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β-CD-catalyzed multicomponent domino reaction: synthesis, characterization, in silico molecular docking and biological evaluation of pyrano[2,3-*d*]-pyrimidinone derivatives

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Abstract Simple and green synthetic procedures constitute an important goal in organic synthesis. The combination of multicomponent reactions (MCRs) and unconventional solvents has become a new research direction, which enables simultaneous growth of both MCRs and green solvents toward ideal organic synthesis. In this paper, we have summarized recent results of MCRs obtained in unconventional media using water and β-cyclodextrin, as supramolecular catalyst, for the synthesis of pyrano[2,3-*d*]-pyrimidinone (**4a–q**) derivatives. The compounds were evaluated for their in vitro antimicrobial activity. Among the synthesized compounds, compounds **4h**, **4m** and **4p** exhibited higher antimicrobial activity than ciprofloxacin used as the reference drug. Most of the synthesized compounds have good to excellent antimicrobial activity. Furthermore, \ molecular docking study was performed to help understand binding interactions of the most active analogs with C_{30} carotenoid dehydrosqualene synthase enzyme.

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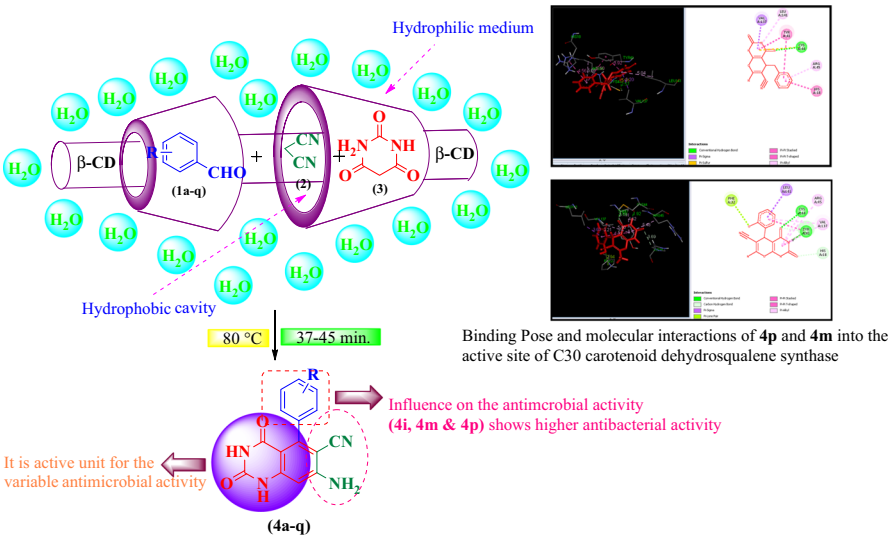
Graphical Abstract



Keywords Pyrano[2,3-*d*]-pyrimidinones · Multicomponent reaction · β-Cyclodextrin · Supramolecular catalysis · Antimicrobial activity · Molecular docking

Introduction

The development of new biomolecules of therapeutic interest by searching and optimizing a simple, environmentally benign and established synthetic route has been an interesting part of contemporary medicinal chemistry research [1–5]. Although nature is the best source of biomolecules, its inadequacy in supplying desired quantities of materials sometimes becomes a limiting factor in the widespread use of such valuable compounds (i.e., the isolation of taxol from treebarks). The use of complicated, lengthy and expensive procedures to isolate biomolecules from natural sources has been another drawback. Synthetic chemists are therefore important for the development of molecular frameworks, analogous either totally or partially to that of biomolecules which can be synthetically accessed.

Pyrano[2,3-*d*]pyrimidinone(thione) derivatives are very important because of their essential biological properties such as antihypertensive, antihepatotoxicity [6], antitumor [7, 8], antibronchitic and anticardiotonic activities [9]. These compounds are also available as important units of some natural products, such as polyethers, antibiotics, carbohydrates, phormones, iridoids and alkaloids [10, 11].

There are several reports on the synthesis of 7-amino-6-cyano-5-aryl-5*H*-pyrano[2,3-*d*]pyrimidine-(1*H*,3*H*)-2,4-diones in which arylidene malononitrile with

barbituric acid have been reacted under either traditional thermal condition [12] or microwave irradiation [13]. Also, several catalysts have been reported wihch are capable of the promotion of the formation of pyrano[2,3-*d*]pyrimidinone (thione) derivatives, including $[DABCO](SO_3H)_2(HSO_4)_2$ [14], nano-sawdust-OSO_3H [15], Al-HMS-20 [16], silica-bonded *N*-propyltriethylenetetramine [17], nano-Al_2O_3, [18], $CaHPO_4$ [19], DABCO [20], KBr [21], choline chloride. $ZnCl_2$ [22], alum [23], Zn[L-proline]$_2$ [24], L-proline [25], succinimidinium hydrogensulfate [26], nano-CuO/ZnO [27], trichloroisocyanuric acid [28], nano-titania sulfuric acid and $B(OH)_3$ [29], *n*-butyl-3-methylimidazolium tetra-fluoroborate [30], nanocatalysts [31], and ionic liquid $[H_2$-DABCO$][H_2PO_4]_2\}$ [32]. Each of these methods has its own merit, with at least one of the limitations of drastic conditions to apply novel conditions, long reaction times and effluent pollution. Thus, new routes for the synthesis of these molecules have attracted considerable attention in the search for a rapid entry to those heterocycles and their diverse biological properties.

