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Synthesis Of Pyrrolo-Dipyrimidine-Dione Derivatives And Their Microbial Evaluation

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Abstract

In this synthetic protocol urea **1** and substituted diethylidene- N-substituted pyrrolidine-2,5-dione **2a-d** on condensation in presence of catalytic amount of base catalyst resulted in to pyrrolo-dipyrimidine-dione **3a-d**. The structures of all synthesized compounds have determined by FTIR, ^{1}H NMR and ^{13}C NMR spectral techniques. All synthesized compounds were tested for their antibacterial and antifungal activities against pathogenic bacteria and fungal species by disc diffusion method. The results have compared with standard antibacterial and antifungal drugs. The results showed that N-substituted pyrolidin-dione not showed activities when it converted to pyrrolo-dipyrimidine-dione showed anti- bacterial as well as anti-fungal activities.

Key words

N-substituted pyrolidin-dione, pyrrolo-dipyrimidine-dione, Michael acceptor

1. Introduction

Structural modification is a massive practice in the field of synthetic organic chemistry. It plays an important role for development of new active pharmaceutical ingredient. In modern period molecular docking throw light on structure activity and relationship with their pharmaceutical properties. Many heterocyclic compounds bearing diverse pharmacological activities among them pyrimidine are important class of six member heterocyclic compounds that attracted attention of many researcher to explore them. The Antibiotics acts on bacterial cells that are different from human cells, antibiotics have selective actions like toxicity (destroying the bacteria without harm to human cells). Similar to antibacterial antifungal destroy fungal cell membrane [1]. The pyrimidine is an important class of six member heterocyclic compound .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 [2]. 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 pyrimidine molecule has and Anti tubercular and Cytotoxic activity [3-4], Antibacterial activity [5]. The compound incorporated pyrimidine moiety Shows Anti-oxidant [6], Analgesic [7] and Anti-inflammatory activities [8-9]. The pyrimidine 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 [10]. The pyrimidine derivatives like Zidovudine (AZT) and Idoxuridine are important class of anti-viral drugs which act as anti-HIV agents [11] by considering this applications of pyrimidine in this work we synthesized some pyrimidine derivatives and selected them for antibacterial and antifungal screening from results made an attempt to investigate structure activity relationship of synthesized derivative and also effect of structural modification of previously synthesized compounds showed antibacterial and antifungal activities.

2. Material and Method

The melting points of synthesized compounds were taken in to open capillaries and are uncorrected. The I.R spectrum were recorded on FTIR shimazdu spectrophotometer using KBr disc method. The N-substituted pyrrolidine-2,5-dione **2a-d** were synthesized by our previously reported microwave assisted solid phase synthetic method [12]. The compounds 2a-d condensed with urea in presence of catalytic amount KOH afforded to pyrrolo dipyrimidine dione **3a-d**. The reaction was monitored by thin layer chromatography by using pre-coated silica gel aluminum plates and mixture of n-hexane: ethyl acetate 7:3 proportion was used as mobile phase. The ^{1}H NMR spectrum were recorded on Bruker 500 MHz instrument and TMS is used as an internal standard the chemical shift were recorded in terms of δ value relative to TMS in solvent DMSO-d_6. The identification of spots was done by visualizing plate in U.V chamber and the chemicals used in this work was an analytical grade and of high purity.

2.1 General procedure for synthesis of dipyrimidine dione derivatives

Initially 0.2 moles of compound **2a-d** and 0.4 moles of Urea **1** have taken in mortar then catalytic amount of KOH is added in above mixture and manually grinding it for 5 min with the help of pestle then whole reaction mixture taken in round bottom flask and in presence of ethanol refluxed for 2 to 3 hours thus dipyrimidine dione derivatives **3a-d** obtained and recrystalised it from ethyl alcohol.

4,5,9-tri-p-tolyl-3,4,4a,4b,5,6-hexahydro-2H-pyrrolo[2,3-d:5,4-d']dipyrimidine2,7(9H)-dione (3a):

M.F: $C_{29}H_{27}N_5O_2$ Physical appearance: light yellow crystals M.P (^{0}C): 160-162; M.W: 477.57 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 . ^{1}H NMR (500 MHZ; DMSO d_6 ;δ ppm): 2.34 (s, 3H, -CH_3) , 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).

