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Research Article

Cp_2ZrCl_2: AN EFFICIENT CATALYST FOR MULTICOMPONENT SYNTHESIS OF CAROTENOID DEHYDROSQUALENE SYNTHASE INHIBITING PYRANO[2,3-d]PYRIMIDINEDIONES

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ABSTRACT

Objectives: The present protocol deals with zirconocene dichloride (Cp_2ZrCl_2) catalyzed synthesis of pyrano[2,3-d]pyrimidinediones through one-pot multicomponent reactions of aromatic aldehydes with malononitrile and barbituric acid at ambient temperature. All the synthesized compounds were characterized and evaluated for antibacterial, antifungal, and antioxidant activities. Furthermore, a molecular docking was carried out to reveal the atomic insights between synthesized compounds and carotenoid dehydrosqualene synthase (PDB ID: 3ACX).

Methods: All the synthesized compounds were evaluated for their *in vitro* antimicrobial activity by diffusion method. Antioxidant activities such as 1,1-diphenyl-2-picrylhydrazyl and radical scavenging activity. A mixture of barbituric acid 1 (1 mmol), malononitrile 2 (1 mmol), benzaldehyde 3a (1 mmol), ethanol (5 mL), and Cp_2ZrCl_2 (5 mol %) was stirred at ambient temperature for specified time. After completion of reaction as indicated by thin-layer chromatography, the obtained crude product was filtered and purified by column chromatography on silica gel (Merck, 60–120 mesh) using ethyl acetate:pet. ether to afford pure product which was then characterized by spectroscopic methods such by FTIR, nuclear magnetic resonance (^{1}H NMR), ^{13}C NMR, and mass spectroscopy.

Results: All the synthesized pyrano[2,3-d]pyrimidinediones were characterized by spectroscopic analysis. The results revealed that pyrano[2,3-d] pyrimidinediones (4 a-k) displayed the zone of inhibition in the range of 3–13 mm. The most active compound 4b displayed largest zone of inhibition of 13 mm for *Escherichia coli* (NCIM-2832) and 9 mm for *Bacillus subtilis* (NCIM-2635). The antifungal and antioxidant activity of all synthesized pyrano[2,3-d]pyrimidinediones (4a-k) showed moderate to good activity. Molecular docking studies suggest that pyrano[2,3-d]pyrimidinediones might inhibit the carotenoid dehydrosqualene synthase activity.

Conclusion: All the synthesized pyrano[2,3-d]pyrimidinediones display moderate to good antibacterial, antifungal, and antioxidant activity. This molecular docking studies supported that pyrano[2,3-d]pyrimidinediones might inhibit the carotenoid dehydrosqualene synthase (PDB ID: 3ACX).

Keywords: Zirconocene dichloride, Pyrano[2,3-d]pyrimidinediones, Antimicrobial, Antioxidant, Carotenoid dehydrosqualene synthase, Molecular docking.

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INTRODUCTION

Zirconocene represents an important class of organometallic compounds in which zirconium is sandwiched between two cyclopentadienyl rings. Due to high reactivity and feeble acidity, zirconocenes have attracted substantial applications in the area of catalysis [1]. Initially, the zirconocene catalyst was limited to the olefin polymerization. However, recent reports concerning to successful applications of zirconocene in synthetic chemistry have been demonstrated their versatility in organic synthesis which has spurred a resurgence of interest in this class of compounds [2]. Zirconocene dichloride (Cp_2ZrCl_2) is an air and moisture stable and non-hazardous, d^0 zirconocene that has been subject of immense interest in catalytic technology due to its Lewis acidic character. It is one of the most efficient and widely employed catalysts in Ziegler–Natta polymerization [3]. Organometallic Lewis acids play an important role in green chemistry and sustainable development [4]. Recently, Cp_2ZrCl_2 has been explored for the synthesis of carbonyl group transformation reactions [5], bis(indole)methanes [6], intramolecular coupling of alkyne, EtMgBr (ethylene or CO) [7], quinozolin-4(3H)-ones [8], 1-amidoalkyl-2-naphthols [9], and benzimidazoles [10]. In addition, Cp_2ZrCl_2 has also been employed for acetylation of phenols/alcohols/amines [11], coupling of terminal alkynes, and intramolecular coupling of amines and alkynes. Significant application of zirconium in organic synthesis mainly includes Cp_2Zr(II) species, the so-called zirconocene [12] and Reformatsky and Barbier reactions [13].