During the past two decades, scientists have paid more and more attentions to the preparation of functional polyrotaxanes using various macrocyclic molecules, such as cyclodextrins [33, 34], crown ethers [35–39], cucurbiturils [40–42], and so on. Among them, cyclodextrins (CDs) have attracted increasing interest for their diverse binding selectivities. CDs are cyclic oligosaccharides, first reported by Villiers in 1891, consisting of six or more D-glucopyranose units connected through a-(1,4) glycosidic linkages [43]. There are three well-known, naturally produced CDs: α-CD, β-CD, and γ-CD, made up of six, seven, or eight glucopyranose units, respectively [44]. β-CD with the advantages of space geometry, cavity size, availability and low cost has gained widespread application and presently accounts for more than 95% of the number of CDs [45]. β-CD takes the form of a truncated cone or torus with the primary hydroxyl functions (C_6-OH) oriented to the narrower end of the torus, and the secondary hydroxyl functions (C_2, 3-OH) oriented towards the wider end [46, 47]. The outer surface of the cavity is hydrophilic due to the assembly of hydroxyl groups, whereas the void cavity, composed of hydrogen atoms of C-3, C-5 and oxygen atoms of a glycosidic linkage, shows lipophilic behavior [48].

As part of our program for applying green catalysts to multi-component reactions [49–51], we have employed a tandem Knoevenagel–Michael reaction in our ongoing research for the one-pot synthesis of various pyrano[2,3-*d*]-pyrimidinone derivatives. We report here on β-CD-catalyzed tandem Knoevenagel–Michael reactions of various substituted aromatic aldehydse with malonitrile and barbituric acid in aqueous medium as a new, robust, greener and highly efficient procedure for the synthesis of pyrano[2,3-*d*]-pyrimidinone derivatives. The synthesized compounds have also been tested for their antimicrobial activities and we also performed molecular docking of the synthesized compounds with the dihydrofolate reductase (DHFR) receptor.

Results and discussion

Chemistry

Initially, the readily available benzaldehyde **1a**, malonitrile **2** and barbituric acid **3** were chosen as model substrate (Scheme 1), and extensive investigations were carried out to define the optimal reaction conditions. As shown in Table 1, without any catalyst and using ethanol as a solvent, the target product was afforded in 40% yield (Table 1, entry 1). Then, the investigatory experiment was performed to improve the yield of the product by varying the reaction conditions, i.e., solvent, temperature and supramolecular catalyst. All the reaction products were purified by recrystallization using ethanol as a solvent. The observations (Table 1) led us to the conclusion that the supramolecular catalyst has an obvious effect on the reaction. Among the selected catalysts (Table 1, entries 2–3, 9, 12–13), the commercially available catalyst β-CD was proved to be the most suitable (Table 1, entry 9) as it gives a single product. The reaction of benzaldehyde **1a**, malonitrile **2** and barbituric acid **3** in the presence of β-CD was completely specific in affording only the one product, whereas the other reported catalysts also yielded byproducts.

Several solvents were investigated (Table 1, entries 4–11) of which water was the best (Table 1, entry 9), although aq. ethanol also produced comparable results (Table 1, entry 8). The stoichiometry ratio of 15 mol% of the catalyst was chosen to be the best for performing the reaction since further increases had no impact on the yield of the reaction (Table 2, entry 4). Thus, the best result was obtained when

Table 1 Optimization of the catalyst for the preparation of pyrano[2,3-*d*]-pyrimidinones

Entry	Solvent	Catalyst	Mole (%)	Temp (°C)	Time (min)	Yield[a] (%)
1.	EtOH	No catalyst	15	70	5–6 h	NR[b]
2.	Aq. EtOH	Et_3N	15	70	110	73
3.	CH_3COOH	P_2O_5	15	135	6–8 h	71
4.	CH_3CN	β-CD	15	80	40	65
5.	Toluene	β-CD	15	80	35	78
6.	Chloroform	β-CD	15	80	45	81
7.	DMF	β-CD	15	80	50	76
8.	Eq. ethanol	β-CD	15	80	40	85
9.	Water	β-CD	15	80	37	90
10.	Ethanol	β-CD	15	80	45	82
11.	Methanol	β-CD	15	80	56	71
12.	Water	α-CD	15	80	65	65
13.	Water	γ-CD	15	80	85	55

All reactions were performed using aldehyde (1 mmol), malonitrile (1 mmol), and barbituric acid (1 mmol), and solvent at 80 °C

[a]Isolated yield after filtration

[b]*NR* No reaction

Table 2 Optimization of catalyst (%) for the synthesis of pyrano[2,3-*d*] pyrimidinones

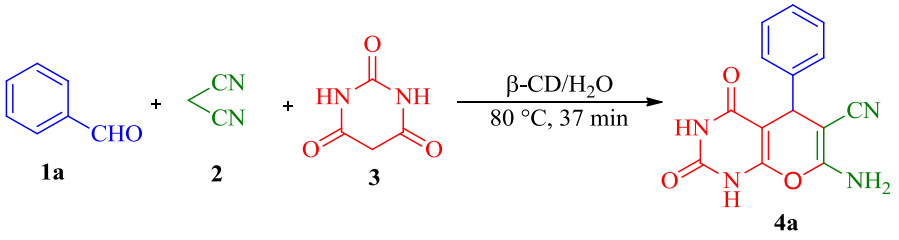
Entry	Catalyst (mol%)	Time (min)	Yield[a] (%)
1	5	40	65
2	10	60	72
3	15	65	90
4	20	72	90

All reactions were carried out using aldehyde (1 mmol), malonitrile (1 mmol), and barbituric acid (1 mmol), and solvent at 80 °C

[a]Isolated yield

benzaldehyde **1a**, and malonitrile **2** were heated for 30 min in the presence of 15 mol% of β-CD as a catalyst for the in situ generation of intermediates of the Knoevenagel condensation, followed by the Michael addition of barbituric acid **3** to provide a precipitate which was filtered off and recrystallized from ethanol to yield the desired product **4a** in 90% yield.

