Bull. Chem. Soc. Ethiop. **2024**, 38(4), 1103-1118.
DOI: https://dx.doi.org/10.4314/bcse.v38i4.23
© 2024 Chemical Society of Ethiopia and The Authors
ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

IN SILICO AND IN VITRO STUDY OF PYRIMIDONES SYNTHESIZED WITH ETHYLENEDIAMINE-MODIFIED B-CYCLODEXTRIN: POTENTIAL AGAINST ESBL *E. COLI*

Manojkumar Rajapriyan[1], Javed Masood Khan[2], Imran Khan R.[3], Ayyiliath M. Sajith[4] and Syed Ali Padusha M.[1*]

[1]Postgraduate & Research Department of Chemistry, Jamal Mohamed College, Affiliated to Bharathidasan University, Tiruchirappalli, Tamilnadu – 620 020, India
[2]Department of Food Science and Nutrition, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia
[3]National Postdoctoral Fellow, Department of Organic Chemistry, Indian Institute of Science, Bangalore, Karnataka – 560 012, India
[4]Postgraduate & Research Department of Chemistry, Kasaragod Government College, Kannur University, Kasaragod, Kerala 671123, India

(Received January 16, 2024; Revised March 18, 2024; Accepted March 23, 2024)

ABSTRACT. Modern organic synthesis is primarily focused on developing environmentally benign synthetic protocols by employing green chemistry principles. Accordingly, in our recent research work, we herein report the use of modified supramolecular host cyclodextrin as an effective solid based green catalyst for accessing structurally diverse and medicinally relevant pyrimidone architectures. The catalyst and the synthesized compounds **4** (**a-r**) were characterized using FT-IR, NMR and GC-mass spectroscopy. Major highlights of the reported work include the economical atom process, remarkably gentler reaction conditions, ease of operation, high isolated yields, and excellent catalyst turnover numbers. The molecular docking studies suggest that the compound 4n has hydrogen bonding, hydrophobic and π-pair interactions with the active site of the CXT M 15 receptor. Further, the *in-vitro* antibacterial study as well as the screening of anti-biofilm activity resulted in a BIC value of 76.79 ± 0.785% at a concentration of 10 µg/mL and morphological alterations induced by DHPMs against the ESBL *E. coli* strain aggregated with a good result.

KEY WORDS: Ethylenediamine modified β-CD, Multicomponent reaction, 3,4-dihydropyrimidin-2(1*H*)-ones DHPM derivatives, Solvent-free conditions, Reusability, Antibactercidal

INTRODUCTION

After the initial discovery of cyclodextrins (CD) in 1891 by Villiers, the use of these oligosaccharides for various catalytic applications was disclosed by various researchers across the globe [1]. In recent years, CD have been extensively serving as host molecules in supramolecular chemistry. They offer several advantages over other host molecules, including wide availability from renewable sources, good water solubility, biocompatibility and simplicity in chemical modification. Their molecular recognition of guest molecules is also well recognized [2]. These "molecules of holes" have lately gained favour as building blocks for the self-assembly of supramolecular structures with varied porosity [3]. The primary characteristic of CD is its conical configuration, which creates a hydrophobic interior cavity capable of accommodating a diverse range of guest molecules possessing suitable polarity and sizes. In addition, the hydrophilic outer surface of the CD can function as a molecular vehicle, allowing hydrophobic molecules to be transferred to the aqueous phase [4].

CDs are classified as α-CD with six D-glucose units, β-CD with seven D-glucose units, and γ-CD with eight D-glucose units, which are the most prevalent [5]. The hydroxy functional groups

*Corresponding authors. E-mail: sp@jmc.edu
This work is licensed under the Creative Commons Attribution 4.0 International License

on CD dictate the majority of their chemical characteristics, say solubility in specific. Particularly, the primary and secondary hydroxy groups on β-CD have the potential to form a high intramolecular H-bonding network, which results in the reduction of water solubility. Thus, the hydroxy group on β-CD is replaced by the methoxy group which reduces the H-bonding strength eventually and emphasizes the water solubility [6].

In modern drug discovery approaches, introducing multiple diversity points in a molecule has immense potential for generating hit molecules for various therapeutic areas. Among the various synthetic approaches, MCRs play a vital role in accessing this structural diversity at multiple points. Such reactions explore the synthesis of bioactive molecules and also facilitate the difficult task of pharmaceutical and therapeutic chemists. By this fact, the Biginelli reaction has been utilised for the synthesis of several bioactive compounds known as 3,4-dihydropyrimidin-2(1H)-ones (DHPMs) [7].

The importance of heterocyclic frameworks is well documented in literature as depicted by their importance in various fields like material science, agrochemicals and drug discovery. DHPMs are found to possess a diverse range of pharmacological properties, including anti-tubercular, antimicrobial, anti-consultant, anti-cancer, and anti-viral properties [8].

Our current research is mainly focused on employing cyclodextrins as catalysts for Biginelli condensation to access the pyrimidone analogues. It has been found that assorted catalysts and several methods were employed for the same. Many catalytic systems, such as the use of strong protic acids H_2SO_4 [9], HCl [10], various Lewis and Bronsted acids like $Bi(NO_3)_3$ [11], Sulfated silica tungstic acid [12], sulfated tungstate [13], SiO_2-polyphosphoric acid (SiO_2-PPA) [14], silica sulfuric acid [15], bismuth subnitrate [16], cellulose sulfuric acid [17], sulfated polyborate [18], Al_2O_3-$MeSO_3H$, $Al(HSO_4)_3$, Al_2O_3-SO_3H, sulfated zirconia, zeolites, and metal trifles [19] were successfully employed. Further methods involved are ultrasonic [20], microwave-assisted [21] and ionic liquids employing different green catalysts [22]. However, most of these reported methodologies have several limitations, including low yields, prolonged reaction times and the use of metal catalysts.

While native cyclodextrins can operate as catalyst for diverse range of organic reactions, the accession of acidity or basicity of the functional groups allows for the precise characterization their functions. Per-6-amino-cyclodextrin, a successful modified form of cyclodextrin contains basic amino groups function as a catalyst and supramolecular ligand for reactions such as asymmetric Michael addition [23], Mizoroki-Heck coupling [24], N-arylation [25], cyanation [26] and also for the multicomponent synthesis of pyranopyrazoles and 2-amino-4H-benzo[*b*]pyrans [27]. The enhanced solubility in the reaction medium is a major hallmark of homogeneous catalytic systems thereby increasing their catalytic activity and substrate accessibility to the catalytic site when compared to heterogeneous catalysts [28].

The synthetic methodologies developed have several limitations such as the use of costly catalysts, hazardous chemicals, higher temperatures, longer reaction time, time-consuming procedures and lower yields. To fulfil the need for developing more environmentally benign protocols, our research was focused on the use of modified cyclodextrins as catalysts for their operational simplicity, enhanced yields, cost effectiveness, faster reaction rates, etc., for the synthesis of DHPMs derivatives.

