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Research Paper

Microwave assisted Synthesis and microbial evaluation of novel dipyrimidone derivatives

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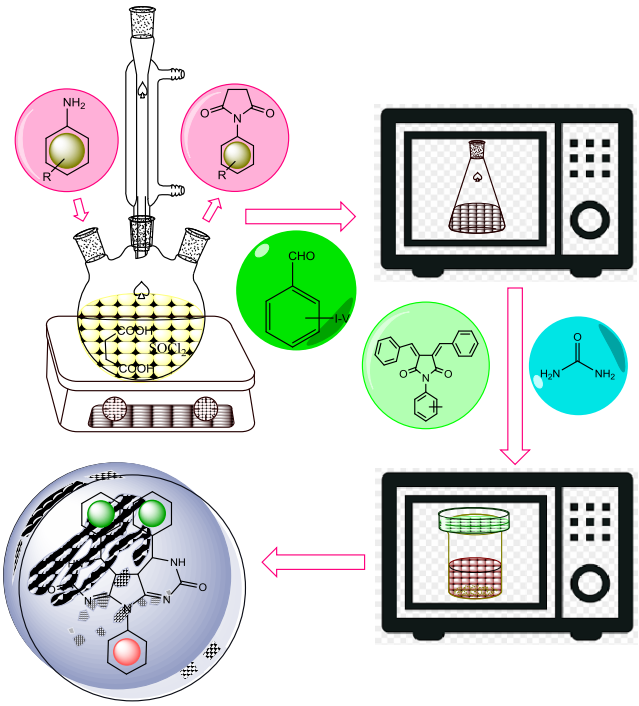
ABSTRACT : In this study synthesis of dipyrimidone derivatives have been synthesized form chalcones 1ai-v and 1a-e



KEYWORDS: *dipyrimidone derivatives,dinucleophilic nitrogen, chalcones, Antimicrobial Activities*

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I. INTRODUCTION

The pyrimidone or pyrimidine is one of the simple member of pyrimidone family .It is heterocyclic compounds which consist of four carbon atom and two nitrogen atoms in their cyclic ring structure. The pyrimidine was isolated between 1837 and 1864. Some important and well known biologically valuable compounds are Cytosine, Thymine, and Uracil. The Cytosine is very important nitrogenous base derived from pyrimidine which is present in nucleic acid. The pyrimidone molecule has Antitubercular activity [1-3], Antibacterial activity [4-5] some of its derivatives act as Antianginal drug [6] particularly used in the treatment of heart disease usually occurs due to not sufficient blood flow to heart. The compound containing pyrimidone functionality shows Antihypertensive effect [7] and Antiplatelate and Antithrombic activity[8] particularly used for preventing myocardial infarction and to reduce formation of blood clot[9]. The Thymine is the ring shaped

pyrimidine molecule and it was discovered by Albrecht Kossel and Albert Newman in year 1893. The Thymine was isolated from thymus glands. It has ability to form two hydrogen bonding with Adenine this leading to formation of DNA molecule. If the natural Structure of Thymine is defected in presence of ultraviolet light then mutation of DNA is take place. The mutated structure of DNA is not natural structure of DNA this affect the function of DNA so Cell growth is not regulated properly and hence possibility to growth of cancer cell. The compound incorporated pyrimidone moiety Shows Anti-oxidant [10], Analgesic [11-12] and Anti-inflammatory activities [13-14].The third important derivative of pyrimidone is Uracil. It is important naturally occurring pyrimidine derivative. The German chemist Robert Barnad synthesized uracil from uric acid derivative. The uracil is nucleo base nucleic acid of RNA. The uracil molecule helps to many necessary enzymes for functioning of cells. The fluorination of uracil forms fluorinated uracil which is act as anticancer drug. The fluorinated uracil inhibit the function of RNA replication enzyme due to this blocking of RNA synthesis is take place and stops the growth of cancer. The pyrimidone moiety is also present in vitamin B-9 i.e. folic acid is a valuable dietary supplement and important for healthy development of fetus during pregnancy and it act as Calcium Chanel modulator [15].

The pyrimidine derivatives like Zidovudine (AZT) and Idoxuridine are important class of anti-viral drugs which act as anti-HIV agents [16]. Such great application and lifesaving importance of pyrimidine derivatives stimulates to researcher to synthesize dipyrimidine -dione derivatives from N-phenyl substituted succinimidechalcone.In this study we have attempted synthesis of di-pyrimidone derivatives from our reported chalcone derivatives. We have developed green efficient method of formulation of di-pyrimidone derivatives here the combinatorial synthesis technique is used which involving grinding and irradiation by microwave radiation techniques. This method gave remarkable results that reduce reaction time.