With these results in hand, we then investigated the substrate scopes and limitations of the synthesis of pyrano[2,3-*d*]pyrimidinones. First, we started the reaction with barbituric acid **3** by varying the aldehydes **4a**–**q** and malonitrile **2** to afford pyrano[2,3-*d*]pyrimidinones (Scheme 2); the results are listed in Table 3. Aromatic aldehydes having electron-donating groups like 4-methoxy benzaldehydes (Table 3, entry 8) reacted with lower rates and with lower yields compared with those substituted with electron-withdrawing groups like 4-fluoro, 4-chloro and 4-bromo benzaldehydes giving higher yields (Table 3, entries 4–6). Various heterocyclic aldehydes such as thiophene-2-carbaldehyde and 1*H*-pyrrole-2-carbaldehyde were also examined (Table 3, entries 11–12). In addition, our methodology has been used successfully in orders of aliphatic materials such as propionaldehyde, butyraldehyde and iso-butyraldehyde, and corresponding pyrano[2,3-*d*]pyrimidinones were obtained in excellent yields without the formation of any byproducts (Table 3, entries 9, 14–15). As is clear from the obtained results, the presented methodology can be used in oxygen-, nitrogen- and sulfur-containing heteroaromatic aldehydes. Thus, the reaction was found to be general and to have a broad scope due to its applicability to a variety of substrates, and the products (**4a**–



Scheme 1 Standard model reaction

Table 3 Synthesis of 7-amino-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1*H*-pyrano[2,3-*d*]pyrimidine-6-carbonitrile[a] **4(a–q)** under optimized conditions

Entry	Aldehyde	Product	Time (min)	Yield[b] (%)	M.P. (°C) (found) [27]
1.	-Ph	**4a**	37	90	225–228
2.	4-OH,3-OMe-Ph	**4b**	38	78.5	275–278
3.	4-Cl-Ph	**4c**	48	75	228–230
4.	4-F-Ph	**4d**	38	95	220–222
5.	3-OH,4-OMe-Ph	**4e**	42	95.2	250–252
6.	4-Br-Ph	**4f**	45	88.5	> 300
7.	2-NO_2^-Ph	**4g**	48	87	228–230
8.	4-OMe-Ph	**4h**	35	86.5	140–142
9.	CH_3-CH_2-CHO	**4i**	38	75	260–262
10.	2,4-di-OMe-Ph	**4j**	42	82.5	238–240
11.	Thiophene-2 carbaldehyde	**4k**	48	78.5	280–282
12.	1*H*-Pyrrole-2-carbaldehyde	**4l**	32	89	280–283
13.	2-OH-Ph	**4m**	35	93.5	178–180
14.	C_3H_8-CHO	**4n**	45	83.5	260–262
15.	C_3H_6-CHO	**4o**	38	78.5	255–258
16.	Ph-CH_2-CHO	**4p**	48	82.5	280–283
17.	4-OH-Ph-CHO	**4q**	43	89.5	140–142

[a]All reactions were performed using aldehyde (1 mmol), malonitrile (1 mmol), and barbituric acid (1 mmol), β-CD (15 mil%), and H_2O (15 mL), at 80 °C

[b]Isolated yield after filtration

q) were isolated in excellent yields (75–95%) under milder reaction conditions. The results are summarized in Table 3.

Analytical results

The structure of all the prepared compounds were elucidated with the aid of IR, ^{1}H and ^{13}C NMR,and mass spectroscopy and elemental analysis data have been discussed for a representative compound, **4a**. The IR spectrum of **4a** exhibited a sharp peak at 3379 cm^{-1} which corresponds to the –NH stretching of the $–NH_2$ group, and peaks at 2191 and 1679 cm^{-1} correspond to the nitrile and amide carbonyl, respectively. The ^{1}H NMR spectra of **4a** shows three singlets at δ 10.98, 10.80 and 6.82 for the –NH and $–NH_2$ protons, clearly indicating the incorporation of both moieties in the product. In the ^{13}C NMR spectra, the two carbonyl carbon atoms display a signal at δ 160.39 and 151.36 and the amide carbonyl carbon atom resonated at δ 153.91 and the nitrile carbon at δ 118.21. The mass spectra also exhibit a distinguishing peak at *m/z* 283.08 [M+H$^+$]. The elemental analysis data of **4a** correlate with the calculated value which is useful in the assignment of the structure of **4a**, which is shown in supplementary material of **4a**. The structures of all pyrano[2,3-*d*]pyrimidinones were consistent with the above-mentioned data.

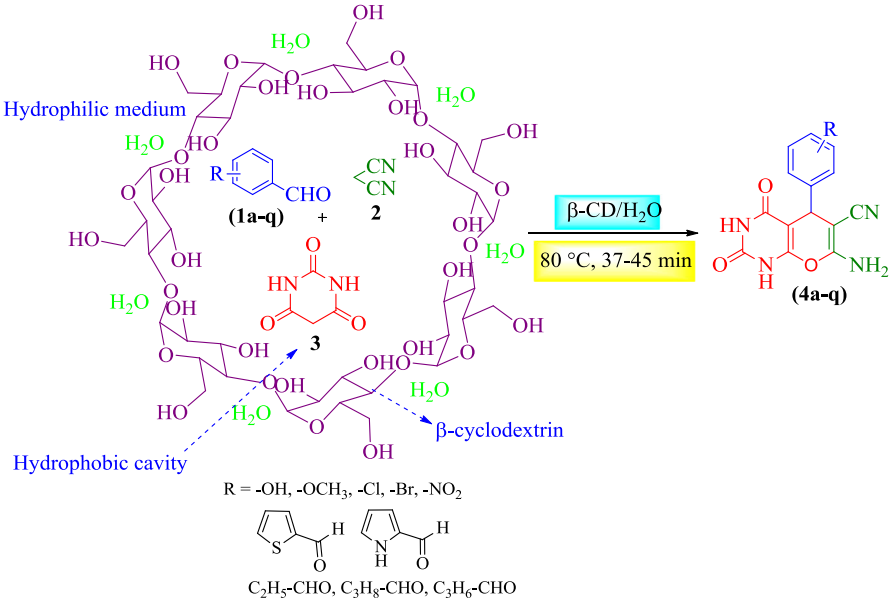
The recyclability of β-CD was examined using the model reaction of benzaldehyde, malonitrile and barbituric acid. After each run, ethyl acetate was added to dissolve the product, and the catalyst was separated by an extraction process. After a simple wash with ethyl acetate and subsequent vacuum removal of residual solvent, the catalyst was reused for the next run. To our delight, the catalyst was highly reusable under the investigated conditions, preserving its initial catalytic activity almost unaltered after three uses, as is shown in Fig. 1. The purification of the β-CD catalyst after the recycling was confirmed by the IR spectra shown in Fig. 2.

