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Synthesis, anti-microbial activity and docking study of cinnamic acid Esters of Salicylanides

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ABSTRACT

In recent days, spread of fungal, viral and microbial infections and their resistance to antibiotics compel us to develope new, powerful and effective pharmaceutics. Thus cinnamic acid esters of salicylanides are synthesized and evaluated for their antimicrobial activity in vitro. All the compounds are characterized by spectroscopic techniques such as FTIR, H^1 NMR, C^{13} NMR and Mass. Molecular docking of these compounds is carried out in silico. Molecular docking of these compounds is carried out with enzyme β-Ketoacyl-acyl carrier protein synthase III from Escherichia coli (ecKAS III pdb id: 1HNJ) as a receptor responsible for growth of bacteria. The results showed that all Cinnamic acid esters of salicylanides are good inhibitors of β-Ketoacyl-acyl carrier protein synthase III.

Keywords: Salicylanides, cinnamic acid esters, Molecular docking, inhibitors, antimicrobial activity etc.

INTRODUCTION

Due to the frequent utilization of antibiotics, microorganism's develope resistance against these antibiotics and cause health hazards to human beings and their livestock's. Therefore there is a need to develope new and effective antibiotics with very less side-effects.

Salicylanides and their derivatives are the important class of compounds which show broad spectrum of biological activities [1-21], Salicylanides and their derivatives are substantial building blocks in medicinal and pharmaceutical chemistry [22]. The expunging property of Salicylanide derivates makes it significant constituent of pharmaceutical chemistry. The wide range of biological properties of Salicylanides make them crucial synthetic molecular targets for many researchers. In previous paper, we have mentioned different salicylanides and also investigated their antibacterial activity in vitro [23].

In the present study, Cinnnamic acid esters of Salicylanides are synthesized using coupling agent DCC and screened for their antimicrobial activity in vitro. Esterification is carried out by converting hydroxyl group of salicylanide into ester group to investigate the effect of functional group transformation on antimicrobial activity. These compounds are exposed to four bacterial strains including two gram positive, two gram negative bacteria and two fungal strains.

Molecular docking is a tool to know the binding interaction of protein molecule with target molecule. From binding score (includes E value, E force and hydrogen bond) we can predict the activity of the target molecule. In present study for molecular docking, we have taken the enzyme from Escherichia coli β -Ketoacyl-acyl carrier protein synthase III [24] (ecKAS III pdb id: 1HNJ) as a receptor responsible for growth of bacteria and our synthesized derivatives as ligands.

MATERIALS AND METHODS

All the reagents and solvents are purchased from Sigma-Aldrich and they are used as received. Melting points are determined using open capillaries method and the reported values are uncorrected. Infrared spectra are recorded on FT-IR spectrometer Shimdzhu 8400S FT-IR in the range of 4,000–400cm−1. The NMR spectra are recorded on a 500MHz instrument at ambient temperature using deuterated dimethylsulfoxide (DMSO-*d6*) solutions of the samples. The chemical shifts δ are given in ppm, with respect to tetramethylsilane as an internal standard. The structures of compounds are drawn with the help of chem. draw 8.0. The mass spectra are recorded on 6460 Triple Quadruple LC/MS model. For Molecular docking, we used bioinformatics tools, biological databases like Drug Bank, PDB (Protein Data Bank) and software's like Hex, Biova discovery studio 4.5visualizer.Chem. draw is used for effective drawings of 2D-3D structures of compounds and it helps the chemists to draw molecules, reactions and schematic diagrams, calculate chemical properties conveniently. The 2D-3D structures of cinnamic acid esters of Salicylanides are constructed on chem. draw 8.0.Then these chem. draw files are converted into protein data bank files using Biova discovery studio 4.5visualizer. We obtained crystal structure of β-Ketoacyl-acyl carrier protein synthase III (pdb id: 1hnj) for antibacterial activity from the Protein Data Bank. All these structures are utilized for molecular docking process using software Hex 6.0.

Procedure: Synthesis of cinnamic acid ester derivatives of Salicylanides

A solution of Cinnamic acid (5 mmol), DCC (5.5 mmol), Salicylanide (5 mmol) and DMAP (5 mmol) in dichloromethane (50 mL) is refluxed for 4 hours. The *N*,*N*'-dicyclodexylurea is filtered off and the filtrate is washed with water(3650 mL), 5% acetic acid solution (3650 mL) again with water (3650 mL) and then dried over anhydrous sodium sulphate. The solvent is evaporated under reduced pressure to give the ester, which is chromatographed over a column of silica gel using petroleum ether diethyl ether (95:5, vol/vol) as eluent.

