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Synthesis, Antimicrobial Activity and Docking Study of Substituted Bis (2-(Phenyl Carbamoyl) Phenyl) Phthalate

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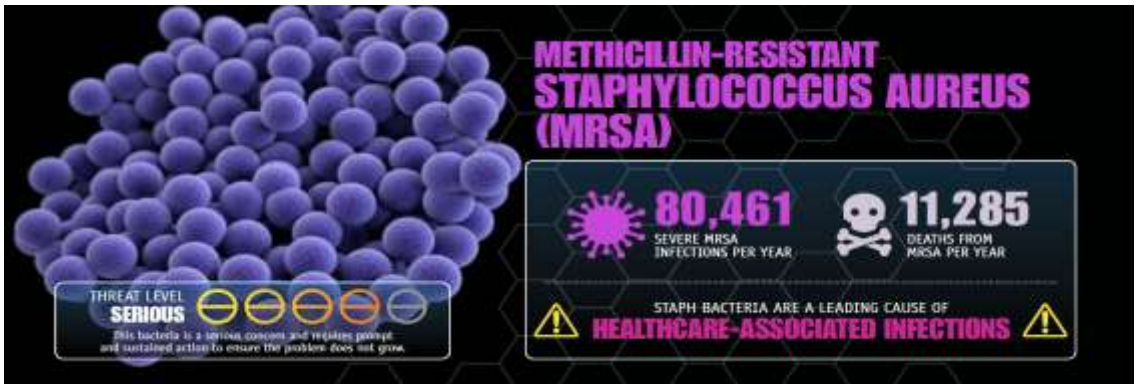
Abstract: *Considering the increase in resistance of pathogens against antibiotics, we have synthesized the new Salicylanides ester derivatives of phthalic acid. All the synthesized derivatives are characterized by using spectroscopic techniques such as IR, H^1 and C^{13} NMR. The antimicrobial activity of all derivatives is investigated against four bacterial and two fungal strains in vitro. Also compared the antimicrobial activity of these Salicylanides ester derivatives of Phthalic acid with their parent Salicylanides; to get knowledge about the effect of functional group substitution on antimicrobial activity. Molecular docking of synthesized compounds is also performed in silico with enzyme from Escherichia coli β -Ketoacyl-acyl carrier protein synthase III [24] (ecKAS III pdb id: 1HNJ) as a receptor which is responsible for growth of bacteria.*

Keywords: *Phthalic acid, Salicylanides, antimicrobial activity, enzyme, receptor, esters.*

I. Introduction

The raise of viral, fungal and bacterial diseases is a major concern of world since last two decades. Due to infections of microbes, diseases like malaria and tuberculosis cause millions of death every year [1]. Microbes such as *Staphylococcus aureus* , *Escherichia Coli, Bacillus subtilis* and *Pseudomonas aeruginosa* tremendously affect on human health. The infections due to *E. coli* causes the diseases like enteric/diarrhoeal disease, urinary tract infections (UTIs) and sepsis/meningitis, hemorrhagic colitis, hemolytic-uremic syndrome and thrombotic thrombocytopenic purpura [2, 3]. Another disease causing microbe is *Bacillus subtilis*. This is food poisoning organism leads to gastrointestinal diseases like diarrhoea and hepatic failure respiratory infections, septicaemia and endocarditis, and even central nervous system infections [4]. The yeast, *candidia albicans* cause severe intestinal and skin problems. The infections seen in the patients suffer from leukomia due to weakened immune system [5]. Different Candida species causes the diseases like invasive candiasis, mucosal cadiasis, pneumonia and septicaemia [6, 7]. Recent examples of disease causing microbes are methicillin resistant *Staphylococcus aureus* (MRSA) bacteria, West Nile virus, and 2009 H_1N_1 influenza virus [8].*Staphylococcus aureus* (MRSA) is a major concern amongst all pathogens due to their rise in drug resistance [9]. *Staphylococcus aureus* causes skin, soft tissue infections and nosocomial infections [10, 11]. The figure indicates the number of deaths causes by the infections of *Staphylococcus aureus* (MRSA) per year [12].



Antibiotics such as lincosamides, cotrimoxazole, linezolid and quinupristin/dalfopristin are used on the treatment of infections caused by *Staphylococcus aureus* (MRSA), but these are expensive [13].

