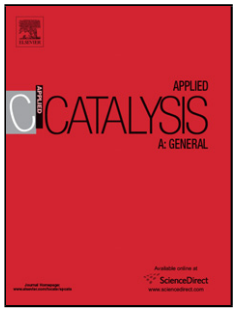

Phosphotungstic acid - Jeffamine® Hybrid Catalyst for One-Pot Biginelli Reaction Starting from Benzyl Alcohol

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Phosphotungstic acid - Jeffamine® Hybrid Catalyst for One-Pot Biginelli Reaction Starting from Benzyl Alcohol

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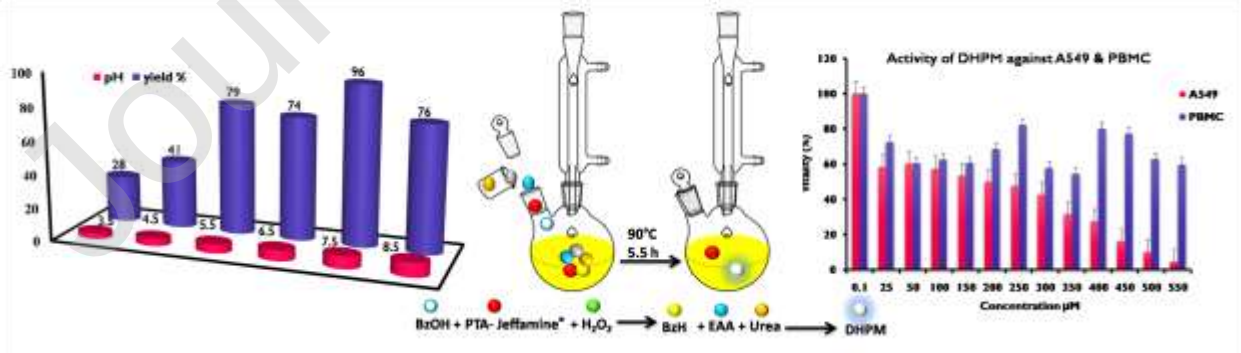
Graphical Abstract:



Efficient conversion of Biginelli product in one-pot one-step process with Phosphotungstic acid immobilized over Jeffamine as supported catalyst

Highlights

- In the present manuscript we have for the first time successfully prepared Biginelli compounds at almost neutral pH (7.5) with the PTA-Jeffamine® catalyst in aqueous medium in a one-pot one-step process.
- The catalyst was effective both in the two-step as well as one-step one pot reaction methodologies starting from BzOH.
- Both processes showed appreciable results with the one-pot one-step protocol demonstrated appreciable yield (87%) of DHPM within 5.5 h at pH 7.5.
- No acid was added and the pH was adjusted by controlling ratio of PTA/ Jeffamine®.
- The catalytic results were compared with literature reports for one-pot one-step Biginelli reactions starting from benzyl alcohol.
- The catalyst was easily separated from the products though a simple freezing process.
- DHPM obtained from the one-pot two-step process demonstrated anti-cancer activities. The PTA-Jeffamine® catalyst showed better performance compared to other POMs having Keggin structure.
- The Biginelli products were well characterized with UV-Vis, FTIR and ^{1}H and ^{13}C NMR spectroscopy.
- The use of hydrogen peroxide as oxidant deserves a special mention in the present manuscript. It can replace conventional oxidizing agents containing metal salts like nitrates.

- The work presented in this manuscript may pave ways to the development of industrial catalysts and environmentally friendly reaction protocols.

Abstract

Herein, we report the catalytic action of phosphotungstic acid - Jeffamine® hybrid (PTA-Jeffamine®) for the synthesis of dihydropyrimidinones (DHPMs) starting from benzyl alcohol by Biginelli reaction in water in a one-pot system. Hydrogen peroxide was used as oxidizing agent instead of the conventional nitrate salts. A two-step and a one-step protocol was used to evaluate the catalytic activity. Both processes showed appreciable results with the one-pot one-step protocol demonstrating appreciable yield (87%) of DHPM within 5.5 h at pH 7.5. No acid was added and the pH was adjusted by controlling ratio of PTA/ Jeffamine®. Catalytic results were compared with literature reports for one-pot one-step Biginelli reactions starting from benzyl alcohol. Biginelli products were characterized by UV-visible, FT-IR, NMR spectroscopy. DHPMs formed from the two-step process demonstrated appreciable anticancer activity. The present work may lead to the development of industry-friendly, non-toxic and scalable catalyst for one-pot Biginelli reactions.

Keywords: Phosphotungstic acid; Jeffamine; Catalyst; Biginelli; Dihydropyrimidinones; One-pot reaction

INTRODUCTION

Biginelli reaction is an industrially important process used for preparation of dihydropyrimidinones (DHPMs), commonly known as Biginelli compounds. These are well known for their pharmacological and biological applications [1, 2]. They are used in the field of therapeutics [3], and bio-organic chemistry [4]. Most of the Biginelli derivatives are used for their antifungal, antiviral, anticancer, antibacterial, anti-inflammatory, and antihypertensive properties [2]. In 1893, the Italian chemist Pietro Biginelli reported the acid-catalyzed cyclo condensation reaction of benzaldehyde, ethyl acetoacetate and urea to form 3, 4-dihydropyrimidine-2 (1H)-one (DHPM) with 60-70% yield, in ethanol medium [5]. Concentrated HCl was used as catalyst. Since then, Biginelli reactions have come up a long way with extensive literature reports in terms on the effects of substituent, solvents, reactions conditions and catalysts. Notably like many other reactions, the Biginelli reaction has its own drawbacks or limitations. The reaction suffers from low yields, long reaction time and harsh reaction conditions [4]. Many research groups have tried variety of acid catalysts like Lewis [6], Bronsted acids [7], ionic liquids [8,26], magnetic catalysts [9], magnetic ionic liquids [10] and also various natural catalysts like Pineapple Juice [11], apple, pomegranate, grape juice [12], Garlic Glove [13], animal bone [14], heterogeneous catalysts [15,27,28,29,30] and zeolites [16]. Additionally, a large number of homogeneous catalysts have been reported such as Mg $(NO_3)_2$ [17], Pb $(NO_3)_2$ [18], $LaCl_3.7H_2O$ [19], P_2O_5 [20]. Biginelli reactions in fruit juice medium was reportedly the most eco-friendly method for the synthesis of DHPM [12,13].

With the advent of green chemistry, there is considerable emphasis on developing environmental friendly protocols. Hence, an effective one pot protocol for Biginelli reaction can be industrially feasible. Many one-pot reactions have been tested for Biginelli with various catalysts [27, 31-35]. It has been observed in literature reports that for reactions starting from

benzyl alcohol (BzOH), the one-pot one-step process shows no yield at all [27, 31-35]. Alternatively, a one-pot two-step process has been shown to work well as it allows full conversion of the BzOH into benzaldehyde (BzH) followed by the addition of urea and Ethyl acetoacetate (EAA) [31-35]. Hence development of an efficient catalyst that favors one-pot one-step process will be a great value addition to the well established Biginelli reactions.