Pyrano[2,3-d]pyrimidinediones are heterocyclic scaffolds with multifarious biological applications. They are typical annelated uracils used in the treatment of B16 melanoma and P388 leukemia [14]. In addition, they possess antibronchitic [15], cardiotonic [16], antifungals [17], antimalarial [18], antihypertensive [19], analgesics [20], and antiviral [21]. Moreover, many of its derivatives are used in natural products, carbohydrates, alkaloids, polyether antibiotics, pheromones, antihypertensives, cardiotonic, bronchodilator, antibronchitic and antitumor activity, anti-inflammatory activity, antiallergic, and antibronchitic [22-25]. Due to intriguing structure and diverse biological properties, considerable efforts have been devoted for the development of efficient methods for the synthesis of pyrano[2,3-d]pyrimidinediones [26]. Among several approaches developed for this purpose, one-pot multicomponent reaction of aromatic aldehydes with active methylene compounds and barbituric acid represents the most efficient and powerful process for synthesis of pyrano[2,3-d]pyrimidinediones [27]. Several techniques such as ultrasound, microwave irradiation, as well as ionic liquids have been reported to carry out this reaction [28,29]. However, despite impressive progress, there is a still scope to develop new protocol for synthesis of

pyrano[2,3-d]pyrimidinediones, especially using metallocene-based catalyst related to applications of metallocenes in organic synthesis [30].

Considering aforementioned discussion, we report herein Cp_2ZrCl_2 catalyzed synthesis of pyrano[2,3-d]pyrimidinediones and their biological activities including molecular docking studies.

MATERIALS AND METHODS

Material and physical measurements

The chemicals and reagents used for the synthesis were obtained from commercial sources. All other chemicals and solvents were of analytical grade. Fourier transform infrared (FT-IR) spectra were recorded with Perkin Elmer FT-IR spectrophotometer (KBr disc, 4000–400 cm^{-1}). The samples were examined as KBr discs (ca 5% w/w). Nuclear magnetic resonance (^{1}H NMR) and ^{13}C NMR spectra were recorded on a Brucker AC (400 MHz for ^{1}H NMR and 100 MHz and ^{13}C NMR) using dimethyl sulfoxide (DMSO)-d_6 and $CDCl_3$ as solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts are expressed in parts per million (ppm) with TMS as an internal reference and coupling constants are expressed in hertz (Hz). Mass spectra were recorded in Shimadzu QP 2010 GCMS.

Experimental

General procedure for synthesis of pyrano[2,3-d]pyrimidinediones

A mixture of barbituric acid 1 (1 mmol), malononitrile 2 (1 mmol), benzaldehyde 3a (1mmol), ethanol (5 mL), and Cp_2ZrCl_2 (5 mol %) was stirred at ambient temperature for specific time. After completion of the reaction, as indicated by thin-layer chromatography, the obtained crude product was filtered and purified by column chromatography on silica gel (Merck, 60–120 mesh) using ethyl acetate:pet. ether to afford pure product which was then characterized by spectroscopic methods such by FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy.

Spectral data of representative compounds

7-Amino-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile (Table 1, Entry a)

Yellow solid; yield: 95%; MP: 223–225°C (Lit. MP 224–226°C) IR (KBr cm^{-1}): 3374 (-NH_2), 3305, 3215 (-NH), 3117 (C-H), 2131 (-C≡N), 1698 (-C=O), 1576 (-C=C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6); δ =10.99 (s, 1H, NH); 10.83 (s, 1H, NH); 7.34 (t, *J* = 7.6 Hz, 2H, Ar-H); 7.18–7.09 (m, 3H, Ar-H), 6.86 (s, 2H, NH_2), 4.28 (s, 1H, CH) ppm. ^{13}C NMR ^{13}CNMR (100 MHz, DMSO-d_6); δ =161.38, 144.95, 128.69, 128.47, 128.65, 118.25, 50.86, ppm; Ms (m/z):283.09 $[M+H]^+$.

