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RESEARCH ARTICLE

In Vitro Investigation of Antibacterial Activity of Novel 3-Acetylcoumarin Schiff Bases and Their Molecular Docking Studies

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ABSTRACT

A new series of Schiff bases, SB1 to SB6 were synthesized from (1) and (2) with different acid hydrazides. The structures of the synthesized compounds were established on the basis of physical and spectral data. They shows a prominent absorption of -(C=N-) in FTIR. Antibacterial activity of these compounds was performed on Gram +ve and Gram –ve bacteria. SB1, SB4, SB5 and SB6 were found active against Gram +ve and Gram –ve bacteria. Molecular docking study of these derivatives in the IsdH protein cavity was also conducted. SB1 and SB4 establish the best binding energy of –10.3 and –10.5 Kcal/mol. A survey of existing literature revealed that there are no reports describing the synthesis of such hydrazones.

KEYWORDS

Molecular docking, 3-Acetylcoumarin, Salicylaldehyde, Ethylacetoacetate, acid hydrazide, Antibacterial and Schiff Base

INTRODUCTION

Coumarins have been synthesized as a well-known naturally occurring heterocyclic compounds isolated from various plants. They belong to the family of lactones having 1-benzopyran-2-one system[1]. Coumarin is a versatile pharmacophore which exhibits wide variety of biological activities[2] like antibacterial[3-4] and antimicrobial[5]. Coumarins is a class of compounds, belongs to the flavonoid class of plant secondary metabolite, which exhibit a variety of biological activities, usually associated with low toxicity and have raised considerable interest because of their potential beneficial effects on human health[6].

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The synthesis of coumarin (2-oxo-2H-chromene) derivatives has attracted considerable attention of organic and medicinal chemists due to its wide usage in food additives, fragrances, pharmaceuticals, and agrochemicals. Furthermore, the pharmacological and biochemical properties as well as therapeutic applications of coumarins depend upon the pattern of substitution[7]. Coumarin derivatives have been reported for anticoagulant, anti-inflammatory[8], antimicrobial[9], antiHIV, antioxidant[10], antiallergic, anticancer[11] and anti-proliferative and antiviral[12] activities. Isoxazole derivatives with coumarin moiety have analgesic[13], anti-inflammatory, anti-microbial, anti tumour, antiHIV, herbicidal, fungicidal[14] and CNS stimulant[15] activities. It was found that when one biodynamic heterocyclic system was

coupled with another heterocyclic system, enhanced biological activity was produced.

IsdH is a surface iron binding protein. The pathogen Staphylococcus aureus uses iron-regulated surface determinant (Isd) proteins to scavenge the essential nutrient iron from host hemoproteins. The IsdH protein (also known as HarA) is a receptor for hemoglobin (Hb), haptoglobin (Hp), and the Hb-Hp complex. It contains three NEAT (NEAr Transporter) domains: IsdH(N1), IsdH(N2), and IsdH(N3). Here, we show that they have different functions; IsdH(N1) binds Hb and Hp, whereas IsdH(N3) captures heme that is released from Hb. The staphylococcal IsdB protein also functions as an Hb receptor. Primary sequence homology to IsdH indicates that it will also employ functionally distinct NEAT domains to bind heme and Hb[16].

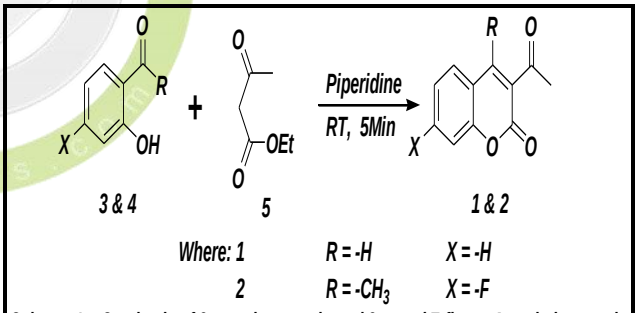
The work in this paper is an extension of our published work[17]. We extended our work by synthesizing the hydrazones of 3-acetyl,7-flouro,4-methylcoumarin with three acid hydrazides such as 3-hydroxy-2-naphthoic acid hydrazide, 4-hydroxy benzoic acid hydrazide and isoniazide. An in-vitro antibacterial activity was also performed on the synthesized compounds against Gram +ve ***(Staphylococcus aureus)*** and two Gram –ve species (***Escherichia coli*** and ***Pseudomonas aeroginosa***). We conducted a molecular docking study of these derivatives in the IsdH protein cavity by using AutodockVina. A survey of existing literature revealed that there were no reports describing the synthesis of such hydrazones.