Until now many metal based catalysts have been tested for one-pot one-step Biginelli reaction. These catalysts often suffer from the leaching metals [36, 37]. It has been found that presence ~ 5% metal leachates in the product can cause toxicity issues as Biginelli compounds are more used in pharmaceutical studies. In this context, polyoxometalates (POMs) or inorganic polyacids may be preferred over other metal based catalysts. These polyacids are interesting metal based catalysts [21, 22]. The advantage of POMs over other inorganic/organic catalyst is the stability of the metal center present in the cage. It does not show leaching of metal ions in the reaction medium and hence can be superior compared to other metal-ligand complexes as catalysts [23]. Cost and scale of usage often limit their applications. In 2004 Tong-Shou Jin et al., have reported phosphotungstic acid (PTA) catalyst in presence of ethanol solvent to produce the desired Biginelli compound (Yield 94%) [24]. Mishra et al. have been reported in PTA used as a catalyst for one-pot synthesis of Biginelli reaction under microwave irradiation (Yield 92%) [25]. Zhong Zhang et al. highlighted $[Cu_{12}(BTC)_8][H_3PW_{12}O_{40}]$ as catalyst for Biginelli reaction and showed 90% yield in solvent free condition [38]. Recent report by Chavan et al. featured $H_4[PMo_{11}V_1O_{40}]$ as catalyst which on ultra sonication shows 93% yield where the reaction was performed in ethanol solvent system [39]. Such homogeneous catalysis often suffers from drawbacks related to separation and purification of products. Recently, various supported-POM based catalysts were tested in Biginelli reaction. J. S. Ghomi et al. reported Biginelli reaction with

dendrimer-attached PTA nanoparticles immobilized on nano silica as catalyst under ultrasonication [40]. Few of the listed POM based catalysts with their respective yield % are following: $H_{14}NaP_5W_{29}MoO_{110}@SiO_2$ (PASi) [82%], ZIF@9(NH_2) [32%], PTA@ZIF 9(NH_2) [85%] [41] , PTA@MIL-101 [90%] [42] were reported. The advantage of supported POM catalyst is the minimum usage of POM often dispersed all over the support. Hence a small amount of POM is effective as supported catalyst and thus industrially affordable.

Though many reports have shown POMs working as efficient catalyst for Biginelli reactions, there are very few reports where POM based catalyst have shown efficiency to catalyze one-pot one-step process starting from BzOH. Hence in this manuscript, we focus our work on the development of POM based catalyst that can show efficient catalytic effect in a one-pot one-step process starting from BzOH. The efficiency of the catalyst was tested at various pH values in a two-step and a one-step one-pot reaction methodology. The easy availability of the supporting polymer (Jeffamine®) as industrial byproduct and the efficient utilization of POMs in the catalyst formulation might help in the development of smarter industrial catalysts.

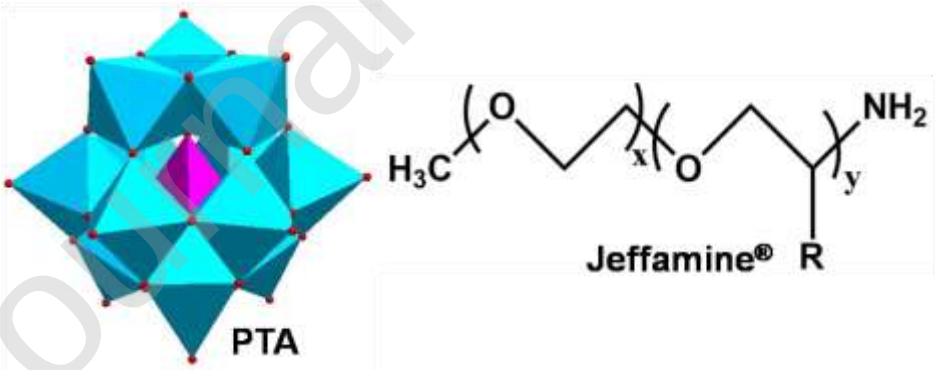


FIGURE 1. Structure of PTA and Jeffamine®.

RESULTS AND DISCUSSION

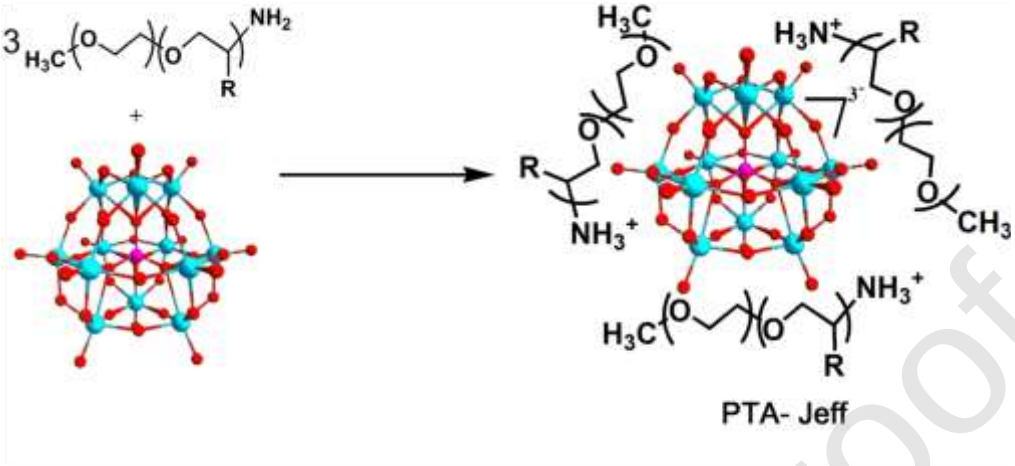


FIGURE 2. Pictorial representation of the liquid-catalyst of PTA- Jeffamine® mixture

For heterogeneous catalysis, often the disadvantage is the interface of the reaction phase with that of catalyst [43]. We have recently reported a novel catalyst that works well in the interface of aqueous and non-aqueous solvents. The catalyst is a hybrid of POM and a low molecular weight co-polymer, Jeffamine [44]. The POM immobilized with Jeffamine as support demonstrated stable structure-property relationship at a wide range of pH and temperature. Amphiphillic nature of the polymer support allowed the POM to work as effective catalyst in both the aqueous and organic phase. We showed that the Venturello ion formed due to a complex between the POM and peroxide stabilized by Jeffamine participated in the reaction and performed well as a phase transfer catalyst too [44]. The material was also shown to perform well as a multifunctional catalyst for various types of reactions like selective oxidation of BzOH to BzH or the synthesis of porphyrin in water [44, 45]. In the current manuscript, we have tried to demonstrate the activity of the POM-polymer catalyst and its effectiveness for Biginelli reactions.

Characterization of the catalyst

Jeffamine® belongs to a class of green di-block copolymer with ethylene oxide (EO) and propylene oxide (PO) moieties. The polymer is a class of low molecular weight polyetheramine obtained as byproduct from industry. Jeffamine® consist of temperature dependant phase separation properties, wide range of molecular weights, amine functionality, type of repetitive unit distribution and good low temperature properties. The preparation process of the catalyst is easy and scalable. Our group has reported that the PTA-Jeffamine® can catalyze the selective oxidation of BzOH to BzH in water. However, best conversion and selectivity of BzH was achieved at pH 7.5 [44]. Strong electrostatic interactions between the ammonium cation of Jeffamine® and anionic oxygen of PTA may be responsible for the enhanced solubility and stability of PTA in water phase [14]. The final pH of the medium was dependent upon ratio of POM/Jeffamine®. No additional acid or base was added to the reaction medium.

The prepared catalytic mixtures of PTA-Jeffamine® were characterized by various techniques. UV-Visible spectrophotometer was used for absorbance measurement in aqueous medium in the range of 200-800nm. The structure of PTA remained almost unchanged during catalyst preparation as evident in UV-Vis and FTIR data in Figure S5 (SI). Further structural analysis was done with X-ray diffraction and Raman spectroscopy. The morphological studies by FE-SEM (Figure S6) have already been reported in our recent work [44, 45]. Other POM-Jeffamine® catalyst were also prepared and characterized (Figure S7).

The XRD patterns of PTA and PTA-Jeffamine® catalysts are shown in Figure 3. XRD patterns of PTA represent the typical Keggin polyanion characteristics. The primary structure of PTA and PTA-Jeffamine® catalysts seemed identical when observed in their XRD patterns (Figure 3). PTA-Jeffamine® prepared at different pH may cause variations in the intensity of the peaks. The XRD patterns for the catalyst prepared at pH 7.5 and 8.5 indicated presence of PTA crystallites

well dispersed in the Jeffamine®. Sharp diffraction peaks at 2Θ values of 21°, 26°, 33° and 36° were indexed with reported data (JCPDS 50-0304 and other reported data JCPDS 50-0657) represents the cubic structure of Keggin PTA [46]. In Figure 3, the Jeffamine® alone occurs that an observable diffraction peak around 2θ 20° confirms the amorphous structure. A few not noticeable peaks of PTA crystal and mixtures suggest that the attached PTA molecule has monolayer dispersion on the surface of Jeffamine® core [46].