Salicylanides and their ester derivates are the important class of compounds possessing excellent antimicrobial properties [14-17] .They are also good anti-tubercular and antifungal agent [18-22]. In previous papers, we have mentioned different Salicylanides, their cinnamic acid esters and also investigated their

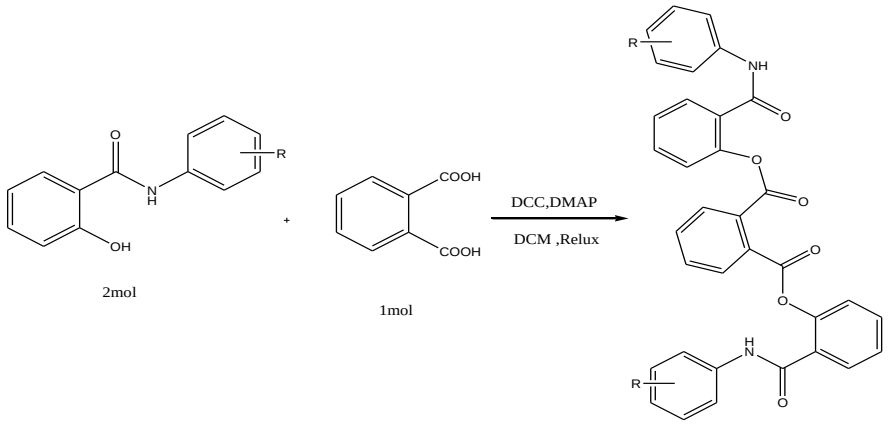
antibacterial activity in vitro [23.24]. Molecular docking is a method of getting information about drug activity against protein responsible for disease causing organism to inhibit the active sites with the help of computational chemistry. It facilitates to find the binding interactions of drug molecule with protein. In the present study, Phthalic acid esters of Salicylanides are synthesized using coupling agent DCC and screened for their antimicrobial activity in vitro. The aim of this study is to synthesize esters of Salicylanides with different acids, to compare the antimicrobial activities in vitro and to perform molecular docking with enzyme sterol 14α-demethylase (PDB entry: 3GW9) which is responsible for growth of yeast and our synthesized derivatives as ligands [25].

II. 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.

III. Results and Discussion

Esterification of Salicylanides with Phthalic acid was performing by using dicyclohexyl carbodiimide (DCC) [26] as a coupling agent, dimethyl amino pyridine (DMAP) as a base in dichloromethane refluxed for four hours.



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Scheme I: Synthesis of Substituted Bis (2-(Phenyl Carbamoyl) Phenyl) Phthalate

3.1 In vitro Antimicrobial Evaluation

Antimicrobial properties of thirteen Phthalic acid esters of salicylanide derivatives are assayed against bacterial strain. This testing is carried out using disc diffusion method. Various bacterial strains - *Bacillus subtilis (*NCIM 2250), *Staphylococcus aureus* (NCIM 2079), *Pseudomonas aeruginosa (*NCIM 2036*), Escherichia coli* (NCIM 2109), 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.