1. (E)-2-(2-chlorophenylcarbamoyl) phenyl cinnamate (5a)

Yield: 67% m.p.-92-94^{0}C, IR Cm-1:1739.85 (CO ester), 1680 (CO amide), 3390 (N-H stretch), ^{1}HNMR: 7.95-7.0 (m, 13H), 7.64(d, 1H), 6.74 (d, 1H), 8.9 (bs , 1H, NH), ^{13}C NMR: 164.9, 163.2, 148.1, 134.8, 132.6, 130.9, 129.07, 128.5, 127.8,127.7,126.5, 124.8,123.4 121.9, 116.2.MS:400 C, Anal. Calcd. For $C_{22}H_{16}ClNO_3$; 69.94; H, 4.27; Cl, 9.38; N, 3.71; O, 12.70 Found 69.27; H, 5.07; Cl, 9.20; N, 3.51; O, 12.41.

2. (E)-2-(3-methoxyphenylcarbamoyl) phenyl cinnamate (5b)

Yield: 69% m.p.-145-147^{0}CIR Cm-1: 1751.42 (CO ester), 1637.62(CO amide), 3346.61(N-H stretch) ^{1}H NMR: 8.0-7.0 (m, 13 H) , 7.6(d, 1H), 6.7 (d, 1H), 3.7 (s, 3H), 8.3 (bs, 1H, NH).^{13}C NMR: 165.29, 163.5, 160.2, 148.2, 147.7, 139.0, 133.7, 132.2, 131.2, 130.0, 129.7, 129.0, 128.9, 128.5, 126.6, 123.3, 116.0, 112.0, 110.6, 112.0, 105.5, 55.2. Anal. Calcd. For $C_{23}H_{19}NO_4$ C, 73.98; H, 5.13; N, 3.75; O, 17.14 Found C, 73.87; H, 5.21; N, 3.34; O, 17.21

3. (E)-2-(phenyl carbamoyl) phenyl cinnamate (5c)

Yield: 64% m.p.-148-150^{0}C IR Cm-1: 1720.56 (CO ester), 1654.98(CO amide), 3338.89(N-H stretch)^{13}C NMR: 165.3, 163.6, 148.2, 147.7, 137.8, 133.7, 132.1, 131.1, 130.3, 129.1, 129.0, 129.0, 128.5, 126.6, 124.6, 123.3, 120.0, 116.0.^{1}H NMR: 7.56-7.1 (m, 14H), 7.9(d, 1H), 6.69(d, 1H), 8.3 (bs , 1H, NH) Anal. Calcd. For $C_{22}H_{17}NO_3$ C, 76.95; H, 4.99; N, 4.08; O, 13.98.Found C, 76.38; H, 4.37; N, 4.11; O, 13.50.

4 (E)-2-(3-chlorophenylcarbamoyl) phenyl cinnamate (5d)

Yield: 60% m.p.-188-190^{0}CIR Cm-1: 1718.63 (CO ester), 1654.98(CO amide), 3331.18 (N-H stretch) ^{1}H NMR: 7.96-7.08 (m, 13H) , 7.64(d, 1H), 6.69 (d, 1H), 8.3 (bs , 1H, NH) ^{13}C NMR: 165.3, 163.6, 148.4, 148.0, 138.9, 134.7, 133.6, 132.4, 130.0, 128.9, 128.8, 128.5, 128.4, 126.2, 126.6, 124.6, 122.7, 120.06, 119.3, 115.8. Anal. Calcd. For $C_{22}H_{16}ClNO_3$ C, 69.94; H, 4.27; Cl, 9.38; N, 3.71; O, 12.70.found C, 70.35; H, 3.73; Cl, 9.50; N, 3.52; O, 12.65

5 (E)-2-(4-bromophenylcarbamoyl) phenyl cinnamate (5e)

Yield: 66% m.p.-176-178^{0}C IR Cm-1: 1720.56 (CO ester), 1649.19(CO amide), 3309.96 (N-H stretch) ^{1}H NMR: 7.96-7.1 (m, 13H), 7.7(d, 1H), 6.68 (d, 1H), 8.24 (bs , 1H, NH) ^{13}C NMR: 165.3, 165.0, 148.4, 147.7, 136.8, 132.8, 132.4, 132.4, 129.1, 129.0, 128.9, 128.7, 126.6, 126.2, 123.3, 122.7, 119.4, 115.8. 6 Anal. Calcd. For $C_{22}H_{16}BrNO_3$ C, 62.57; H, 3.82; Br, 18.92; N, 3.32; O, 11.37 Found C, 62.97; H, 3.53; Br, 17.98; N, 3.12; O, 11.86.

