Synthesis, Characterization Of Pyrazolo-Pyrimidine Derivatives By Using Nano-Zno Catalyst And Study Of Their Antibacterial Activity

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ABSTRACT
So many literatures reported on the work of Pyrazolo-pyrimidine derivatives by different methods. Different moieties of these compounds have been synthesized by simple and convenient steps. We are reported the synthesis of Pyrazolo-pyrimidinone derivatives and structures were confirmed by FT-IR, ^{1}H NMR Spectra and ^{13}C NMR Spectra. In this work, we studied In-vitro antibacterial activity of all synthesized compounds against Candida albicans, Aspergillus flavus and Streptococci.

KEY WORDS: Pyrazolo-pyrimidine derivatives, Nano-ZnO, Ultrasonication, In-vitro screening and antibacterial activity

INTRODUCTION

So many literatures reported on the work of Pyrazolo-pyrimidine derivatives by different methods. Different moieties of these compounds have been synthesized by simple and convenient steps. In this work, we synthesized novel Pyrazolo-pyrimidinone derivatives by adopting green chemistry. In reaction **scheme-6.1.1**, Pyrazolo-pyrimidinone derivative (1) was made to react with hydroxyl amine in presence of Zinc oxide catalyst to **form (2).** In the Reaction **scheme-6.1.2,** the formation of **(3)** by the reaction of (1) with Benzamide. In reaction **scheme-6.1.3**, (1) was reacted with urea to form and then undergo reduction to **form (4).** Some literatures used Ethylene glycol as a green solvent[1-5]. A green solvent Ethylene glycol was used under ultrasonication for entire schemes [6-7]. All the synthesized compounds obtained by the ultrasonic method which afforded good yield as well as the reactions completed more quickly than conventional method. We focused on the ultrasonic method which is very simple to handle under room temperature.

MATERIALS AND METHODS

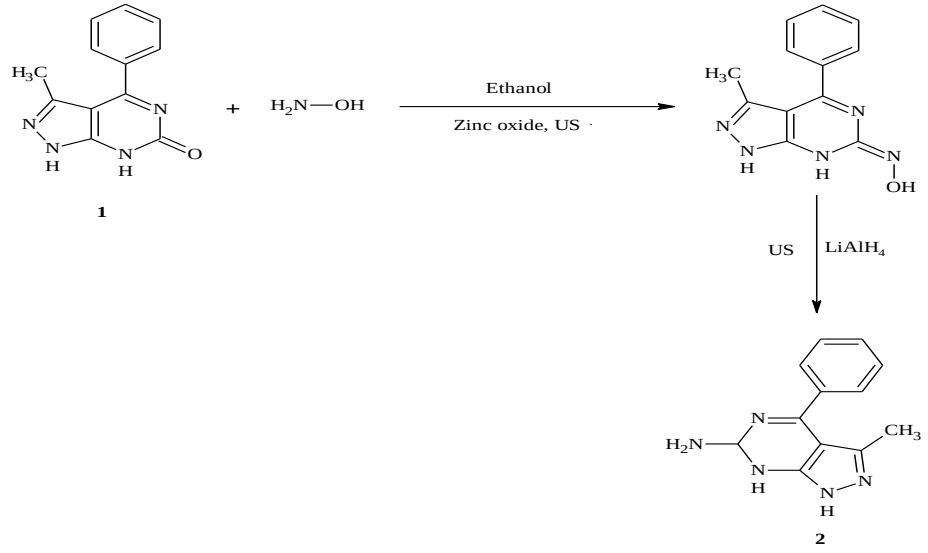
All chemicals purchased from SDFCL Company, Reactions were conducted by Ultrasonic bath (170VAC270VAC), Buchi B-540 melting point apparatus for melting point determination. Antibacterial activity was studied by well diffusion method.Our work showed interest to carry out

reactions of Pyrazolo-pyrimidinone derivatives at room temperature. In this case we used ultrasonic method is a convenient and efficient one for the reaction completion at short span of time and yields are high.