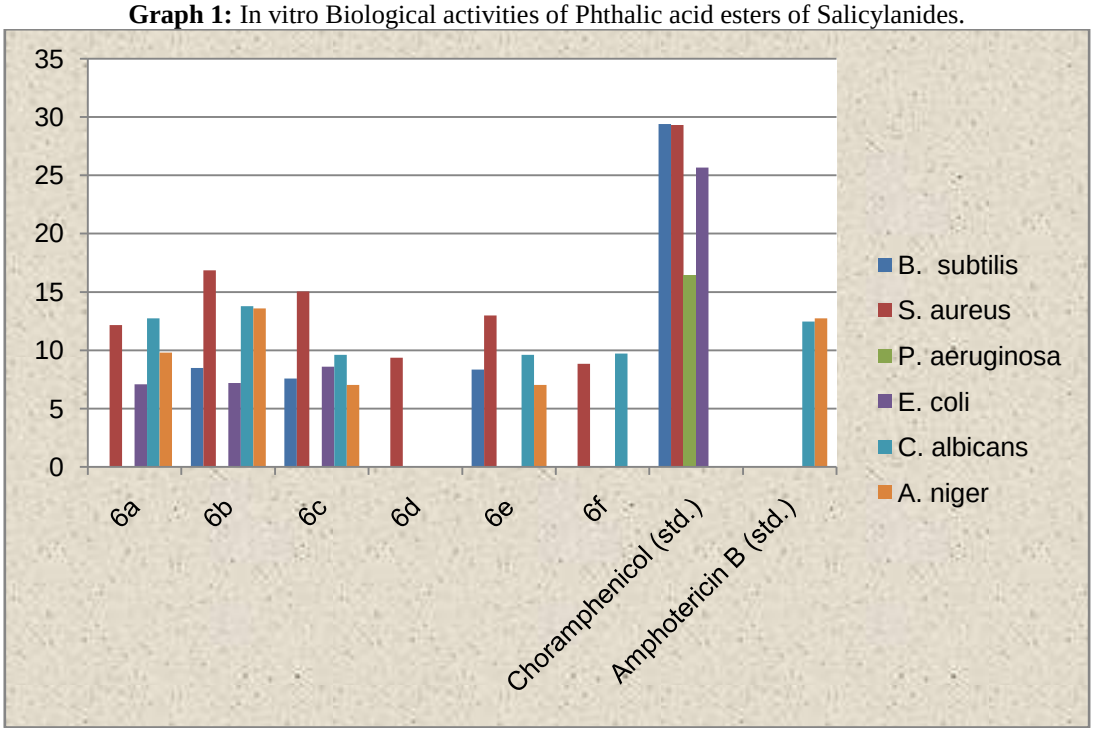
Table1: In vitro antibacterial activity and their Phthalic acid esters of Salicylaindes towards bacteria and fungi.

Sr. No	Code	R Group	B. subtilis	S. aureus	P. aeruginosa	E. coli	C. albicans	A. niger
1	6a	3-methoxy aniline	-	12.16	-	7.08	12.73	9.80
2	6b	aniline	8.48	16.85	-	7.20	13.79	13.59
3	6c	4-bromoaniline	7.59	15.05	-	8.59	9.62	7.02
4	6d	p-Toludine	-	9.35	-	-	-	-
5	6e	o-toludine	8.34	12.98	-	-	9.61	7.03
6	6f	4-methoxy aniline	-	8.85	-	-	9.71	-
7	Choramphenicol (std.)		29.40	29.33	16.43	25.66	NA	NA
8	Amphotericin B (std.)		NA	NA	NA	NA	12.46	12.75

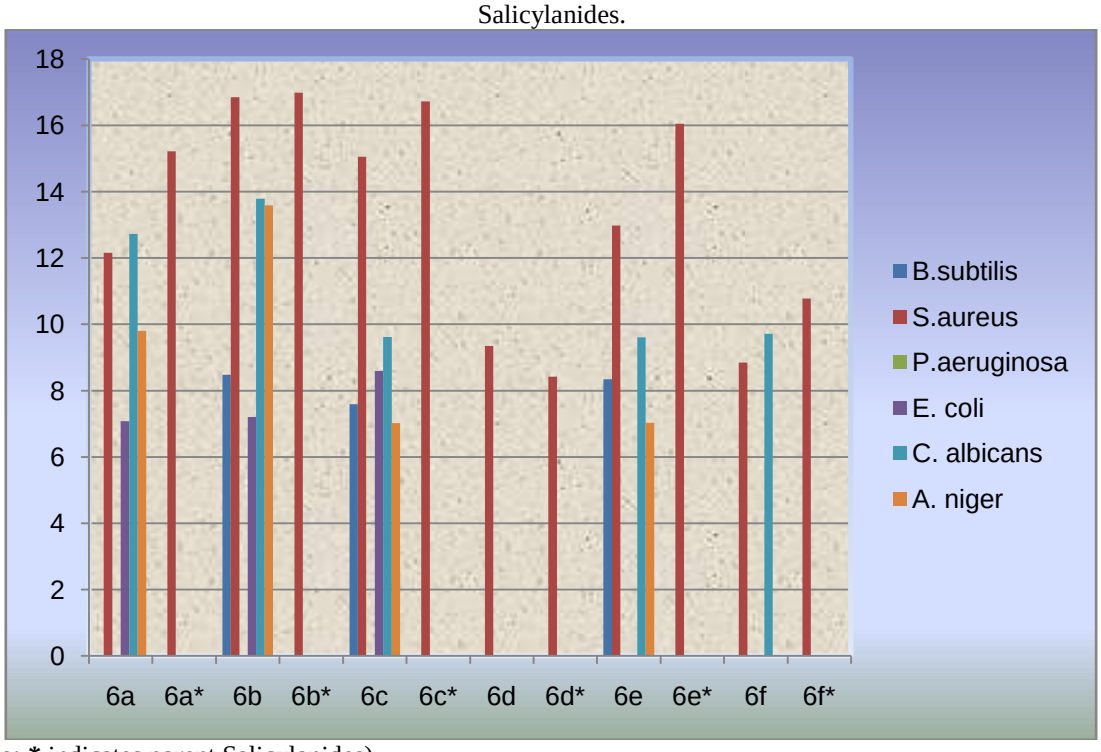
In above table the experimental value of antimicrobial activities of synthesized compounds is shown. In all derivatives compounds 6b, 6c and 6e showed antibacterial activity against *B. subtilis*. All compounds possessed antibacterial activity , while compounds 6b and 6c showed almost half of the antibacterial activity as compared to standard drug choramphenicol. against *Staphylococcus aureus*. On the other hand compounds **6a**