To explain the mechanism of this tandem reaction, a postulated reaction course is depicted in Scheme 3. In the first step, malonitrile **2** undergoes a condensation reaction with aldehyde promoted by β-CD with the removal of water molecules and affords the intermediate 2 benzylidenemalononitrile. In the second step, Michael addition of barbituric acid **3** to the product gives the intermediate 2-(phenyl(2,4,6-trioxopiperidin-3-yl)methyl)malononitrile. β-CD protonates the intermediate and, after cyclization and intramolecular condensation, the product **4a** is produced.

Pharmacology

In vitro antimicrobial activity

Finally, all synthesized compounds were screened for antimicrobial activity. The microorganisms used in this study were *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative bacteria) *Bacillus subtilis*, and *Staphylococcus aureus* (Gram-



Scheme 2 General scheme for the synthesis of 7-amino-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1*H*-pyrano[2,3-*d*]pyrimidine-6-carbonitriles

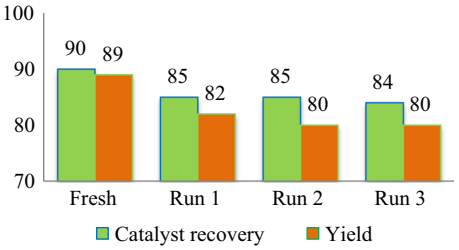


Fig. 1 Reuse and recovery of β-CD and its effect on yield

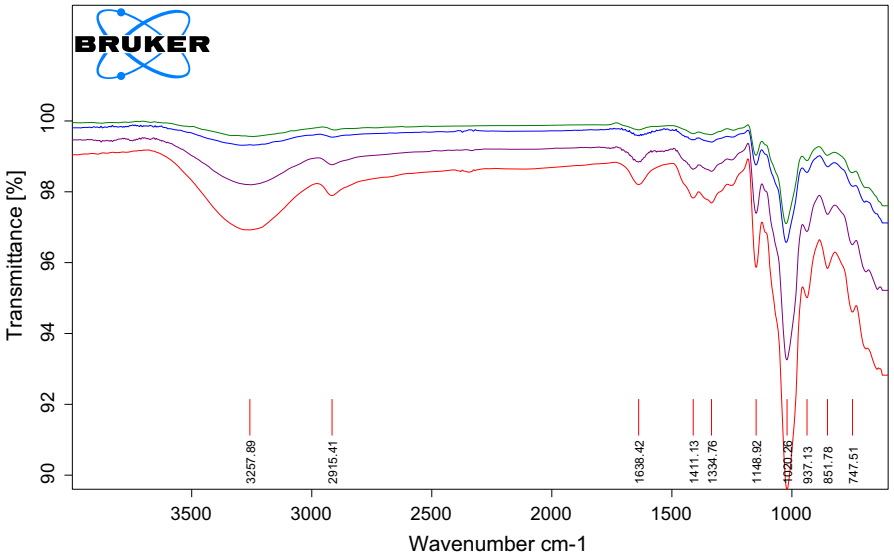
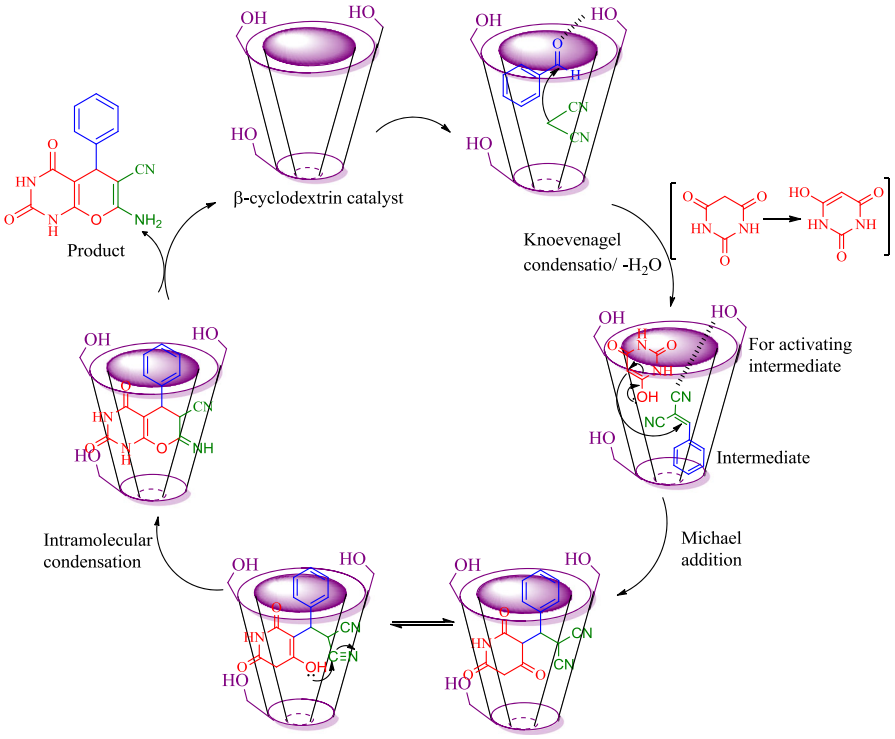