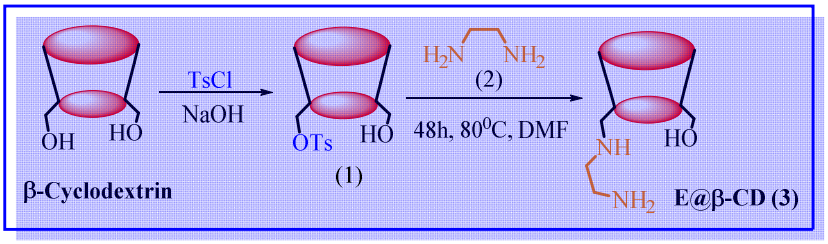
There is a significant increase in bacterial infections in recent years. Bacteria have been scientifically established as the primary catalyst for the most fatal illnesses and massive outbreaks in the annals of human society. Investigations into protein-compound interactions are crucial in understanding biological processes and are considered the second stage in the development of rational drug design. Proteins play a crucial role as macromolecules that provide a wide range of tasks within living organisms. The activities of proteins mostly rely on their precise three-dimensional structures, which are influenced by several non-covalent interactions including π–π interactions, hydrogen bonds, van der Waals contacts, electrostatic interactions, and hydrophilic/hydrophobic effects.

Gram-negative bacteria that possess extended-spectrum beta-lactamases (ESBLs) exhibit resistance to a wide range of penicillin and cephalosporin medications, as well as other medicines. Escherichia coli (*E. coli*) is the primary generator of extended-spectrum beta-lactamases (ESBLs) that are commonly linked to urinary tract infections. In certain cases, these infections may escalate to more severe conditions such as blood poisoning, posing a significant risk to an individual's life. ESBLs, which confer resistance to third-generation cephalosporins, are now globally detected in all Enterobacteriaceae species, with a particular emphasis on *Escherichia coli* [29]. Bacterial biofilms have a complex and dynamic architecture, manifesting on various inanimate substrates including plastic, glass, metal, and minerals, as well as animate substrates such as plants, animals, and humans [30].

In this study, we focused on a series of 3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs) synthesized using aromatic aldehydes, heterocyclic aldehyde, acetoacetate (Ethyl, Methyl & Ethyl-4-chloro), and Urea with modified β-cyclodextrins-ethylene diamine (E@β-CD) as a carrier. The synthesized compounds were further evaluated for, the molecular docking study which was performed using docking tool AutoDock Tool version 1.5.6, against the ESBL-producing *E. coli* receptors (CTX-M-15). The compounds were chosen as per the results obtained from the *in-silico* study to administer in the in-vitro studies. The study also compiles the screening of anti-biofilm activity and morphological alterations induced by DHPMs against the multidrug resistant ESBL *E. coli* strain.

RESULTS AND DISCUSSION

In the current work, we have employed E@β-CD (3), an ace supramolecular host for the synthesis of three-component pyrimidine derivatives under solvent-free conditions at ambient temperature. The reusability of the E@β-CD (3) for multiple times highlights the significance of the developed protocol in terms of green chemistry perspective. In Scheme 1, the protocol for the synthesis of E@β-CD (3) is illustrated. Tosylation was assisted to develop mono-6-tosyl-β-cyclodextrin (1) from commercially available β-CD and the intended product, E@β-CD (3) was produced as a yellow solid after treatment with ethylenediamine (2). E@β-CD (3) was successfully synthesized in a single step with a 78% yield. NMR and ESI-MS spectra validated the structure of E@β-CD (3).



Scheme 1. Synthesis of ethylenediamine modified β-cyclodextrin E@β-CD (3).

ESI-MS spectrum showed an m/z peak at 1177.89 which corresponds to [M+1 adduct]. The chemical shift values of the ^{1}H NMR and ^{13}C NMR spectra are found to be in good agreement with the synthesized E@β-CD (3).

Insight II molecular modeling studies were used to conduct energy minimization analyses to ascertain the prospective inclusion of ethylenediamine in E@β-CD (3) of the β-CD cavity. Δ^{a}Ea (kcal.M^{-1}) values of Mode A and Mode B revealed that the existence of ethylenediamine group outer side of the E@β-CD (3) cavity is more preferred than inside.

E@β-CD catalyzed for multi-component reactions

The reaction was optimized using Urea, ethyl acetoacetate and benzaldehyde as substrates and the results are shown in Table 1.

Table 1. Optimized reaction conditions in E@β-CD (3) catalyzed by pyrimidones[a,b].

Entry	Catalyst	Medium	Time (h)	Yield (%)[b]
1	β-CD	Water	24	28
2	Ethylenediamine (2)	-	8	26
3	Methanol	-	8	28
4	Ethanol	-	8	32
5	Methylamine	-	8	35
6	Diethylamine	-	8	33
7	Triethylamine	-	8	34
8	Pyridine	-	8	36
9	E@β-CD (3)	DMF	24	58
10	E@β-CD (3)	DMSO	24	60
11	E@β-CD (3)	-	5 min	96
[c]12	E@β-CD (3)	-	5 min	96[b]
[d]13	E@β-CD (3)	-	5 min	96[c]

[a]Reaction conditions: benzaldehyde (1 mmol, 1 equiv), urea (1.2 equiv) and ester (1.2 equiv), E@β-CD (3) (0.2 mol%) RT, 5 min. [b]Isolated Yield, [c]0.01 mmol of catalyst and [d]0.001 mmol of catalyst.

The investigation of catalyst loading on reaction time and yield was carried out. The minimal yield was observed when the plain β-CD was administered as a catalyst in an aqueous medium at ambient temperature (entry 1). Subsequently, the use of ethylenediamine (2) as a catalytic system resulted in poor product yields (entry 2). Besides, a wide range of organic bases were employed as catalysts under solvent-free conditions to see the outcome of reaction, such as methanol (entry 3), ethanol (entry 4), methylamine (entry 5), diethylamine (entry 6), triethylamine (entry 7) and pyridine (entry 8) as catalysts (entries 3-8). Surprisingly, lower yields were observed in these cases. Similar suboptimal yields were observed when E@β-CD (3) was initially employed as a catalyst in this process while using DMF and DMSO as solvents (entries 9 and 10).

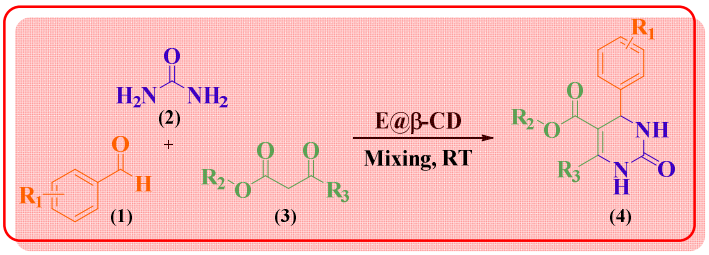
To our surprise, an excellent yield of 96% was observed (entry 11) at a shorter reaction time of 5 min when the catalyst is used with the substrates in solvent-free conditions. Similarly (entry 12), the addition of successive amounts of urea, ethyl acetoacetate, and aromatic aldehydes led to a quantifiable yield of pyrimidones even with a 0.01 mmol amount of E@β-CD (3) and the reaction was completed in 5 min. There was no traceable change in the yield after admitting 0.001 mmol of catalyst reaction (entry 13). The protocol developed was highly economical and excellent isolated yields were observed. The impact of this efficient supramolecular host E@β-CD (3) has been demonstrated in this study with a high quantitative yield and the facile feature of reusability.

To further expand the substrate scope of the developed methodology, electronically biased aldehydes, β-ketoesters and urea were employed. The observed results are listed in Table 2.