and **6b** exhibited excellent antifungal activities against *C. albicans* and *A. niger* as compared to standard drug Amphotericin B. These compounds can replace the standard drug Amphotericin B.

Graph 1: In vitro Biological activities of Phthalic acid esters of Salicylanides.



Graph 2: Comparison of biological activities of Phthalic acid esters of SalicylanidesWith their parent Salicylanides.



(**Note: *** indicates parent Salicylanides)

From graph 2 we can compare the antimicrobial activities of Phthalic acid esters of Salicylanides with their parent Salicylanides.

From the above graph it is clear that the parent salicylanides showing antibacterial activity against S. aureus only, while their Phthalic acid esters exhibits antibacterial and antifungal activity against all strains except *Pseudomonas aeruginosa*.

3.2 Molecular Docking

For docking study we have used here two docking software's hex 6.0 and iGEMDOCKv2.1. Hex is an interactive molecular graphics program for calculating and displaying possible docking modes of protein and drug molecules utilized for rigid protein- ligand docking [27]. GEM-dock is a generic evolutionary method for molecular docking [28] which gives the predicted poses of the ligand with respect to the active binding site of the protein [29]. It is the flexible protein- ligand docking protein which generates protein-compound interaction Profiles of electrostatic (E), hydrogen-bonding (H), and Van der Waal's (V) interactions [30] . For Molecular docking, we used bioinformatics tools, biological databases like Drug Bank, PDB (Protein Data Bank) and software's like Hex, GEM-dock, Pymol and Biova discovery studio 4.5 visualizer. Chem. Draw is used for effective drawings of 2D-3D structures of compounds. The 2D-3D structures of pthallic 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 the enzyme sterol 14α-demethylase (PDB entry: 3GW9) which is responsible for growth of yeast from Protein Data Bank.

3.2.1 Molecular Docking using Hex 6.0

Following parameters are considered to perform docking process.
Correlation Type ... Shape + Electrostatics
FFT Mode ... 3D
Post Processing ... MM Energies
Grid Dimension ... 0.6
Solutions ... 2000
Receptor Range ... 45
Receptor Step Size ... 7.5
Ligand Range ... 180
Ligand Step Size ... 7.5
Twist Range ... 360
Twist Step Size ... 5.5
Distance Range ... 40
Scan Step ... 0.8
Substeps ... 1
Steric Scan ... 20
Final Search ... 20

In this process sterol 14α-demethylase (PDB entry: 3GW9) docked with all synthesized compounds and form complex with minimum interaction energy and intermolecular hydrogen bonding interaction.