General synthetic procedure for Pyrazolo-pyrimidinone derivatives:

A mixture of pyrazolo-pyrimidinone compound (10 Mmol), NH_2OH/ Benzamide/ urea/ Thiourea/acetanilide/nitro-toluene/hydrazine (10 Mmol) were taken in a round bottom flask and add 10-20 ml of Ethylene Glycol as a solvent. Zinc oxide (0.1%) was added to the mixture as a catalyst. Allow the mixture for the reaction to conduct under ultrasonic condition at room temperature for 5-10 minutes. The preliminary test of the reaction was examined by TLC using pet. Ether and Ethyl acetate. The precipitate thus obtained was filtered off, Wash with hot water (10x3 ml) and recrystallized by Ethanol (10x3 ml) to get pure product. Further, this compound was reduced by $LiAlH_4$ to afford Pyrazolo-pyrimidine derivative.

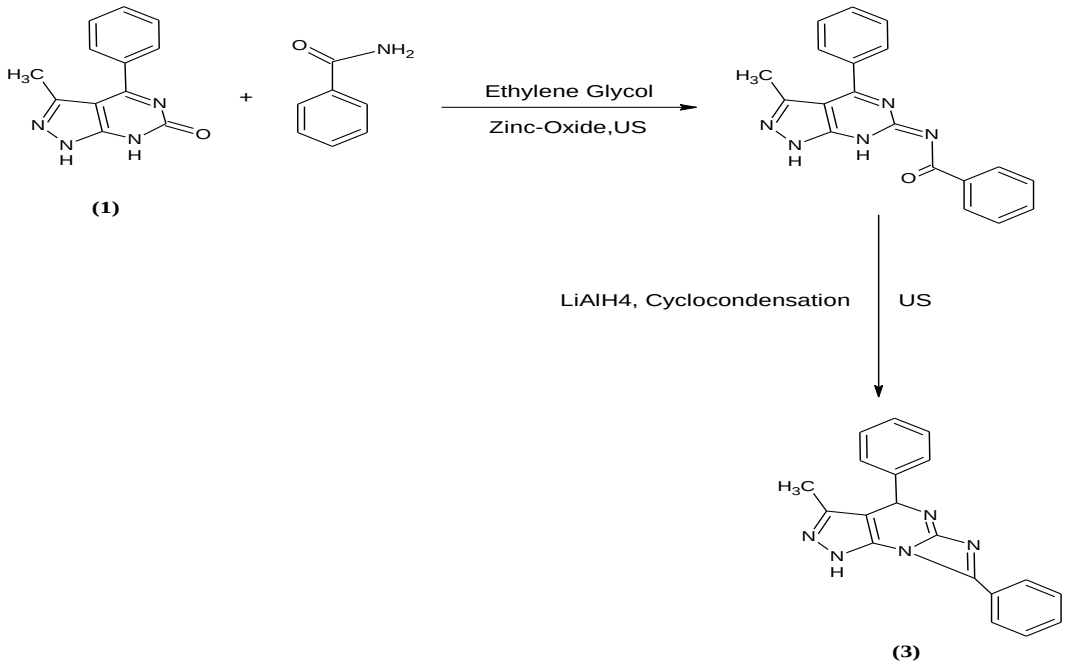
Scheme-6.1.1:



1

2

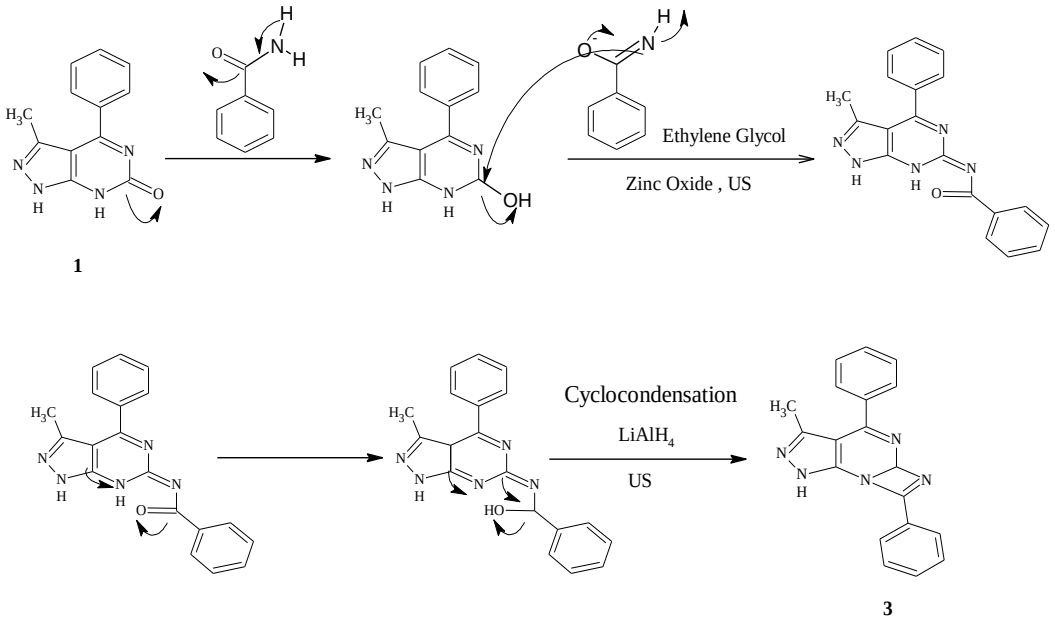
Scheme-6.1.2



(1)

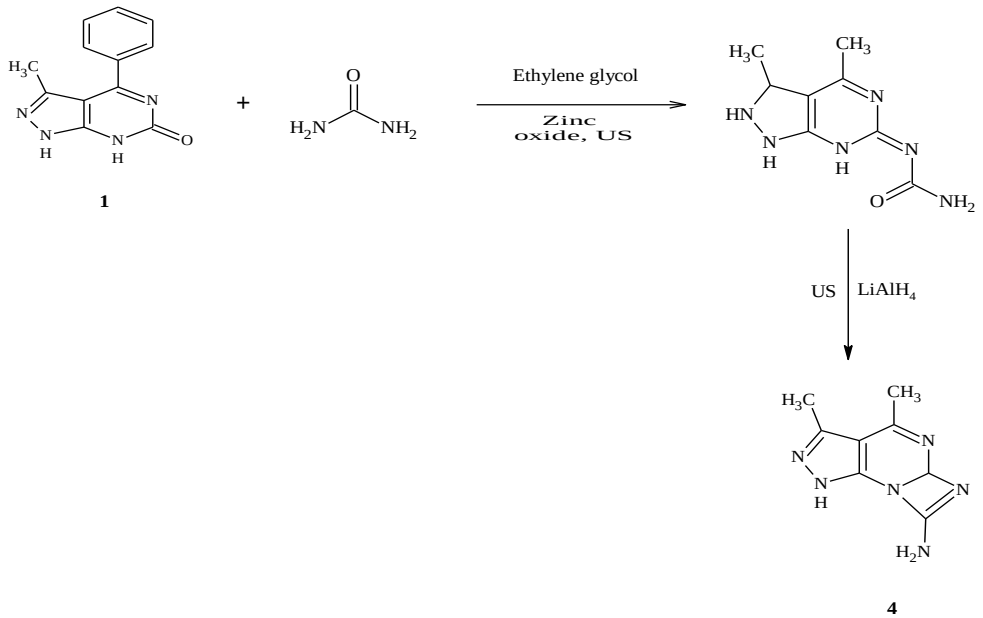
(3)