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

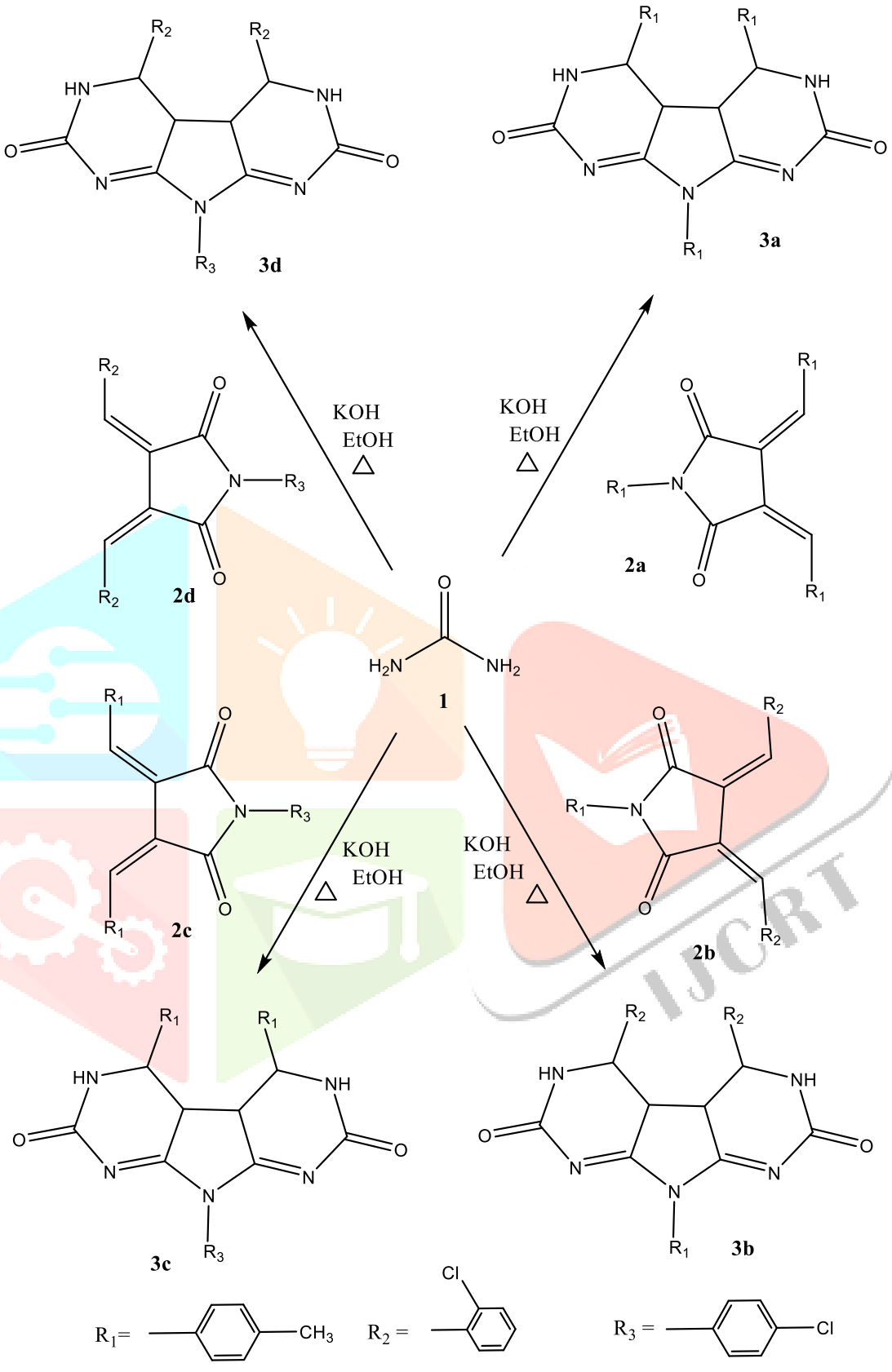
M.F: $C_{27}H_{21}Cl_2N_5O_2$ Physical appearance: Yellow crystals M.P (^{0}C): 154-156; M.W: 518.40; Yield (%): 70.51; C H N Analysis: Cal.: C, 62.56; H, 4.08; N, 13.51 Obs.: C, 62.26; H, 4.38; N, 13.61. ^{1}H NMR (500 MH_Z; DMSO d_6 ;δ ppm): 2.3 (s, 3H, -CH_3), 2.76 (d, 1H, -CH, pyrimidone), 3.34 (d,1H, -CH, pyrimidine),7.29-7.1(m, 6H, Ar-H), 8.76 (s, 1H, -NH). **,** ^{13}C NMR (400 MH_Z; DMSO d_6 ;δ ppm) 20.68,28.39,40.11, 126.84,129.23,130.08,137.55(C=O), 176.97(C=N).