The substrates chosen furnished the corresponding 3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs) with good to excellent yield and a shorter reaction time as shown in Table 2. The addition of electron-withdrawing substituents, such as nitro groups, to aromatic aldehydes resulted in a high yield across extended reaction durations (Table 2, entries 8 and 15), whereas halogen substituents resulted excellent yield in shorter reaction time (Table 2, entries 4, 6, 9, 10, 12, 13 and 16). Electron donating substituents like 4-methylbenzaldehyde showed a moderate yield in the shorter reaction time, whereas the methoxy and di-methoxy substituents require the longest time and their yields are also moderate (Table 2, entries 3, 5, 11 and 14). These, phenomena may be attributed to their affinity for electron release. The bulkier aldehydes like α-napthaldehyde and

heterocyclic aldehydes like thiophene-2-carboxaldehyde (Table 2, entries 17 and 18) resulted in good yield. The optimized methodology exhibits a high degree of tolerance towards a diverse array of functional groups, hence providing a wider scope for the Biginelli reactions. The present systems offer various advantages: (i) the catalyst can be recycled without experiencing a substantial decrease in its catalytic activity. (ii) readily available, (iii) excellent yield in short reaction times, (iv) simple and easy separation.

Table 2. E@β-CD (3) catalyst multicomponent reactions of aldehydes, amine and acetoacetate (methyl, ethyl/ 4-chloro).



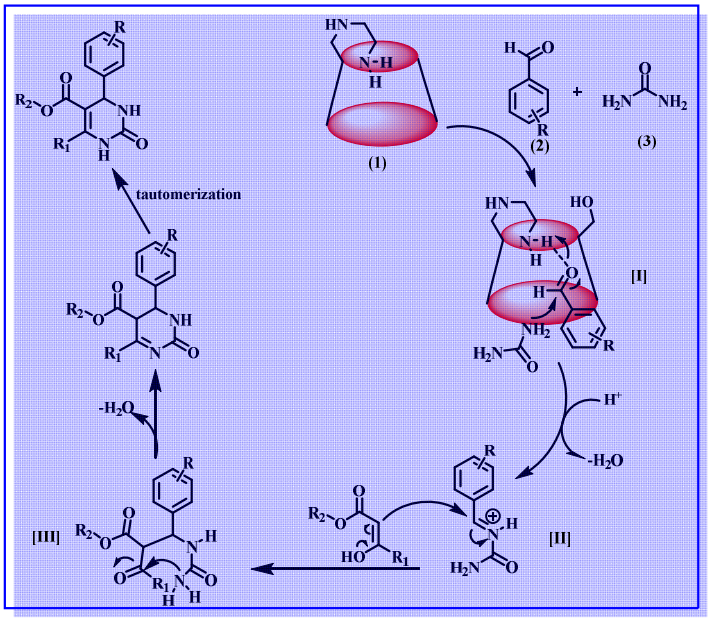
Entry	R_1 in aldehydes	R_2 and R_3 in esters	Product	Yield (%)
1	C_6H_5-CHO	$C_5H_8O_3$	**4a**	86
2	p-CH_3-C_6H_4-CHO	$C_5H_8O_3$	**4b**	88
3	p-CH_3O-C_6H_4-CHO	$C_5H_8O_3$	**4c**	88
4	p-Br-C_6H_4-CHO	$C_5H_8O_3$	**4d**	93
5	p-CH_3O-C_6H_4-CHO	$C_6H_{10}O_3$	**4e**	85
6	p-Cl-C_6H_4-CHO	$C_6H_{10}O_3$	**4f**	92
7	p-CH_3-C_6H_4-CHO	$C_6H_{10}O_3$	**4g**	88
8	p-NO_2-C_6H_4-CHO	$C_6H_{10}O_3$	**4h**	94
9	o-Cl-C_6H_4-CHO	$C_6H_{10}O_3$	**4i**	98
10	p-F-C_6H_4- CHO	$C_6H_{10}O_3$	**4j**	97
11	o,p-OCH_3-C_6H_3- CHO	$C_6H_{10}O_3$	**4k**	83
12	p-Br-C_6H_4- CHO	$C_6H_{10}O_3$	**4l**	93
13	m-Cl-C_6H_4- CHO	$C_6H_{10}O_3$	**4m**	98
14	p-OCH_3-C_6H_4- CHO	$C_6H_9ClO_3$	**4n**	89
15	m-NO_2-C_6H_4-CHO	$C_6H_9ClO_3$	**4o**	94
16	p-Cl-C_6H_4-CHO	$C_6H_9ClO_3$	**4p**	96
17	$C_{10}H_8$-CHO	$C_6H_{10}O_3$	**4q**	93
18	C_4H_3S-CHO	$C_6H_{10}O_3$	**4r**	95

[a]Reaction conditions: Aldehyde (1 equiv), urea (1.2 equiv) and ester (1.2 equiv), E@β-CD (3) (0.2 mol%) RT, 5 min. [b]Isolated yield in solvent-free conditions for 5 min at room temperature.

The systematic investigation of the reaction mechanism, which holds significance in elucidating the precise process, was conducted in accordance with prior reports. [31]. A presumptive mechanistic pathway for the assemblage of 3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs) derivatives catalyzed by E@β-CD (3) is presented in Scheme 2.

According to the proposed mechanism, E@β-CD (3) primarily supports activating the carbonyl group of aldehydes to give an intermediate (I), the nucleophilic addition of urea followed by cage elimination under acid conditions, then dehydration takes place to form the intermediate (II), wherein the ethyl acetoacetate was added. The final product 3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs) is to be obtained immediately and we speculated that intermediate (III) can be obtained through the reaction between intermediate (II) and the enolized ethyl acetoacetate.

Finally, intermediate (III) underwent cyclisation followed by dehydration quickly and afforded the target product.



Scheme 2. Proposed mechanism for the synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs) derivatives.

Once the reaction is complete, the catalyst is removed by washing the mixture with acetone from the substrates and the product is washed with EtOH, EtOAc and n-hexane, respectively. In Figure 1, the acquired white coloured powder was subsequently dried in an oven and utilised in additional runs. Another crucial feature of this eco-friendly, effective and active heterogeneous catalyst is its recyclable nature.

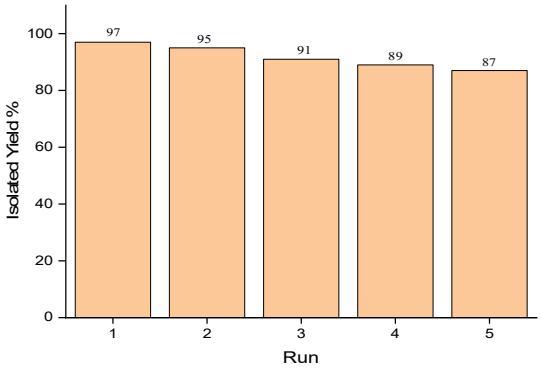


Figure 1. Reusability of the E@β-CD (3) for the synthesis of DHPM.

Our catalyst serves better than other catalysts that have been reported for the Biginelli reactions are between aromatic benzaldehydes, β-ketoesters and urea, details are presented in Table 3. The highlights of the developed protocol include good to excellent yield, involves reaction in mild condition and simple work up, recyclable and reusable nature of the catalyst.

Table 3. Comparative analysis of catalyst performance across various supported catalytic methods employed in the synthesis of pyrimidines.

