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ECO-FRIENDLY AND EFFICIENT SYNTHETIC PROTOCOL FOR BIOLOGICALLY ACTIVE DERIVATIVES OF PYRIMIDOPYRIMIDINE USING IONIC LIQUID TEAA: A GREEN AND EFFECTIVE CATALYST

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ABSTRACT

Green and efficient protocol is designed and implemented for the synthesis of biologically active derivatives of pyrimidopyrimidine promoted by the use of an effective and green ionic liquid TEAA, as a catalyst and solvent. Multi-component one-pot condensation reaction of aryl aldehyde, barbituric acid and urea/thiourea resulted in high yield of pure products in short reaction time in presence of TEAA catalyst is carried out and finally catalyst is easily recovered and reused for many trials. The structural features of new derivatives were characterized by IR, ^{1}HNMR, ^{13}CNMR, Mass spectroscopic techniques. All newly synthesized compounds were evaluated for anti-bacterial activities against gram +ve bacteria and gram –ve bacteria and anti-fungal activities using disc diffusion method.

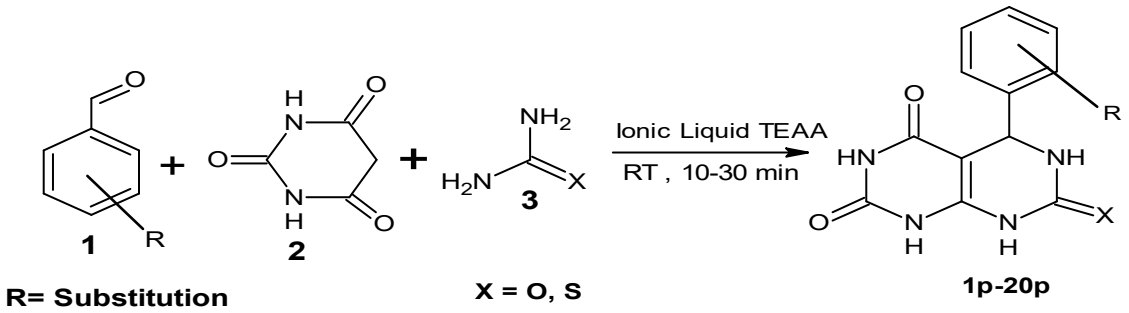
Keywords: *Pyrimidopyrimidines; Biginelli reaction; Ionic Liquid Triethyl ammonium acetate (TEAA); Green Chemistry; Anti-microbial activities*

1. INTRODUCTION

Pyrimidopyrimidinesis also known as pyrimidine-2,4,6-trione or pyrimido[4,5-*d*] pyrimidine-2,4,6(*1H,3H,6H*)-trione are basically poly fused ring compounds synthesized by multicomponent condensation reaction of aryl aldehyde, barbuturic acid and urea/thiourea. This class of Biginelli compounds represents biologically and pharmaceutically active area of heterocyclic arena.[1, 2]There is large scope for this scaffold to obtain novel and biologically efficient derivatives by simple modification or introduction of large number of functional groups.[3, 4]These derivatives represent a large number of biological and pharmaceutical activities such as antiallergic,[5] antiviral,[6] antibacterial,[7] antioxidant,[8] hepatoprotective[9] properties, antitumor agents,[10] herbicide antidotes[11] and diuretics.[12]Further, fused pyrimidines represent a large number of biologically active compounds such as pyrano,[13] pyrido,[14]pyrido[2,3-d,4,5-d]dipyrimidines[15] and pyrimido[4,5-d]pyrimidines,[16]but pyrimido[4,5-d]pyrimidines are most versatile compounds and a considerable amount of literature is available for synthetic pathway of pyrimido[4,5-d]pyrimidines[17-18] The first fundamental synthetic method for the synthesis of these compounds was proposed by Biginelli[19] by the use multi-component one pot condensation method under drastic reaction conditions resulted in very less product yield in long reaction duration. Keeping in view, their vast applicability, numerous improved

synthetic protocols were reported by using variety of acidic, basic, natural catalysts and different solvents,[20-24]which suffer by many drawbacks. Some more advanced and eco-friendly techniques were also used such as microwave assisted irradiation, ultrasound sonication, mortar pestle grinding, etc.[25-27] to overcome all deficiencies and drawbacks of reported methods and work in accordance with green chemistry principles.