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 (3c):

M.F: $C_{28}H_{24}ClN_5O_2$ Physical appearance: Yellow crystals; M.P (^{0}C): 139-141 M.W: 497.98 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), ^{1}H NMR (500 MH_Z; DMSO d_6 ; δ ppm): 2.38 (s, 3H, -CH_3), 2.77 (d, 1H, -CH), 3.40 (d, 1H, -CH, pyrimidone), 7.57-7.29 (m,6H, Ar-H), 8.57 (s, 1H, -NH).

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 (3d):

M.F: $C_{26}H_{18}Cl_3N_5O_2$; Physical appearance: light yellow solid; M.P (^{0}C): 118-120 M.W: 538.81 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)., ^{1}H NMR (500 MH_Z; DMSO d_6 ; δ ppm): 2.38 (s, 3H, -CH_3), 2.77 (d, 1H, -CH), 3.40 (d, 1H, -CH, pyrimidone), 7.57-7.29 (m,6H, Ar-H), 8.57 (s, 1H, -NH).


3d
3a
KOH
EtOH
KOH
EtOH
2d
2a
1
KOH
EtOH
KOH
EtOH
2c
2b
3c
3b
R1= CH3
R2 = Cl
R3 = Cl

SCHEME-1

3. Result and Discussion:

3.1 Chemistry

The of dinucleophilic nitrogen atoms of Urea molecule which used for condensation reaction with two carbonyl carbon atoms for the formulation of six member heterocyclic pyrimidine-dione derivatives. The various pyrrolo dipyrimidine-dione derivatives have been synthesized from Micheal receptor moiety, which have synthesized from our reported substituted succinamide. The substituted diethylidene- N-substituted pyrrolidine-2,5-dione **2a-d.**

Pyrrolo-dipyrimidine-dione **3a-d** derivatives have been synthesized from substituted diethylidene- N-substituted pyrrolidine-2, 5-dione **2a-d** and urea **1** refluxed in round bottom flask in presence of KOH in ethanol as solvent. This recation leads to formation of ring closure reaction and formation of six member dipyrimidine-dione ring which is shown in (Scheme-1). The IR spectra of compound **3c** showed stretching frequency at 3381.21 cm^{-1} corresponds to NH group and 1489.08 cm^{-1} indicates presence of C=N groups this distinguishing IR bands give an evidence of arrangement of dipyrimidine ring gave strong evidence of formation of six member dipyrimidone derivatives. The ^{1}H NMR Spectrum in DMSO d_6 of compound **3c** the peak of 8.57 δ singlet is due to presence of 1H of –NH group and peak appeared around 2.77 δ doublet is due to 1H doublet of -CH of pyrrolo ring. The peak of 3.40 δ doublet is for 1H of -CH of pyrimidione ring. The peaks obtained around 7.59-7.29 δ multiplates is due to presence 6 H of Ar-H and one peak obtained at 2.34 δ singlet 3H due to presence of $-CH_3$ group. So spectral data obtained is in good agreement with considered structures of synthesized compounds.

3.2 Mechanistic Pathway

The substituted diethylidene- N-substituted pyrrolidine-2,5-dione possesses α-β un saturated carbonyl functionality Michael acceptor which is electrophilic in nature . The one nitrogen atom of urea first attacked on carbon-carbon double bond of Michael acceptor leading to formation of 1,1'-((2,5-dihydroxy-1-methyl-1H-pyrrole-3,4-diyl)bis(ethane-1,1-diyl))diurea as an intermediate in first step. Then the second nitrogen atom of urea molecule makes Michael acceptor attack on carbonyl carbon of which converted in 8a,9a-dihydroxy-4,5,9-trimethyldecahydro-1H-pyrrolo[2,3-d:5,4-d']dipyrimidine-2,7-dione the second intermediate which on dehydration with loss of water molecule then formation of 4,5,9-trimethyl-3,4,4a,4b,5,6-hexahydro-2H-pyrrolo[2,3-d:5,4-d']dipyrimidine-2,7(9H)-dione with ring closure.