Entry	Catalyst	Reaction condition	Time (h)	Yield (%)	Ref
1.	E@β-CD / SF	Rt	5 min	97	This work
2.	KSF/SF	130 ºC	48 h	74–88	[32]
3.	Lanthanide triflate/ SF	100 ºC	1–1.5 min	81–91	[33]
4.	strontium(II) triflate/ SF	70 ºC	4 h	85–97	[19]
5.	Mg-Al-CO_3 hydrotalcite/SF	80 ºC	30–60 min	71–74	[34]
6.	DBSA (5 mol %) / SF	80 ºC	2.5–3h	81–94	[35]
7.	β-CD SF	100 ºC	3 h	85	[36]
8	β-CD-SO_3H SF	100 ºC	2 h	83	[37]
9	β-CD-HCl	EtOH/reflux	8 h	92	[38]
10	nano-γ-Fe_2O_3-SO_3H SF	60 ºC	3 h	90	[39]
11	PS-PEG-SO_3H	Dioxane/80 ºC	10 h	86	[40]
12	Fe_3O_4/PAA-SO_3H SF	RT	120 min	90	[41]
13	Bentonite/PS-SO_3H SF	120 ºC	30 min	89	[42]
14	Tartaric acid	EtOH/reflux	4 h	92	[43]
15	Citric acid	EtOH/reflux	4 h	96	[44]
16	Lactic acids	EtOH/reflux	2.5 h	92	[45]
17	Ascorbic acid	Solvent free	6 h	85	[46]
18	Imidazole-1yl-acetic acid	Water/reflux	30 min	94	[47]
19	Sulfanilic acid	Water	3 h	98	[48]
20	Phenyl Phosphonic acid	ACN/reflux	4 h	97	[49]

SF – Solvent free; DBSA – *p*-Deodecylbenzene sulfonic acid; β-CD-SO_3H – Cyclodextrin modified propyl sulfonic acid; PS-PEG-SO_3H – Polysterene-poly(ethylene glycol) sulfonic acid; PAA-SO_3H – Pheylaceticacid sulfonic acid.

In-silico analysis

According to reports, CTX-M-15 hydrolyzes cefotaxime with high catalytic efficiency causing bacterial resistance to cefotaxime. The ligands (**4a**, **4e**, and **4n**) that were investigated exhibited significant interactions with the active regions of the CTX-M-15 receptor through the formation of hydrogen bonds and hydrophobic interactions.

The ligand-receptor interaction of the compound **4n** shows five hydrogen bonds. The interaction of three hydrogen bonds is between the oxygen atom of the ligand and the hydrogen atom of the receptors ASN10, SER 130 and THR 235 (O---H = 2.12 Å, dihedral angle = 142.45º; O---H = 2.10 Å, dihedral angle = 140.82º; O---H = 1.8 Å, dihedral angle = 169.9º), respectively. The rest of the hydrogen bonds are between the hydrogen atom of the ligand and the oxygen atom of the receptors THR 235 and SER 130 (H---O = 2.19 Å, dihedral angle = 131.59º; H---O = 2.2 Å, dihedral angle = 129.3º). Results of **4e** show that there are three hydrogen bonds in which all the interactions are between the oxygen atom of the ligand and the hydrogen atoms of the receptors GLN 93, LYS 137 and HIS 141 (O---H = 2.4 Å, dihedral angle = 97.40º; O---H = 1.78 Å, dihedral angle = 154.74º; O---H = 1.77 Å, dihedral angle = 156.08º). The hydrogen bond interaction for compound **4b** possesses the same residues as that for compound **4d**, but with different bond distance and dihedral angle. The residues involved in the interactions are GLN 93, LYS 137 and

HIS 141 (O---H = 2.01 Å, dihedral angle = 141.72°; O---H = 1.8 Å, dihedral angle = 142.49°; O---H = 2.04 Å, dihedral angle = 153.3°).

Furthermore, the binding models were reinforced by the hydrophobic interactions between the ligands (**4a**, **4e** and **4n**), in addition to the hydrogen bonds and the residues SER 70, SER 237, TYR 105, GLY 236, ASN 170, ARG 94, GLU 96, VAL 95, THR 116, ARG 94, VAL 195(A) and GLU 96(A) of the CXT M 15 receptor molecule.

The validation of the best docking poses and binding affinity of the compounds **4a**, **4e** and **4n**, are found within the active sites of CXT M 15 receptor. Results show that, there exists a similarity in hydrogen bonding found in CXT M 15 and ceftoxamine [50] and between **4n** and CXT M 15.

Antimicrobial activity of DHPMs

Agar well diffusion assay

The antibacterial efficacy of DHPMs **4a**, **4e** and **4n** were assessed using the well-cut method against the ESBL *E. coli (U655)* bacterial strain. The diameter of the growth inhibition zone (mm) for the bacterial strain under investigation is presented in Table 4. The results demonstrate that all the DHPMs exhibit efficacy against the tested ESBL bacterium strain. At a concentration of 30 µg/mL of DHPMs, compound **4n** had the highest level of inhibitory action against MRSA, as evidenced by a zone of inhibition measuring 18 mm. Then, the remaining compounds, namely **4a** and **4e** exhibited zone of inhibition measurements of 8 and 10 mm, respectively. These values were observed to be lower in comparison to compound **4n**. Multiple reports have demonstrated that DHPMs possess antibacterial properties against enterococci, enteric bacterial pathogens and a wide range of antimicrobial activity against certain multidrug resistant human pathogens. The current study unequivocally demonstrates that the introduction of an antimicrobial efficacy of DHPMS against ESBL *E. coli (U655)*. The increased antimicrobial activity of substituted DHPMs, in comparison to other substitutions alone, it may be attributed to the methoxy group's ability to penetrate the cell membrane, resulting in cell death.

Table 4. Inhibition zone and MIC zone of DHPMs against ESBL *E. coli (U655).*

Compounds	Inhibition zone (mm)	MIC (µg/mL)
4a	8	180.85
4e	10	62.8
4n	18	130.56
Cefotaxime	2	10

MIC of DHPMs

The MIC values for the DHPMs **4a**, **4e** and **4n** were assessed against the ESBL *E. coli (U655)* pathogen, and the results are presented in Table 4. The self-assembled DHPMs exhibited antibacterial activity at comparatively lower doses. The ESBL *E. coli (U655)*, when applied at the same concentration as the DHPMs, resulted in different inhibitory diameters. Dihydropyrimidinones (DHPMs) exhibited the most potent inhibitory effect against ESBL germs. Pyrimidone derivatives have demonstrated efficacy against both Gram positive and Gram-negative pathogens in various investigations. The variation in antibacterial effects on these bacteria is mostly attributed to changes in their structures and cell wall densities. Species-specific activity has been observed among several infections, including ESBL. DHPMs demonstrate significant bactericidal efficacy by inducing the production of reactive oxygen species (ROS) or toxic chemicals that induce cellular damage or death, as well as by direct interaction with pathogens. Moreover, the DHPMs can potentially interact with bacterial development and have

fatal effects, depending on the strain and dosage employed. The present study substantiated this by demonstrating the antibacterial effectiveness of substituted methoxy DHPMs **4n** against ESBL *E. coli (U655)*, with minimum inhibitory concentrations (MICs) of 180.85 µg/mL. In addition, several other DHPMs with another substitutions shown bactericidal activity against ESBL *E. coli (U655)* at minimum inhibitory concentrations (MICs) of 62.8 and 130.56 µg/mL for compounds **4a** and **4e**, respectively. These compounds effectively suppress the growth of ESBL (Table 4).