MATERIALS AND METHOD

Solvents for synthesis were reagent grade and dried by standard procedures. The starting materials such as Salicylaldehyde,7-Flouro,2-hydroxyacetophenone, 4-hydroxy benzoic acid hydrazide, 3-hydroxy naphthoic acid hydrazide and isoniazide were obtained from Sigma-Aldrich chemicals and Ethylacetoacetate, Piperidine, acetone, methanol, ethanol and dichloromethane were obtained from SD-FCL Chemical Limited, Mumbai, India. All compounds were routinely checked by TLC on silica gel-G plates using petroleum ether/ethyl acetate (7:3; 6:4; 5:5 by V/V) as solvent system and the developed plates were visualized by UV light and iodine vapours. The detailed synthesis has been shown in Scheme 1.

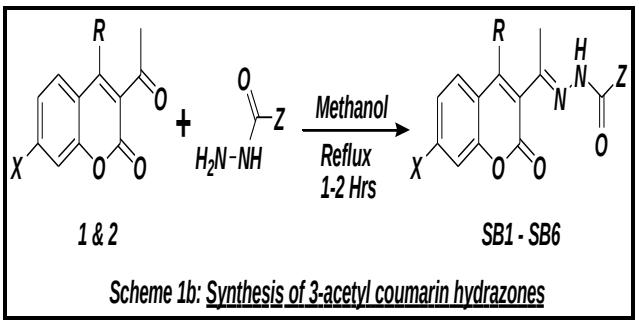
Melting points of synthesized compounds were determined with open capillary tube on a VEEGO melting point apparatus. The H^1-NMR and Liquid chromatography mass spectra (LCMS) were obtained from NCL, Pune and purity was checked by "HPLC—Systronics". IR spectra were recorded by "FT- IR Jasco" spectrometer at the center.

3-Acetylcoumarin(1) and 3-acetyl,7-flouro,4-methylcoumarin(2) was synthesized by reported method[17]. A mixture of Salicylaldehyde/7-Flouro, 2-hydroxyacetophenone (3/4, 1 eq.), ethyl acetoacetate (5, 1 eq.) and a few drops of piperidine were mixed for 5 min. at room temperature without any solvent. Reaction was neutralized with HCl (1M) and finally the product was isolated by filtration. The final compound was then recrystallized in EtOH (Scheme 1a).



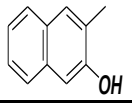
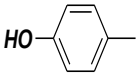
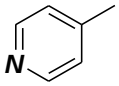
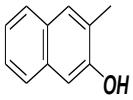
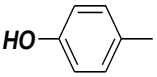
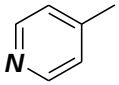
Scheme 1a: Synthesis of 3-acetyl coumarin and 3-acetyl,7-flouro,4-methylcoumarin

The Schiff's bases were synthesized by condensing 3-acetyl coumarin and different acid hydrazides as explained by Anees Pangal et al[18] (**Scheme 1b)**.



Scheme 1b: Synthesis of 3-acetyl coumarin hydrazones

Where,

Sr. No.	Name of Hydrazones	Z	X	R
1	SB1		H	H
2	SB2		H	H
3	SB3		H	H
4	SB4		F	-CH_3
5	SB5		F	-CH_3
6	SB6		F	-CH_3

The *in vitro* antibacerial activity was performed according to procedure explained by Arpit et al[19]. A standardize inoculums were inoculated with the help of a sterile cotton swab on the surface of the agar plate. Disc of antimicrobial agents were placed on the surface of agar plate. The plates were incubated at 37°C for 24 hours and susceptibility is determined on the basis of zone of inhibition. A standard and control strain was also tested for comparison. The diameter of the zone of growth inhibition around each disc were measured and compared with zones of inhibition of standard and control.

