$_3$-CH=CH-CH=), 7.51 (d, 2H, ArH), 7.89 (d, 2H, ArH), 5.2 (d, CH of CH-C=O), 2.03 (d, 3H, CH$_3$)

3-Ethoxy-4-hydroxybenzylidenehexa-2,4-dienehydrazide (6)
Mp (^{o}C) 220-223; yield–75.7%. IR (KBr pellets): cm^{-1} 3207.65 (NH str.), 3034.43 (C-H str., aromatic), 2974.33 (C-H str., aliphatic), 1631.83 (C=O str. amide), 1514.17 (C=C str., aromatic), 1246.06 (C-O str., phenol), 1041.60 (C-O str., ethoxy). H^1 NMR (400 MHz, DMSO) δ: 8.3 (s, 1H, CH of N=CH), 8.1 (s, 1H, NH), 7.4 (d, 2H, ArH), 6.8 (s, 1H, ArH), 5.7-7.3 (m, 3H, CH of CH$_3$-CH=CH-CH=), 5.23 (s, OH), 5.0 (d, CH of CH-C=O), 4.1 (t, 2H, CH$_2$ of OCH$_2$-CH$_3$), 2.05 (d, 3H, CH$_3$), 1.3 (m, 3H, CH$_3$ of OCH$_2$-CH$_3$).

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4-Dimethylaminobenzylidenehexa-2,4-dienehydrazide (7)
Mp (°C) 213-215, yield–71.1%, IR (KBr pellets): cm^{-1} 3203.29 (NH str.), 3031.57 (C-H str., aromatic), 2908.75 (C-H str., aliphatic), 1600.97 (C=O str. amide), 1519.96 (C=C str., aromatic), 1363.72 (CH$_3$ bending). H^1 NMR (400 MHz, DMSO) δ: 8.2 (s, 1H, CH of N=CH), 7.9 (s, 1H, NH), 5.60-7.60 (m, 7H, ArH), 5.1 (d, CH of CH-C=O), 2.53 (s, 6H, CH$_3$ of –N(CH$_3$)$_2$), 2.04 (d, 3H, CH$_3$). MS ES+ (ToF): *m/z* 295.23 [M$^+$ + K].

3-Ethoxy-4-methoxybenzylidenehexa-2,4-dienehydrazide (8)
Mp (°C) 212-215, yield–68.8%, IR (KBr pellets): cm^{-1} 3031.47 (C-H str., aromatic), 2960.83 (C-H str., aliphatic), 1624.12 (C=O str., amide), 1579.75 (C=C str., aromatic), 1259.56 (C-O-C str. asymmetric), 1141.00 (C-O-C str. symmetric). H^1 NMR (400 MHz, DMSO) δ: 8.4 (s, 1H, CH of N=CH), 8.09 (s, 1H, NH), 5.7-7.9 (m, 6H, ArH), 4.9 (d, CH of CH-C=O), 4.1 (m, 2H, OCH$_2$), 3.73 (s, 3H, OCH$_3$), 2.05 (d, 3H, CH$_3$), 1.30   (t, 3H, CH$_3$ of –O-CH$_2$-CH$_3$). MS ES+ (ToF): *m/z* 326.13 [M$^+$ + K].

4-Hydroxybenzylidenehexa-2,4-dienehydrazide (9)
Mp (°C) 216-219, yield–50.9%, IR (KBr pellets): cm^{-1} 3380.42 (OH str., phenol), 3040 (C-H str., aromatic), 2950.83 (C-H str., aliphatic), 1608.69 (C=O str. amide), 1591.33 (C=C str., aromatic). H^1 NMR (400 MHz, DMSO) 8.3 (s, 1H, CH of N=CH), 8.02 (s, 1H, NH), 5.5-7.2 (m, 7H, ArH), 5.23 (s, OH), 5.1 (d, CH of CH-C=O), 2.00 (d, 3H, CH$_3$).

Evaluation of antibacterial activity
The antibacterial activity of synthesized sorbic acid hydrazide derivatives (**3-11**) was preformed against Gram-negative-bacterium: *E. coli,* Gram-positive bacteria: *S. aureus, B. subtilis* and *S. epidermidis* by tube dilution method [30]. The test compounds were dissolved in DMSO to give a concentration of 100 µg/ml. The test and standard compounds were diluted in double strength nutrient broth –I.P. for bacterial growth [31]. The samples were incubated at 37°C for 24 hr and the results were recorded in terms of MIC (the lowest concentration of test substance which inhibited the growth of microorganisms) by turbidity method.