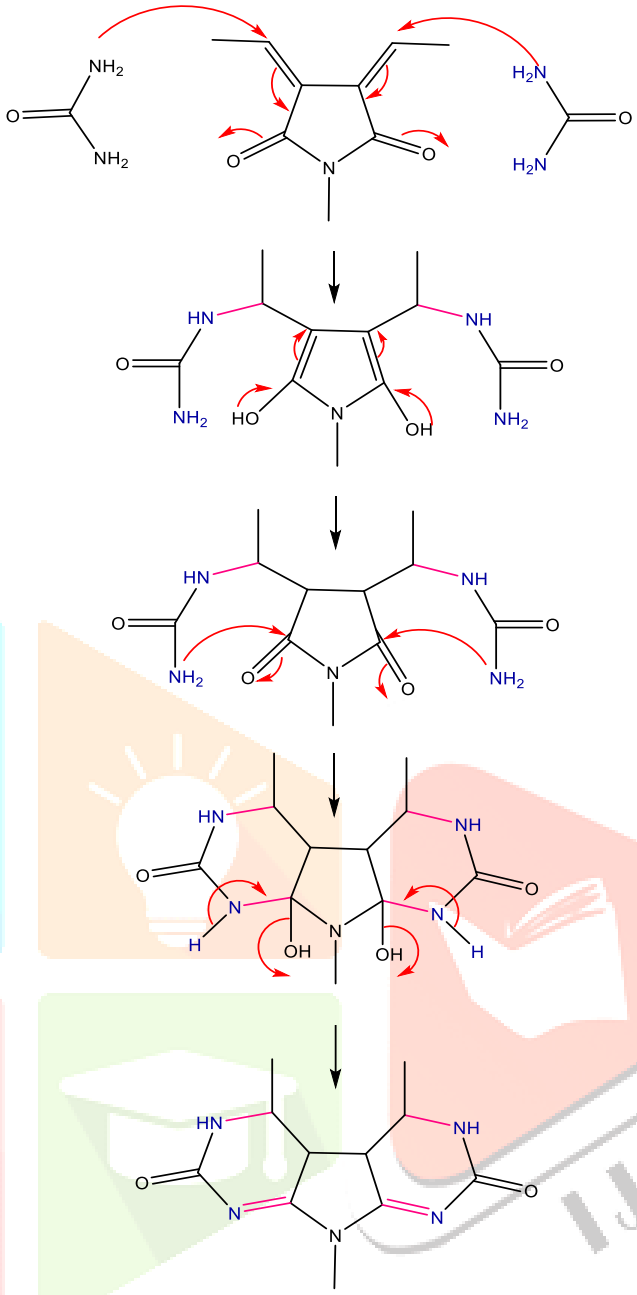


Figure:- Shows Possible mechanism of formation of pyrimidone derivatives

3.3 Antibacterial and Antifungal Evaluation

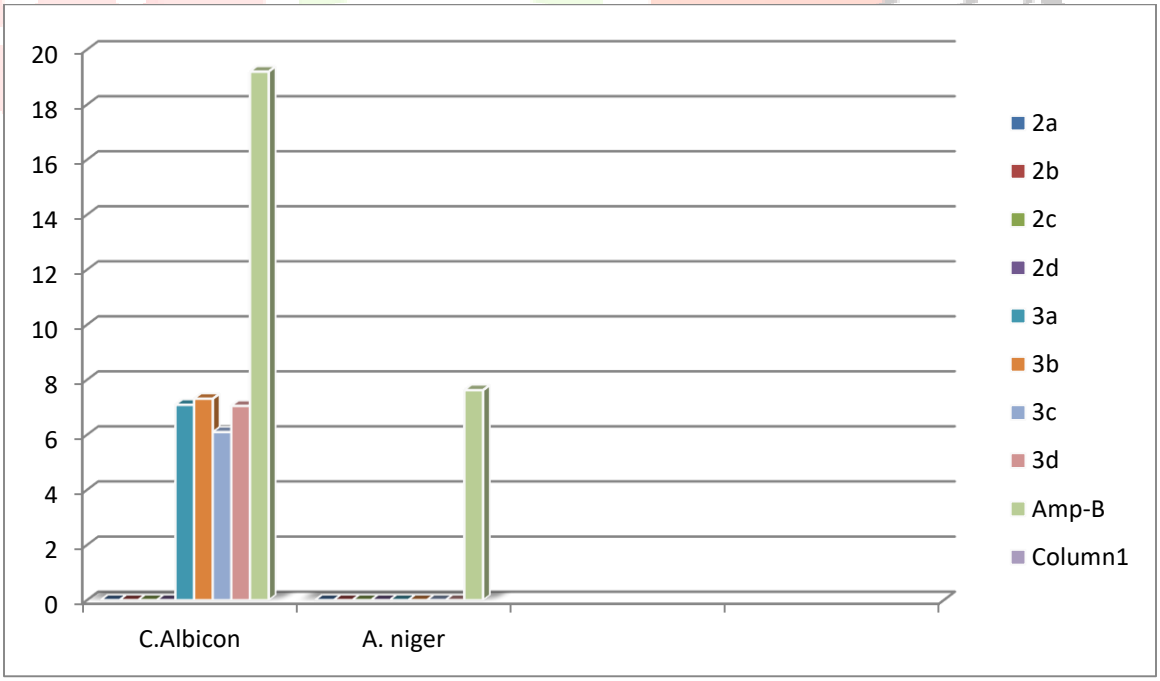
We have tested our synthesized compounds against fungal strain and bacterial strains the compounds 2a-d have not shown any activity but when structural modifications of these compounds have carried out then their derivatives showed promising activities which is mentioned in table-1