Fig. 2 FTIR spectral analysis of recycling of: **a** β-cyclodextrin fresh; **b** β-CD first run; **c** β-CD second run; **d** β-CD third run

positive bacteria). The minimum inhibitory concentration (MIC) of the synthesized compounds was determined by well diffusion method [52] and compared to two commercial antibiotics (Table 4). In addition, the antifungal activity against three fungal species, *Candida albicans*, *Aspergillus flavus* and *A. niger*, was tested against Miconazole. The results are compiled in Table 3. From the screening results, the MIC of the most active compounds toward the microorganisms showed that the MICs ranged between 25 and 50 μg/mL, with most of compounds exhibiting good to potent in vitro antimicrobial activity against both Gram-positive and Gram-negative strains. The obtained results, depicted in Table 4, reveal that compounds **4c**, **4h**, **4i**, **4k**, **4m** and **4p** could effectively inhibit the growth of most tested bacterial strains, while compound **4i** exhibited good activities against all the tested fungal strains. Compounds **4h**, **4m** and **4p** with a 4-OCH_3, 2-OH and –CH_2 derivative attached to the pyrano[2,3-*d*]-pyrimidinones backbone gave stronger antibacterial efficacies and broader bioactive spectra than ciprofloxacin with quite low MIC values, (MIC = 25 ± 0.25, 25 ± 0.43, 25 ± 0.49 and 25 ± 0.88 μg/mL)



Scheme 3 Proposed mechanism for the multicomponent reaction using β-CD catalyst for the synthesis of pyrano[2,3-*d*]-pyrimidinones

against *E. coli* and *P. aeruginosa*, while compounds **4m** and **4p** also exhibited excellent antibacterial activity with quite low MIC values, (MIC = 25 ± 0.77, and 25 ± 0.75 μg/mL) against *S. aureus* compare with ciprofloxacin. Almost all of the compounds were found to be more active than ciprofloxacin against all the tested strains. On the other hand, in other cases of each class of products, the types of substitution on the aldehyde moiety have affected the antibacterial activity.

In silico molecular docking study

A molecular docking study was performed to predict the possible binding modes and to rationalize the observed biological activity. The docking study was carried out using the Surflex–Dock module of the Sybyl 2.1.1 package following the standard procedure [53].

In silico pharmacokinetics evaluation

An in silico absorption, distribution, metabolism, and excretion–toxicity (ADMET) study was also performed to predict the pharmacokinetic and toxicity profile of the synthesized compounds. Using ADMET software. The FAF Drug2 tool was used for

Table 4 Antimicrobial activity of of pyrano[2,3-*d*]-pyrimidinones (**4a–q**) compounds

Compd.	MIC values in μg/mL[a]				MIC values in μg/mL[a]		
	Antibacterial activity				Antifungal activity		
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*	*A.niger*	*A. flavus*
4a	75 ± 0.32	62.5 ± 0.42	100 ± 1.27	75 ± 0.55	175 ± 0.34	*	200 ± 0.29
4b	50 ± 0.34	125 ± 0.47	100 ± 0.43	150 ± 0.22	225 ± 0.09	*	225 ± 0.17
4c	75 ± 0.49	125 ± 0.95	**50 ± 0.62**	75 ± 0.29	175 ± 0.18	*	200 ± 0.37
4d	187.5 ± 0.09	200 ± 0.30	175 ± 0.01	125 ± 0.38	*	225 ± 1.02	225 ± 0.69
4e	100 ± 0.62	125 ± 0.93	150 ± 0.07	125 ± 0.23	237.5 ± 0.11	225 ± 0.51	*
4f	162.5 ± 0.49	150 ± 0.04	175 ± 0.37	225 ± 0.74	*	*	175 ± 0.07
4g	75 ± 0.77	75 ± 0.14	125 ± 0.07	87.5 ± 0.17	225 ± 0.34	*	200 ± 0.18
4h	**25 ± 0.25**	87.5 ± 0.47	75 ± 0.33	62.5 ± 0.87	*	187.5 ± 0.84	175 ± 0.45
4i	50 ± 0.24	100 ± 0.35	**50 ± 0.87**	**25 ± 0.39**	125 ± 0.28	187.5 ± 0.44	175 ± 0.39
4j	125 ± 0.30	150 ± 0.36	150 ± 0.40	175 ± 0.41	225 ± 0.24	*	200 ± 0.27
4k	**37.5 ± 0.51**	87.5 ± 0.56	87.5 ± 0.41	75 ± 0.99	200 ± 0.74	225 ± 0.09	187.5 ± 101
4l	50 ± 0.51	75 ± 0.48	75 ± 10.01	125 ± 0.50	*	*	150 ± 0.11
4m	75 ± 0.69	**25 ± 0.49**	**25 ± 0.77**	62.5 ± 0.40	*	162.5 ± 0.40	187.5 ± 0.08
4n	125 ± 0.82	137.5 ± 0.44	225 ± 0.39	112.5 ± 0.59	225 ± 0.09	200 ± 0.10	*
4o	62.5 ± 0.50	87.5 ± 0.44	87.5 ± 1.24	100 ± 0.71	175 ± 0.12	*	*
4p	**25 ± 0.43**	**25 ± 0.88**	**25 ± 0.75**	**37.5 ± 0.14**	150 ± 0.03	187.5 ± 0.07	*
4q	75 ± 0.21	87.5 ± 0.05	125 ± 0.14	100 ± 0.67	225 ± 0.03	175 ± 1.23	225 ± 0.81
Ampicillin	100 ± 1.24	100 ± 2.14	250 ± 2.99	250 ± 0.88			
Ciprofloxacin	25 ± 1.00	25 ± 1.15	50 ± 1.44	50 ± 0.96	*	*	
Miconazole	*	*	*	*	25 ± 1.17	2.5 ± 1.11	12.5 ± 0.98

*Antifungal activity not reported up to 400 μg/m

[a]Values are average of three readings

in silico ADMET predictions. These programs computed pharmacokinetic parameters such as molecular percent absorption %ABS, MW, log*P*, N-ROTB, *H*/*C*, PSA, HBA, and HBD, etc. The values obtained are summarized in (Table 5).