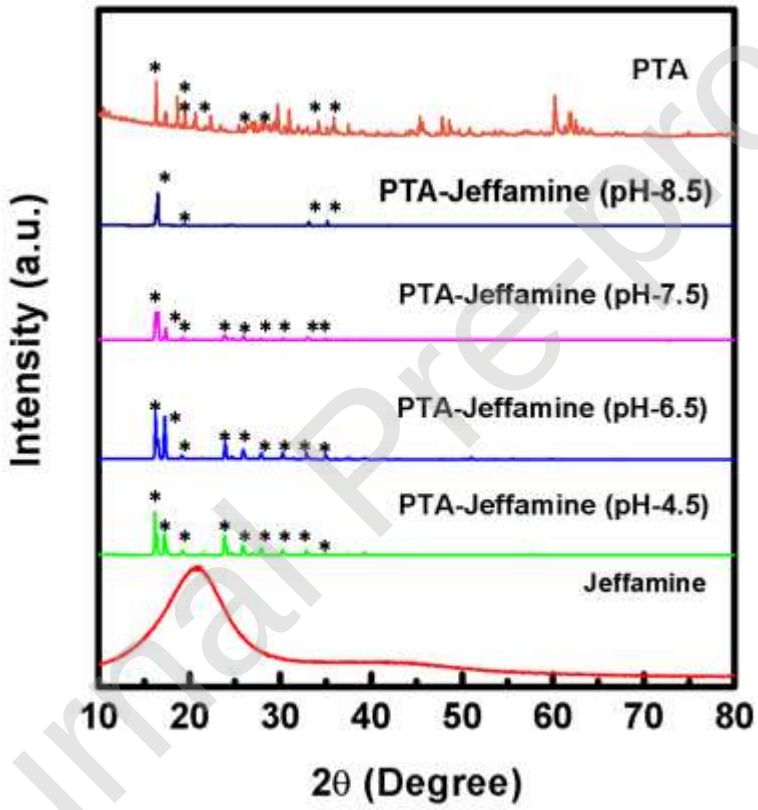


FIGURE 3. XRD patterns of PTA and PTA-Jeffamine® mixtures at different pH ratio.

PTA and PTA-Jeffamine® catalysts were further characterized by Raman spectroscopy to identify the structural changes of the prepared catalysts. The Raman spectrum of the Keggin heteropoly acid of pure PTA exhibits a strong band between 909 and 1005 cm^{-1} (Figure 4). Similar

observation was reported by S .R. Matkovic et al. and co-workers [47]. The Raman band at 1005 cm^{-1} attributed to the symmetrical stretching of the terminal tungsten oxygen (W=O) within the octahedral WO_6 unit was observed in the spectrum. The presence of PW_{11} unit was indicated from the band at 909, 513 and 218 cm^{-1} corresponding to Vs (W = Od), Vas (W-Od), Vas (W-Ob-W), Vs (W-Oc-W) and V (W-Oa) respectively. Where Oa, Ob, Oc and Od are the oxygen atoms attached to phosphorus and tungsten. In case of addition of Jeffamine® to PTA, certain spectral changes were observed (Figure 4). The red shifts of the Raman peaks may be caused by the surface modification of PTA by Jeffamine®. Peaks were observed in all mixtures in the spectral ranges between 113 and 191 cm^{-1}. It shows the lattice vibration in crystals and strong Raman peaks.

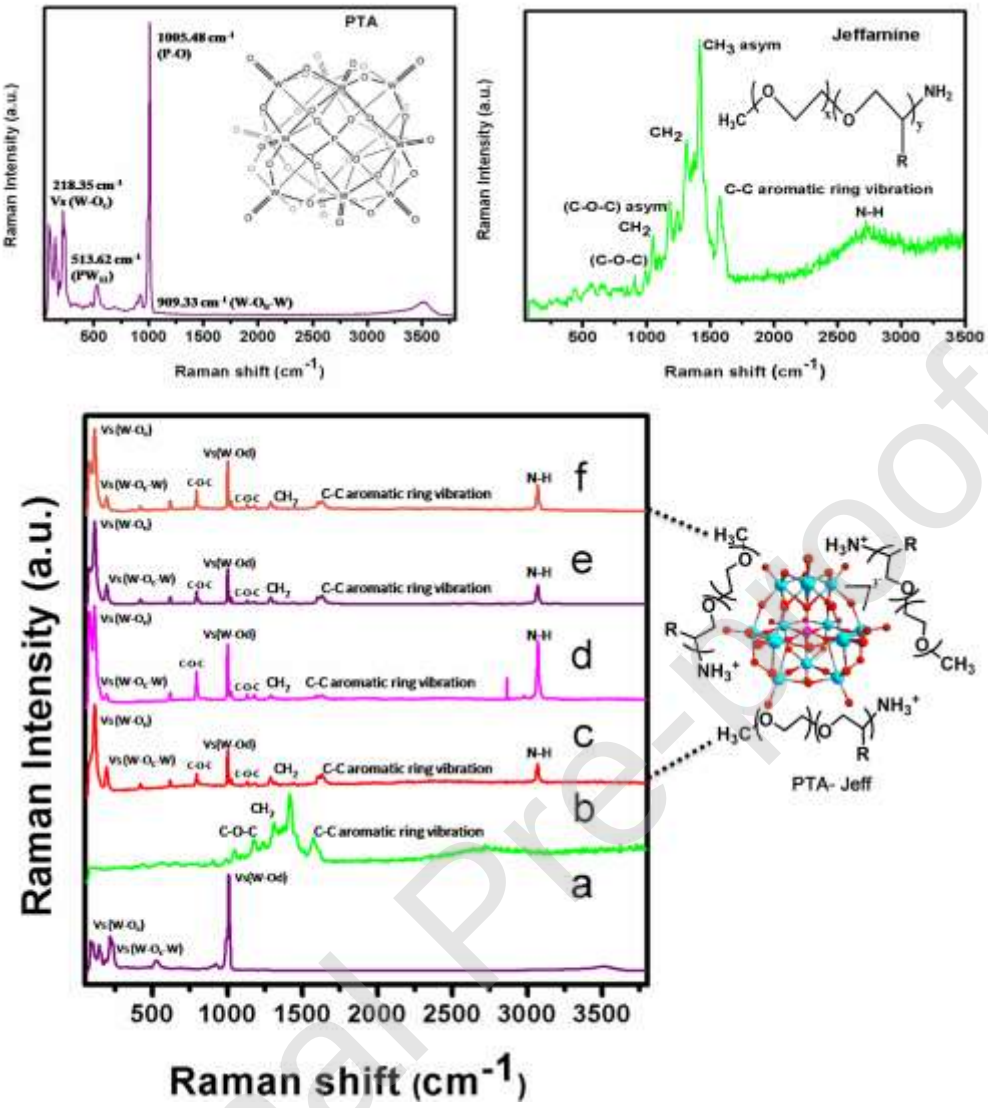


FIGURE 4. Raman spectra of a) PTA b) Jeffamine® c) pH 4.5, d) pH 6.5, e) pH 7.5 and f) pH 8.5 of PTA-Jeffamine® mixtures.

Catalytic performance of PTA-Jeffamine® in two step one-pot reaction system

In the two step reaction pathway, BzH was prepared in the first step by oxidation of BzOH in water using PTA-Jeffamine® catalyst and hydrogen peroxide as oxidant. After 1 h, to the same

mixture, proportionate amounts of urea and EAA were added. All the three components were added in a one-pot condition as shown schematically in Figure 5. Subsequent addition of ethanol to the reaction medium resulted in the formation of DHPM.