For microbial evaluation disc diffusion method was employed, initially compounds **(2a-d)** and **(3a-d)** were dissolved in DMSO solvent for preparation of solution. The assay was carried out by taking 100 µgm per disc

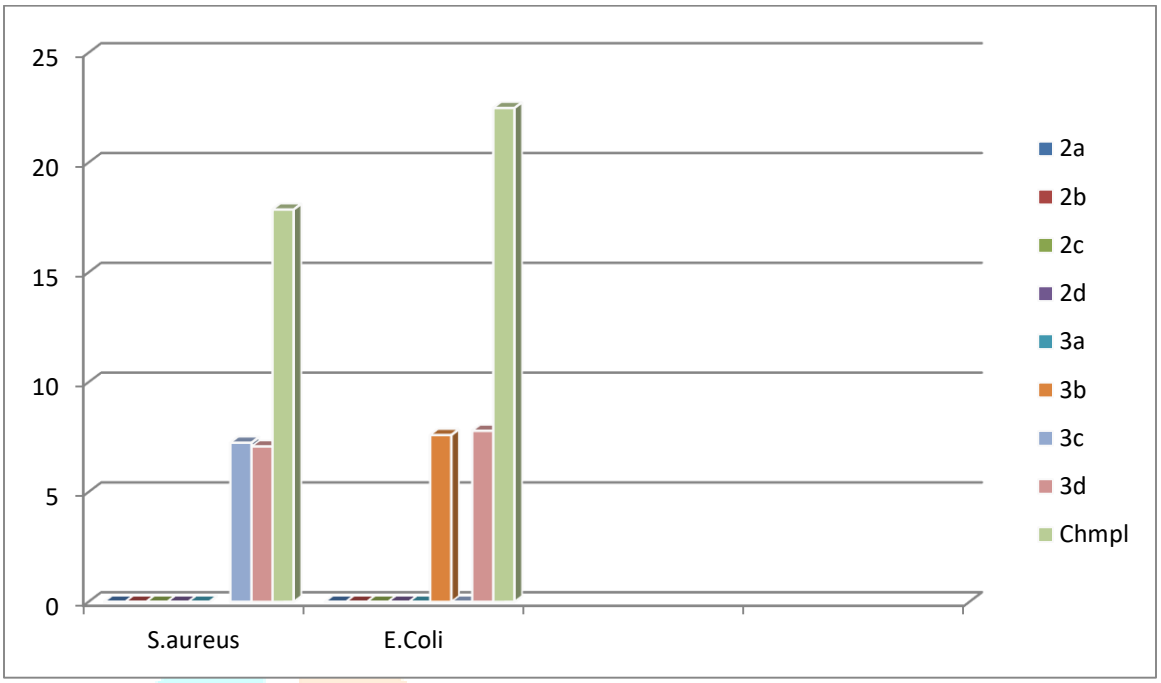
by using disc diffusion method, for this purpose nutrient agar media was employed. The results were obtained in the form of zone of inhibition and noted after period of incubation (at 37^0C for 24-28 hrs).The zone of inhibition was measured in mm with help of venire caliper and it has compared with standard antibiotic drug 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-1.