6 (E)-2-(4-chlorophenylcarbamoyl) phenyl cinnamate (5f)

Yield: 62% m.p.-180-184^{0}C IR Cm-1: 1720.56 (CO ester), 1636.55(CO amide), 3321.53(N-H stretch) ^{1}H NMR: 7.92-7.2 (m, 13H) , 7.64(d, 1H), 6.65 (d, 1H), 8.2 (bs , 1H, NH) ^{13}C NMR: 165.3, 163.6, 148.4, 147.7, 136.3, 132.3, 133.6, 130.2, 129.5,129.1, 128.7, 128.5, 126.6, 123.3, 121.1, 115.8. Anal. Calcd. For $C_{22}H_{16}ClNO_3$ C, 69.94; H, 4.27; Cl, 9.38; N, 3.71; O, 12.70, Found C, 69.23; H, 4.12; Cl, 9.28; N, 3.42; O, 12.62.

7 (E)-2-(p-tolylcarbamoyl) phenyl cinnamate(5g)
Yield: 59% m.p.-140-144^{0}C IR Cm-1: 1718.63 (CO ester), 1654.98(CO amide), 3329.25(N-H stretch) ^{1}H NMR: 7.96-7.1 (m, 13H) , 7.64 (d, 1H), 6.6 (d, 1H), 8.1 (bs , 1H, NH), 2.19 (s, 3H).^{13}C NMR: 168.4, 165.3, 164.9, 148.1, 147.7, 135.3, 134.2, 132.0, 132.0, 129.7, 129.5, 128.9, 128.8, 128.5, 128.1, 126.5, 126.1, 123.2, 122.3, 119.7, 116.4, 21.2. Anal. Calcd. For $C_{23}H_{19}NO_3$ C, 77.29; H, 5.36; N, 3.92; O, 13.43. Found C, 76.99; H, 5.12; N, 3.32; O, 13.21.

8 (E)-2-(naphthalen-1-ylcarbamoyl) phenyl cinnamate (5h)
Yield: 67% m.p.-186-188^{0}C, IR Cm-1: 1739.85 (CO ester), 1631.83 (CO amide), 3267.52(N-H stretch)^{13}C NMR: 165.3, 164.3, 148.3, 147.9, 134.4, 134.2, 134.1, 132.3, 128.9, 128.8, 128.6, 128.4, 126.9, 126.8, 126.5, 126.3, 127.05, 126.0, 125.8, 125.6, 123.3, 121.6, 120.7, 119.0, 116.0 ^{1}H NMR: 8.1- 7.0 (m, 16H), 7.7(d, 1H), 6.6 (d, 1H), 8.7 (bs, 1H, NH) Anal. Calcd. For $C_{26}H_{19}NO_3$ C,79.37; H, 4.87; N, 3.56; O, 12.20. Found C, 78.87; H, 4.54; N, 3.45; O,12.32.

9 (E)-2-(o-tolylcarbamoyl) phenyl cinnamate (5i)
Yield: 55% m.p.-144-146^{0}C, IR Cm-1: 1730.21 (CO ester), 1641.48(CO amide), 3315.74(N-H stretch), ^{1}H NMR: 7.94-6.85 (m, 13H), 3.77 (s, 3H), 7.7(d, 1H), 6.6 (d, 1H), 8.2(bs , 1H, NH),^{13}C NMR: 165.3, 163.5, 156.5, 148.1, 133.7, 132.0, 131.1, 130.9, 130.1, 129.5, 128.9, 128.8, 128.5, 128.5, 126.5, 123.2, 121.8, 116.1, 114.9. Anal. Calcd. For $C_{23}H_{19}NO_3$ C, 77.29; H, 5.36; N, 3.92; O, 13.43. Found 78.20; H, 5.14; N, 4.12; O, 13.25