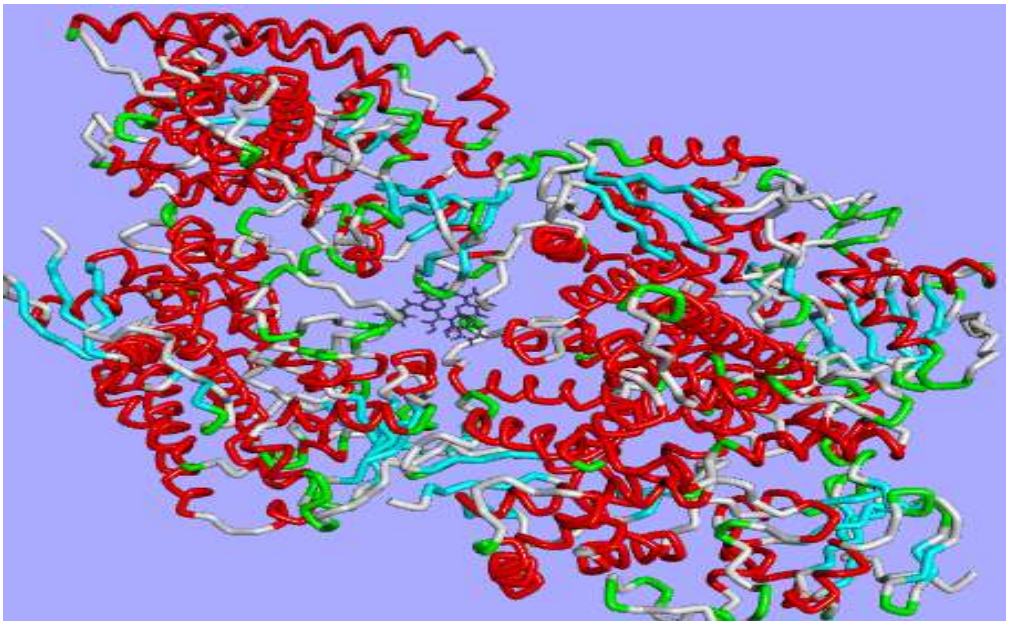
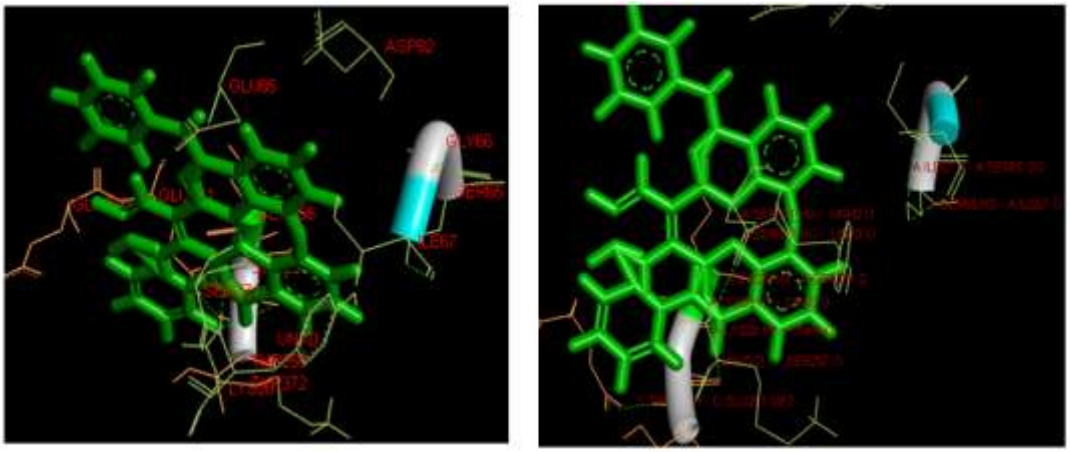


Fig 2: X-ray structure of complex of compound 6b with sterol 14α-demethylase (pdb id: 3GW9)



(A) **(B)**

Fig 3: Docking of compound 6b with active binding sites of sterol 14α-demethylase **(A)** and Intermolecular hydrogen bonding within amino acids residues and compound 6b **(B)**

Table 2: binding energies of Salicylanides esters of Phthalic acid using hex

Sr. No	Compound Code	E-Value	No. of H bonds
1	6a	-6165.3	1,GLU250
2	**6b**	**-11586.9**	**5,1 SER257,2 SER371, 2LYS28,**
3	6c	-	-
4	6d	-6224.9	3,1 TYR372,1 SER371,1 ASP82,
5	6e	-	-
6	6f	-7682.9	3, 1 GLY418,2 SER256

According to hex, among all derivatives compound **6b** shows very less binding energy with highest hydrogen bonding interactions i.e.5. Likewise compounds 6a, 6d and 6f exhibited less binding energy with 1, 3 and 3 hydrogen bonding interactions of amino acids residues of enzyme sterol 14α-demethylase. All compounds, except 6c and 6e are acts as inhibitors of enzyme sterol 14α-demethylase.