Biological section

In vitro antibacterial activity

All the synthesized compounds (**4a–q**) showed moderate to very good antibacterial activity.

It has been noted that for *E. coli*, compounds **4h** and **4p** were found to be equipotent, i.e., 25 ± 0.25 and 25 ± 0.43 μg/mL a,s compared to ciprofloxacin, i.e., 25 ± 1.00 μg/mL. The compounds **4a**, **4c**, **4m** and **4q** have been noted to be highly potent as compared to ampicillin. Compounds **4b**, **4i**, **4l** and **4o** showed maximum potency, i.e., **50 ± 0.24** μg/mL, as compared to ampicillin, i.e., 100 ± 1.24 μg/mL, as well as against *P. aeruginosa*. Compounds **4m** and **4p** were found to be equipotent against *P. aeruginosa*, i.e., 25 ± 0.49 and 25 ± 0.88 μg/mL, compared to ciprofloxacin, i.e. 25 ± 1.15 μg/mL, and compounds **4a**, **4g**, **4h**, **4k**, **4l**, **4o** and **4q** showed higher potency compared to *P. aeruginosa* and compared to ampicillin, i.e., MIC 100 ± 2.14 μg/mL. Compounds **4c**, **4i**, **4m** and **4p** were highly potent and compounds **4h**, **4k**, **4l** and **4o** showed maximum potency against *S.*

Table 5 Molecular docking results of pyrano[2,3-*d*]-pyrimidinones analogues (**4a–q**)

Compd.	Molecular docking score		
	Total score (− log *Ki*)	Crash score	Polar score
4a	4.9804	− 1.2586	2.2293
4b	3.5209	− 1.8443	1.2225
4c	4.3198	− 1.0598	2.1235
4d	2.2607	− 2.7613	2.885
4e	4.0916	− 0.9534	2.0091
4f	3.0113	− 1.6559	1.6576
4g	4.0734	− 1.0279	2.1996
4h	4.7445	− 2.7954	1.1486
4i	5.0558	− 1.6234	1.4042
4j	3.6243	− 0.5049	0.921
4k	4.6797	− 1.9226	2.0557
4l	4.2783	− 1.7266	1.5441
4m	5.1109	− 0.5016	1.3076
4n	3.8353	− 1.3838	2.3866
4o	4.133	− 0.5843	1.2786
4p	5.2555	− 0.7034	2.5962
4q	3.7112	− 1.4132	1.7355
Ampicillin	1.9548	− 3.4237	0.3824
Ciprofloxacin	3.707	− 3.3287	0.8515

Total score total docking score; *Crash score* degree of inappropriate penetration by the ligand into the protein and of interpenetration between ligand atoms that are separated by rotatable bonds; *Polar score* contribution of the polar non-hydrogen bonding interactions to the total score

aureus compared to ampicillin, i.e., 250 ± 2.99 μg/mL. Compounds **4c** and **4i** exhibited the same influence as compared to ciprofloxacin. Compounds **4m** and **4p** were found to have excellent potency against *S. aureus* compared to that of ciprofloxacin. Also, against *B. subtilis*, compounds **4a**, **4c**, **4g**, **4h**, **4k** and **4m** showed maximum potency and compounds **4i** and **4p** were found to be more active (25 ± 0.39 and 37.5 ± 0.14 μg/mL) than ampicillin (250 ± 0.88 μg/mL). Compounds **4i** and **4p** showed maximum potency compared with that of ciprofloxacin, i.e., MIC 50 ± 0.96 μg/mL, against *B. subtilis*. The results suggest that compound **4m** may be a potent candidate for a new class of antibacterial agents in the future.

Structure–activity relationship (SAR) studies for antibacterial activity revealed that the substitution at the aromatic ring positively affects the antimicrobial activities of most of the compounds, and especially those with electron-withdrawing and also electron-donating properties such as the methoxy and hydroxyl groups. Replacement of heterocyclic aldehydes **4k** and **4l** also increases the antibacterial activity. The introduction of the 2-hydroxy group **4m** compared to the 4-hydroxy group **4q** on the benzaldehyde ring led to the most active compound of the synthesized library and showed an increase in antibacterial activity. The introduction of a bromo group at the 4th position of benzaldehyde **4f** led to a decrease in activity compared with compound **4m**.

In vitro antifungal activity

The synthesized compounds (**4a–q**) were screened for their antifungal activity. The MIC (μg/mL) of all compounds was determined using Miconazole as a standard drug. However, none of the compounds exhibited good activity against the fungal strains. The results of the antifungal activity of all compounds (**4a–q**) are shown in Table 4.

In silico molecular docking study

In order to explore the binding affinity, binding mode and molecular interactions of the synthesized agent, a molecular docking study was carried out against its X-ray crystal structure. Bacterial C_{30} carotenoid dehydrosqualene synthase was a potential target in the antibacterial drug design and drug discovery process [54, 55]. In bacterial systems, such as *S. aureus* formation, a pigment system such as staphyloxanthin was carried out with the incorporation of two molecules of farnesyl diphosphate in a condensation reaction by enzyme dehydrosqualene synthase. Farnesyl diphosphate was again been converted into dehydrosqualene by the enzyme dehydrosqualenesynthase which on conversion results in staphyloxanthin and acts as a virulence factor, and its inhibition results in the development of potential antibacterial agents.