7-Amino-S-(3-nitrophenyl)-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitril (Table 1, Entry b)

White powder, yield 85%; MP: 256–257°C (Lit. MP 263–264°C) IR (KBr cm^{-1}): 3317 (NH_2), 3306, 3248 (NH), 2945 (C-H), 2214 (C=N), 1609 (C=O), 1476 (C=C). ^{1}HNMR (400 MHz, DMSO-d_6); δ = 12.05 (s, 1H, NH), 10.25 (s, 1H, NH) 8.37 (s, 1H, Ar-H), 8.24 (d, *J* = 6.4 Hz, 2H, Ar-H), 6.85 (s, 2H, NH_2); 3.95 (s, 1H, CH) ppm; ^{13}CNMR (100 MHz, DMSO-d_6); δ = 166.12, 160.73, 157.01, 151.56, 150.57, 146.11, 129.23, 123.08, 119.45, 104.42, 79.32, 71.10 ppm; Ms (m/z): 328.2 M+Hj.

7-Amino-S-(4-bromophenyl)-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitril (Table 1, Entry f)

White powder yield: 87% MP 236–239°C (Lit MP 235–238°C) IR (KBr, υ cm^{-1}): 3376 (NH_2) 3347, 3193 (NH), 3085 (C-H) 2225 (C=N), 1685 (C=O), 1568 (C=C). ^{1}H NMR (400 MHz, DMSO- d_6): δ = 14.16 (s, 1H, NH), 13.04 (s, 1H, NH), 7.78 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.36 (d, *J* 7.2 Hz, 2H, Ar-H) 6.87 (s, 2H, NH_2) 3.97 (s, 1H, CH) ppm; ^{13}CNMR (100 MHz, DMSO-d_6): δ = 173.49, 160.39, 153.97, 145.96, 138.56, 131.97, 129.69, 124.07, 121.32, 105.28, 69.65, 50.14. ppm; Ms (m/z): 384.15 (M+Na).

7-Amino-5-(4-nitrophenyl)-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile (Table 1, Entry g)

White powder, MP: 234–238°C (Lit. Mp: 238–239°C) yield: 95% IR (KBr ϑ cm^{-1}): 3415 (-NH_2), 3208, 3166 (-NH) 2988 (-CH), 2208 (-C≡N), 1737 (-C=O), 1465 (-C=C),^{1}HNMR (400 MHz, DMSO-d_6): δ = 13.04 (s, 1H, NH): 8.97 (s, 1H, NH), 7.97 (d, *J* = 7.2 Hz, 2H Ar-H) 7.47 (d *J* = 7.2 Hz, 2H, Ar- H), 6.77 (s, 2H, NH2), 4.98 (s, 1H, CH) ppm; ^{13}CNMR (100 MHz, DMSO-d_6): δ = 177.48, 170.61, 153.96, 153.80, 150.37, 147.46, 128.98, 123.18, 120.45, 104.47, 75.54, 70.73. ppm, MS, (m/z), 329.2 $(M+1)^+$.

MATERIALS AND METHODS

Antioxidant study

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (RSA) of synthesized pyrano[2,3-d]pyrimidinediones (4 a-k) was determined using DPPH assay as described by Lee *et al*. [31] with slight modification. In short, the stock reagent DPPH solution was prepared by dissolving 24 mg of DPPH in 100 mL methanol and stored at −20°C for further use. The working reagent was prepared by adding 10 mL of stock DPPH solution with 45 mL methanol, to obtain an absorbance 1.1±0.02 O. D. at 517 nm using spectrophotometer. The extracted samples with known concentrations were allowed to react with 3 mL of DPPH solution. The reaction mixture was then incubated in dark condition at room temperature for 3 h and absorbance was measured at 517 nm. A control sample with no added extract was analyzed and the results are expressed as RSA in percent.

$$\% \text{ RSA} = (A_{control}\text{-}A_{sample}) \times 100/\text{Control}$$

Where, A = absorbance at 517 nm.