3.2.2 Molecular Docking using gem dock

Docking Accuracy settings (GA parameters)
Population size: 200
Generations: 70
Number of solutions: 2
Default setting: Standard docking
Docking Scoring Function: Gemdock Scoring Function
Ligand Preference
Hydrophobic preference: 1.00
Electrostatic preference: 1.00

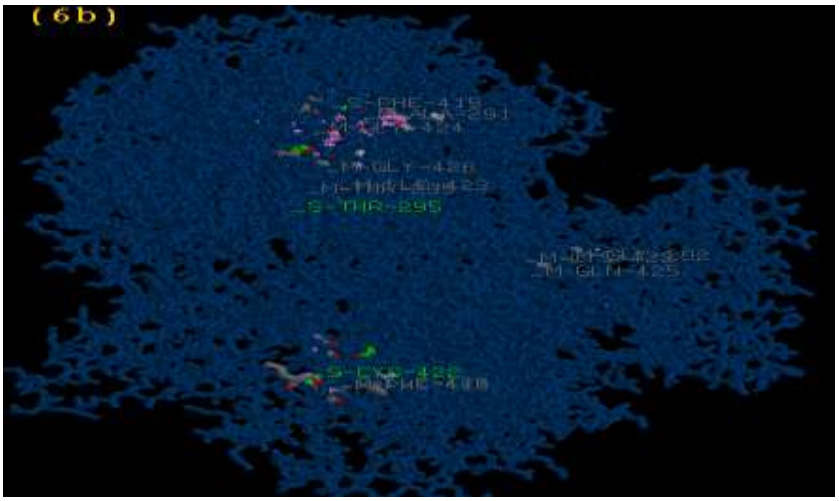


Fig 4: Complex of compound **6b** with sterol 14α-demethylase (PDB entry: 3GW9)