II. PRESENT WORK

The synthesis of six member heterocyclic compound from N-substituted succinamidechalcone for that purpose urea is an important reagent which has dinucleophilic nitrogen atoms used for synthesis of pyrimidone derivatives. The various 2H-pyrrolo [2, 3-d: 5, 4-d'] dipyrimidine-2, 7(9H)-dione derivatives have been prepared from chalcone 7a-e and 8a-e. The two moles of Urea 24 reacted with one mole of chalcone 7a-e in presence of neutral alumina and irradiated it in microwave oven converted in to p-tolyl-2H-pyrrolo[2,3-d:5,4-d']dipyrimidine-2,7-(9H)-dione derivatives 25 a-e. By use of same procedure one mole of chalcone 8a-e reacted with two moles of Urea 24 converted in to 4-chlorophenyl-2H-pyrrolo [2,3-d:5,4-d'] dipyrimidine-2,7-(9H)-dione 26 a-e.

2.1PREPARATION OF PYRROLO DIPYRIMIDINE

General procedure for Synthesis of 4,5-bis-(p-tolyl)-3,4,4a,4b,5,6-hexahydro-2Hpyrrolo[2,3-d:5,4-d']dipyrimidine-2,7(9H)-dione (3ai-v):

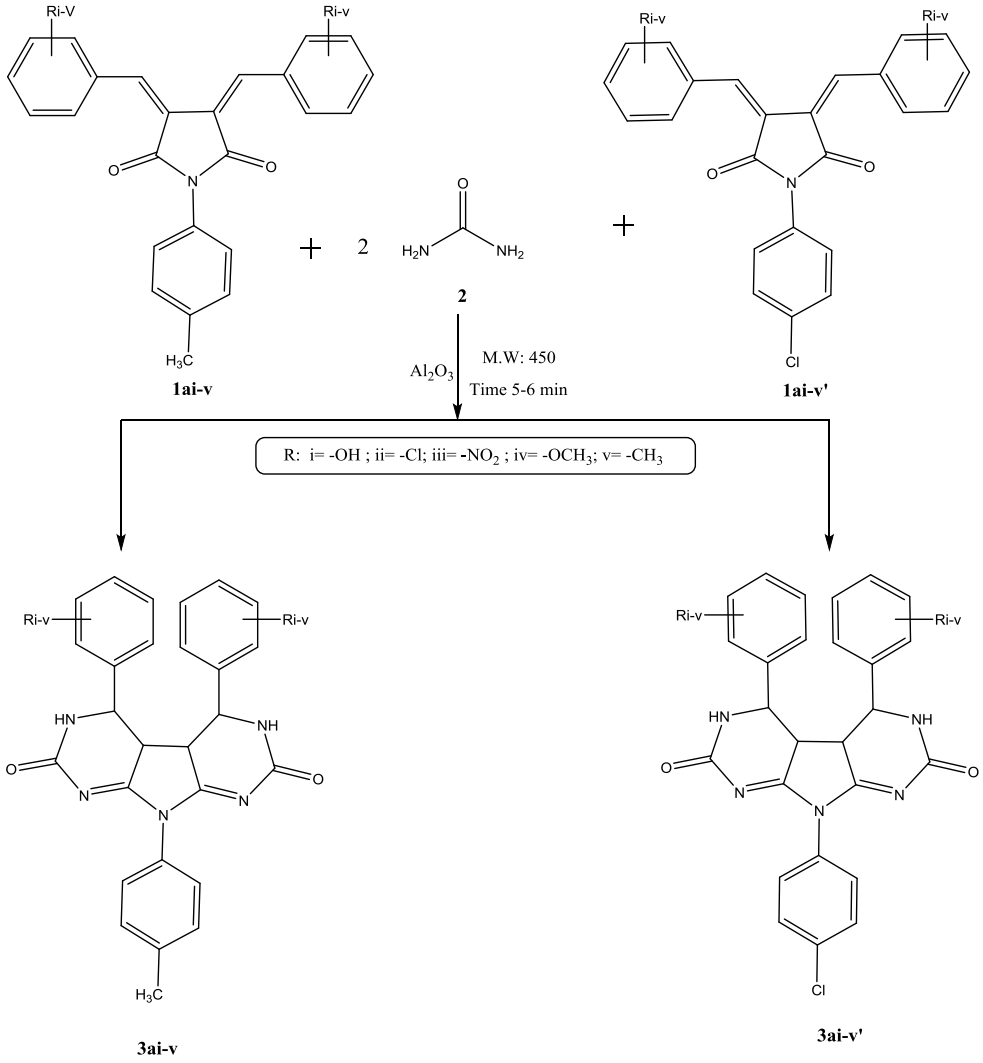
The 5 milimole of chalcone 7a-e and 10 milimole of urea taken in presence of 2 gm of neutral alumina then homogenized this mixture in mortar and taken in 100 ml borosilicate glass beaker covered it with glass petridish then irradiated reaction mixture in microwave oven at 450 watt power for 5-6 min as solvent free condition thus fused solid of 4, 5 –bis-(p-tolyl) - dipyrimidine-2, 7(9H)-dione obtained and recrystalised it from ethyl acohol.