Sr. No	Comp	substituent's			Fungal strain		Bacterial strains	
		R1	R2	R3	C.Albican	A.niger	S.aureus	E.Coli
1	2a	p-Me-phenyl	o-Cl-phenyl	p-Cl-phenyl	--	--	--	--
2	2b				--	--	--	--
3	2c				--	--	--	--
4	2d				--	--	--	--
5	3a				7.09	--	--	--
6	3b				7.31	--	--	7.59
7	3c				6.10	--	7.24	--
8	3d				7.05	--	7.07	7.78
9	Chmpl				NA	NA	17.86	22.48
10	Amp-B				19.20	7.62	NA	NA

Table: 1- Shows Zone of inhibition measured in mm, '--' means no activity, NA means Not Applicable



Graph:-1. Shows antifungal activity and comparison zone of inhibition of synthesized compounds



Graph:-2. Shows antibacterial activity and comparison zone of inhibition of synthesized compounds

3.4 Structure Activity Relationship

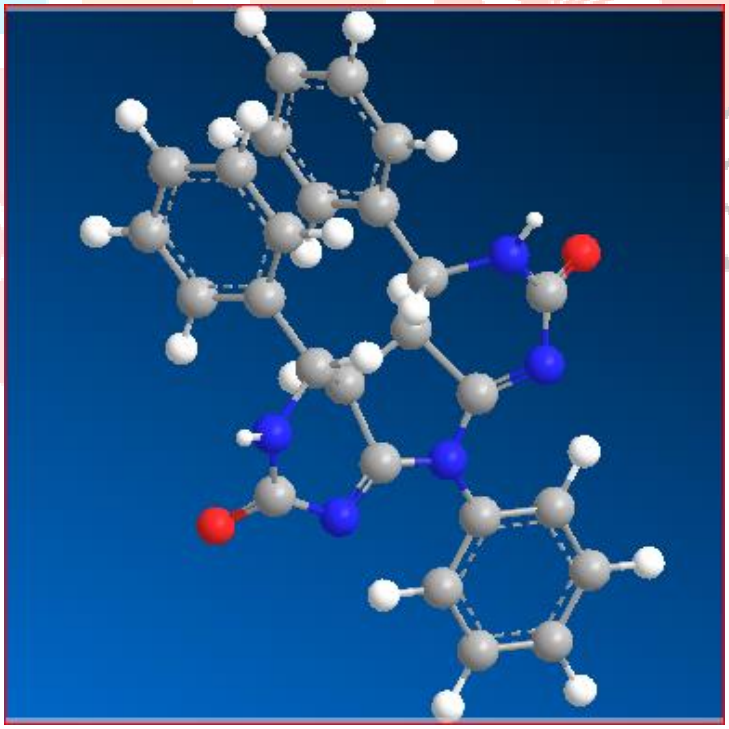


Figure: 3D image of dipyrrolo-dipyrimidine-dione generated by Chem. Draw 16 professional software

We have selected various pathogenic microorganisms' bacterial and fungal strain for conducting this study. We already synthesized substituted pyrrole derivative incorporating with Michael receptor functionality and

we have tested this compounds against pathogenic fungal strain and bacterial strain the result reveals that the compound have not any potential efficacy against Microorganism.

When the molecule incorporating the Michael receptor have allowed to reacted with Ammonia derivative Urea in a basic condition afforded to Pyrimidine derivative. The ring closer reaction provides structural modification. The introduction pyrimidine functionality in pyrrole molecule and tested against the same microorganisms then the promising result were obtained and result revealed that the rate of inhibition improved.

The substituted phenyl rings having chlorine and methyl substituent's are electron donating groups and which are present on para and ortho positions on benzene ring which makes activation of benzene ring and increases the electron density but presence of Michael receptor α-β unsaturated carbonyl functionality (compounds **2a-d**) which is electrophilic in nature and having electron withdrawing inductive effect so it probably decreases the binding effect of molecule with receptors which are present on cell wall on bacteria and fungi so dose not shown inhibition activity. When the series of compounds **2a-d** have converted in to pyrimidine derivatives with same substituted phenyl rings and compounds **3a-d** have tested then the zone of inhibition have increased. This indicated that after formation pyrimidine the number of nitrogen atoms increases and the antibacterial and anti-fungal activity increases because nitrogen have loan pair of electron and it has electron donating effect so it increases the bacterial and antifungal activities

Conclusion

In this research work we have develop the combinatorial method of synthesis grinding and refluxing of reactant that reduces reflux timing of condensation reaction. The SAR studies suggested that after formation of pyrimidine the zone of inhibition is increases. From spectral analysis the compounds **3a-d** have been successfully synthesized by using this synthetic protocol. The series of pyrrolo-dipyrimidine-dione 3a-d shows promising antifungal activites against C.albicans.

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