Docking calculations were carried out on IsdH protein model. Essential hydrogen atoms, Kollman united atom type charges and salvation parameters were added with the aid of AutoDock 4.2 tools[20].The molecular docking was conducted in the IsdH protein cavity by using AutodockVina[21].

RESULTSAND DISCUSSION

The structures of the synthesized compounds are established on the basis of physical and spectral data. They shows a prominent absorption of -(C=N-) in FTIR. It also shows a common peak of imine at 7.24 ppm in the form of singlet. The detailed physical and spectral properties are discussed below.

3-Acetyl coumarin: COLOUR: Pale Yellow. YEILD: 98%, M. P.:120-122 °C.PURITY (HPLC): 99.6%, MASS (LCMS): 188, 189(M+1), FTIR(cm^{-1}):1095(C-O), 1710 (-C=O).H^1-NMR(*DMSO*) (δ, ppm,): 2.75 (s, 3H, -$COCH_3$), 7.67 (m, 2H), 7.40 (m, 2H), 8.55 (s, 1H).IUPAC name:3-Acetyl-2H-chromen-2-one.

SB1: COLOUR: Pale yellow. YEILD: 89%, M. P.: 244-248 °C. PURITY (HPLC): 99.6%, MASS (LCMS): 372, FTIR(cm^{-1}):1095(C-O), 1710 (-C=O), 3265.86 (-NH), 2958.27 (-CH), 1660.41 (-C=O), 1647.56 (-C=N), 1057.03 (-N-N). H^1-NMR (*DMSO*) (δ, ppm,): 2.05 (s, 3H, -$COCH_3$), 7.67 (m, 2H), 7.40 (m, 2H), 8.55 (s, 1H), 7.24 (s, 1H, -NH), 7.36 (s, 4H), 7.66 (m, 6H, naphthoicHs), 9.73(s, 1H, exchangeable –OH). IUPAC name: (13E)-3-hydroxy-N'-(1-(2-oxo-2H-chromen-3-yl)ethylidene)naphthalene-2-carbohydrazide.

SB2: COLOUR: Yellow. YEILD: 85%, M. P.:212-216 °C. PURITY (HPLC): 98%, MASS (LCMS): 322, FTIR(cm^{-1}):1095(C-O), 1710 (-C=O), 3265.86 (-NH), 2958.27 (-CH), 1660.41 (-C=O), 1647.56 (-C=N), 1057.03 (-N-N). H^1-NMR (*DMSO*) (δ, ppm,): 2.75 (s, 3H, -$COCH_3$), 7.67 (m, 2H), 7.40 (m, 2H), 8.55 (s, 1H), 7.24 (s, 1H, -NH), 7.84 (dd, J=8Hz, 2H), 7.68 (dd, J=8Hz, 2H), 9.75 (s, 1H, exchangeable –OH). IUPAC name: (13E)-4-hydroxy-N'-(1-(2-oxo-2H-chromen-3-yl)ethylidene)benzohydrazide.

SB3: COLOUR: Yellow. YEILD: 90%, M. P.: 170-174 °C. PURITY (HPLC): 99%, MASS (LCMS): 307, FTIR(cm^{-1}):1095(C-O), 1710 (-C=O), 3265.86 (-NH), 2958.27 (-CH), 1660.41 (-C=O), 1647.56 (-C=N), 1057.03 (-N-N). H^1-NMR (*DMSO*) (δ, ppm,): 2.75 (s, 3H, -$COCH_3$), 7.67 (m, 2H), 7.40 (m, 2H), 8.55 (s, 1H), 7.24 (s, 1H, -NH), 7.86 (d, 2H), 8.66 (d, 2H).IUPAC name: (13E)-N'-(1-(2-oxo-2H-chromen-3-yl)ethylidene)nicotinohydrazid.