Ferric reducing/antioxidant power (FRAP) assay

The ferric reducing power of synthesized pyrano[2,3-d] pyrimidinediones (4 a-k) was investigated using the method of Benzie and Strain with a slight modification [32]. The working FRAP reagent was prepared by the addition of 300 mM acetate buffer (pH 3.6), 2, 4, 6-tripyridyl-s-triazine (10 mm) in 40 mm HCl and 20 mm $FeCl_3$ $6H_2O$ in 10:1:1 ratio and heated at 37°C in water bath for 10 min. Respective samples with known concentrations were allowed to react with 2.7 mL of the FRAP reagent. The final volume of the reaction mixture was adjusted to 3 mL with D/W. The reaction mixture was then incubated at room temperature for 30 min in dark condition and absorbance was measured spectrophotometrically at 593 nm, and the results were directly expressed in terms of increase in O. D.

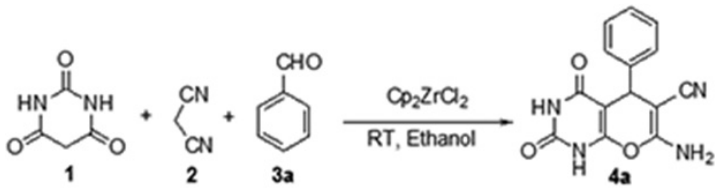
Antifungal activity

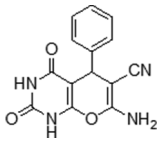
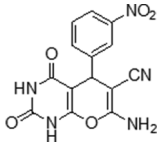
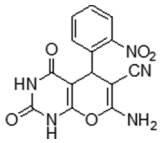
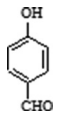
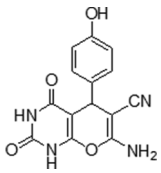
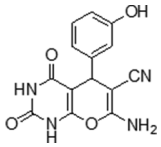
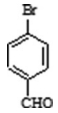
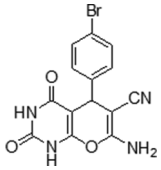
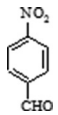
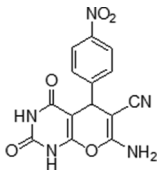
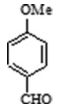
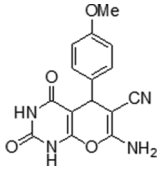
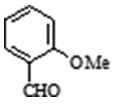
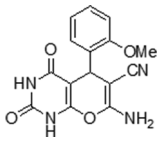
The antifungal activity of synthesized pyrano[2,3-d]pyrimidinediones (4 a-k) was investigated using strains such as *Candida albicans* (NCIM-3466) and *Saccharomyces cerevisiae* (NCIM-3495). The inoculums of *C. albicans* (NCIM-3466) and *S. cerevisiae* were spread on the sterile potato dextrose agar (PDA) plates. The wells in PDA plates were prepared with the help of sterile steel borer. The pyrano[2,3-d] pyrimidinediones (4 a-k) were added in each respective wells, and then, these plates incubated at 37°C for 24 h. The results were assessed after completion of incubation time.

Antimicrobial activity

The synthesized pyrano[2,3-d]pyrimidinediones (4 a-k) were assessed for their antimicrobial activity against bacterial strains, namely *Bacillus subtilis* (NCIM-2635) and *Escherichia coli* (NCIM-2832), as per earlier methodology [33]. Inoculums of target bacterial cells were prepared using sterile saline water. These inoculums were then spread on a sterile nutrient agar plats using spread plate techniques sterile steel borer was used to prepare wells in the plates. Finally, pyrano[2,3-d] pyrimidinediones (4 a-k) were added in each wells containing *B. subtilis* (NCIM-2635) and *E. coli* (NCIM-2832), respectively. All the plates were incubated at 37°C for 24 h.

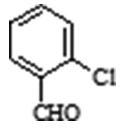
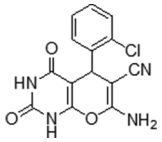
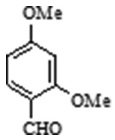
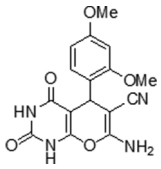
Table 1: Cp_2ZrCl_2 catalyzed synthesis of pyrano[2,3-d] pyrimidinediones[a]



Entry	Aldehyde (3)	Product (4)	Time (min)	Yield[b] (%)	Melting point observed (°C)	Melting point (Lit.) (°C)
a			30	95	223–225	224–226 [31]
b			30	85	261–263	263–264 [31]
c			40	80	259–261	261–264 [31]
d			35	87	160–162	163–164 [27]
e			40	85	168–170	170–174 [30]
f			30	87	235–237	235–238 [20]
g			30	95	235–238	238–239 [26]
h			30	88	287–288	289–293 [30]
i			40	87	230–234	230–231 [41]

(Contd...)