10 (E)-2-(4-methoxyphenylcarbamoyl) phenyl cinnamate (5j)
Yield: 58% m.p.-225-227^{0}C IR Cm-1: 1751.30 (CO ester), 1637.62(CO amide), 3346.61(N-H stretch) ^{1}H NMR: 8.0-7.0 (m, 13 H) , 7.6(d, 1H), 6.7 (d, 1H), 3.7 (s, 3H), 8.3 (bs, 1H, NH).^{13}C NMR: 165.29, 163.5, 160.2, 148.2, 147.7, 139.0, 133.7, 132.2, 131.2, 130.0, 129.7, 129.0, 128.9, 128.5, 126.6, 123.3, 116.0, 112.0, 110.6, 112.0, 105.5, 55.2 Anal. Calcd. For $C_{23}H_{19}NO_4$ C, 73.98; H, 5.13; N, 3.75; O, 17.14. Found C, 72.90; H, 5.54; N, 3.65; O, 17.10.

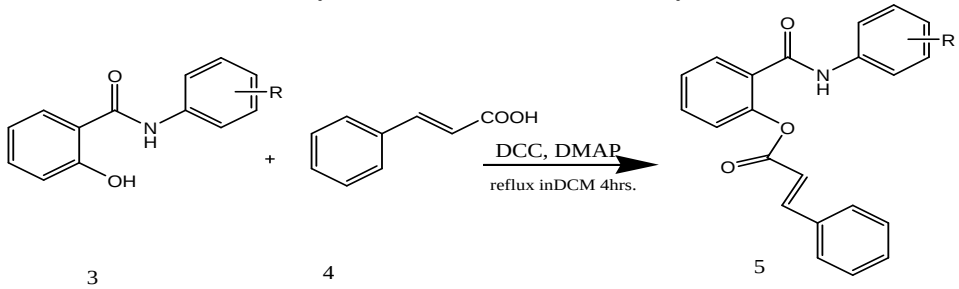
11 (E)-2-(4-nitrophenylcarbamoyl) phenyl cinnamate (5k)
Yield: 50% m.p.-charred 265^{0}C, IR Cm-1: 1724.42(CO ester), 1626.05(CO amide), 3327.32(N-H stretch), ^{1}H NMR: 8.05- 7.2 (m, 13 H), 7.7(d, 1H), 6.5 (d, 1H).^{13}C NMR: 169.9, 169.5, 151.9, 150.2, 147.6, 138.6, 135.7, 132.7, 130.6, 129.4, 129.3, 127.9, 124.4, 124.2, 121.4. Anal. Calcd. For $C_{22}H_{16}N_2O_5$ C, 68.04; H, 4.15; N, 7.21; O, 20.60.Found C, 67.53; H, 3.85; N, 7.43; O, 20.51.

12 (E)-2-(3-nitrophenylcarbamoyl) phenyl cinnamate (5l)
Yield: 54% m.p.-charred 250^{0}C, IR Cm-1: 1705.13(CO ester), 1672.34 (CO amide), 3329.25(N-H stretch), ^{1}H NMR: 8.6- 7.3 (m, 13 H), 7.64 (d, 1H), 6.8 (d, 1H), 8.7 (bs, 1H, NH) ^{13}C NMR:165.15,148.4, 148.38,147.18,140.64,134.16, 132.5, 131.43,130.64,129.7,129.7, 129.4, 126.5,126.14,123.85, 118.6,114.2 Anal. Calcd. For $C_{22}H_{16}N_2O_5$ C, 68.04; H, 4.15; N, 7.21; O, 20.60. Found C, 68.19; H, 4.22; N, 7.22; O, 21.11
13 (E)-2-(cyclohexylcarbamoyl) phenyl cinnamate(5m)
Yield: 72% m.p.-70-72^{0}C, IR Cm-1: 1724.42 (CO ester), 1633.76(CO amide), 3309.96(N-H stretch), ^{1}H NMR: 7.91- 7.16 (m, 9 H), 7.64(d, 1H), 6.64 (d, 1H).7.9(s,1H, NH), 1.9-1.1 (m, 10H), 2.18(d, 1H),^{13}C NMR: 165.3, 164.7, 156.8, 147.8, 147.6, 133.8, 131.5, 129.4, 129.1, 128.4, 127.7, 126.4, 116.3, 48.4, 33.9, 32.9, 29.3, 24.9, 24.6. Anal. Calcd. For $C_{22}H_{23}NO_3$ C, 75.62; H, 6.63; N, 4.01; O, 13.74. Found C, 75.82; H, 6.32; N, 4.21; O, 12.95.