3-Acetyl,7-flouro,4-methylcoumarin:
COLOUR: White. YEILD: 99%. M. P.: 246°C. PURITY (HPLC): 99.6%. MASS (LCMS): 220. FTIR(cm^{-1}): 1095(C-O), 1710 (-C=O), 1673.91(-C=O), 1500 to 1600 (Aromati region). H^1-NMR(*DMSO*) (δ, ppm,): 2.5 (s, 3H, -$COCH_3$), 2.6 (s, 3H, olefinic), 6.8 (d, 2H), 8 (t,

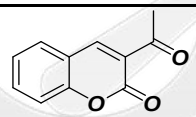
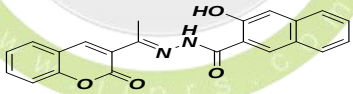
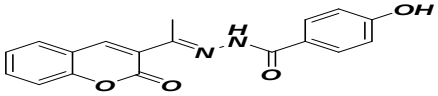
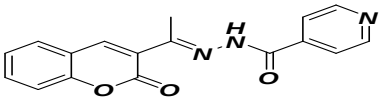
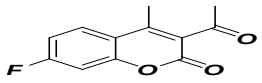
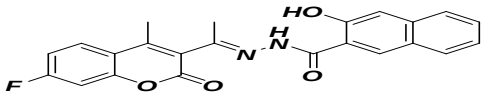
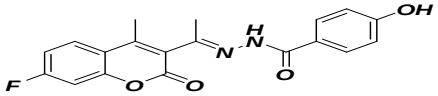
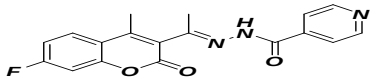
1H). IUPAC name: 3-acetyl-7-fluoro-4-methyl-2H-chromen-2-one.

SB4: COLOUR: Pale yellow. YEILD: 89%. M. P.: 294-298°C. PURITY (HPLC): 99.6%. MASS (LCMS): 404. FTIR(cm^{-1}): 1095(C-O), 1647.56 (-C=N), 1617.98(-C=O), 3265.86 (-NH), 3081.69(-OH), 2958.27(-CH), 1736.58(-C=O), 1057.03 (-N-N), 1500 to 1600 (Aromati region). H^1-NMR (*DMSO*) (δ, ppm,): 2.5 (s, 3H, $-COCH_3$), 2.6 (s, 3H, olefinic), 6.8 (d, 2H), 7.6 (t, 1H), 11.6 (s, 1H, -NH), 7.66 (m, 6H, naphthoicHs), 9.73(s, 1H, exchangeable –OH). IUPAC name:(13E)-N'-(1-(7-fluoro-4-methyl-2-oxo-2H-chromen-3-yl)ethylidene)-3-hydroxynaphthalene-2-carbohydrazide.

SB5: COLOUR: Dark green. YEILD: 88%. M. P.: 260-262°C. PURITY (HPLC): 97%, MASS (LCMS): 354 FTIR(cm^{-1}):1095(C-O), 1647.56 (-C=N), 3265.86 (-NH), 3321.78(-OH) 2274.63 (-CH), 1660.41 (-C=O), 1057.03 (-N-N), 1500 to 1600 (Aromatic region). H^1-NMR (*DMSO*) (δ, ppm,): 2.5 (s, 3H, $-COCH_3$), 2.6 (s, 3H, olefinic), 6.8 (d, 2H), 7.6 (t, 1H), 7.24 (s, 1H, -NH), 6.9 (d, 2H), 7.8 (d, 2H), 9.75 (s, 1H, exchangeable –OH). IUPAC name: (13E)-N'-(1-(7-fluoro-4-methyl-2-oxo-2H-chromen-3-yl)ethylidene)-4-hydroxybenzohydrazide.