Docking Studies
Molecular docking studies of synthesized compounds were carried out on the target protein using *E. coli* FabH-CoA complex structure (pdb id: 1HNJ) [32]. The ligands were drawn in ChemBioDraw Ultra 12.0 followed by MM2 minimization of ligands (using ChemBio3D Ultra 12.0) by keeping a check on the connection error in the bonds. The co-crystallized protein-ligand complex structure (pdb id: 1HNJ) was used from RCSB Protein Data Bank (PDB) and refined as per requirement of docking study. Protein and Grid preparation was carried out using Autodock Vina 1.1.2 and used to perform molecular docking [33].

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RESULTS AND DISCUSSION

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The synthesis of intermediates (**2 and 3**) and target sorbic acid benzylidene hydrazides (**4-11**) was carried out according to reaction summarized in Scheme 1. Ethyl ester of sorbic acid was synthesized by refluxing sorbic acid with ethanol in the presence of conc. sulphuric acid. The ethyl ester was refluxed with hydrazine hydrate in ethanol to synthesize hexa- 2,4-dienehydrazide (**3**), which was then refluxed with corresponding aromatic aldehyde/ acetophenone to yield the target sorbic acid hydrazone derivatives (**4-11**). The physiochemical characteristics of synthesized compounds are presented in Table 1.

Chemical structures of synthesized compounds were ascertained on the bases of their IR, mass and ^{1}H NMR spectral data. The appearance of lower absorption band (due to resonance in –CO-NH-) in the range of 1595.18-1631.83 cm^{-1} indicated the presence of C=O of amide group (**4-11**), whereas the IR spectra of sorbic acid (**1**) showed the higher C=O str. at 1695.49, which confirmed the synthesis of hydrazide bond. The presence of IR band around 3200 cm^{-1} indicated the presence of N-H linkage of amide bond in synthesized hydrazides. The aromatic nitro stretching at 1346.46 cm^{-1} (symmetrical stretching) and 1521.89 cm^{-1} (asymmetrical stretching) showed the presence of NO$_2$ groups in the compound **4**. The appearance of absorption band around 2950 cm^{-1}, indicated the presence of aliphatic C-H moiety, whereas the appearance of stretching in the range of 3030-3040 cm^{-1} indicated the presence aromatic ring in synthesized derivatives. The presence of stretching at 702.11 cm^{-1} indicated the presence of chlorine group in synthesized compound (**5**). The appearance of absorption band at 1141.00 cm^{-1} (C-O-C symmetric str.) and 1259.56 cm^{-1} (C-O-C asymmetric str.) showed the presence of methoxy groups (C-O-C streching) in compound **8**. The presence of phenol hydroxyl group in compound **6** demonstrated by stretching band at 1246.06 cm^{-1}.

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Table 1 Physiochemical characteristics of synthesized sorbic acid hydrazide derivatives

Comp.	Mol. Formula	M. Wt	Mp/Bp* (^{o}C)	R_f^a	% yield
1	$C_6H_8O_2$	112.13	135-137	0.63	-
2	$C_8H_{12}O_2$	140.18	156-159	0.82	67.0
3	$C_6H_{10}N_2O$	126.08	84-87*	0.75	65.4
4	$C_{13}H_{13}N_3O_3$	259.26	210-213	0.70	73.6
5	$C_{13}H_{13}ClN_2O$	248.71	214-217	0.65	56.2
6	$C_{15}H_{18}N_2O_3$	274.13	220-223	0.71	75.7
7	$C_{15}H_{19}N_3O$	257.30	213-215	0.60	71.1
8	$C_{16}H_{20}N_2O_3$	288.34	212-215	0.85	68.8
9	$C_{13}H_{14}N_2O_2$	230.26	216-219	0.80	50.9
10	$C_{14}H_{15}BrN_2O$	307.19	131-133*	0.75	60.4
11	$C_{14}H_{15}N_3O_3$	273.29	151-153*	0.71	65.7

^{a}Mobile phase- Hexane: Ethyl acetate (1:1)