General procedure for Synthesis of 9-(4-chlorophenyl)-3,4,4a,4b,5,6-hexahydro2H-pyrrolo[2,3-d:5,4-d']dipyrimidine-2,7(9H)-dione (3ai-v'):

The 5 milimole of chalcone 8a-e and 10 milimole of urea taken in presence of 2 gm of neutral alumina then homogenized this mixture in mortar and taken in 100 ml borosilicate glass beaker and covered with glass petridish then irradiated in microwave oven at 450 watt power for 5-6 min as solvent free condition thus fused solid of 9-(4-chlorophenyl) -2H-pyrrolo [2,3-d:5,4-d']dipyrimidine-2,7(9H)-dione derivatives of 26a-e obtained and recrystallized it from ethyl alcohol.

2.1.1 4,5-bis(2-hydroxyphenyl)-9-(p-tolyl)-3,4,4a,4b,5,6-hexahydro-2H-pyrrolo[2,3d:5,4-d']dipyrimidine-2,7(9H)-dione (3ai):

Molecular formula: C27H23N5O4 Physical appearance: Radish yellow solid Melting Point (0C): 140-142 Molecular weight: 481.51 Percent yield: 72.12 % C H N Analysis: Cal.: C, 67.35; H, 4.81; N, 14.54 Obs.: C, 67.65; H, 4.91; N, 14.84 FTIR (KBr, Cm-1): 3734.19 (-OH), 3456.44 (-NH), FTIR (KBr, Cm-1): 3734.19 (-OH), 3456.44 (-NH), 1512.19 (C=N), 1705.07 (C=O), 2918.30 (CH3, Ar-CH3). ^{1}H NMR (500 MH_Z; DMSO d6 ;δ ppm) 2.34 (s ,3H, -CH_3), 2.76 (2H, dd, *J* = 8.1, 2.2 Hz), 3.33 (2H, d, *J* = 2.2 Hz), 6.89 (4H, ddd, *J* = 8.0, 1.3, 0.5 Hz), 7.10 (4H, ddd, *J* = 8.0, 1.3, 0.5 Hz), 7.32-7.52 (4H, 7.38 (ddd, *J* = 8.1, 1.3, 0.5 Hz), 7.46 (ddd, *J* = 8.1, 1.7, 0.5 Hz)).



SCHEME 1

2.1.2 4,5-bis(3-nitrophenyl)-9-(p-tolyl)-3,4,4a,4b,5,6-hexahydro-2H-pyrrolo[2,3-d:5,4-d'] dipyrimidine-2,7(9H)-dione (3aii):

Molecular formula: C27H21N7O6 Physical appearance: brown Melting Point (^{0}C): 198-200 Molecular weight: 539.51 Percent yield: 73.29 % C H N Analysis: Cal.: C, 60.11; H, 3.92; N, 18.17 Obs.: C, 60.71; H, 3.44; N, 18.57 FTIR (KBr, Cm-1) : 1529.55 (C=N), 3336.85 (-NH), 1788.01 (C=O), 2916.37(CH3), 1384.24 (Ar-NO2).