In the same context, the main purpose of our research was to develop an effective and eco-friendly alternative method for the synthesis of biologically important derivatives of pyrimidopyrimidine under desired reaction conditions catalyzed by the ionic liquid *TEAA*, a green and highly efficient catalyst which also serves best energy transfer media.



2. MATERIALS AND METHODS

2.1 Materials

All chemicals and solvents used in this work were of analytical grade quality and purchased from Merck and Loba. Ionic Liquid *TEAA* has been prepared in laboratory. The completion of reaction was checked by thin layer chromatography on silica-gel-G coated plates and spots were visualized by exposure to UV chamber. The recrystallized products were identified and characterized by IR,^{1}H NMR and MASS Spectroscopy. All melting points were measured in open capillary method and were uncorrected.

2.2 General procedure

2.2.1 Method A

Synthesis of triethylammonium ionic liquid (*TEAA*)

The synthesis of ionic liquid was carried out in a 250 mL round-bottom flask, which was immersed in a water-bath and fitted with a reflux condenser. 90.1g Acetic acid (1.5mol, 86.03mL) was dropped into 101.2g triethylamine (1mol, 139.4 mL) at 70°C within 1 hr according to the reported procedure.[28]

2.2.2 General Method B

2.2.3 Ionic Liquid TEAA catalyzed synthesis of substituted dihydrpyrimidines

A solution of aryl aldehyde (10 mmol), **1**barbituric acid (10 mmol) **2** and urea/thiourea (10 mmol), **3a/b** were taken in a round bottom flask. 5mL *TEAA* added to this and the mixture was stirred at 25°C mixture for 10-30 minutes. The completion of the reaction was monitored by TLC. Upon completion of the reaction, cold water was added to facilitate the precipitation of product. The aqueous layer consisting of Ionic Liquid was subjected to distillation at 80°C and 5 mm Hg for 1 hr to remove water, and was further recycled. The crude product was subjected to column chromatography on silica gel. The products thus isolated, were purified by recrystallization from 90 % ethanol.