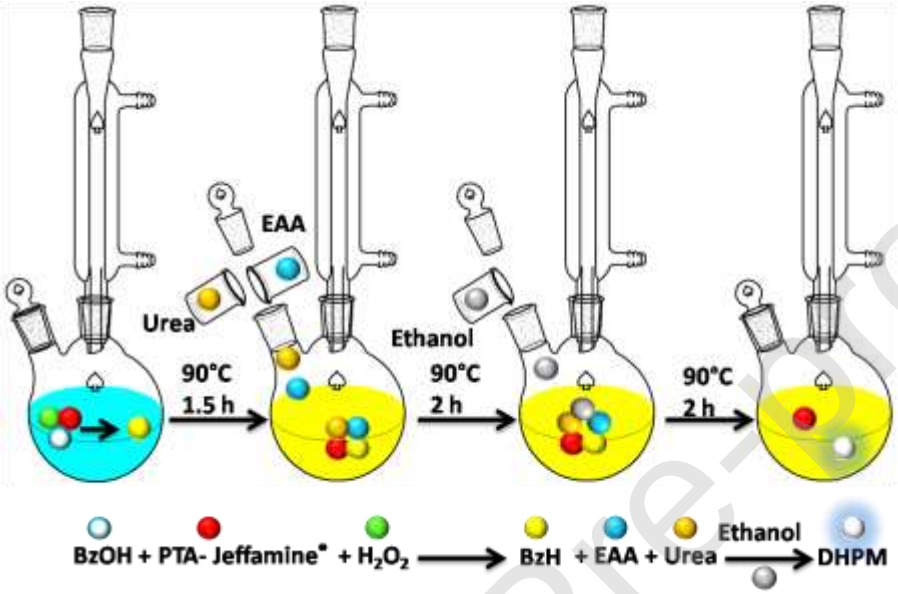


FIGURE 5. Schematic illustration of the catalytic behavior of PTA-Jeffamine® mixtures in dihydropyrimidinones synthesis via BzOH oxidation reaction process in two step one-pot condition.

However the yield of the DHPM varied depending up on the pH of the medium, that is, the amount of Jeffamine® as shown in table 1. Effect of pH on the reaction was studied by keeping PTA constant and varying the volume of Jeffamine®. The reason behind the pH dependency may be related to the structural changes of the catalyst as observed in our previous report at pH 7.5. The phase transfer behavior of the catalyst at pH 7.5 may also contribute to the better yield.

Table 1. The yield calculations of 3, 4-dihydropyrimidinones at different PTA-Jeffamine® pH ratio

S.No	Different pH catalyst Solutions	DHPM	Yield %	M.P
1.	PTA-Jeffamine® pH -3.5	White crystal	28%	200˚C
2.	PTA-Jeffamine® pH -4.5	White crystal	41%	196˚C
3.	PTA-Jeffamine® pH -5.5	White crystal	79%	182˚C
4.	PTA-Jeffamine® pH -6.5	White crystal	74%	202˚C
5.	PTA-Jeffamine® pH -7.5	White crystal	96%	193˚C
6.	PTA-Jeffamine® pH -8.5	White crystal	76%	190˚C
7.	PTA	White crystal	69%	196˚C
8.	Jeffamine®	White crystal	32%	165˚C

Characterization of DHPM

FT-IR spectra of DHPM obtained from Figure 6 shows two peaks for NH at first and third position in the range 3244 - 3115 cm^{-1}. The ester carbonyl stretching frequency was observed in the range of 1600- 1750 cm^{-1}. FT-IR peak for C=O was observed in the range of 1450-1600 cm^{-1}. The C-N bond in dihydropyrimidinones ring corresponds to peaks in the range of 1150-1350 $cm^{-1}$and for C-O-C stretch at 1290.38 and1240 cm^{-1} respectively. FT-IR spectroscopic data for DHPM obtained at different pH is shown in Figure 6a. The experimental procedure of controlled reaction is provided in SI and spectral data Figure S1. Table S1 shows the FT-IR spectral ranges for pure DHPM and catalyzed reaction products of DHPMs at pH-7.5. The results from the reactions separately done as controlled reactions with pristine PTA and Jeffamine® are shown in table 1. The products obtained from the controlled reactions were characterized by IR, UV and NMR spectroscopic studies (Figure S3&S4). Controlled reaction in absence of POM was also performed (Table S2). In the UV spectrum, the λ_{max} values of the DHPMs were observed at 284 and 245nm. The absorption maximum occurs at 284nm because the pair of electrons on nitrogen atom is in conjugation with the pi bond system of the benzene ring. The peaks may originate from $n\rightarrow\pi^*$ and $\pi\rightarrow\pi^*$ transitions from C=O group respectively. The pure DHPM after recrystallization

shows λ_{max} at 284 nm. The absorption peak at 284 nm observed for pure DHPM and synthesized DHPM products at different pH 3.5, 4.5, 5.5, 6.5, 7.5 and 8.5 are shown in Figure 6b.

NMR studies were performed to determine formation of DHPM and to determine if impurities are present (Figure 6c). The quartet of two protons and the triplet of three protons were seen around 4.08 ppm and 1.17 ppm, respectively. Both had a coupling constant (J) equal to 7.2 Hz, indicating presence of ethyl groups. The singlet of three hydrogen atoms around 2.34 ppm indicated the presence of the isolated methyl group. At 8.17 ppm and 7.26 ppm, multiplets made of two protons were indicative of an aromatic system, may be the pyrimidine aldehyde. A multiplet of three protons was observed around 7 ppm, again suggesting aromaticity. The peak for 7 H atoms at 7.26 ppm is due to peaks from the benzylic H and also from the two amide hydrogen. At 3.7 ppm the observed singlet consisting of 6 protons was indicative of the two CH_3 groups making up the dimethylamino moiety, while the singlet around 2.3 ppm made up of 3 protons indicated presence of an isolated methyl group. At 7 ppm aromatic protons around with each peak being comprised of 2 H atoms. It should be noted that around 8 ppm and 7.32 ppm, where there should be probable indication of singlet's consisting of only one H-proton, there are small peaks; however, their diminished sizes warranted no significant integration value, despite their apparent presence. The products were analyzed with NMR spectroscopy for structural elucidation (Figure 6c). Table S4 shows the Chemical shift values of DHPM pH 7.5. In the ^{13}C NMR spectrum (Figure S5), the chemical shift at δc 166.1 and 152.9 were due to carbonyl carbon, The chemical shift at δc 152.9, 148.6, 144.8, 128.9, 128.0 and 126.6, were on account of aromatic carbon. The chemical shift at δc 100.1 indicated presence of C=C. The chemical shift at 54.3, due to C-4 carbon and δc 60.0 for $-CH_2$ carbon was observed. The chemical shifts at δc 18.0, and 14.3 due to methyl carbon. The ^{13}C NMR and GC-MS spectrum data were shown in Figure S2. Mass spectrum of the DHPM

compound catalyzed by pH 7.5 recorded and its molecular ion at m/z 256, 213, 185, 171, 129, 73, 60, 43, 41.