SB6: COLOUR: Yellow. YEILD: 91%. M. P.: 292-296°C. PURITY (HPLC): 98%. MASS (LCMS): 339. FTIR(cm^{-1}): 1095(C-O), 1647.56 (-C=N), 3199.33(-NH), 2958.27 (-CH), 1673.91(-C=O), 1057.03 (-N-N), 1500 to 1600 (Aromati region). H^1-NMR (DMSO) (δ, ppm,): 2.56 (s, 6H), 6.8 (d, 2H), 7.7 (dd, 1H), 11.4 (s, 1H, -NH), 7.86 (d, 2H), 8.66 (d, 2H). IUPAC name:(13E)-N'-(1-(7-fluoro-4-methyl-2-oxo-2H-chromen-3-yl)ethylidene)-isonicotinohydrazide

Table-1: Summarized Results

Sr. No.	Name of Hydrazones	Structure of Hydrazones	% Yield	M. P. °C
1	1		98	120-122
2	SB1		89	244-248
3	SB2		85	212-216
4	SB3		90	170-174
5	2		99	246-248
6	SB4		89	294-298
7	SB5		88	260-262
8	SB6		91	292-296

Antibacterial Discussion

The chemically synthesized compounds were tested for antimicrobial activity. Strains of both Gram positive and Gram negative bacteria were used. Amoxicillin (25 $\mu g/mL^{-1}$) was used as standard which showed a zone of inhibition of 8mm. The compounds were serially diluted and different dilutions were tested against three organisms such as *Staphylococcus aureus, E. coli* and *Pseudomonas aeroginosa*. Out of the six tested compounds four compounds such as SB3, SB4, SB5 and SB6 showed a good activity against both Gram positive and Gram negative bacteria whereas compounds SB2 and SB2 didn't exhibit any antibacterial activity.

Among the two Gram negative strains compounds were found to be more effective against *P. aeroginosa* than *E. coli*. However, if antibacterial activity is to be compared between Gram positive and Gram negative bacterial, the results are more promising against Gram positive organisms. The disc diffusion method was used for determining the antibacterial activity of the compounds and the results obtained were summarized in following Table-2.

The results clearly indicate that the compounds have selective action on Gram negative organisms. The basic difference between Gram positive and Gram negative organism lies in organization. The compounds seem to interfere or inhibit the cell wall organization or may be they do not allow the synthesis of cell wall or one of its components in Gram positive bacterial. Also the zone of inhibition seen around the antibiotic disc seems to increase in diameter with the increase in concentration of the drug.

Molecular Docking Studies

SB1, SB4, SB5 and SB6 when docked into the active site of IsdH proteins were found to give a good fit allowing additional hydrogen bonding interactions (Fig. 1, Table 3).

The best binding energy was exhibited by SB1 and SB4 followed by SB5, SB6, SB3 and SB2 respectively. SB1 undergoes hydrogen bonding interactions with three amino acid residues, viz. SER – 45, THR – 117, THR -125 respectively in the IsdH cavity. Five H-bonding interactions are seen for SB4 with amino acid residues, viz. TYR – 127, HIS – 42, PHE – 44, and SER – 45. SB3 and SB4 shows four H-bonding interactions each with amino acid residues such as THR – 67, TYR – 127, SER – 45, THR – 70 and TYR 129, THR – 130, LEU – 32 respectively. These interactions lead to stabilization of the compounds in the protein cavity. Based on these considerations, compound SB1, SB4, SB5 and SB6 have better stabilities in the IsdH protein cavity than SB2 and SB3 and anticipated to show enhanced antibacterial activity.