Table 1: Characterization data for some selected newly synthesized compounds

Entry	IR(CM^{-1}), 1H NMR (300 MHz,DMSO), :
1p	IR (cm^{-1}): 3567 (NH str.), 2970 (Ar. C-C str.), 2831(alip. C-C str),1741 (C=O str.), 1358 (C=C str.),1054 (C-C bend.) ; ^{1}H NMR (300 MHz, DMSO): δ: 5.42 (s,1H,CH),7.39-6.91(m, 5H Ar.), 8.71 (s,1H,NH), 10.62(s,1H,NH),11.31(s,1H,NH)11.38(s,1H,NH) :; Mass
2p	IR (cm^{-1}): 3233 (NH str.),3092 (C-C arom. str.), 2997(C-C alip. str.), 1696(C=O str.) 1413(C=C str.),1082(C-C bend.); ^{1}H NMR (300 MHz,DMSO): δ: 5.47 (s,1H,CH), 7.35-6.82 (m, 5H Ar.),8.82 (s,1H,NH),10.71(s,1H,NH), 11.24(s,1H,NH), 11.36(s,1H,NH) ;Mass
3p	IR (cm^{-1}): 3388 (NH str.3087(C-C arom. str.), 2979 (C-C alip. str.),1749 (C=O str.),1377 (C=C str.),1043 (C-C bend.); ^{1}H NMR (300 MHz,DMSO): δ: 5.43 (s,1H,CH),7.31-6.88(m, 5H Ar.), 8.62 (s,1H,NH), 10.59(s,1H,NH),11.22(s,1H,NH)11.37(s,1H,NH) ; Mass
4p	IR (cm^{-1}): 3213& 1679 (NH str. & def.),3115 (C-C arom. str.),2915 (C-C alip. str.),1748 (C=O str.), 1430 (C=C str.),1083 (C-C bend.); ^{1}H NMR (300 MHz,DMSO):): δ: 5.47 (s,1H,CH),7.38-6.91(m, 5H Ar.), 8.65 (s,1H,NH), 10.62(s,1H,NH),11.21(s,1H,NH)11.41 (s,1H,NH) ; Mass
6p	IR (cm^{-1}): 3203 (NH str.), 3089 (Ar. C-C str.), 2835(alip. C-C str),1750 (C=O str.), 1440 (C=C str.),1034 (C-C bend.); ^{1}H NMR (300 MHz, DMSO): δ: 5.48 (s,1H,CH),7.37-6.93(m, 5H Ar.), 8.68 (s,1H,NH), 10.60(s,1H,NH),11.16(s,1H,NH)11.41(s,1H,NH); Mass
10p	IR (cm^{-1}): 3567 (NH str.), 2970(C-C Ar str.), 2831(C-C alp. str.), 1741 (C=O str.) 1443 (C=C str.),1054(C-C bend.); ^{1}H NMR (300 MHz,DMSO): δ: 5.44 (s,1H,CH), 3.41 (s,3H,OCH_3),7.35-6.98(m, 5H Ar.), 8.23 (s,1H,NH),10.59(s,1H,NH), 11.14 (s,1H,NH), 11.26 (s,1H,NH) ; Mass
13p	IR (cm^{-1}): 3189 (NH str.), 3073 (C-C arom. str.), 2799 (C-C alip. str.),1742 (C=O str.) 1441 (C=C str.), 1031(C-C bend); ^{1}H NMR (300 MHz,DMSO): δ: 5.40 (s,1H,CH), 7.42-6.56 (m, 5H Ar.), 8.64(s,1H,NH),10.66(s,1H,NH), 11.27 (s,1H, NH), 11.36(s,1H,NH); Mass

3. RESULTS AND DISCUSSION

Keeping the pharmaceutical and biological proficiency in consideration, a simple and efficient method is proposed for biologically important pyrimidopyrimidines(1p-20p) by the multicomponent condensation reaction of variety of substituted aryl aldehydes, barbituric acid and urea/thiourea catalyzed by ionic liquid TEAA with excellent yields in a short period of time (Table 4).

Ionic liquid TEAA, as a cheap and green catalyst and /or solvent offers best synthetic alternative. TEAA belongs to special category of ionic liquid family which have unique physio-chemical properties such as low melting points, negligible vapor pressure, easy recyclability, low-toxicity, repeated reusability, have high thermal and chemical stability. Triethylammonium acetate ionic liquid has Lewis acidity of the $[Et_3NH]^+$cation, which makes the CH bond weaker, enhancing the nucleophilicity of carbon for addition to electron-deficient alkenes. It is noteworthy that the use of TEAA as catalyst guarantees high product yield, environment friendly nature, eliminating the need of volatile organic solvents revealed by reported methods.[29, 30]

It has been found that reusability and recyclability of catalyst alters the product yield greatly and it has been analyzed that upon 5-6 times reuse of TEAA worked effectively without apparent loss of catalytic reactivity and yield products. Generally different attempts reveal that rate of reaction and yield were fairly decreased over the regular reuse of this ionic liquid. The results are given in Table 2.