The appearance of multiplet signals in the range of δ 5.70-8.40 ppm in NMR spectra revealed the presence of aromatic protons in synthesized derivatives. Singlet proton signal in the range of δ 7.9-8.1 ppm in synthesized derivatives (**4-11**) showed the presence of NH moiety, confirmed the attachment of aromatic aldehyde/acetophenone moieties with sorbic acid hydrazide scaffold. Multiplet signals around δ 5.70-7.40 ppm is due to presence of –CH-groups in sorbic acid. The presence of NMR signals in the range of δ 2.00-2.05 ppm indicated the presence of terminal methyl group of sorbic acid in the synthesized compounds (**4-11**). Signals at δ 3.73 ppm (singlet for OCH_3), δ 4.10 ppm (multiplet for OCH_2) and δ 1.10 ppm (triplet for CH_3 of $-O-CH_2-CH_3$) depicted the presence eight protons of OCH_3 and OC_2H_5 groups in compound **8**. Moreover, the absence of singlet signal at δ 11.2 ppm of carboxylic acid proton, confirmed the synthesis of hydrazide-hdrazone derivatives (**3-11**). The appearance of a peak in mass spectrometer at 295.23 m/z (M^++K) confirmed that compound **7** have been synthesized, likewise the appearance of a peak at 326.13 m/z (M^++K) confirmed the synthesis of compound **8**.

Evaluation of antibacterial activity
In present study, antibacterial screening of synthesized sorbic acid hydrazide derivatives was performed against Gram-negative *E. coli* and Gram-positive *B. subtilis, S. aureus* and *S. epidermidis* by tube dilution method [30] Nutrient broth I.P. was used as a media for the growth of bacteria [31]. The results of antibacterial activity (in µM/ml) are presented in Table 2.

Table 2 MIC values of synthesized sorbic acid hydrazide derivatives (µM/ml)

Minimum Inhibitory Concentration (MIC in µM/ml)			
Comp.	*E. coli*	*B. subtilis*	*S. aureus*	*S. epidermidis*
1	0.111	0.111	0.111	0.111
2	0.089	0.089	0.178	0.089
3	0.099	0.198	0.099	0.099
4	0.023	0.023	0.046	0.046
5	0.101	0.201	0.050	0.201
6	0.051	0.051	0.051	0.051
7	0.049	0.049	0.049	0.097
8	0.043	0.043	0.043	0.043
9	0.054	0.054	0.054	0.109
10	0.041	0.041	0.041	0.041
11	0.046	0.046	0.023	0.023
Ciprofloxacin	0.004	0.004	0.004	0.004

For *E. coli,* compounds **4**, **7**, **8**, **10** and **11** exhibited the highest activities among the synthesized compounds having MIC value range from 0.023-0.049 µM/ml (Table 2). Compounds **4** and **10** showed a very good activity against *B. subtilis,* with an MIC value of 0.023 and 0.041 µM/ml, respectively. This may be due to the presence of electron withdrawing *p*-NO_2 and *p*-Br groups present in the compounds **4** and **10**, respectively. Compounds **8**, **10**, and **11** showed better activity against Gram-positive *S. aureus* as compared to other synthesized derivatives with MIC values 0.043, 0.041 and 0.023 µM/ml, respectively (Table 2). The highest activity (MIC= 0.023 µM/ml)) of compound **11** against *S. aureus* may be due to the presence of electron withdrawing *p*-NO_2 group. Similarly, in case of *S. epidermidis*, compounds **8**, **10** and **11** exhibited the highest potency among synthesized derivatives, with MIC value ranging from 0.023-0.043 µM/ml (Table 2). The results of antibacterial activities indicated that the compounds

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having electron withdrawing groups *viz.* NO_2 (**4** and **11**), Br (**10**) and electron donating groups *viz. p*-OCH_3, *m*-OC_2H_5 (**8**) are more active against tested bacterial strains as compared to other synthesized derivatives.

Structure activity relationship

1. The above results (Table 2) indicated that the presence of an electron withdrawing (NO_2, Br) group, increases the antibacterial activity of the synthesized compounds (**4**, **10** and **11**). These observations are similar to results reported by Kumar *et al.* [34].

2. The analysis of the results depicted that the presence of electron donating group (OCH_3 and OC_2H_5), enhances the antibacterial activity (MIC = 0.043 µM/ml) of compound **8** and interpretations are corresponds to a results observed by Emami *et al.*[35].