Table 1: (*Continued*)

j			40	89	216–218	215–217 [41]
k			35	90	309–310	303–306 [27]

*Optimal condition: Barbituric acid 1 (1 mmol), malononitrile 2 (1 mmol), and benzaldehyde 3a (1 mmol) in solvent (5 mL) at ambient temperature

RESULTS AND DISCUSSION

Our initial studies were directed toward the optimization of reaction conditions. In this context, multicomponent reaction between barbituric acid 1 (1 mmol), malononitrile 2 (1 mmol), and benzaldehyde 3a (1 mmol) was chosen as a model reaction. To obtain the best results, the model reaction was examined in the presence of various quantities of Cp_2ZrCl_2 in ethanol. In the presence of 5 mol% Cp_2ZrCl_2, the reaction proceeded smoothly furnishing desired 7-amino-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1H-pyrano[2,3-*d*]pyrimidine-6-carbonitrile (4a) in 85% yield. When the quantity of Cp_2ZrCl_2 was increased from 5 mol% to 10 mol%, the yield of corresponding product was elevated from 90 to 95% (Table 2, entry 1 and 2). However, further increase in catalyst quantity beyond 10% did not significantly influence yield of the product (Table 2, entries 3, 4).

Next, we investigated the solvent effect on the model reaction by employing an array of solvents. Good yields were obtained in polar protic solvents such as methanol and ethanol (Table 3, entries 1, 2), while comparatively lower yields were obtained in non-polar solvents such as CH_3CN, CH_2Cl_2, 1,4-dioxane, $CHCl_3$, and toluene (Table 3, entries 3-7). The reaction could not be initiated to synthetically useful degree in DMF (Table 3, entry 8). Among all the screened solvents, ethanol was found to furnish excellent yield of the corresponding product in shorter reaction time (Table 3, entry 2).

After optimization of reaction conditions, we investigated the scope of reaction by reacting barbituric acid 1 (1 mmol) and malononitrile 2 (1 mmol), with structurally diverse aldehydes (3 a-k). The results are summarized in Table 1. The present methodology is flexible to the presence of different functional groups on aromatic ring in aryl aldehydes and provided the anticipated products in moderate to excellent yields. It is noteworthy to mention that aryl aldehydes possessing electron withdrawing as well as electron-donating substituents did not affect the product yield.

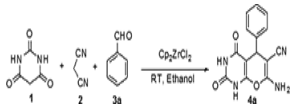
The plausible mechanism for the formation of pyrano[2,3-d] pyrimidinediones (4 a-k) using Cp_2ZrCl_2 is depicted in Scheme 1. Initially, Cp_2ZrCl_2 activates carbonyl group of aryl aldehyde through coordination of Zr with carbonyl oxygen [8]. This facilitates nucleophilic attack of malononitrile on carbonyl group, leading to the formation of 2-benzylidenemalonitrile, on which there is a conjugate addition of barbituric acid, leading to the formation of a cyclic imine intermediate that undergoes subsequent cyclization furnishing the desired product.

To compare the applicability and efficiency of present protocol with reported methods for the synthesis pyrano[2,3-d]pyrimidinediones, we have summarized the results of Cp_2ZrCl_2 with reagents (Table 4). The results reveal that Cp_2ZrCl_2 is better catalyst both in terms of reaction time and yield of the products.