REACTION MECHANISM FOR (**3)**



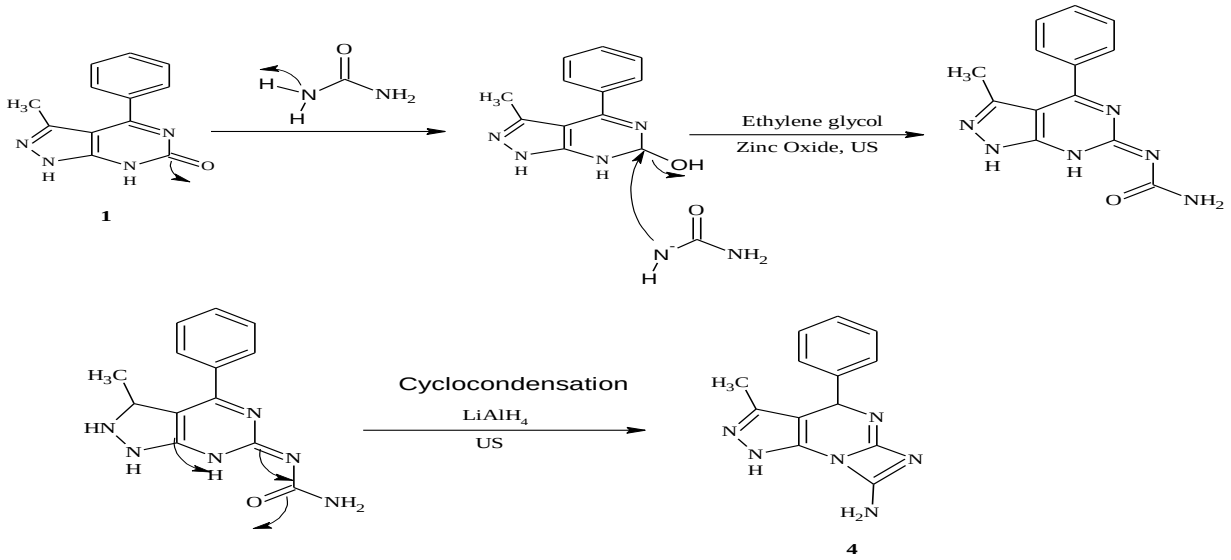
1 **3**

Scheme-6.1.3



1 **4**

REACTION MECHANISM FOR **(4)**



1

4

3-methyl-4-phenyl-6,7-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (2): M.P 156-158 ℃, yield(%): 84.00; FT-IR: υ cm^{-1} 3068 (=C-H, Ar stretch, strong), 1620 (-N-H, bending),1468 (C=C, Ar), 1634 (C=N, bend);^{1}H-NMR (DMSO, 300MHz): 2.48(s, NH_2, 2H), 7.4 (Ar-H, s), 8.97 (ddd, J=8.2, 1.1, 0.5Hz, 2H), 12.00 (N-H, pyrazole).^{13}C-NMR (100 MHz, DMSO-*d_6) δ*: 38.8, 39.0, 39.3, 39.5,39.7, 39.9 (C=N, C_3, C_4 and C_5,Pyrazolo-pyrimidine), 40.1 (C_3-CH_3, pyrazole, 62.75 (C_{10}-C_4=N, pyrimidine).

3-methyl-4,7-diphenyl-1,5a-dihydro[1,3]diazeto[1,2-*a*]pyrazolo[4,3-*e*]pyrimidine (3): M.P 254-256 ℃, yield(%): 89.00; FT-IR: υ cm^{-1} 3068(=C-H, Ar stretch, strong), 1613(-N-H, bending), 1049(C=N, bend); ^{1}H-NMR (DMSO, 300MHz):7.4 (Ar-H, s), 8.92 (ddd, J= 8.2,1.1,0.5Hz, 2H); ^{13}C-NMR (100MHz,DMSO-*d_6) δ*: 38.8,39.0,39.3, 39.5,39.7,39.9 (C=N,C_3,C_7andC_9, Pyrazolo-pyrimidine),40.1(C_3-CH_3,pyrazole,127.7, 128.1, 130.1, 130.4, 133.1, 134.6 (Ar, C_{12},C_{13},C_{14},C_{15}, pyrimidine), 158.148 (C_{16} and C_{17}, pyrimidine).