RESULTS AND DISCUSSION

Cinnnamic acid esters of Salicylanides are prepared by esterification of Salicylanides with cinnamic acid using dicyclohexyl carbodiimide (DCC) as a couling agent and dimethyl amino pyridine as a base refluxed for four hours [25] in anhydrous dichloromethane (scheme1).

Scheme 1: Synthesis of cinnamic acid esters of Salicylanides.



3 4 5

In vitro Antimicrobial Evaluation:

Antimicrobial properties of thirteen Cinnamic acid esters of salicylanide derivatives are assayed against bacterial strain. This testing is carried out using disc diffusion method. Various bacterial strains - *Staphylococcus aureus* (NCIM 2079), *Bacillus subtilis (*NCIM 2250), *Escherichia coli* (NCIM 2109), *Pseudomonas aeruginosa (*NCIM 2036*)* and fungal strains *Candida albicans* (NCIM 3471), *Aspergillus niger (*NCIM *545)* are used as test microorganism to evaluate the antimicrobial testing of newly synthesized compounds.

Pure culture of test bacterial strain is picked with a loop, and the growth is transferred into a tube containing 5 ml nutrient broth medium, while pure culture of test fungal strain is transferred into a tube containing 5 ml of a MGYP medium. The broth culture is incubated at 37°C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually to 6 hours). The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain turbidity optically comparable to that of the 0.5 McFarland standard. This resulting suspension contains 2 x 10^8 CFU/ml of microbial cells.

Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab is rotated several times and pressed firmly on the inside wall of the tube above the fluid level. The surface of a nutrient agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking several times, rotating the plate approximately through 60° each time to ensure an even distribution of inoculum. Stock solution [1000 microgram per ml] of each newly synthesized compounds are prepared in dimethylsulfoxide (DMSO).

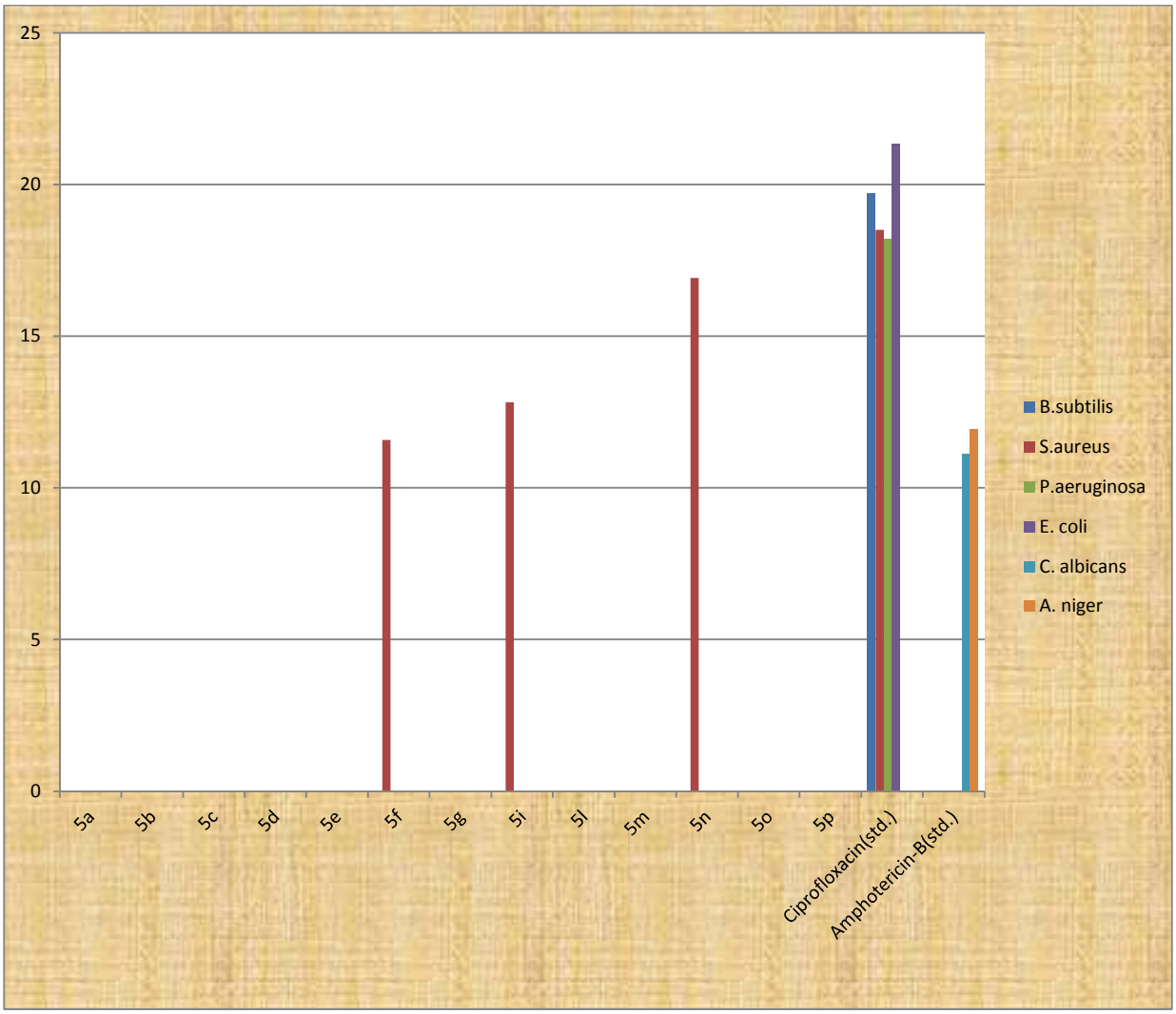
The sterile discs of 6 mm diameter are used in this assay. The disc diffusion assay is carried out by taking concentration 100 microorganism per disc**.** The discs immersed with compounds are dispensed onto the surface of the inoculated agar plate. Also, Ciprofloxacin (10 microgram/disk, Amphotericin-B (100 units/disk) [Hi-media, Mumbai, disc diameter 6 mm] moistened with DMSO are placed on agar plate as standard. Each disc is pressed down to ensure complete contact with the agar surface. The plates is placed in a refrigerator at to 8°C for 30 minutes and then incubated in incubator at 37°C for 24 hours. After 24 hours of incubation, each plate is examined. The diameters of the zones of complete inhibition including the diameter of the disc are measured using Vernier calliper, which is held on the back of the inverted Petri plate. Table 1overviews the antibacterial and antifungal properties.