Antibiofilm activity of DHPMs

The global prevalence of infection caused by microorganisms that are resistant to multipile drugs is a major health concern, leading to higher rates of illness and death. As to the National Institute of Health and the Centre for Disease Control, over 80% of infections are attributed to biofilm that is linked to biomaterials like catheters and implants. Biofilms are intricate assemblages of microorganisms that are encased in an extracellular matrix, or slime, made up of proteins, lipids, and polysaccharides. This matrix enables the microbes to attach themselves to biomaterials. Eliminating biofilm remains a formidable task due to their antibiotic resistance and ability to withstand the host's defense system. The ineffectiveness of current treatment methods for biofilm-related infections necessitates the development of an improved drug delivery system that can overcome the drawbacks of traditional antibiotics. This has motivated researchers to explore alternative therapies. The present investigation is aimed to evaluate the antibiofilm activity of DHPMs **4n** against the biofilm-forming ESBL *E. coli (U655)* strain, based on the provided reports.

Dose dependent inhibition of biofilm formation by DHPMs

Antibiofilm efficacy of DHPMs and control was evaluated in vitro by quantifying the binding of crystal violet to the adhered cells of ESBL *E. coli (U655)* on 24-well microtitre plates. The presence of biofilms is readily demonstrated through the quantitative experiment, which involves measuring the absorbance values at a wavelength of 540 nm. The chemical 4n exhibited the greatest decrease in biofilm formation in ESBL *E. coli (U655)*, with a BIC value of 76.79 ± 0.785% at concentration of 10 µg/mL. The control group exhibited well-developed biofilm formation as observed through light microscopic investigations. In contrast, the group treated with DHPMs **4n** had the most significant decrease in biofilm formation (Figure 2).

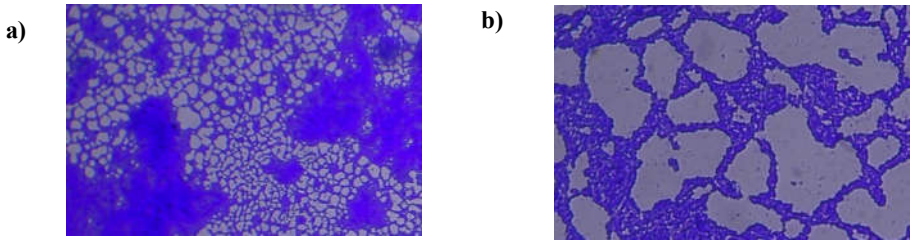


Figure 2. Light microscopy images of a) control ESBL *E. coli (U655)* and b) treated ESBL *E. coli (U655)* against **4n** DHPMs.

Changes in bacterial morphology against DHPMs

The alterations in the physical structure of ESBL *E. coli (U655)* induced by the administration of the compound **4n** DHPMs were examined using scanning electron microscopy (SEM) (Figure 3). The ESBL treated with DHPMs (Figure 3b) and the untreated control (Figure 3a) showed a noticeable difference in the number of bacterial cells. The control sample exhibited a high abundance of both micro and macroconidia of ESBL, but they were scarce in the treated sample. The disparity in the shape of the bacterial cells between ESBL subjected to DHPMs and the control group that was not treated was also observable. The morphology of the ESBL cells after treatment exhibited a skinny, deformed, and rough appearance, in contrast to the well-formed and smooth bacterial cells observed in the control group (Figure 3a).

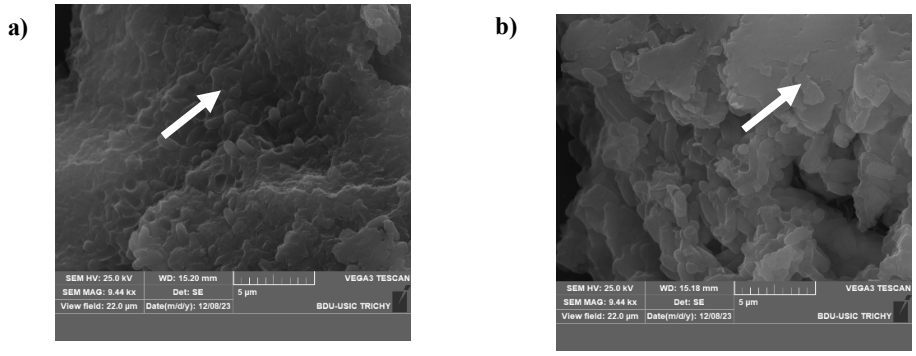


Figure 3. SEM images of a) Control ESBL *E. coli (U655)* and b) treated ESBL *E. coli (U655)* against **4n** DHPMs.

EXPERIMENTAL

Aerobic conditions were used for all reactions. All compounds utilised in this study were procured from Sigma Aldrich and were employed without undergoing any further purification, unless explicitly specified differently. NMR spectra were obtained at a frequency of 400 MHz using several solvents, including DMSO-d_6 and $CDCl_3$, on a Bruker spectrometer. TMS was used as the internal standard. A BRUKER ALPHA II ECO-ATR instrument was used to measure the FT-IR spectrum from 4000–550 cm^{-1} range. Thermo Fisher Instruments Limited's (US) LCQ Fleet recorded Electrospray ionization mass spectrometry (ESI-MS) was used, under negative ion mode.

General procedure for the synthesis of 3,4-dihydropyrimidin-2(1H)-ones DHPMs (4 a-r)

E@β-CD (0.001 mmol) was taken without solvent in a round-bottom flask, aldehydes (1 equiv), β-keto-ester (1.2 equiv) and urea (1 equiv) were added. The mixture was subsequently agitated for 30 minutes at ambient temperature. TLC was used to ensure the completion of the reaction with ethyl acetate and n-hexane (1:4) as the eluent system. Following this procedure, the mixture underwent two rounds of extraction using ethyl acetate (2 x 20 mL). The organic component of the extract underwent concentration, followed by a washing procedure using distilled water and subsequent drying using anhydrous Na_2SO_4. Without performing any additional purifications, the resulting solid was recrystallized with ethyl acetate. The aqueous layer of the separation is treated with acetone to recover the catalyst (E@β-CD) used for the reaction.

Molecular docking studies

Molecular docking studies with the AutoDock Tool docking programme, version 1.5.6, the binding affinities of the synthesised compounds 3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs) (**4a**, **4e**, and **4n**) and the crystal structure of CTX-M-15 (PDB: 4HBU) were assessed. Using CHEMSKETCH, the heterocyclic ligand's structure was sketched in mol format, which was later translated to PDB format by OPENBABEL. The Protein Data Bank, located at https://www.rcsb.org/PDB, was used to download the CTX-M-15 protein in PDB format. The heterocyclic compounds (**4a, 4e** and **4n**) and the receptor (CTX-M-15) files were created using AutoDock tools. After removing all heteroatoms and water molecules, the receptor molecule was then assigned with polar hydrogen atoms and Kollman charges. Subsequently, the rotation of all remaining bonds was allowed, while the ligand molecules were allocated the rotatable bonds. A box with a rigid spacing of 0.372 Å and 60 points each of the three dimensions (60 x 60 x 60 in *X* x *Y* x *Z* dimensions) was used to wrap the CTX-M-15 molecule. The Lamarckian evolutionary algorithm, as implemented in AutoDock, was used for the docking computations, and all other parameters were left at their default values. Using the DISCOVERY studio client, docked positions were shown in three dimensions, while LIG-PLOT plus was utilized to see hydrogen bonds and hydrophobic interactions in 2D.