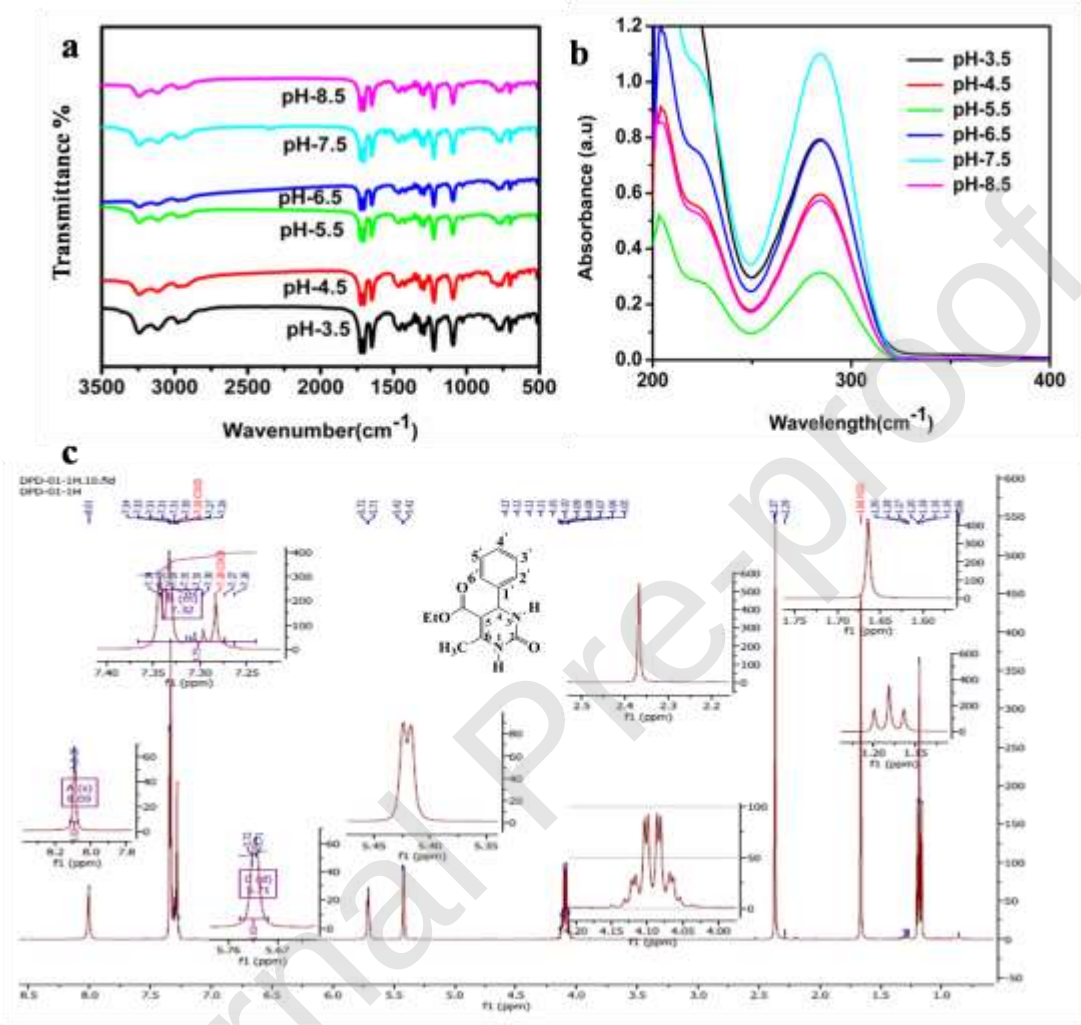


FIGURE 6. a) IR spectra of compound DHPM and different pH catalyzed reaction products of DHPMs b) UV-Visible spectra of compound DHPM and different pH catalyzed reaction products of DHPMs c) NMR spectra of compound DHPM and different pH catalyzed reaction products of DHPMs.

The above mentioned FT-IR and NMR analysis of the products obtained from the two step one pot reaction suggest that PTA-Jeffamine® derivative was more effective than only PTA as catalyst. Because in only presence of PTA in oxidation reaction there was no formation of benzaldehyde and the detailed section was reported in our previous work [44]. It was assumed that may be PTA was working efficiently towards the first step of the reaction (i.e., oxidation) in presence of Jeffamine®, it was also capable to formulate the second step (Multicomponent) more efficient. The controlled reaction also carried out without any catalyst for 5.5h, monitored and found that there is no product formation. The reaction mixture was characterized by UV-visible, FT-IR, ^{1}H and ^{13}C NMR spectroscopy mentioned in SI (Figure S1).

Anti-cancer analysis

Non-small cell lung carcinoma (NSCLC) is a devastating cancer arising in the lung epithelial cells. NSCLC accounts for about 85% of lung cancer [48]. This cancer remains aggressive with increasing incidence and poor survival rate despite of current multimodal treatment. It usually grows and spreads slowly compared to small cell lung cancer and remains as a common lung carcinoma [49]. DHPM compound and its derivatives were mostly tested on various cancer cell lines including breast, liver, ovarian, gastric, kidney, skin, colorectal, prostate, central nervous system, cervical, endothelial, pancreas, blood, lymphoma, myeloma, Pheochromocytoma and lung [50]. The U251 and OVCAR-03 were found to be more sensitive to DHPM derivatives which affected the cell growth at IC50 <10µg/mL [51]. In this manuscript, the DHPM synthesized by the PTA-Jeffamine® catalyst (pH 7.5) was tested against A549 cell lines as a model for NSCLC. This was also done to test the efficiency of the synthesized DHPM. Results of the DHPM test compound produced a dose and time dependent cell cytotoxicity against A549

cell line. After 24 h and 48 h of treatment, the compound induced up to 50% cell death at different concentrations in A549 cell line. A significant decrease in cell viability at higher concentrations (i.e., above 200µM) was observed in A549 cell line in Figure 7. To establish the optimal treatment concentration for the drug, its IC50 values were calculated and compared. The half maximal inhibitory concentration of DHPM compound against A549 cells was shown at 202.6µM, with an R^2 value of 0.944. Analyzing the percentage of viability and percentage of growth inhibition of DHPM compounds shows that the test compounds are dose dependent and decreases the cell viability as the dose is increased. PBMCs viability was found to remain more than 70% even after 72 h of drug treatment. The percentage of cell viability at different concentrations of DHPM at PBMCs was calculated to identify the specificity of DHPM compound with cancer cells (Figure 7). A549 non-small cell lung cancer cell line untreated control cells and cells treated with 100µM DHPM were shown in Figure 7a & b. There was no significant higher cytotoxic effect observed in PBMCs at IC50 concentration against A549 cells. Rather, the percentage of viability remained higher in PBMCs at all concentrations compared to untreated A549 cells. Though there were few variations in the pattern of cell growth inhibition at different concentrations, the percentage ranged between 20% - 35% (rarely >35%). The time dependent cytotoxicity of DHPM compound was evaluated by treating the cells with drugs for 24 and 48 h. Cell deaths in 48 hrs treatment were higher when compared to 24 h treatment as shown in Figure 7d.