Table 1:In vitro antibacterial activity and their cinnamic acid esters of Salicylaindes towards bacteria and fungi.

Sr. No	**Code**	**Amines**	***B.subtilis***	***S.aureus***	***P.aeruginosa***	***E. coli***	***C. albicans***	***A. niger***
1	5a	2-chloroaniline	-	-	-	-	-	-
2	5b	3-methoxy aniline	-	-	-	-	-	-
3	5c	aniline		-	-	-	-	
4	5d	3- chloroaniline	-	-	-	-	-	-
5	5e	4-bromoaniline	-	-	-	-	-	-
6	5f	4- chloroaniline	-	11.58	-	-	-	-
7	5g	p-Toludine	-	-	-	-	-	-
8	5h	Naphthyl amine	-	12.82	-	-	-	-
9	5i	o-Toludine	-	-	-	-	-	-
10	5j	p-anisidine	-	-	-	-	-	-
11	5k	4-nitroaniline	-	**16.92**	-	-	-	-
12	5l	3-nitroaniline	-	-	-	-	-	-
13	5m	Cyclohexyl amine	-	-	-	-	-	-
14	Ciprofloxacin(std.)		19.7	18.5	18.19	21.32	NA	NA
15	Amphotericin-B(std.)		NA	NA	NA	NA	11.12	11.92

In case of cinnamic acid esters of Salicylanides, compounds 5f, 5h and 5k show good antibacterial activity only against S.aureus. In all above ester derivatives, compound **5k** shows significant antibacterial activity against S.aureus. As we compare antimicrobial activity of Salicylanides with their cinnamic acid ester derivatives, from all the above observations it is clear that all Salicylanides show better antibacterial activity than their cinnamic acid ester derivatives.

Graph 1: Biological activities of Cinnamic acid esters of salicylanide



Molecular docking

Following parameters are used for the molecular docking process.

- Correlation type – Shape + Electrostatics
- FFT Mode – 3D
- Post Processing–MM Energies
- Grid Dimension – 0.6
- Receptor range – 180
- Ligand Range – 180
- Twist range – 360
- Distance Range – 40

Using all these parameters cinnamic acid esters of Salicylanides are docked against with β-Ketoacyl-acyl carrier protein synthase III (ecKAS III).