2.1.3 4,5-bis(2-chlorophenyl)-9-(p-tolyl)-3,4,4a,4b,5,6-hexahydro-2H-pyrrolo[2,3-d:5,4-d']dipyrimidine-2,7(9H)-dione (3aiii):

Molecular formula: C27H21Cl2N5O2 Physical appearance: yellow crystals Melting Point (^{0}C): 154-156 Molecular weight: 518.40 Percent yield: 70.51 % C H N Analysis: Cal.: C, 62.56; H, 4.08; N, 13.51 Obs.: C, 62.26; H, 4.38; N, 13.61 1H NMR (500 MHZ; DMSO d6 ;δ ppm): 2.3 (s, 3H, -CH3), 2.76 (d, 1H, -CH, pyrimidone), 3.34 (d,1H, -CH, pyrimidone),7.29-7.1(m, 6H, Ar-H), 8.76 (s, 1H, -NH). 13C NMR (400 MHZ; DMSO d6 ;δ ppm):20.68,28.39,40.11, 126.84,129.23,130.08,137.55(C=O),176.97(C=N)

2.1.4 4,5-bis(4-methoxyphenyl)-9-(p-tolyl)-3,4,4a,4b,5,6-hexahydro-2H-pyrrolo[2,3- d:5,4-d']dipyrimidine-2,7(9H)-dione(3aiv):

Molecular formula: C29H27N5O4 Physical appearance: light brown crystals Melting Point (^{0}C): 143-145 Molecular weight: 509.57 Percent yield: 71.04 % C H N Analysis: Cal.: C, 68.36; H, 5.34; N, 13.74 Obs.: C, 68.86; H, 5.74; N, 13.64 ; 1H NMR (500 MHZ; DMSO d6 ;δ ppm): 2.34(s,1H, -CH3), 2.76(d,1H, -CH),3.35 (d,1H, -CH), 3.85 (s,1H, -OCH3), 7.29-7.11(6H, Ar-H), 8.41 (s,1H, -NH)

2.1.5 4,5,9-tri-p-tolyl-3,4,4a,4b,5,6-hexahydro-2H-pyrrolo[2,3-d:5,4-d']dipyrimidine- 2,7(9H)-dione (3av):

Molecular formula: C29H27N5O2 Physical appearance: light yellow crystals Melting Point (^{0}C): 160-162 Molecular weight: 477.57 Percent yield: 72.29% C H N Analysis: Cal.: C, 72.94; H, 5.70; N, 14.66 Obs.: C, 72.74; H, 5.40; N, 14.36 1H NMR (500 MHZ; DMSO d6 ;δ ppm): 2.34 (s, 3H, -CH3) , 2.76 (s, 1H, -CH pyrimidone), 3.33 (s, 1H, -CH) , 8.43 (s, 1H, -NH of pyrimidone), 7.29-7.11 (m, 6H, Ar-H).

2.1.6 9-(4-chlorophenyl)-4,5-bis(2-hydroxyphenyl)-3,4,4a,4b,5,6-hexahydro-2Hpyrrolo[2,3-d:5,4-d']dipyrimidine-2,7(9H)-dione (3ai'):

Molecular formula: $C_{26}H_{20}ClN_5O_4$ Physical appearance: Yellow solid Melting Point (^{0}C): 98-100 Molecular weight: 501.93 Percent yield: 73.21 % C H N S Analysis: Cal.: C, 62.22; H, 4.02; N, 13.95 Obs.: C, 62.52; H, 4.32; N, 13.35 FTIR (KBr, Cm-1): 3534.19 (-OH), 3446.44 (-NH) , 1512.19 (C=N) , 1708.07 (C=O), 745.11 (Ar-Cl).

2.1.7 9-(4-chlorophenyl)-4,5-bis(3-nitrophenyl)-3,4,4a,4b,5,6-hexahydro-2Hpyrrolo[2,3-d:5,4-d']dipyrimidine-2,7(9H)-dione (3aii'):

Molecular formula: $C_{26}H_{18}ClN_7O_6$ Physical appearance: Yellow solid Melting Point (^{0}C):151-153 Molecular weight: 559.92 Percent yield: 71.68 % C H N Analysis: Cal.: C, 55.77; H, 3.24; N, 17.51 Obs.: C, 55.87; H, 3.44; N, 17.71 FTIR (KBr, Cm-1) : 3381.21 (-NH), 1708.86 (C=O), 1489.08 (C=N), 825.53(Ar-Cl).