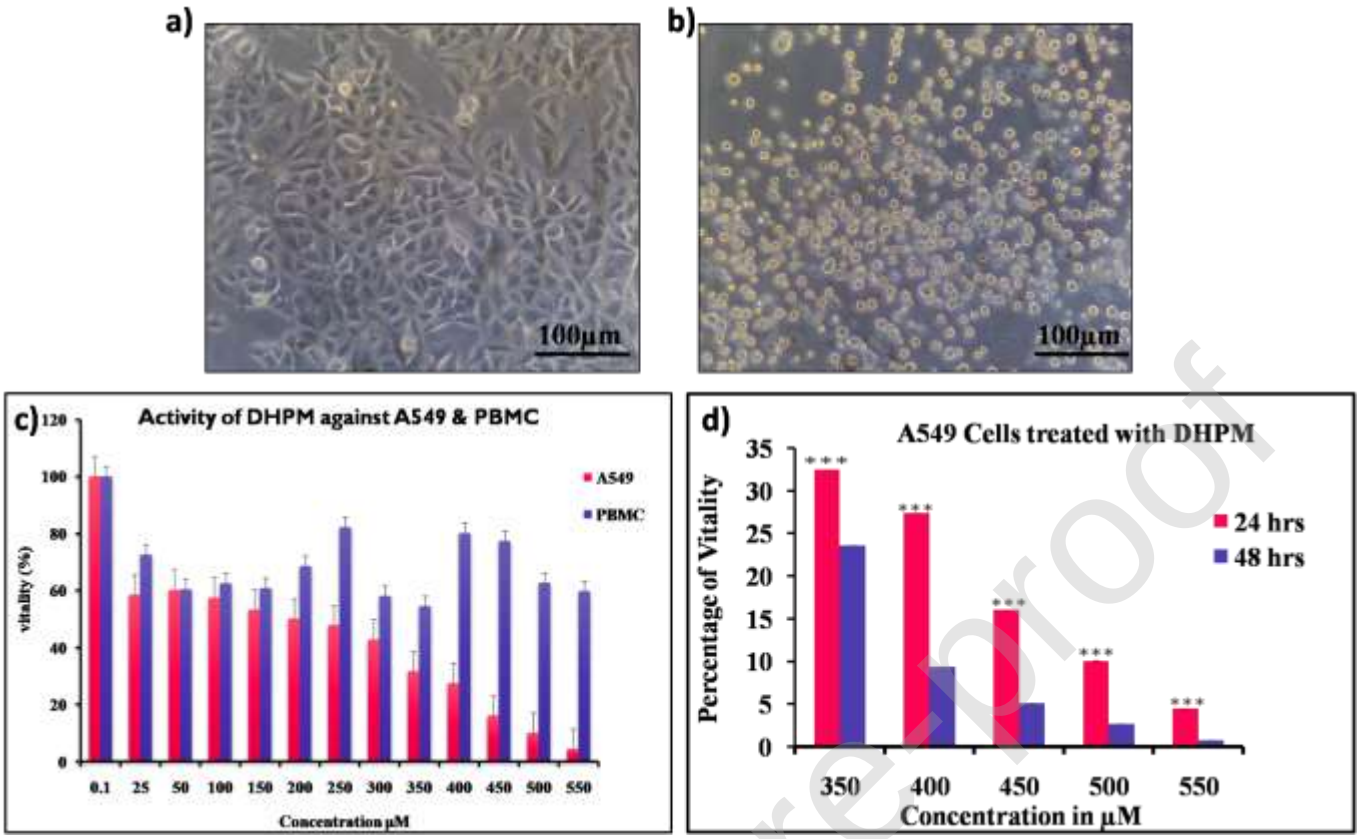


FIGURE 7. A549 non-small cell lung cancer cell line a) Untreated control cells b) Cells treated with 100µM DHPM. c) Dose dependent cytotoxicity of DHPM against A549 and PBMC cell lines d) Time dependent cytotoxicity of DHPM against A549 cell lines. [(***- extremely statistically significant, **- statistically very significant)]

An *in vitro* examination of cytotoxicity induced by DHPM compound was performed. Results indicate that the drugs induce a dose dependent cell death. The cell viability decreased gradually as the concentration has been increased. The percentage of cell viability and half maximal inhibitory concentration significantly reduced in 48 h treatment compared to 24 h treatments. We used healthy PBMCs to evaluate any cytotoxicity of DHPM against healthy host cells. DHPM analogue was non-toxic to PBMCs on comparing the activity of DHPM to A549. In

the published work of Amany S. Mostafa, Khalid B. Selim, 2018, the DHPM derivatives exhibited lower toxicity against MRC-5 cells at IC50 44.16 and 32.04µM [52]. But the toxicity of our novel synthesized DHPM remained low even at the half maximal inhibitory concentration (<20%) and more than 80% of cells remained viable after the treatment of 72 h. In the research work published by Venugopala *et al*., the DHPM derivatives exhibited up to 20% growth inhibition at 50µg/mL [53].

Comparing the DHPM derivatives treated against breast cancer cell line MCF7 and MDA-MB-231, the novel synthesized DHPM molecule has proven to show significant decrease in cell viability gradually in all concentrations tested [54]. The half maximal inhibitory concentrations have also fallen between 200µM - 250µM, whereas in the research work published by Guido *et al*., the concentrations used were up to 1Mm [54]. The DHPM derivatives exhibited over 80% of cell growth inhibition with IC50 around 6 to 35µM. The monastrol mimic Biginelli DHPM derivatives exhibited cytotoxicity against HepG2 with half maximal inhibitory concentration of 120.62µg/mL and it exhibited weak toxicity towards HeLa cell lines with IC50 200µg/mL [55].

Though the DHPM compound obtained by the PTA-Jeffamine® catalyst was found to have moderate activity against cancer cell lines at lower concentrations, it is more specific only to cancer cells. At the same concentrations, the compound exhibited very low toxic effect on normal cells (PBMCs). In addition to these, further studies can be done in order to elucidate the molecular pathways in NSCLC cancer cell line death induced by DHPM compound.

Catalytic reaction with other POMs

In order to compare the catalytic activity of PTA based Jeffamine® catalyst with other Keggin structure POMs, the following reactions were performed as shown in table 2. The two step one-pot Biginelli reaction starts with 1.9M of BzOH, 30% H_2O_2 (3.9 M) in presence of POM-Jeffamine® catalyst with refluxing temperature of 90^0C and time 1.5 h. In that same pot condition, addition of 2.1M of ethyl acetoacetate and 1.4M of urea was done. After 2h of the reaction, 20ml of ethanol was added to the above reaction mixture and again reflux at 90˚C for 2 h. The yellow color of the homogeneous reaction mixture was kept for crystallization. The PMA-Jeffamine® catalyst produces the 37% yield and the other two POM catalysts showed no product formation. The results indicated that the Keggin structure of PTA supported on Jeffamine® is the better catalyst for DHPM production in water.

Table 2. The yield calculations of 3, 4-dihyropyrimidinones at different POM-Jeffamine® pH ratio

S.No	Different pH; catalyst Solutions	DHPM	Yield %	M.P
1.	PMA-Jeffamine® pH-5.7 Phosphomolybdic acid	White crystal	37%	196˚C
2.	STA-Jeffamine® pH-7.8 Silicotungstic acid	No product formation		
3.	$H_3PMO_{12}.SiO_2$-Jeffamine® pH-6.7	No product formation		

Catalytic performance of PTA-Jeffamine® in one-step one-pot reaction system

After successfully testing two step one-pot process, the PTA-Jeffamine® catalyst was tested for the one-pot one-step process starting from BzOH. The reaction was initiated with the addition of 1.9M of BzOH, 30% H_2O_2 (3.9 M), 2.1M of ethyl acetoacetate, 1.4M of urea and catalyst in a

single reaction vessel followed by refluxing at 90^0C for 5.5 h (Figure 8). Three different ratio of PTA-Jeffamine® was tested as catalyst separately. PTA was kept constant and the amount of Jeffamine® was varied resulting in three different pH (6.5, 7.5 & 8.5). No acid was added and the pH was adjusted by controlling ratio of PTA/ Jeffamine®. The clear yellow colored homogeneous reaction mixture was kept overnight for crystallization. The white crystal mixture obtained was washed thoroughly with ethanol and water mixture (2:1) until the product turned pure white and was further recrystallized from ethanol.

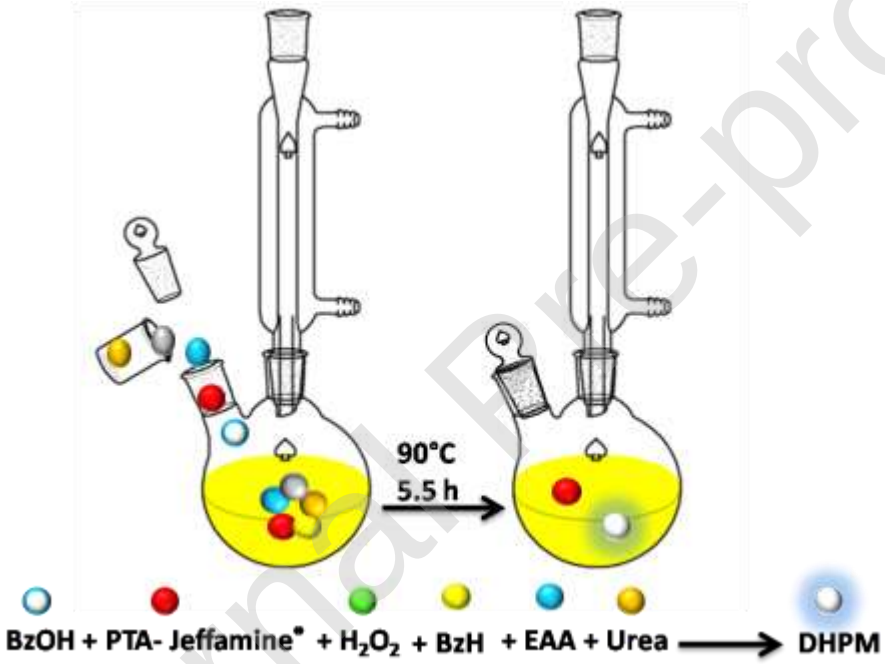


FIGURE 8. Schematic illustration of the catalytic behavior of PTA-Jeffamine® mixtures in DHPM synthesis via BzOH oxidation reaction process in one-pot one-step protocol.