Table 2: Catalyst ionic liquid TEAA effectiveness on several runs

Sr. No.	No. of Run	Time (min.)	Isolated Yield (%)
1	1	15	96
2	2	15	94
3	3	15	93
4	4	15	90
5	5	15	89

Reaction condition: 4-methoxy, benzaldehyde (10mmol), Barbituric acid (10mmol) and Urea (10mmol), at room temp in Ionic Liquid TEAA (5 ml in all cases)

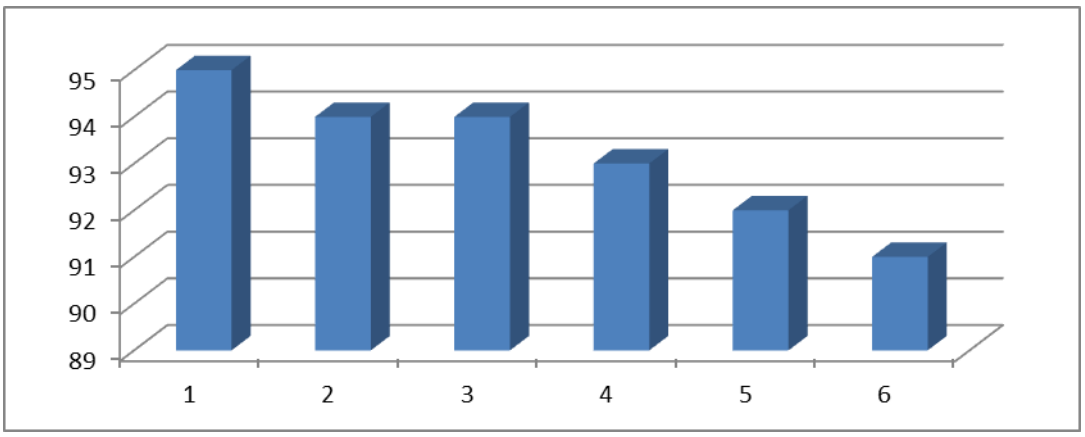
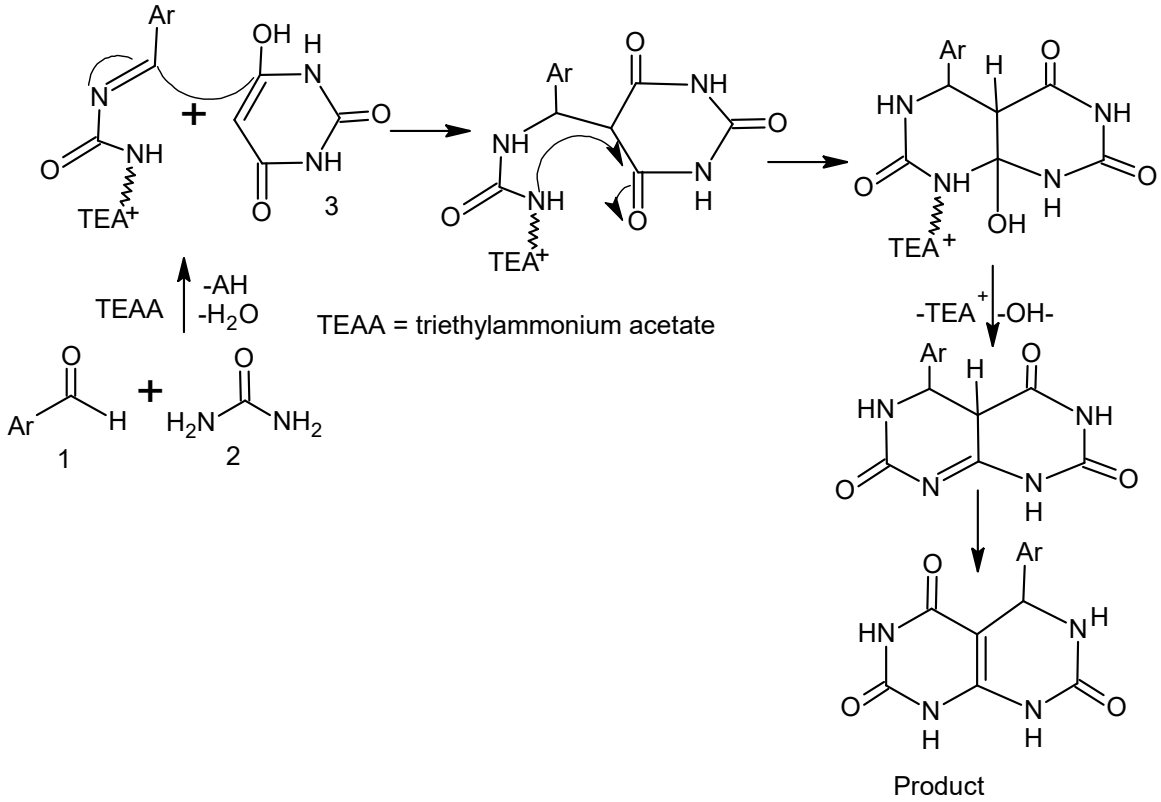