Anti-microbial activity of DHPMs against ESBL E. coli

Bacteria and culture conditions

The Gram-negative Clinical isolated bacterial strain was acquired from the microbial type culture collection at Doctors Diagnostic Centre, Tiruchirappalli. The stock cultures were cryopreserved at a temperature of −80 °C in a solution containing 60% glycerol. The bacterial strain ESBL *E. coli (U655)* was kept on nutrient agar slopes at a temperature of 40 °C for experimental purposes. It was regularly transferred to new culture media every 2 weeks. The confirmation of ESBL *E. coli* was conducted using a combination disc (Hexa disc), and the interpretations were documented in accordance with the recommendations set forth by the Clinical Laboratory Standard Institute (CLSI). Accordingly, the indicators employed in the study consisted of ceftazidime (30 mg), cefotaxime (30 µg), and cepfodoxime (10 µg) either alone or in conjunction with clavulanic acid (10 µg). The presence of ESBL was detected when the combined inhibition zone of cephalosporin/clavulanic acid exceeded 5 mm (using the combination method) and above 8 mm (using the E-test), as opposed to the inhibition zone observed with cephalosporin alone.

Antimicrobial activity of DHPMs

Microbial resistance to antimicrobial agents DHPMs were conducted using the well diffusion method. Cefatoxamine was employed as a positive control to assess the effectiveness of DHPMs. Prepared sterile Muller Hinton agar plates and perforated them with a well borer to create 6 mm diameter wells. A 0.1% concentration of ESBL *E. coli (U655)* was evenly spread across the Muller Hinton agar surface using a swab. The well was treated with a solution of DHPMs at a concentration of 1 mg/mL. Subsequently, the plates were subjected to incubation at a temperature of 37 °C for a per-hour period. The quantification of the clear zone was performed by measuring the zone of inhibition, and its diameter was recorded in mm.

Minimal inhibitory concentration (MIC) determination

The microbial strains were introduced into MH broth and standardized to a concentration of 0.5 McFarland standards (10^8 cells/CFU). A microdilution test was conducted following the modification of the inoculum to determine the minimum inhibitory concentration (MIC). In this

experiment, a 96-well plate was utilized. A solution was prepared by combining 0.5 mg of DHPMs, 2 mL of ultra-pure water, and 0.1 Mm acetic acid. A solution mix was prepared by preparing serial dilutions that ranged from 100 to 6.25 µg/mL. 5 wells were filled with 180 µL of freshly prepared Mueller Hinton broth. 10 microliters of the modified inoculum and 10 microliters from each solution mix were introduced into the wells. An additional six wells were included in the experiment using MH broth. Among these, two wells were infected with the same inoculum to serve as negative controls, two were treated with cefatoxamine antibiotics as positive controls, and two were left unfilled to serve as sterility controls. Additionally, the well plate was incubated overnight at a temperature of 36 ºC. Following incubation, the wells that appeared clear were identified as having an inhibited culture, whereas the wells that appeared muddy were determined to be negative for inhibition. The minimum inhibitory concentration (MIC) values were determined by quantifying the concentration of the tested solution required to block bacterial growth. This was done by spreading the bacterial culture onto a plate containing Mueller-Hinton agar (MHA) and observing for suppression of bacterial growth. The plates lacking apparent development were considered to be favorable.

Assessment of antibiofilm activity of DHPMs

The impact of DHPMs on the production of biofilm by ESBL *E. coli (U655)* was evaluated using the culture that had been cultured overnight was diluted to a concentration of 10^{-8} colony-forming units per millilitre (CFU/mL) in Tryptic Soy Broth (TSB) with 0.5% glucose. The experiment was conducted both with and without DHPMs (10-50 µg/mL) and the samples were then kept at a temperature of 37 °C for a duration of 24 hours. The planktonic cells from each well were removed and subsequently rinsed three times with distilled water after the incubation period. Subsequently, the adhered cells were subjected to a 0.4% crystal violet (CV) solution and allowed to incubate for a duration of 3 min. The excess CV was disposed of and the wells underwent two rounds of rinsing with distilled water. A solution of 20% glacial acetic acid was used to dissolve the CV extracted from the pigmented biofilms for a period of 10 min. The spectrophotometer was utilised to evaluate the intensity of the colour at a specific wavelength of 540 nm.
The percentage of eradication was calculated by using the formula as follows:

$$\% \text{ of Eradication} = \text{Absorbance of control}_{(OD630)} - \text{Absorbance of sample}_{(OD630)} / \text{Absorbance of control}_{(OD630)} \times 100$$

Light and confocal microscopic analysis

In order to observe the biofilm using light microscopy, bacterial colonies with a density of 1×10^6 cfu/mL were cultivated on glass slides (1×1 cm). These slides were then placed on 24 well polystyrene plates, each holding 1 mL of nutrient broth. The experiment was conducted both with and without DHPMs, and the samples were incubated for 24 hours at a temperature of 30 ºC. The glass slides were subsequently rinsed three times with distilled water and then treated with a solution containing 0.4% CV. The stained glass slides were examined using a light microscope (Nikon Eclipse Ti 100, Tokyo, Japan) at a magnification of 400X.

Observation of bacterial morphology against DHPMs

The morphological alterations of ESBL *E. coli (U655)* were examined using a scanning electron microscope (SEM). In summary, the bacterial cells from two groups (control and treated) were gathered and preserved using 2.5% glutaraldehyde at a temperature of 4 ºC for the duration of one night. Afterwards, the samples underwent three washes with PBS and were then fixed with a 1% solution of OsO_4 in PBS for a duration of 1-2 hours. Subsequently, the samples underwent

additional dehydration using a sequence of ethanol concentrations and were then coated with a gold-palladium mixture using the Hitachi Model E-1010 ion sputter for a duration of 4-5 min. The samples were subsequently examined using the Hitachi Model SU-8010 scanning electron microscope (SEM) located in Tokyo, Japan.

CONCLUSION

In summary, the synthesis of Ethylenediamine modified β-CD (E@β-CD (3)) using mono-tosy-β-CD is used as a promoter for the first time in the Biginelli reaction. The catalyst and the synthesized compounds **4** (**a-r**) were characterized using FT-IR, NMR and GC-mass spectroscopy. It's been evident and demonstrated that E@β-CD (3) is a very effective and recyclable transition metal-free catalyst for the one-pot three-component synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs) derivatives under solvent-free conditions. The amount of catalyst, the temperature and the solvent all had a substantial impact on the reaction system. Short reaction periods, high to exceptional yields, elimination of toxic transition metals or organic solvents, ease of workup, reusability of the catalyst and ease of product purification are the key benefits of this procedure. Further, the docking results reveal that the binding modes of compound 4n are in close approximation with the active sites of cefotaxamine hydrolysis sites with four common amino acids involved in the hydrogen bonding with CXT M 15 receptor. As per the docking result, compounds **4a**, **4e** and **4n** possess good antibacterial inhibition against ESBL *E. coli (U655).* In addition, compound 4n was administered to assess its biofilm activity, resulting in a BIC value of 76.79 ± 0.785% at a concentration of 10 µg/mL. The morphological alterations in ESBL give rise to a novel perspective in which methoxy substituted DHPMs can be regarded as a potential solution for combating multi-resistant human diseases.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Researchers Supporting Project number (RSP2024R360), King Saud University, Riyadh, Saudi Arabia for supporting this research. The authors also thanks to DST-FIST and DBT Star College Scheme (Government of India) for providing instrumentation facilities.