Recrystallized compound was characterized by IR, NMR and mass spectroscopy (Figure S9a & b). The yield % calculated for the three reactions indicate product formation only at pH 7.5.Repeated reactions reproduced the same results. Such selective formation of DHPM can be

related to the activity of PTA-Jeffamine® catalyst prepared at pH 7.5. To explain the observation we consider a few factors that may have influenced the reaction. We may assume that the Venturello ion has a considerable effect on stabilizing the product as has been shown in our previous report on the selective conversion of BzOH to BzH. Secondly we understand that the very fast production of BzH from BzOH in the reaction medium due to the catalyst can contribute substantially to the yield [44]. Following Le Chatelier's principle, if the concentration of substrate at any point of time in the reaction medium is high, the forward reaction also proceeds fast to completion. As the rate determination step for Biginelli reaction is either the formation of the iminium ion or the carbenium ion intermediate, the fast build up of BzH in the reaction medium can influence the equilibrium leading up to the intermediate formation [56,57]. High concentrations of the intermediates may subsequently control the overall kinetics of the Biginelli process. The stabilizing effect of the Venturello ion against over oxidation of BzH to BzAc provides effectively high concentrations of BzH in the reaction medium that favors the may favor the above equilibrium [44]. Further we may also predict that the worm-like micelles of the catalyst formed at pH 7.5 (as shown in our previous reports 44, 45) may be the reason for the catalyst to be more active for the one step one-pot reaction. Table 3 shows a comparative study with literature reports on one-pot one-step processes starting from benzyl alcohol. Though the yield of our reaction is high compared to the other reports and our reactions conditions are more environmentally friendly (pH 7.5) compared to other reaction conditions as shown in table 3.

Table 3. Reaction starting from benzyl alcohol studies on two-step and one-step one pot system.

S.No	Catalyst	pH	Time/temp °C	Oxidizing agent	Yield %	Ref
Two-step one pot system						
1	$[MIMPS]_3PW_{12}O_{40}$	acidic	10-20 min, 700W	$NaNO_3$	90	[31]
2	$CuSO_4$ x H_2O in PBS	5.2	24 h 60	-	81	[32]
3	$[Hmim]HSO_4$	acidic	2-4 h 80	$NaNO_3$	94	[33]
4	$Bi(NO_3)_3.5H_2O$	-	3h 90	$Bi(NO_3)_3$	82	[34]
5	$Al(NO_3)_3 \cdot 9\ H_2O$	-	15 min, 80	-	93	[35]
6	PTA-Jeffamine® pH -7.5	7.5	5.5 h 90	H_2O_2	96	Present manuscript
One-step one pot system						
7	PTA-Jeffamine® pH -7.5	7.5	5.5h 90	H_2O_2	87	Present manuscript

Characterization of DHPM from one-pot one-step process

FTIR, NMR and ESI-Mass spectra were carried out to characterize the Biginelli product obtained from one-pot one-step process performed at pH 7.5 FTIR and NMR spectral characterization data were provided in Figure S9. The ESI-Mass spectra of pH 7.5 catalyzed reaction product of DHPM were shown the molecular ion at m/z 102, 165, 187, 188, 203, 266, 351, 360 and their mechanistic representations of the mass spectra were shown in Figure 9.

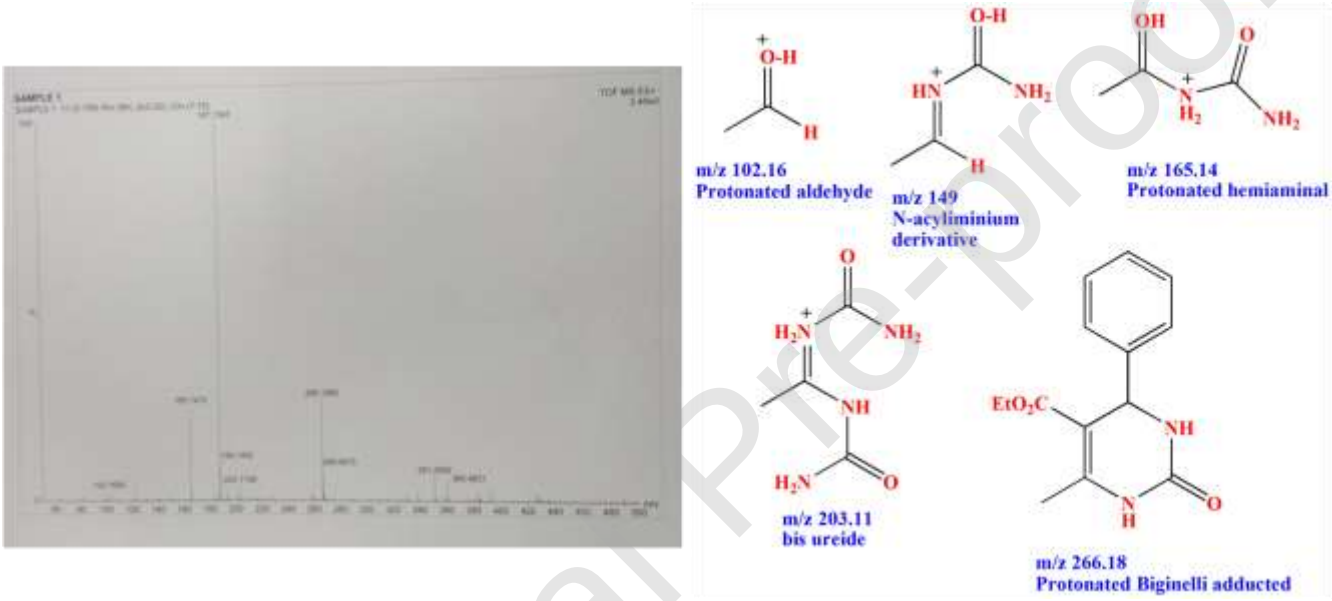


FIGURE 9. ESI Mass spectra of one-pot DHPM

Characterization of the PTA- Jeffamine® catalyst after reaction

After the Biginelli reaction was over, the phase separated aqueous part containing the PTA-Jeffamine® catalyst was kept under observation in normal laboratory condition. The aqueous part separated after first reaction cycle dried and used for characterization (Figure 10a). Due to strong electrostatic interactions with the PTA molecules, Jeffamine® can remove most of the PTA molecules from organic part and bring them into the aqueous part. FTIR spectroscopy was used to

detect the presence of PTA along with Jeffamine® in the phase separated aqueous part (Figure 10b). FTIR data shows peaks at 1247 cm^{-1} (ether) and 3435 cm^{-1} (amine) that indicate presence of Jeffamine® along with four characteristic bands of PTA at 1087,948,883,842 cm^{-1}. The four FTIR peaks of PTA were observed to be slightly blue shifted as compared to that of pure PTA-Jeffamine® catalyst. The shift in FTIR peak position indicates changes in characteristics of the bridge-oxygen present in PTA. The characteristic changes observed in the FTIR data of PTA can be attributed to the presence of trace amounts of BzA in the aqueous part. BzA can be detected in the phase separated aqueous part from FTIR data. Sharp FTIR peak at 1714 cm^{-1} corresponding to –CO of COOH. Presence of BzA resulted in lower pH values of the extracted aqueous part from the first reaction cycle (pH 7.5). The same may also interfere in the catalytic process resulting in lower conversion of BzOH and lower selectivity towards BzH. After recover of catalysts from separation, the Tollen's tests performed with the used catalyst present in the phase separated organic part in Figure S8.