3,4-dimethyl-1,5a-dihydro[1,3]diazeto[1,2-*a*]pyrazolo[4,3-*e*]pyrimidin-7-amine (4):

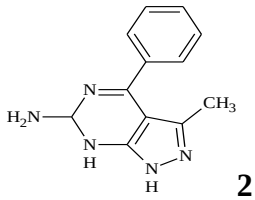
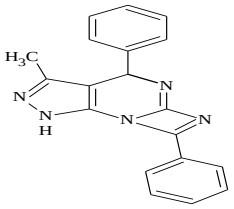
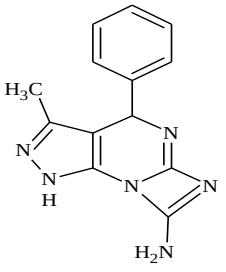
M.P 242-244 ℃, yield(%): 88.00; FT-IR: υ cm^{-1} 3068(=C-H, Ar stretch, strong), 1613 (-N-H, bending),1436 (-CH_3,bending); ^{1}H-NMR (DMSO,300MHz): 2.49 (s,CH_3,3H), 8.92 (ddd, J=8.2,1.1,0.5Hz, 2H); ^{13}C-NMR(100MHz, DMSO-*d_6) δ*: 38.8, 39.0, 39.3, 39.5, 39.7, 39.9 (C=N,C_3,C_7andC_9, Pyrazolo-pyrimidine), 40.1 (C_3-CH_3, pyrazole, 127.7, 128.1, 130.1, 130.4, 133.1, 134.6 (Ar, C_{12},C_{13},C_{14},C_{15}, pyrimidine), 158.148 (C_{16} and C_{17}, pyrimidine).

RESULTS AND DISCUSSION

Eight compounds were synthesized in the reactions. Pyrazolo-pyrimidinone is a starting material to prepare different derivatives of it by using Ethylene glycol as a solvent and Nano zinc oxide as a catalyst. Reactions are carried out in presence of catalyst afforded compounds with lesser time and higher yield. In absence of catalysts, compounds formed with higher reaction time and lower yield. Mainly in the reaction **scheme-6.1.2, 6.1.3,** there was a tri-cyclic formation via the cyclo-condensation to afford four membered *diazeto heterocycle* fused to pyrimidine moiety[12]. $LiAlH_4$ is a good reducing agent was mainly responsible for the formation of tri-cyclic molecules **3 and 4.**

We made some literature studies on the synthesis of Pyrazolo-pyrimidines in presence of nano metal- oxide catalysts by efficient methods[13-16].
The reactions are carried out at room temperature in an ultrasonic method. Literatures reported the reactions which were carried out in different solvents but poor yields are obtained[17-20]. In our present work, Ethylene glycol was used and better yields were obtained. Since this solvent is high polar, yields of the products were good.
We are reported the synthesis of Pyrazolo-pyrimidinone derivatives and structures were confirmed by FT-IR, ^{1}H NMR Spectra, ^{13}C NMR Spectra. All compounds are confirmed their structure by structural analysis.

Table -6.1: Synthesis of Pyrazolo pyrimidine derivatives

Product	Time (Min)		% of yield		Melting Point (^{o} C)
	With Catalyst ZnO	Without Catalyst	With Catalyst	Without Catalyst	
 **2**	13	25	84.00	69.00	155-157
 **3**	10	17	97.00	81.00	254-256
 **4**	12	18	88.00	70.00	242-244

From the **Table-6.1**, in **scheme 6.1.1**., **(1)** was afforded hydroxyl imines which are more unstable and further converted into **(2)** by $LiAlH_4$ reduction. Likewise, in scheme-**6.1.2, 6.1.3-** unstable intermediates were formed and which in turn converted into **(3), (4)** by undergoing reduction using $LiAlH_4$ respectively.

We made comparison study with some literatures that our work completed the reactions more quick under ultrasonicator and afforded higher yield[21-24].

.ANTIBACTERIAL ASSAY:

Inhibition zones were measured in millimetre after the incubation. This experiment was performed in triplicates. We reported In-vitro antibacterial activity of all synthesized compounds tested at 1mg/ml concentration showed low to high activity against Streptococci,

Few literatures that we referred reported the work on antibacterial activity of Pyrazolo [3,4-d] pyrimidines[25-29]. Zone inhibition and MIC of most synthesized compounds were found to exhibit less activity. Some literatures that we studied reported antibacterial activity of Pyrazolo [3,4-d] pyrimidines and found to exhibit less activity.