Fig.1: Reusability cycle and isolated yield

The synthesis follows a simple protocol and precisely involves two step, first step is slow and reaction rate determining, where aldehyde reacts with urea/thiourea results in carbocation, intermediate by the release of water molecule, followed by cyclodehydration reaction of intermediate with barbituric acid, which further loses another water molecule and after due cyclisation give desired product (1p-20p) with excellent yield and purity. *TEAA* when added as a reaction medium, the reaction rate as well as yield of the reaction improved dramatically. This is probably due to the higher solubility of aldehyde/cyclic 1,3-diketone in the ionic liquid. It has been observed that the ionic liquid *TEAA* is the most suitable solvent for barbituric acid and 1,3-cyclohexadienone as synthon for the production of final condensed heterocyclic ring assembly systems. It has been found that the presence of substituents at aromatic ring affects rate of reaction and yield and purity of product considerably. Aromatic aldehydes carrying substituents as electron-donating (Table 4, products-2p, 5p, 10p, etc.) reacts well with high yields of products while as electron-withdrawing group bearing aldehydes reacts slowly with less yield of products shown in (Table 4, products- 3p, 4p, 9p etc.).



Scheme 1: Plausible proposed mechanism for the synthesis of pyrimidopyrimidines

Table 3: Physical data of all synthesized compounds (1p-20p)

Sr. No.	Product	Ar-R	X	m.p.(°C)	Time	Yield (%)
1	1p	Ar-H	O	242-244 [240-242][31]	22	88
2	2p	Ar-4-CH_3	O	264-266 [262-264][31]	14	95
3	3p	Ar-4-Cl	O	281-284 [280-281][31]	18	92
4	4p	Ar-2-OH	O	261-263 [258-260][31]	19	90
5	5p	Ar-2-OCH_3	O	241-243 [244-246][31]	12	95
6	6p	Ar-4-OCH_3	O	253-255 [252-254][31]	10	97
7	7p	Ar-2,4-(OCH_3)	O	261-263	16	92
8	8p	Ar-4-$N(CH_3)_2$	O	302-304 [300-302][31]	28	83
9	9p	Ar-4-NO_2	O	281-283	25	91
10	10p	Ar-4-OH,3-OCH_3	O	298-300	20	94
11	11p	Ar-H	S	223-225 [222-224][31]	24	86
12	12p	Ar-4-CH_3	S	255-257	16	94
13	13p	Ar-4-Cl	S	241-242 [242-244][31]	19	91
14	14p	Ar-2-OH	S	251-252 [250-252][31]	20	89
15	15p	Ar-2-OCH_3	S	264-266	13	93
16	16p	Ar-4-OCH_3	S	247-249 [248-250][31]	11	96
17	17p	Ar-2,4-(OCH_3)	S	257-260	18	90
18	18p	Ar-4-$N(CH_3)_2$	S	293-295	30	85
19	19p	Ar-4-NO2	S	281-284	23	92
20	20p	Ar-4-OH,3-OCH_3	S	294-297	22	92