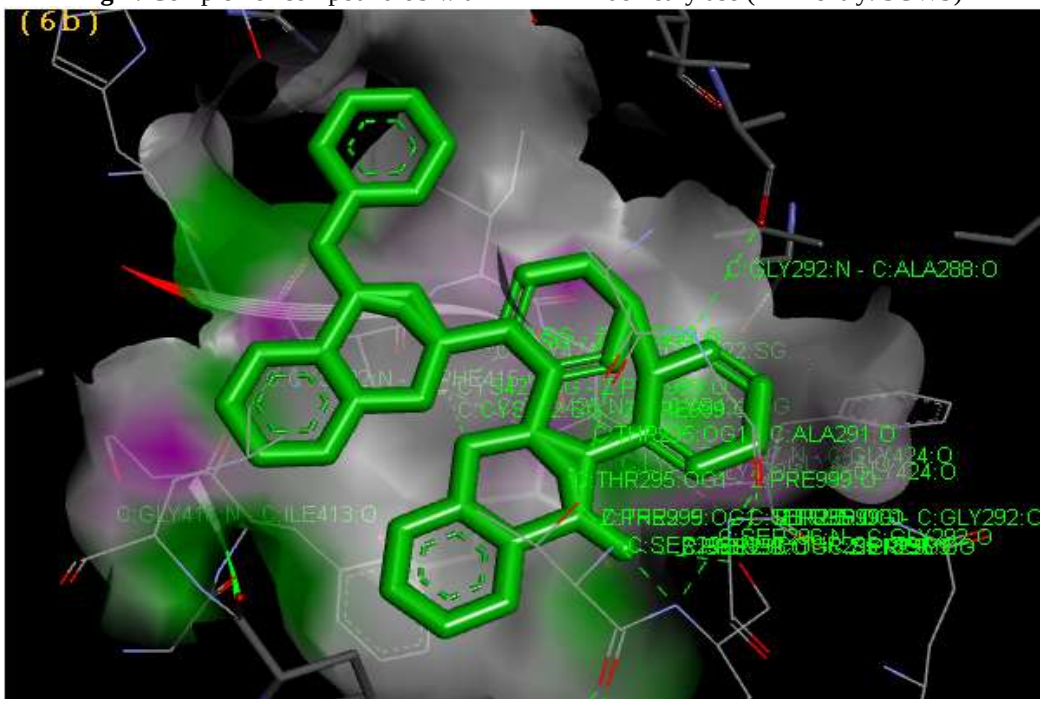


Fig 5: Intermolecular hydrogen bonding within amino acids residues of enzyme sterol 14α-demethylase (PDB entry: 3GW9) with compound 6b visualized in discovery studio visualize 4.5.

Table 2: binding score of Phthalic acid esters of Salicylanides using gem dock

Sr. No.	Compound code	Total Energy	VDW	H Bond Interaction	No. of H bond
1	6a	-164.529	-152.24	-12.289	5, 4CYS 422,1 HIS 420,
2	**6b**	-160.488	-139.969	-20.5188	7, 4CYS 422, 2THR295,1 SER 296,
3	6c	-131.439	-120.379	-11.0597	4, 2CYS 422, 1THR 299, 1TYR 116
4	6d	-152.97	-147.79	-5.18001	8,6CYS 422, 1GLY414, 1HIS420,
5	6e	-157.336	-145.422	-11.9137	8, 7CYS 422, 1HIS 420
6	6f	-127.952	-115.823	-12.1289	5, 4CYS 422, 1HIS 420

From above table it is clear that the all the compounds are good blockers of enzyme sterol 14α-demethylase (PDB entry: 3GW9) as they encompass very good binding score, Vander Waals and hydrogen bonding interactions. Among all derivatives compound **6a** shows very less interaction energy similarly compounds **6b** and **6e** also exhibited very less binding energies. Compounds 6d and 6e more actively bound with eight amino acids residues such as CYS 422, GLY and HIS 420. Derivatives 6b, 6a, 6f and 6c form 7, 5, 5, and 4 hydrogen bonds with amino acid residues respectively. All compounds are excellent inhibitors of enzyme sterol 14α-demethylase. fig 4 shows that these amino acids formed strong intermolecular hydrogen bonding interactions with compound 6b.

3.3 Experimental:

3.3.1 Procedure: Synthesis of Pthallic acid ester derivatives of Salicylanides

A solution of Pthallic acid (1 mol), DCC (1.1 mol), Salicylanide (1 mol) and DMAP (1 mol) in dichloromethane (50 ml) is refluxed for 4 hours. The *N,N*'-dicyclodexylurea is filtered off and the filtrate is washed with water, 5% acetic acid solution again with water 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. Bis (2-(3-metoxyphenyl carbamoyl) phenyl) Pthallate (6a)

Yield: 56%, m.p:108-110^{0}c, IR cm-1:1722.49 (CO ester), 1633.76 (CO amide), 3323.46 (N-H stretch).^{1}HNMR (DMSO-d^6): 8.02-6.5 (m, 20H), 8.0 (dd,2H,2 NH),3.73,(s, 6H, 2 OCH_3)
^{13}C NMR (DMSO-d^6): 169.69,167.39,159.71, 159.51,157.28, 138.66,135.52, 134.10,133.49, 132.82, 130.48, 130.23,129.84,129.34,129.04,128.03,127.66,124.80,123.27,118.55,118.48, 117.37,115.83, 113.48,113.35, 109.86,106.90. LC-MS: 471.3, 382.0, 225.1

2. Bis (2-(phenyl carbamoyl) phenyl) Pthallate (6b).

Yield: 61% m.p: 202-204^{0}C IR cm-1:1763.00 (CO ester), 1627.97(CO amide), 3327.32 (N-H stretch).
^{1}HNMR (DMSO-d^6):7.8-7.2(m,22H),7.9 (dd,2H, 2NH)^{13}C NMR (DMSOd^6):167.32, 161.71, 160.69, 156.90, 152.81, 136.84, 136.52, 134.58, 134.43, 131.78, 131.41, 129.41, 129.14, 128.50, 128.14, 128.07, 126.60, 125.83, 125.66, 125.26, 123.78, 121.27, 118.95, 118.83, 116.64, 114.82. Anal. Calcd. For $C_{34}H_{24}N_2O_6$: C, 73.37; H, 4.35; N, 5.03; O, 17.25, Found C, 72.78; H, 4.86; N, 5.27; O, 16.38.