3. Attachment of hydrazine hydrate moiety decrease the antibacterial activity of the synthesized compound **3** as compared to sorbic acid ester (MIC = 0.89-0.178 µM/ml, **2**). This may be due to the presence of hydrophilic groups i.e hydrazide, which may decrease the penetration through lipophilic cell membrane/cell wall of bacteria.

4. Significant improvement in antibacterial activity was observed on reaction of benzaldehyde/acetophenone with hydrazide scaffold (**3**) and this noticeable increase in the activity may be due to increase in the lipophilicity of the molecule, which permit the entrance of the moleclule in to the microbial membrane.

5. Attachment of methyl group to benzylidene scaffold showed no improvement in antibacterial activity of synthesized derivatives (**9-10**). Structure activity relationship of synthesized derivatives is presented in Fig. 1.

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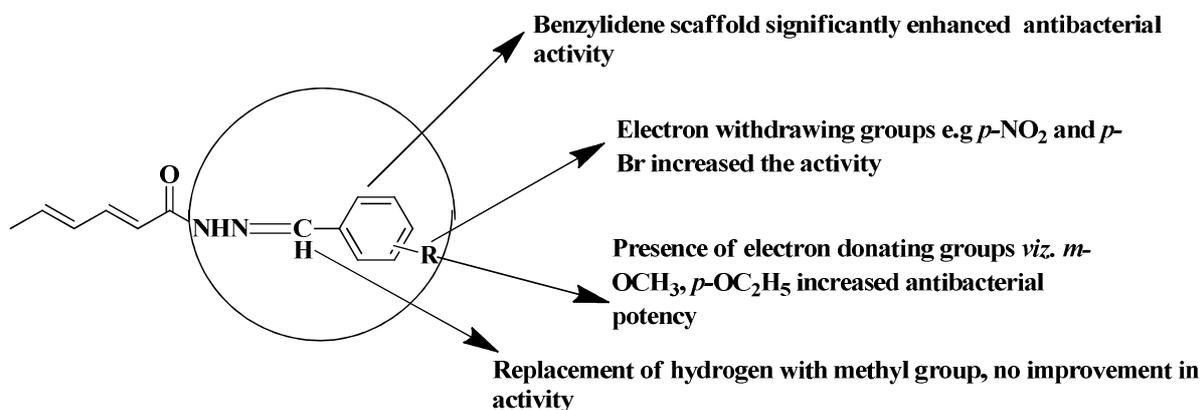
Fig. 1 Structural requirement for antibacterial activities of sorbic acid hydrazide derivatives

Analysis of docking studies

To understand the binding pattern, molecular docking of sorbic acid, ester and their hydrazides (**1-11**) was performed using *E. coli* FabH-CoA complex structure (pdb id: 1HNJ) [32]. Earlier study has been reported that Cys-His-Asn triad and Thr81 are crucial for catalytic activity of FabH in various bacteria [24]. In present docking study Cys112 and Ala246 was found to be responsible for substrate binding and cleavage of alkyl chain of CoA. All the studied compounds (**1-11**) were found to bind in the same binding pocket that of co-crystallized ligand. However, hydrazone derivatives (**4-11**) showed strong binding affinity having binding score ranging from -7.0 to -7.7 Kcal/mol, which is comparable to co-crystallized ligand (-7.6 Kcal/mol) (Table 3). Conformational and binding interaction analysis indicated that >C=O group of compound **6** functions as a H-bond acceptor and involved in two H-bonds formation with thiol group of Cys112 and H_2O molecule. Whereas, *p*-OH group of phenyl ring functions as H-bond donor to the Ala246. Docking results indicated significant role of carbonyl group of hydrazone moiety and electron donating or withdrawing substituents at *p*-position of phenyl ring in binding with target site. Thus, dipolar interactions with amino acid residues (Cys112 and Ala246) and H_2O found to be crucial and comparable with *in vitro* antimicrobial activities of synthesized compounds (Figure 2).

CONCLUSION

A series of sorbic acid derivatives *i.e. N'*-benzylidene/*N'*-(1-phenylethylidene)hexa-2,4-dienehydrazide derivatives was synthesized and screened for their *in vitro* antibacterial potential against Gram-negative *E. coli* and Gram-positive *B. subtilis, S. aureus* and *S. epidermidis* by tube dilution method. Molecular docking study was also

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performed and evaluated as potential FabH inhibitor. The chemical structures of synthesized derivatives were ascertained on the basis of their spectral data (NMR, IR and Mass).