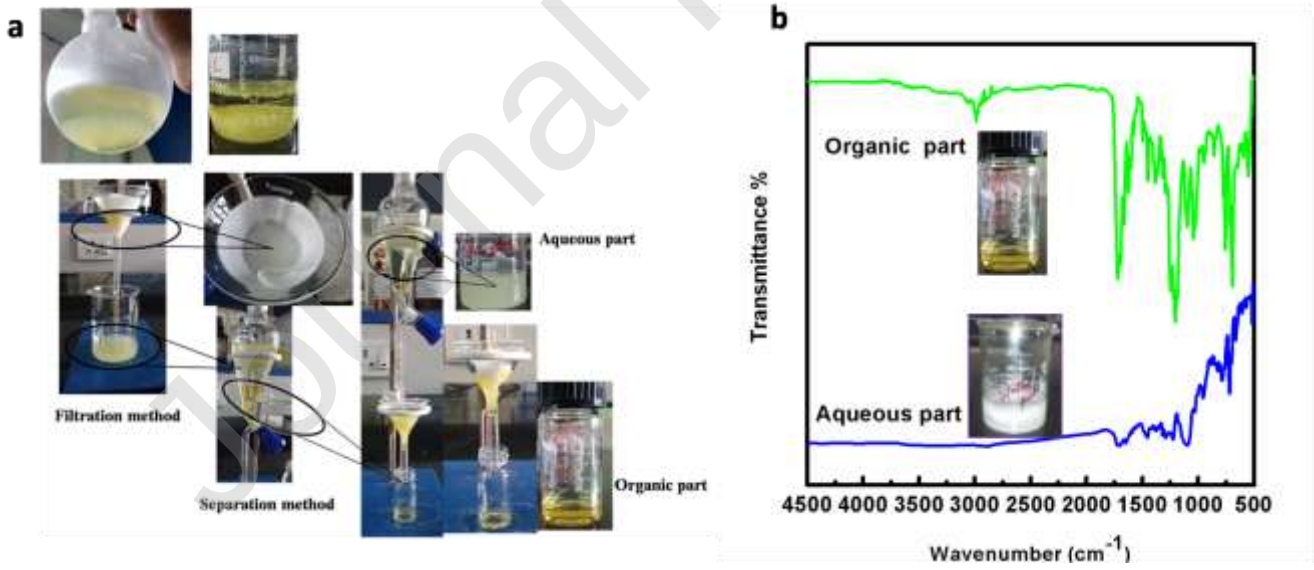


FIGURE 10. a) Catalyst separation process b) IR spectra of the aqueous and organic part of the catalyst recovery process.

CONCLUSIONS

Literature reports have suggested that Biginelli reactions are mostly acid catalyzed. Such dependency on acidic medium may affect the performances of the Biginelli products in anticancer and other biological applications. In the present manuscript we have for the first time successfully prepared Biginelli compounds at almost neutral pH (7.5) with the PTA-Jeffamine® catalyst in aqueous medium in a one-pot one-step process. The catalyst was effective both in the two-step as well as one-step one pot reaction methodologies starting from BzOH. Both processes showed appreciable results with the one-pot one-step protocol demonstrated appreciable yield (87%) of DHPM within 5.5 h at pH 7.5. No acid was added and the pH was adjusted by controlling ratio of PTA/ Jeffamine®. The catalytic results were compared with literature reports for one-pot one-step Biginelli reactions starting from benzyl alcohol. The catalyst was easily separated from the products though a simple freezing process. DHPM obtained from the one-pot two-step process demonstrated anti-cancer activities. The PTA-Jeffamine® catalyst showed better performance compared to other POMs having Keggin structure. The Biginelli products were well characterized with UV-Vis, FTIR and ^{1}H and ^{13}C NMR spectroscopy. The use of hydrogen peroxide as oxidant deserves a special mention in the present manuscript. It can replace conventional oxidizing agents containing metal salts like nitrates. It also helps in forming the active Venturello ion in association with the catalyst that stabilizes BzH. The work presented in this manuscript may pave ways to the development of industrial catalysts and environmentally friendly reaction protocols.

EXPERIMENTAL

Preparation of PTA-Jeffamine®

0.1 M of Jeffamine® was taken and titrates against the 0.01M of PTA, the pH of the solution was adjusted for values of 3.5, 4.5, 6.5, 7.5 & 8.5 and then the mixture was stirred overnight.

Figure 1 shows the structures of PTA and Jeffamine®. In the present work, the catalytic effect of PTA on synthesis of dihydropyrimidinones was studied in presence of Jeffamine® in water.

Procedure for the two-step one-pot synthesis of dihydropyrimidinones

An aqueous solution of PTA (0.01 M) was drop wisely added to 0.1M of Jeffamine® under continuous stirring. pH of the medium depended on the volume of added Jeffamine® as the amount of PTA was kept constant. 1.9M of BzOH, 30% H_2O_2 (3.9 M) was added to the catalyst solution and refluxed at 90^0C for 1.5 h. In that same reaction vessel (one-pot), 2.1M of ethyl acetoacetate and 1.4M of urea were added. After 2h, 20ml of ethanol was added to the above reaction mixture and again refluxed at 90˚C for 2 h. The yellow color of the homogeneous reaction mixture slowly turns to yellowish white solid. The reaction was monitored by TLC (ethyl acetate/ n-hexane). The solid product was kept in the refrigerator overnight for crystallization. Sometimes the reaction mixture was kept at room temperature overnight. The white crystal mixture was washed thoroughly with ethanol and water mixture (2:1) until the product turned pure white and recrystallized from ethanol. The solid product was characterized by FT-IR, UV-Visible spectroscopy, and ^{1}H, ^{13}C NMR spectroscopy.

Material and methods

Benzyl alcohol, hydrogen peroxide, ethyl acetoacetate, urea was purchased from LOBA chemie and were used without purification. Jeffamine® was obtained as gift sample from Huntsman India Ltd. Phosphotungstic acid (PTA), Phosphomolybdic acid (PMA), Silicotungstic acid (STA) was purchased from Sigma and Fluka used as received without further purification. Ethanol was purchased from China chemicals. Milli-Q water was used in all preparation. The standards and the reaction mixture were spotted on TLC Silica gel 60 F_{254} purchased from Merck chemicals. The cytotoxicity analysis materials and methodology mentioned in SI.

Characterization methods

The reaction mixture was spotted on TLC aluminium oxide 150 F_{254} neutral (Solvent: Hexane/chloroform, 9:1) purchased from Merck. The solvent system is 9:1 Hexane/chloroform. UV-Visible spectrum was recorded with Shimadzu UV-1800 spectrophotometer. The samples were placed on sample holder with quartz window of 1cm path-length. The scanning range is 200-800 nm. FT-IR spectra were recorded using Shimadzu IR affinity series 1S in the region of 4500-600cm^{-1}. Samples were analyzed with KBr and ATR accessory. The products were analyzed with NMR spectroscopy for structural elucidation. ^{1}H and ^{13}C NMR spectra were analyzed using Bruker BioSpin GmbH. DMSO and $CDCl_3$ was used as a solvent for analysis. X ray-Diffraction analysis was performed using CuKα radiation, (k = 1.5406 A° at 40 kV and 30 mA, Empyrean, Malvern Panalytical) for the 2θ measurement and scanned from 10-80° for patterning of the samples. Raman spectroscopy was used for the structural elucidation of molecular structure of the samples. The spectral range was scanned from 200-3500 cm^{-1} (WiTec alpha 300, Germany).

Declaration of interests

The authors declare that they have no known competing financialinterestsor personal relationships that could have appeared to influence the work reported in this paper.

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