REFERENCES

1. Gregorio, C. Review: A history of cyclodextrins. *Greg Chem. Rev.* **2014**, 114, 10940–10975.
2. Mitra, B.; Chandra Pariyar, G.; Ghosh, G.P. β-Cyclodextrin: A supramolecular catalyst for metal-free approach towards the synthesis of 2-amino-4,6-diphenylnicotinonitriles and 2,3-dihydroquinazolin-4(1 H)-one. *RSC Adv.* **2021**, 11, 1271–1281.
3. Jie, K.; Zhou, Y.; Yao, Y.; Huang, F. Macrocyclic amphiphiles. *Chem. Soc. Rev.* **2015**, 44, 3568–3587.
4. Hapiot, F.; Monflier, E. Unconventional approaches involving cyclodextrin-based, self-assembly-driven processes for the conversion of organic substrates in aqueous biphasic catalysis. *Catalysts* **2017**, 7, 173.
5. Szejtli, J. Introduction and general overview of cyclodextrin chemistry. *Chem. Rev.* **1998**, 98, 1743–1754.
6. Vyas, A.; Saraf, S.; Saraf, S. Cyclodextrin based novel drug delivery systems. *J. Incl. Phenom. Macrocycl. Chem.* **2008**, 62, 23–42.
7. Xin, J.; Chang, L.; Hou, Z.; Shang, D.; Liu, X.; Feng, X. An enantioselective Biginelli reaction catalyzed by a simple chiral secondary amine and achiral Brønsted acid by a dual-activation route. *Chem. A Eur. J.* **2008**, 14, 3177–3181.

8. Ruamps, M.; Lugan, N.; César, V. A cationic n-heterocyclic carbene containing an ammonium moiety. *Organometallics* **2017**, 36, 1049–1055.
9. Chen, W.Y.; Qin, S.D.; Jin, J.R. Efficient Biginelli reaction catalyzed by sulfamic acid or silica sulfuric acid under solvent-free conditions. *Synth. Commun*. **2007**, 37, 47–52.
10. Venkatesan, K.; Satyanarayana, V.S.V.; Sivakumar, A. Synthesis of pyrimidine carboxamide derivatives catalyzed by uranyl nitrate hexa Hydrate with their antibacterial and antioxidant studies. *Bull. Chem. Soc. Ethiop*. **2016**, 30, 119–128.
11. Zahiri, S.; Mokhtary, M. $Bi(NO_3)_3 \cdot 5H_2O$: An efficient catalyst for one-pot synthesis of 3-((aryl)(diethylamino)methyl)-4-hydroxy-2H-chromen-2-ones and biscoumarin derivatives. *J. Taibah Univ. Sci.* **2015**, 9, 89–94.
12. Ahmed, N.; Siddiqui, Z.N. Sulphated silica tungstic acid as a highly efficient and recyclable solid acid catalyst for the synthesis of tetrahydropyrimidines and dihydropyrimidines. *J. Mol. Catal. A Chem.* **2014**, 387, 45–56.
13. Salim, S.D.; Akamanchi, K.G. Sulfated tungstate: An alternative, eco-friendly catalyst for Biginelli reaction. *Catal. Commun.* **2011**, 12, 1153–1156.
14. Davoodnia, A.; Allameh, S.; Fazli, S.; Tavakoli-Hoseini, N. One-pot synthesis of 2-amino-3-cyano-4-arylsubstituted tetrahydrobenzo[b] pyrans catalysed by silica gel-supported polyphosphoric acid (PPA-SiO_2) as an efficient and reusable catalyst. *Chem. Pap.* **2011**, 65, 714–720.
15. Salehi, P.; Dabiri, M.; Zolfigol, M.A.; Bodaghi Fard, M.A. Silica sulfuric acid: an efficient and reusable catalyst for the one-pot synthesis of 3,4-dihydropyrimidin-2(1H)-ones. *Tetrahedron Lett.* **2003**, 44, 2889–2891.
16. Thirupathi Reddy, Y.; Rajitha, B.; Narsimha Reddy, P.; Sunil Kumar, B.; Rao, V.P. Bismuth subnitrate catalyzed efficient synthesis of 3,4-dihydropyrimidin-2(1h)-ones: an improved protocol for the Biginelli reaction. *Synth. Commun.* **2004**, 34, 3821–3825.
17. Aswin, K.; Mansoor, S.S.; Logaiya, K.; Sudhan, S.P.N. Triphenylphosphine: An efficient catalyst for the synthesis of 4,6-diphenyl-3,4-dihydropyrimidine-2(1H)-thione under thermal conditions. *J. King Saud Univ. Sci.* **2014**, 26, 141–148.
18. Khatri, C.K.; Rekunge, D.S.; Chaturbhuj, G.U. Sulfated polyborate: a new and eco-friendly catalyst for one-pot multi-component synthesis of 3,4-dihydropyrimidin-2(1H)-ones/thiones via Biginelli reaction. *New J. Chem.* **2016**, 40, 10412–10417.
19. Su, W.; Li, J.; Zheng, Z.; Shen, Y. One-pot synthesis of dihydropyrimidiones catalyzed by strontium(II) triflate under solvent-free conditions. *Tetrahedron Lett.* **2005**, 46, 6037–6040.
20. Safaei-Ghomi, J.; Tavazo, M.; Mahdavinia, G.H. Ultrasound promoted one-pot synthesis of 3,4-dihydropyrimidin-2(1H)-ones/thiones using dendrimer-attached phosphotungstic acid nanoparticles immobilized on nanosilica. *Ultrason. Sonochem.* 2018, 40, 230–237.
21. Dong, J.; Liu, M.; Jiang, R.; Huang, H.; Huang, Q.; Wen, Y.; Tian, J.; Dai, Y.; Zhang, X.; Wei, Y. Ultrafast fabrication of fluorescent organic nanoparticles with aggregation-induced emission feature through the microwave-assisted Biginelli reaction. *Dye. Pigment.* **2019**, 165, 90–96.
22. Venkatesan, K.; Satyanarayana, V.S.V.; Sivakumar, A. Microwave-assisted synthesis and evaluation of antibacterial activity of 2,2'-(naph- thalene-2,7-diylbis(oxy))bis(N'-substituted acetohydrazide) derivatives. *Bull. Chem. Soc. Ethiop.* **2012,** 26, 257–265.
23. Suresh, P.; Pitchumani, K. Per-6-amino-β-cyclodextrin catalyzed asymmetric Michael addition of nitromethane and thiols to chalcones in water. *Tetrahedron* **2008**, 19, 2037–2044.
24. Kanagaraj, K.; Pitchumani, K. The aminocyclodextrin/$Pd(OAc)_2$ complex as an efficient catalyst for the Mizoroki-Heck cross-coupling reaction. *Chem. A Eur. J.* **2013**, 19, 14425–14431.
25. Suresh, P.; Pitchumani, K. Per-6-amino-β-cyclodextrin as an efficient supramolecular ligand and host for Cu(I)-catalyzed N-arylation of imidazole with aryl bromides. *J. Org. Chem.* **2008**, 73, 9121–9124.