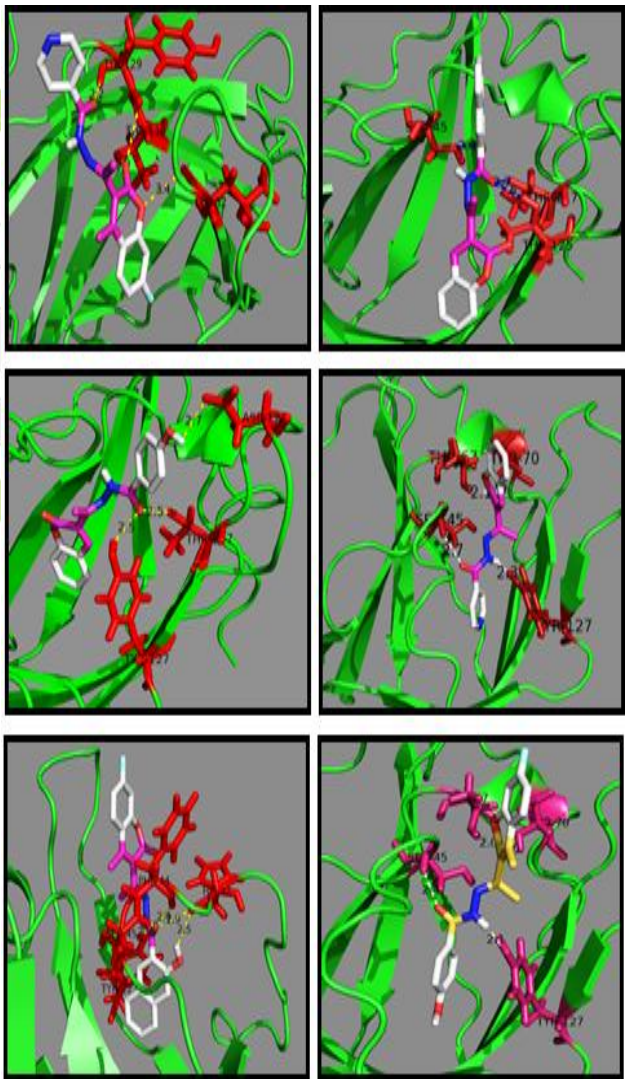


Figure 1: Binding of SB1 – SB6 into active site of IsdH as assessed by molecular docking studies.

Table 2: Results of Antibacterial Activity

Organisms	*Conc.* (µg/mL^{-1})	SB1	SB2	SB3	SB4	SB5	SB6
Staphylococcos aureus	25	-	No Activity	No Activity	-	-	6
	50	6			-	7	6
	75	7			6	7	6
	100	9			7	12	8
	125	10			8	10	16
	150	12			9	14	20
Pseudomonas aeroginosa	25	-			-	-	6
	50	5			-	6	6
	75	5			6	7	6
	100	6			6	9	8
	125	7			7	10	9
	150	8			8	12	10

Table 3: Binding of SB1 – SB6 into active site of IsdH as assessed by molecular docking studies

Schiff Base	Binding Affinity (Kcal/Mol)			Residue	No. of H-bonds	Bond length (A°)
	I	II	III			
SB1	–10.2	–10.0	–10.3	SER – 45, THR – 117, THR -125	3	2.4, 2.7, 2.5
SB2	–8.7	–8.7	–8.8	THR – 117, TYR-127, ASP – 121	3	2.5, 2.5, 2.7
SB3	–8.8	–8.9	–8.8	TYR – 127, SER – 45, THR – 67, THR – 70	4	2.3, 2.7, 2.1, 2.7
SB4	–10.3	–10.7	–10.5	TYR – 127, HIS – 42, PHE – 44, SER – 45	5	2.1, 2.4, 2.0, 2.9, 2.5
SB5	–9.1	–9.1	–8.9	THR – 67, TYR – 127, SER – 45, THR – 70	4	2.0, 2.1, 2.4, 2.9
SB6	–8.8	–9.2	–9.2	TYR 129, THR – 130, LEU – 32	4	2.0, 2.2, 3.2, 3.4

CONCLUSION

We reported the synthesis and structural characterization of some novel Schiff bases of 3-acetylcoumarin. Only four compounds show effective antibacterial activity where as SB2 and SB3 are inactive against Gram +ve and Gram –ve bacteria. All the synthesized compounds are moderately active when compared with standard Amoxicillin. The molecular docking studies are also in best agreement with experimental results. The shown activity can be accounted for the presence of polar interactions in the respective hydrazide. As the synthesized Schiff bases are biological active, study of their other biological activities is of our further interest.

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