To identify the possible identical positions of synthesized pyrano[2,3-*d*]-pyrimidinones analogues as they docked into active sites of the protein, they were further ranked based on total docking score and interactions. To perform molecular docking, a three-dimensional X-ray crystal structure of bacterial C_{30} carotenoid dehydrosqualene synthase enzyme (PDB ID: 3ACX 1.31 Å) in halo form was used

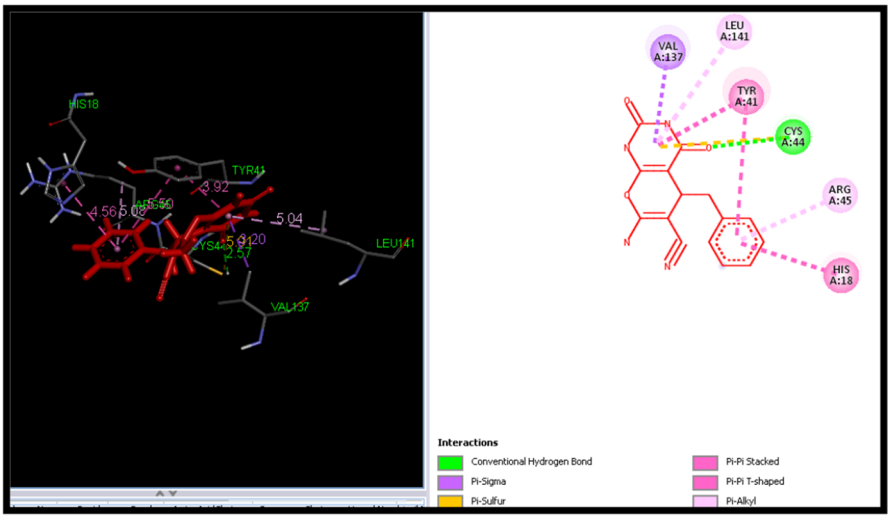
[56]. The number of compounds that have shown high MIC values calculated using the well diffusion method and replicating their potential with a very high total docking score, polar score and low crash score indicate their non-covalent interactions such as hydrogen bond interaction and π interactions [52]. To represent the details of the docking score, the following terms are used: (1) total score is the total docking score, (2) crash score is the degree of inappropriate penetration by the ligand into the protein and of interpenetration between ligand atoms which are separated by rotatable bonds of compounds, and (3) polar score gives an idea about the contribution of the polar non-hydrogen bonding interactions to the total score, as shown in Table 5.

The detailed analysis of binding affinity ($-\log ki$) values and molecular interactions of the most active molecules among the series (**4p**, **4m** and **4i**, ampicillin, and ciprofloxacin) shows they inhibit AmpC. The molecules interact with the active pockets of protein by forming H-bonds. The docking scores of molecules **4p** (5.2555), **4m** (5.1109) and **4i** (5.0558) were excellent as compared to the standard drugs, ampicillin and ciprofloxacin. It has been found that these analogues have a strong potential to inhibit AmpC. The most active pyrano[2,3-*d*]-pyrimidinones, i.e., **4p** (5.2555), **4m** (5.1109) and **4i** (5.0558) show efficient binding modes and penetrate active site cavities by forming thhydrogen bond interactions and π interactions with active site residues. The most active **4p** (5.2555) analogue interacts with the active site amino acid residue CYS44 to form conventional hydrogen bond interactions, and it also interacts with the π electrons of the cloud of diazinane to form an π–sulfur bond interaction of 5.91 Å. The amino acids VAL137, LEU141 and TYR41 interact with the diazinane ring π electron cloud to form π–π stacking, and π–σ and π–π T-shaped interactions. Amino acid residues TYR41, ARG45 and HIS18 interact with the π electrons of the benzyl ring to form π–π stacking, and π–σ and π–π T-shaped and π–alkyl interactions, as shown in Fig. 3a.

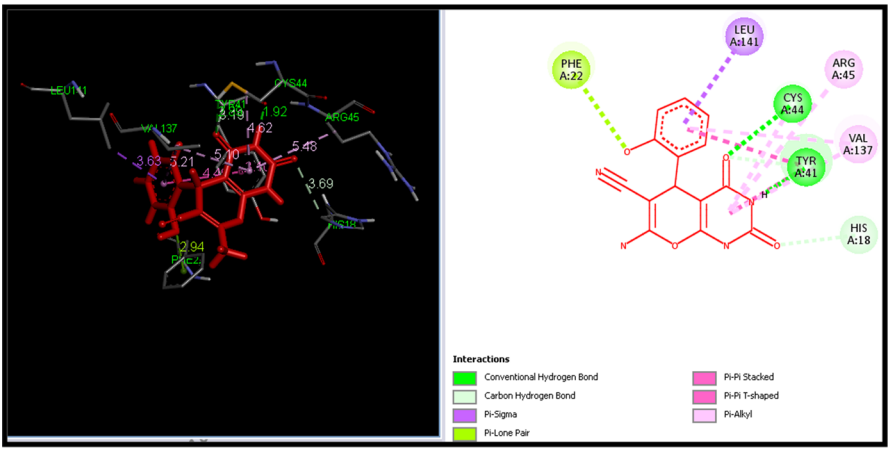
The second most active pyrano[2,3-*d*]-pyrimidinones analogue **4m** (5.1109) forms conventional hydrogen bond interactions with amino acids CYS44 and TYR41 where they interact with nitrogen-substituted hydrogen atoms and carbonyl oxygen of the diazinane ring. Active site amino acid residue HIS18 forms a carbon–hydrogen bond by interacting with the carbonyl oxygen of the diazinane ring with a distance of 3.69 Å. Hydrophobic amino acid PHE22 interacts with hydroxyl oxygen atoms of the phenyl ring to form π–lone pair interactions. Other active site amino acid residues such as ARG45, VAL137 and LEU141 form various kinds of weak π interactions with the π electron cloud of diazinane and the phenyl ring forming π interactions such as π–π stacking, π–σ and π–π T-shaped and π–alkyl, as shown in Fig. 3b.