3. Bis (2-(4- bromophenyl carbamoyl) phenyl) Pthallate (6c).

Yield: 65% m.p:152-154^{0}C IR cm-1:1710.92 (CO ester), 1612.54 (CO amide), 3313.82 (N-H stretch). ^{1}HNMR (DMSO-d^6):7.9-6.9 (m, 20H), 8.5 (s, 2H, 2NH) ^{13}C NMR (DMSOd^6):167.14, 166.94, 161.35,156.96, 136.71 136.08, 134.64,134.59, 132.82, 132.26,132.03,131.53,130.64,129.75,128.45,127.91,126.15, 125.78, 123.84, 122.72,121. 83,119.04, 118.69, 117.85, 116.64, 114.78. LC-MS: 471.3, 225.1, 123.0

4. Bis (2-(p-tolyl carbamoyl) phenyl) Pthallate (6d).

Yield: 68 % m.p:130-132^{0}C IR cm-1:1708.99 (CO ester),1629.90 (CO amide).3323.46 (NH stretch) ^{1}HNMR (DMSO-d^6): 8.1-6.9 9(m, 20H),7.9 (dd, 2H,2 NH), 2.4 (s, 6H, $2CH_3$)^{13}C NMR (DMSO-d^6): 168.25,167.50,161.63,138.23,135.10,134.46,134.32,134.00, 131.74, 129.75,129.62,126.43, 125.62, 123.66, 121.37, 118.87, 118.75, 114.72,26.13,25.48.

5. Bis (2-o-tolyl carbamoyl) phenyl) Pthallate (6e).

Yield: 64% m.p: 126-128^{0}C IR cm-1: 1728.28(CO ester),1629.90(CO amide).3323.46 (N-H stretch) ^{1}HNMR (DMSO-d^6):7.8-6.9 (m, 20 H), 8.06 (dd, 2H,2 NH),2.29 (s, 6H, $2CH_3$) ^{13}C NMR (DMSOd^6):166.10, 158.60, 136.67, 134.16,132.56, 132.19, 131.12, 130.80, 130.03, 129.77, 129.54, 129.34, 126.76, 126.44, 126.42, 125.63, 124.46, 123.32, 119.76, 117.60, 18.23,18.19. Anal. Calcd. For $C_{36}H_{28}N_2O_6$: C, 73.96; H, 4.83; N, 4.79; O, 16.42.Found: C, 73.23; H, 4.05; N, 5.11; O, 16.89.

6. Bis (2-(4-methoxyphenyl carbamoyl) phenyl) Pthallate (6f).

Yield: 59% m.p: Charred at 228^{0}C IR cm-1: 1740.21(CO ester), 1631.83 (CO amide).3321.53 (N-H stretch) ^{1}HNMR (DMSO-d^6):7.8-6.8 (m, 20 H), 7.9 (dd, 2H,2 NH), 3.7 (s, 6H, $2OCH_3$) ^{13}C NMR (DMSOd^6):167.85, 167.47,160.44,157.72,156.64, 136.02,134.50, 133.95, 133.80, 131.69, 130.91, 128.40, 128.13, 128.05, 125.27, 123.58, 123.50, 122.50, 118.81, 117.76, 116.13, 114.37, 113.94, 55.5, 55.4.

IV. Conclusion

We have successfully prepared the substituted bis (2-(phenyl carbamoyl) phenyl) pthallate derivatives. Antimicrobial activity of all the synthesized derivatives is checked in vitro. In all pthallic acid ester derivatives, compounds **6a** and **6b** exhibited excellent antifungal activities against *C. albicans* and *A. niger* as compared to standard drug Amphotericin B. All Parent Salicylanides shows antibacterial activity against *S. aureus* only but their esters shows antibacterial activity against all stains except *P.aeruginosa.* From molecular docking results we can conclude that these derivatives can be used as targets for sterol 14α-demethylase protein as a receptor responsible for growth of yeast (fungi) to inhibit its active sites.

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