3.1 Biological Activity

The antimicrobial activity was screened by using the agar plate disc-diffusion method (Collins and Lyne, 1976) to assess the activity of the chosen compounds. Sterilized filter paper discs (6 mm in diameter) were wetted with 10 L each of a solution of the tested compounds (10 mg/mL of the compound in DMSO). The discs were then allowed to dry and placed on the surface of agar plates seeded with the test organism. Nutrient agar was used for bacterial plating and Sabouraud's dextrose agar for fungi. For conditions of cultivation, Petri dishes were poured with 15 mL of agar medium and then incubated at 37°C overnight. The test organisms were grown in liquid medium for approximately 16 to 24 hr for bacteria and fungi respectively and then poured on pre-incubated Petri dishes containing medium. The test organism was evenly spread over the surface with the help of a sterilized glass spreader. After 15 min the filter paper disc presoaked with test compounds were incubated at 37°C and 26°C for bacteria and fungi respectively. Most of the prepared compounds were tested for their antimicrobial activity against two types of bacteria, one gram-positive *Staphylococcus aureus* and one gram-negative *Escherichia coli*. The antifungal activity was tested using pathogenic yeast stain *Candida albicans* and *Aspergillus niger*. Preliminary testing was carried out by measuring the inhibition zone on the agar plates in mm. The screening results are summarized in **Table-4**. The compounds showed mild inhibition against gram-positive and gram-negative bacteria. Compounds 1p, 4p, 8p, 11p, 19p and 20p exhibit moderate activity while as2p, 3p, 6p, 9p, 12p& 16pexhibit highest activity against *S. aureus;* the compounds 1p, 8p,9p, 11p, 12p & 16pexhibited moderate antibacterial activity and 2p,3p, 4p, 6p, 19p & 20p exhibited highest activity against *E. coli*. Concerning the antifungal activities, the compounds 1p, 4p, 8p,9p,11p,12p&13pexhibited moderate activity and 2p, 3p, 9p,16p, 19p& 20pexhibited highest activity against *A. niger;* the compounds 1p, 8p, 9p, 11p, &16pexhibited moderate activity and 2p, 3p, 12p &20p exhibit the highest activity against *C. albicans*. Ciprofloxacin and Amphotericin B were used as standard antibiotics in this study.

Table 4: Antimicrobial activity of some synthesized compounds

Compound	Zone of inhibition in mm (Antibacterial activity)		Compound	Zone of inhibition in mm (Antifungal activity)	
	S. aureus	***E. coli***		***A. niger***	***C. albicans***
1p	13	14	1p	15	13
2p	18	19	2p	17	18
3p	19	20	3p	18	19
4p	16	18	4p	16	15
6p	19	17	6p	20	21
8p	14	16	8p	15	13
9p	18	14	9p	16	15
11p	14	13	11p	15	14
12p	17	16	12p	16	17
16p	17	16	16p	18	16
19p	16	18	19p	17	16
20p	16	18	20p	18	17
Ciprofloxacin	26	24	Amphotericin B	25	26

4. CONCLUSION

Besides the Synthesis of therapeutically novel derivatives of pyrimidopyrimidines, the new eco-friendly methodology following basic green chemistry principles is the core concern of present work. In this connection, we have successfully developed an eco-friendly and efficient solvent free method for the synthesis of various derivatives of 3,4-dihydropyrimidin-2(1H)-one by using green and versatile ionic liquid TEAA, as efficient catalyst and solvent. The ionic liquids used can be obtained from cheap sources, they are easy to handle and environmentally benign and recovery of products is easy. The use of ionic liquid offers a best methodic alternative and the yields are found to be higher as compared to earlier reported methods. Biological evaluation of these compounds show activeness against selected microbes as bacteria and fungi.

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