In silico pharmacokinetic evaluation

The drug likeness of the synthesized molecules was identified by predicting the pharmacokinetic properties in silico. In particular, we calculated the compliance of the synthesized compounds considering the basic parameters of Lipinski's rule of five, [57], BBB, Caco2, %HIA, etc. The pharmacokinetic parameters were obtained



(a)



(b)

Fig. 3 **a** Binding position and molecular interactions of **4p** into the active site of C_{30} carotenoid dehydrosqualene synthase. **b**. Binding position and molecular interactions of **4m** into the active site of C_{30} carotenoid dehydrosqualene synthase

by the ADMET predictor FAFDrugs2 which runs on the Linux OS. This tool is freely available and used for in silico ADMET filtering [58, 59]. For an orally active compound , two violations of Lipinski's rule are accepted [60, 61]. The molecules of the present study were found to follow the rule with maximum violation of two, thus demonstrating their drug-likeness properties. We have assessed parameters like molecular percent absorption (%ABS > 100), weight (MW > 500), partition coefficient (log$P$ > 5), number of rotatable bonds (> 10) and rthe atio of *H*/

Table 6 In silico drug-like (physicochemical) properties of pyrano[2,3-*d*]-pyrimidinones analogues (**4a–q**) and standard drugs

ID	%ABS	MW	log*P*	PSA	RotatableB	HBD	HBA	Rings	Ratio *H/C*	Toxicity
4a	65.9578	282.2542	0.98168	124.76	1	3	3	2	0.5	NT
4b	55.7941	328.2796	0.69588	154.22	2	4	5	2	0.6	NT
4c	65.9578	316.6993	1.63508	124.76	1	3	3	2	0.571	NT
4d	65.9578	300.2447	1.12078	124.76	1	3	3	2	0.571	NT
4e	55.7941	328.2796	0.69588	154.22	2	4	5	2	0.6	NT
4f	65.9578	361.1503	1.74418	124.76	1	3	3	2	0.571	NT
4g	52.1647	327.2518	1.41308	164.74	2	3	5	2	0.714	NT
4h	62.77345	312.2802	0.99028	133.99	2	3	4	2	0.533	NT
4i	62.77345	340.3333	1.55268	133.99	3	3	4	2	0.47	NT
4j	59.5891	342.3062	0.99888	143.22	3	3	5	2	0.562	NT
4k	56.215	288.2819	1.04318	153	1	3	4	2	0.666	NT
4l	60.51025	271.2316	0.30978	140.55	1	4	3	2	0.666	NT
4m	58.97845	298.2536	0.68728	144.99	1	4	4	2	0.571	NT
4n	65.9578	262.2646	1.12358	124.76	3	3	3	1	0.583	NT
4o	65.9578	248.238	0.73348	124.76	2	3	3	1	0.636	NT
4p	65.9578	296.2808	1.17608	124.76	2	3	3	2	0.466	NT
4q	58.97845	298.2536	0.68728	144.99	1	4	4	2	0.571	NT
Ampicillin	61.37965	349.4048	1.3472	138.03	4	3	5	2	0.5	NT
Ciprofloxacin	99.66775	416.1286	6.4548	27.05	6	0	2	3	0.388	NT

Percent absorption

MW molecular weight, *logP* logarithm of partition coefficient of compound between *n*-octanol and water, *PSA* polar surface area, *n-RotBond* number of rotatable bonds, *n-RigBond* number of rigid bonds, *HBA* hydrogen bond acceptors, *HBD* hydrogen bond donor

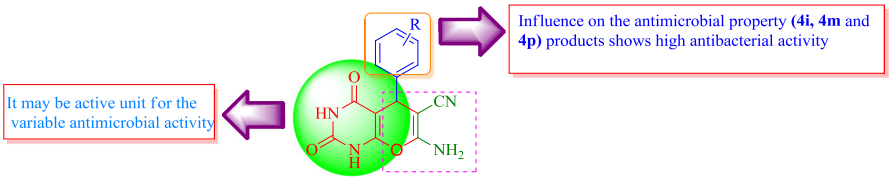


Fig. 4 SAR analysis of synthesized compounds (**4a–q**)

C (> 1). All these parameters signify oral bio-availability and good intestinal absorption [62]. The values obtained are depicted in Table 6.

Topological polar surface area (TPSA), i.e., the surface belonging to polar atoms, and molecular weight are the descriptors which correlate with passive molecular transport through membranes, allowing the prediction of the route of transport of drugs through barrier membranes such as the intestine and the blood–brain barrier. The percentage of absorption (%ABS) was calculated using TPSA by using the formula %ABS = 109 − (0.345 × TPSA) [62]. All the synthesized compounds exhibited a very good %ABS, ranging from 62.93 to 78.64%. The values of the partition coefficient (log*P* > 5), the number of rotatable bonds (> 10), the number of rigid bonds (> 25) and the ratio of *H*/*C* (> 1) determines the absorption performance through the lipophilic phospholipid membranes and the toxicity. Moreover, none of the pyrano[2,3-*d*]-pyrimidinones violated Lipinski's rule of five. All the synthesized compounds, pyrano[2,3-*d*]-pyrimidinones (**4a–q**), followed the criteria for orally active drugs and, therefore, these compounds can be further developed as oral drug candidates. The results of this in silico ADME prediction analysis suggest that the synthesized compounds follow the computational assessment and thus represent a pharmacologically active framework that should be considered for progressing their further potential against bacterial species and may be good candidates for drugs (Fig. 4).

Conclusion

In conclusion, we have used β-cyclodextrin, a green supramolecular catalyst for the synthesis of pyrano[2,3-*d*]pyrimidinone derivatives, as a very important biologically active compound in a green media. No organic solvent was used during the reaction and separation process, the catalyst was removed by simple filtration, no extra purification was necessary for the obtained products, yields of the reaction were high and the catalyst showed excellent reusability. The synthesized compounds were evaluated for their antimicrobial activity against Ampicillin, Ciprofloxacin and Miconazole in vitro. The bioassay results demonstrated that the title compounds exhibited desirable antibacterial activity. A molecular docking study was performed to investigate the possible binding mode of the compounds with the active site of the enzyme. The molecular docking and MIC study revealed good binding energies and potent antimicrobial values, respectively, for synthesized compounds having electron-releasing substituents.

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