2.1.8 9-(4-chlorophenyl)-4,5-bis(2-chlorophenyl)-3,4,4a,4b,5,6-hexahydro-2Hpyrrolo[2,3-d:5,4-d']dipyrimidine-2,7(9H)-dione (3aiii'):

Molecular formula: $C_{26}H_{18}Cl_3N_5O_2$ Physical appearance: light yellow solid Melting Point (^{0}C):118-120 Molecular weight: 538.81 Percent yield: 70.66 % C H N Analysis: Cal.: C, 57.96; H, 3.37; N, 13.00 Obs.: C, 57.76; H, 3.57; N, 13.23 FTIR (KBr, Cm-1): 3361.21 (-NH), 1708.86 (C=O), 1476.08 (C=N), 815.53(Ar-Cl).

2.1.9 9-(4-chlorophenyl)-4,5-bis(4-methoxyphenyl)-3,4,4a,4b,5,6-hexahydro-2Hpyrrolo[2,3-d:5,4-d']dipyrimidine-2,7(9H)-dione (3aiv'):

Molecular formula: $C_{28}H_{24}ClN_5O_4$ Physical appearance: Yellow crystals Melting Point (^{0}C):140-142 Molecular weight: 529.98 Percent yield: 70.41 % C H N Analysis: Cal.: C, 63.46; H, 4.56; N, 13.21 Obs.: C, 63.86; H, 4.86; N, 13.61 1H NMR (500 MHZ; DMSO d6 ; δ ppm):2.77 (d, 1H, -CH, pyrimidone), 3.36 (d, 1H, -CH, pyrimidone) , 3.76 (s, 3H, -OCH3), 7.577.29 (m, 6H, Ar-H), 8.41 (s, 1H, -NH); HRMS (500 MHz): M+ 529.03 (100).

2.1.10 9-(4-chlorophenyl)-4,5-di-p-tolyl-3,4,4a,4b,5,6-hexahydro-2H-pyrrolo[2,3-d:5,4d'] dipyrimidine-2,7(9H)-dione (3av'):

Molecular formula: $C_{28}H_{24}ClN_5O_2$ Physical appearance: Yellow crystals Melting Point (0C): 139-141 Molecular weight: 497.98 Percent yield: 72.61 % C H N Analysis: Cal.: C, 67.53; H, 4.86; N, 14.06. Obs.: C, 67.43; H, 4.76; N, 14.56. FTIR (KBr, Cm-1): 3381.21 (-NH), 1708.86 (C=O), 1489.08 (C=N), 825.53(Ar-Cl).1H NMR (500 MHZ; DMSO d6 ; δ ppm): 2.38 (s, 3H, -CH3), 2.77 (d, 1H, -CH), 3.40 (d, 1H, -CH, pyrimidone), 7.57-7.29 (m,6H, Ar-H), 8.57 (s, 1H, -NH).

III. RESULTS AND DISCUSSION

Chemistry

The possible mechanism of formation of dipyrimidone derivative is shown here and there are four steps. The first step of this mechanism is reaction of urea with succinamidechalcone , the enol form of urea is carbamimidic acid which attacks on chalcone leading in to formation of 1,1'-((2,5-dihydroxy-1-phenyl-1H-pyrrole-3,4-diyl)bis(argiomethylene))diurea. Which is rearranges in to 1,1'-((2,5-dioxo-1-phenylpyrrolidine-3,4-diyl)bis(argiomethylene))diurea. In (b) step The nucleophilic attack of second $-NH_2$ group on carbonyl carbon takes place which results in to formation of 4,5-diargio-8a,9a-dihydroxy-9-phenyldecahydro-1H-pyrrolo [2,3-

d:5,4-d'] dipyrimidine-2,7-dione. And in (d) step the dehydration takes place and formation of 4,5-diargio-9-phenyl-3,4,4a,4b,5,6-hexahydro- 2H-pyrrolo [2,3-d:5,4-d']dipyrimidine-2,7(9H)-dione. This evident that the reaction is carried out in presence of neutral alumina which is an important dehydrating agent which create driving force to dehydration and formation of final product. Thus crude product is obtained and recrystallized if from ethyl alcohol by use of reverse polarity method. In this method all crude product is dissolved in ethanol at 40 ^{0}C temperature below the boiling point of alcohol, in this temperature all crude product was dissolved in ethanol except neutral alumina. With help of filtration alumina have been separated from product and then in solution of ethanol with T.M conductivity water slowly added then formation crystals.