26. Azath, I.A.; Suresh, P.; Pitchumani, K. Per-6-amino-β-cyclodextrin/CuI catalysed cyanation of aryl halides with $K_4[Fe(CN)_6]$. *New J. Chem.* **2012**, 36, 2334.
27. Azath, I.A.; Puthiaraj, P.; Pitchumani, K. One-pot multicomponent solvent-free synthesis of 2-amino-4H-benzo[b]pyrans catalyzed by per-6-amino-β-cyclodextrin. *ACS Sustain. Chem. Eng.* **2013**, 1, 174–179.
28. Quintas, P.C.; Al-Salami, H.; Pfaff, A.; Li, D.; Koks, S. β-Cyclodextrin based nano gene delivery using pharmaceutical applications to treat Wolfram syndrome. *Ther. Deliv.* **2022**, 13, 449–462.
29. Bouchillon, S.; Johnson, B.; Hoban, D.; Johnson, J.; Dowzicky, M.; Wu, D.; Visalli, M.; Bradford, P. Determining incidence of extended spectrum β-lactamase producing Enterobacteriaceae, vancomycin-resistant Enterococcus faecium and methicillin-resistant *Staphylococcus aureus* in 38 centres from 17 countries: The PEARLS study 2001–2002. *Int. J. Antimicrob. Agents* **2004**, 24, 119–124.
30. Hall-Stoodley, L.; Costerton, J.W.; Stoodley, P. Bacterial biofilms: From the natural environment to infectious diseases. *Nat. Rev. Microbiol.* **2004**, 2, 95–108.
31. Sadjadi, S.; Koohestani, F. Composite of cross-linked chitosan beads and a cyclodextrin nanosponge: A metal-free catalyst for promoting ultrasonic-assisted chemical transformations in aqueous media. *J. Phys. Chem. Solids.* **2021**, 156, 110157.
32. Bigi, F.; Carloni, S.; Frullanti, B.; Maggi, R.; Sartori, G. A revision of the biginelli reaction under solid acid catalysis. Solvent-free synthesis of dihydropyrimidines over montmorillonite KSF. *Tetrahedron Lett.* **1999**, 40, 3465–3468.
33. Wang, L.; Qian, C.; Tian, H.; Ma, Y. Lanthanide triflate catalyzed one-pot synthesis of dihydropyrimidin-2(1H)-thiones by a three-component of 1,3-dicarbonyl compounds, aldehydes, and thiourea using a solvent-free Biginelli condensation. *Synth. Commun.* **2003**, 33, 1459–1468.
34. Lal, J.; Sharma, M.; Gupta, S.; Parashar, P.; Sahu, P.; Agarwal, D.D. Hydrotalcite: A novel and reusable solid catalyst for one-pot synthesis of 3,4-dihydropyrimidinones and mechanistic study under solvent free conditions. *J. Mol. Catal. A Chem.* **2012**, 352, 31–37.
35. Aswin, K.; Mansoor, S.S.; Logaiya, K.; Sudhan, P.N.; Ahmed, R.N. Facile synthesis of 3,4-dihydropyrimidin-2(1 H)-ones and -thiones and indeno[1,2-d]pyrimidines catalyzed by *p*-dodecylbenzenesulfonic acid. *J. Taibah Univ. Sci.* **2014**, 8, 236–247.
36. Liberto, N.A.; De Paiva Silva, S.; De Fátima, Â.; Fernandes, S.A. β-Cyclodextrin-assisted synthesis of Biginelli adducts under solvent-free conditions. *Tetrahedron* **2013**, 69, 8245–8249.
37. Gong, K.; Wang, H.; Wang, S.; Ren, X. β-Cyclodextrin-propyl sulfonic acid: a new and eco-friendly catalyst for one-pot multi-component synthesis of 3,4-dihydropyrimidones via Biginelli reaction. *Tetrahedron* **2015**, 71, 4830–4834.
38. Liu, Z.; Larock, R.C. Facile N-arylation of amines and sulfonamides and O-arylation of phenols and arenecarboxylic acids commonly found in a variety of biologically active and natural. *J. Org. Chem.* **2006**, 71, 3198–3209.
39. Kolvari, E.; Koukabi, N.; Armandpour, O. A simple and efficient synthesis of 3,4-dihydropyrimidin-2-(1H)-ones via Biginelli reaction catalyzed by nanomagnetic-supported sulfonic acid. *Tetrahedron* **2014**, 70, 1383–1386.
40. Quan, Z.J.; Da, Y.X.; Zhang, Z.; Wang, X.C. PS-PEG-SO_3H as an efficient catalyst for 3,4-dihydropyrimidones via Biginelli reaction. *Catal. Commun.* **2009**, 10, 1146–1148.
41. Zamani, F.; Izadi, E. Synthesis and characterization of sulfonated-phenylacetic acid coated Fe_3O_4 nanoparticles as a novel acid magnetic catalyst for Biginelli reaction. *Catal. Commun.* **2013**, 42, 104–108.
42. Kalbasi, R.J.; Massah, A.R.; Daneshvarnejad, B. Preparation and characterization of bentonite/PS-SO_3H nanocomposites as an efficient acid catalyst for the Biginelli reaction. *Appl. Clay Sci.* **2012**, 55, 1–9.

43. Suresh; Saini, A.; Kumar, D.; Sandhu, J.S. Multicomponent eco-friendly synthesis of 3,4-dihydropyrimidine-2-(1H)-ones using an organocatalyst Lactic acid. *Green Chem. Lett. Rev.* **2009**, 2, 29–33.
44. de Vasconcelos, A.; Oliveira, P.S.; Ritter, M.; Freitag, R.A.; Romano, R.L.; Quina, F.H.; Pizzuti, L.; Pereira, C.M.P.; Stefanello, F.M.; Barschak, A.G. Antioxidant capacity and environmentally friendly synthesis of dihydropyrimidin-(2H)-ones promoted by naturally occurring organic acids. *J. Biochem. Mol. Toxicol.* **2012**, 26, 155–161.
45. Amarante, G.W.; Coelho, F. Catalytic synthesis of 3,4-dihydropyrimidin-2(1H)-ones under green conditions and by keggin type heteropolyacid catalyst $H_7[PMO_8V_4O_{40}]$. *Quim. Nova* **2009**, 32, 469–481.
46. Sehout, I.; Boulcina, R.; Boumoud, B.; Boumoud, T.; Debache, A. Solvent-free synthesis of polyhydroquinoline and 1,8-dioxodecahydroacridine derivatives through the Hantzsch reaction catalyzed by a natural organic acid: A green method. *Synth. Commun.* **2017**, 47, 1185–1191.
47. Kargar, M.; Hekmatshoar, R.; Mostashari, A.; Hashemi, Z. Efficient and green synthesis of 3,4-dihydropyrimidin-2(1H)-ones/thiones using imidazol-1-yl-acetic acid as a novel, reusable and water-soluble organocatalyst. *Catal. Commun.* **2011**, 15, 123–126.
48. Rajack, A.; Yuvaraju, K.; Praveen, C.; Murthy, Y.L.N. A facile synthesis of 3,4-dihydropyrimidinones/thiones and novel N-dihydro pyrimidinone-decahydroacridine-1,8-diones catalyzed by cellulose sulfuric acid. *J. Mol. Catal. A Chem.* **2013**, 370, 197–204.
49. Ganwir, P.; Gavali, K.; Chaturbhuj, G.U. N-(Phenylsulfonyl)benzenesulfonamide: A new organocatalyst for one-pot, solvent-free synthesis of biginelli's 3,4-dihydropyrimidine-2 (1H)-Thiones. *Polycycl. Aromat. Compd.* **2022**, 56, 1–10.
50. Maryam, L.; Khan, A.U. Structural insight into mode of binding of Meropenem to CTX-M-15 type β-lactamase. *Int. J. Biol. Macromol.* **2017**, 96, 78–86.