The three dimensional structure of ecKAS III enzyme formed complex with cinnamic acid esters of Salicylanides with good binding site. This method helps us to study how these ester molecules fit into the active sites β-Ketoacyl-acyl carrier protein synthase III. From this study we found the binding affinity of protein with target molecule (ligand). We carried out 10 docking operations for each ligand and selected top score for binding affinity of each ligand-enzyme complex.

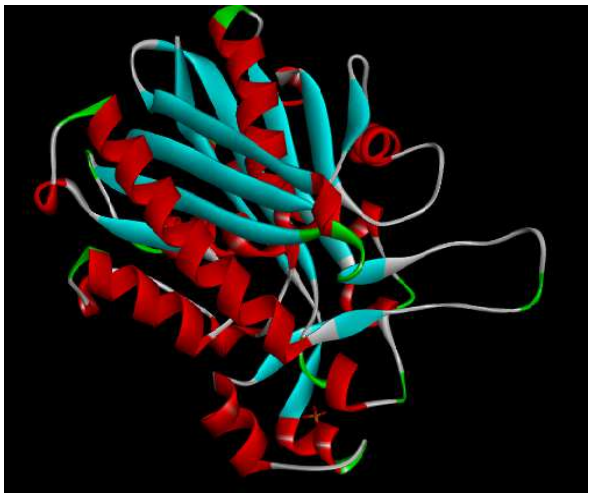


Fig 1: Structure of receptor β-Ketoacyl-acyl carrier protein synthase III (pdb id: 1hnj)

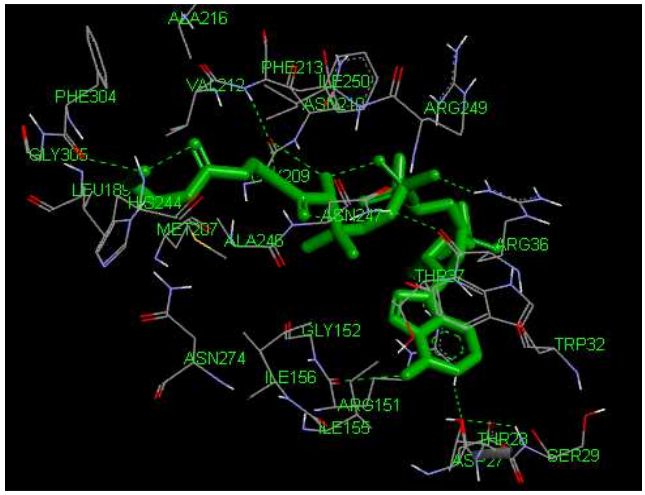


Fig 2: compound 5a with active site of 1hnj

Table 2: Binding energies of cinnnamic acid esters of Salicylanides

Sr. No	Compound code	E-Value	H Bond
1	5a	-144.88	1
2	5b	-55.94	1
3	5c	-107.21	1
4	5d	-67.38	1
5	5e	-56.97	1
6	5f	-56.27	1
7	5g	-101.81	1
8	5h	-119.51	1
9	5i	-60.75	1
10	5j	-78.86	1
11	5k	-55.65	1
12	5l	-124.76	1
13	5m	-135.12	1

Here, we have noted that there is a variation in the results obtained from the experimental and theoretical data. From the above table, the results indicate that cinnamic acid ester derivatives of Salicylanides show moderate binding energy with strong hydrogen bonding. Parent Salicylanides show significant binding energy than all other ester derivatives that is parent Salicylanides form more stable complex with ecKAS III enzyme with minimum energy. Among all the Salicylanides and its cinnamic acid ester derivatives compounds **5a** exhibit good binding affinity with ecKAS III.

CONCLUSION

We have synthesized thirteen cinnamic acid ester derivatives successfully. All the synthesized compounds are characterized and also checked for the antibacterial and antifungal activity of these ester derivatives in vitro. In all Salicylanide esters of cinnamic acid; only compounds **5f, 5h** and **5k** showed antibacterial activity. Further Molecular docking of all these esters is carried out using β Ketoacyl-acyl carrier protein synthase III from Escherichia coli (ecKAS III pdb id: 1HNJ) as a receptor responsible for growth of bacteria in silico. Among all the ester derivatives compound **5a** exhibit good binding interactions with β-Ketoacyl-acyl carrier protein synthase III. Compounds **5f, 5h** and **5k** can be utilised as an antimicrobial agent against S.aureus in future.

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