IV. MICROBIAL EVALUATION OF DIPYRIMIDINE-DIONE DERIVATIVES

The series of hexahydro-2H-pyrrolo [2,3-d:5,4-d']dipyrimidine-2,7(9H)-dione (3ai-v)and (3ai-v')were screened for antibacterial activity in vitro against Gram positive bacteria *Staphylococcus auras* (NCIM 2079) , *Bacillus subtilis*(NCIM 2250) and Gram negative bacteria *Pseudomonas aeruginosa*(NCIM 2036), *Escherichia coli*(NCIM 2109). The solution of all compounds (25a-e) and (26a-e) were prepared in DMSO solvent .The assay was carried by taking 100 µgm per disc by using disc diffusion method for this purpose nutrient agar media was employed. The results were obtained in the form of zone of inhibition and noted after period of incubation (at 37^{0}C for 24-28 hrs).The zone of inhibition was measured in mm with help of venire caliper and compared with standard antibiotic Chloramphenicol (Chmpl). Similarly antifungal evaluation was also carried out in vitro against fungi *Aspergillusniger*(NCIM 545) and *Candida albicans*(NCIM 3471) in Hi-Media at conc. of 100 µgm per disc. The zone of inhibition was measured in mm and compared with standard drug Amphotericin-B. The anti-bacterial and anti-fungal results obtained are mentioned in table-7.

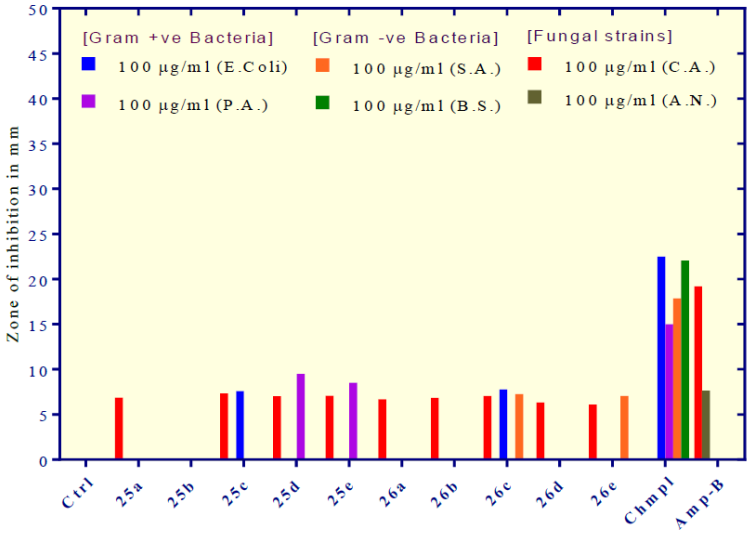
Table 1.1: Antimicrobial activities of the synthesized compounds

Sr. No.	Sample code	Bacterial strains				Fungal strains	
		Gram +ve		*Gram -ve*			
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
1	**Ctrl**	0.0	0.0	0.0	0.0	0.0	0.0
2	**25a**	--	--	--	--	6.88	--
3	**25b**	--	--	--	--	-	--
4	**25c**	--	--	7.59	--	7.31	--
5	**25d**	--	--	--	9.48	7.01	--
6	**25e**	--	--	--	8.52	7.09	--
7	**26a**	--	--	--	--	6.70	--
8	**26b**	--	--	--	--	6.85	--
9	**26c**	7.24	-	7.78	--	7.05	--
10	**26d**	--	--	--	--	6.31	--
11	**26e**	7.07	--	--	--	6.10	--
12	**Chmpl**	17.86	22.05	22.48	15.01	NA	NA
13	**Amp-B**	NA	NA	NA	NA	19.20	7.62

'NA' means not applicable, '--' means no zone of inhibition at 100µgm concentration

All the synthesized compounds were evaluated *invitro*for antibacterial action counter to gram +ve*S.aureus, B.subtilis*and gram –ve*Escherichia coli, P.aeruginosa*bacterial strains with the dilution concentrations of 100µg/ml by using disc diffusion method DMSO solvent and nutrient agar. After 48 hrs of incubation at 37 ºC, the results were achieved by clear zone and noted after the completion of incubation. The inhibition zones were recorded in 'mm' evaluated *in-vitro* likewise for antifungal activity against *Aspergillusniger*and *Candida albicans*fungal strains at the concentration 100µg/ml per disc by paper disc diffusion method using DMSO as solvent. The yeast *Candida albicans*cultured using a malt extract, glucose

yeast extract peptone agar medium (MGYP medium) and for fungi *Aspergillusniger*potato dextrose agar medium was used. After 3-7 days of incubation at 30ºC, the diameters of the zones of inhibition were measured.



Graph 1.1: Antimicrobial activities of the synthesized compounds

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