Compound **4** showed highest activity (21 mm) against Aspergillus Flavus. All the remaining compounds showed moderate anti-bacterial activity. It revealed that the compounds synthesized in the work possessed effective anti-bacterial activity.

MIC:

The compound **4** inhibited Streptococci in lowest (6.785 µ g/ml) inhibitory concentration. The other remaining compounds **2, 3** inhibited Asperigillus Flavus in lowest (7.000 µg/ml). Compound **4** inhibited Candida albicans in lowest (6.785 µg/ml) inhibitory concentration. This concluded that the compounds synthesized have great potential to inhibit/kill the test bacteria in low concentration.

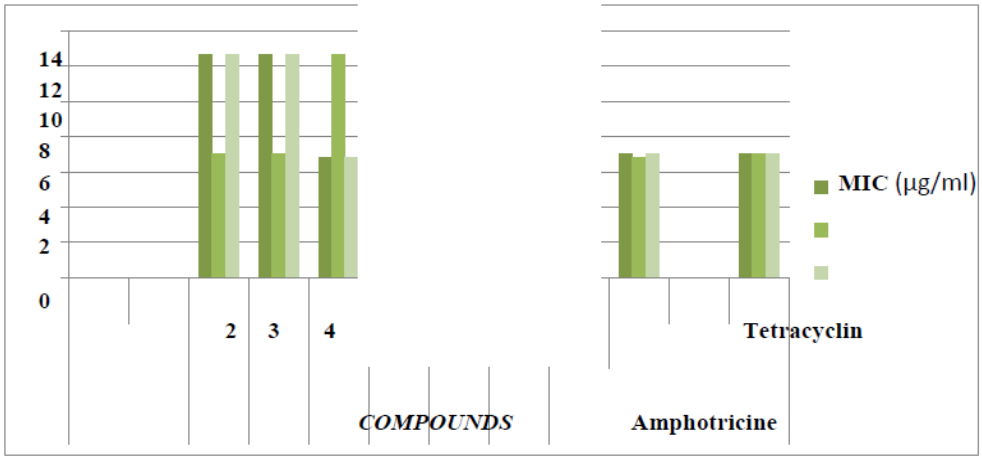
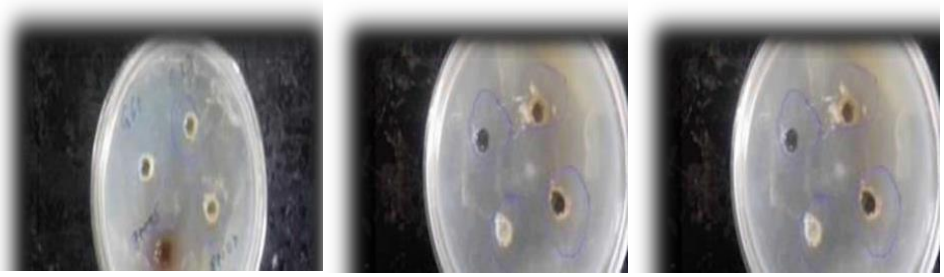


Fig-6.1: Graph showing antibacterial activity of test compounds against Streptococcus, Aspergillus Flavus and *Candida albicans* in comparision with reference antibiotics Amphotricine and Tetracycline.



*Images of Zone of Inhibition of test compounds in Mm against **Streptococcus, Aspergillus Flavus and Candida albicans***

Table-6.2. Minimum Inhibitory Concentration (MIC):

Compound name	MIC (µg/ml)		
	Streptococcus	Aspergillus Flavus	Candida albicans
2	12.625	7.000	12.625
3	12.625	7.000	12.625
4	6.785	12.625	6.785

CONCLUSION

Our report of this work concluded that compounds that we synthesized in presence of Nano ZnO catalyst completed their reaction time more quickly than in absence of catalyst. Also, yield of the compounds are excellent in presence of catalyst. The ultrasonication method is a simple one to conduct the reactions of the chemistry. It completes the reaction at shorter time than the conventional reactions.

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