Table 3 Docking score of compounds (1-11) in β-ketoacyl-acyl carrier protein synthase III FabH, pdb id: 1HNJ)

Compounds	Affinity (Kcal/mol)
1	-5.1
2	-4.6
3	-5.4
4	-7.4
5	-7.0
6	-7.7
7	-7.4
8	-7.4
9	-7.1
10	-7.3
11	-7.2
Co-crystallized ligand	-7.6
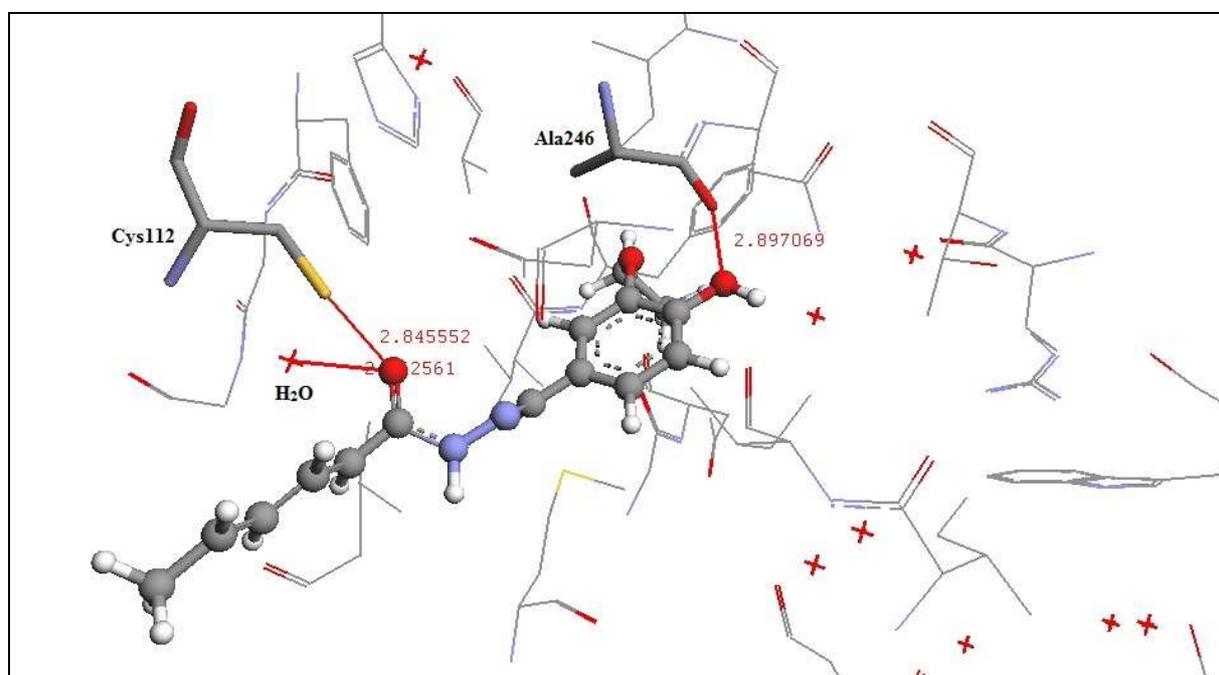
Fig. 2 The dipolar interactions of sorbic acid hydrazide (6) with *E. coli* FabH (pdb id: 1HNJ)

Antibacterial evaluation depiced that compounds having electron withdrawing groups *viz.* *p*-NO$_2$ (**4** and **11**), *p*-Br (**10**) and electron donating groups *viz.* *p*-OCH$_3$, *m*-OC$_2$H$_5$ (**8**) are more active against tested bacterial strains as compared to other synthesized derivatives and may be further developed as antibacterial agent. Furthermore, compound **4** was pointed to be most active against all tested bacterial strains with MIC value from 0.023 to 0.46 µM/ml. However, all the synthesized derivatives were found to be less potent as compared to standard drug, ciprofloxacin (MIC value = 0.004 µM/ml). Docking studies revealed that dipolar interactions with amino acid residues (Cys112 and Ala246) and H$_2$O of FabH found to be crucial and results are comparable with *in vitro* antimicrobial activities of synthesized derivatives.

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