Antimicrobial and antioxidant activity

The antimicrobial activity of pyrano[2,3-d]pyrimidinediones (4a-k) was examined using agar gel diffusion method. The antimicrobial activity

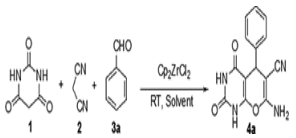
Table 2: Optimization of catalyst loading in the synthesis of pyrano[2,3-d] pyrimidinediones



Entry	Cp_2ZrCl_2 (mol %)	Time (min)	Yield (%)
1	5	35	95
2	10	25	90
3	15	30	91
4	20	35	93

*Optimal condition: Barbituric acid 1 (1 mmol), malononitrile 2 (1 mmol), and benzaldehyde 3a (1 mmol) in ethanol (5 mL) at ambient temperature

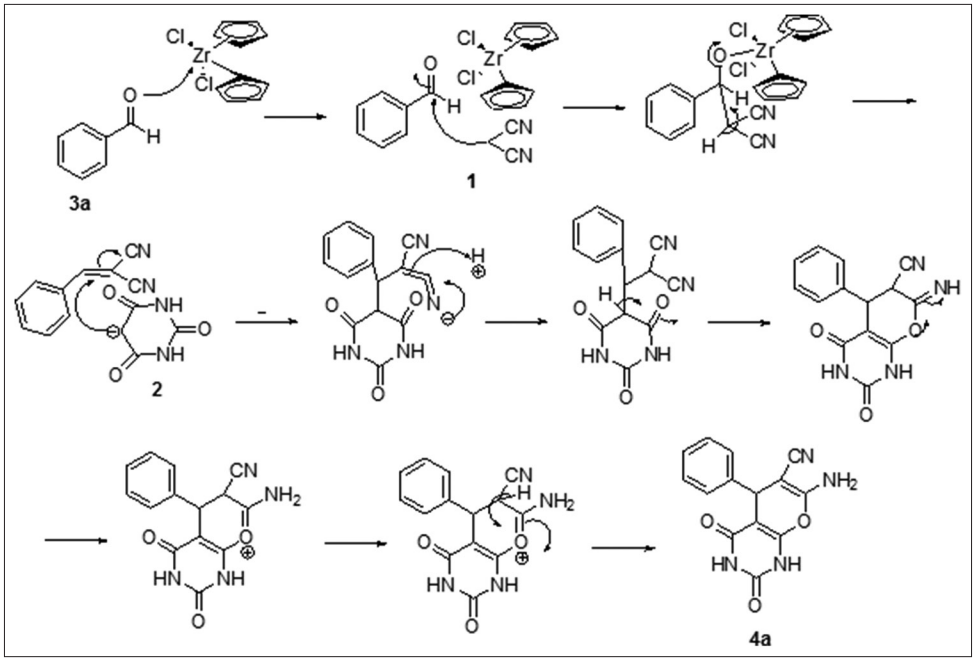
Table 3: Optimization of solvent loading in the synthesis of pyrano[2,3-d] pyrimidinediones



Entry	Solvent	Time (min)	Yield (%)
1	Methanol	45	75
2	Ethanol	30	95
3	CH_3CN	45	55
4	CH_2Cl_2	55	65
5	1,4-Dioxane	90	55
6	$CHCl_3$	55	62
7	Toluene	90	45
8	DMF	70	34

*Optimal condition: Barbituric acid 1 (1 mmol), malononitrile 2 (1 mmol), and benzaldehyde 3a (1 mmol) in solvent (5 mL) at ambient temperature

performed against Gram-negative (*E. coli* NCIM-2832) and Gram-positive (*B. subtilis* NCIM-2635) bacterial strains. The clear zones of inhibition were observed around the wells of *B. subtilis* (NCIM-2635) and *E. coli* (NCIM-2832) plates (Fig. 2). The results revealed that pyrano[2,3-d]pyrimidinedione (4a-k) displayed the zone of inhibition in the range of 3–13 mm (Fig. 2 and Table 5). The most active compound 4b displayed having largest zone of inhibition of 13 mm for *E. coli* (NCIM-2832) and 9 mm for *B. subtilis* (NCIM-2635) (Fig. 2 and Table 5). Hence, antibacterial, of all the screened synthesized compounds pyrano[2,3-d]pyrimidinediones, showed moderate to excellent activity against these bacteria. The antioxidant potential of synthesized derivatives of pyrano[2,3-d]pyrimidinedione was



Scheme 1: Proposed mechanism for the synthesis of pyrano[2,3-d]pyrimidinediones using Cp_2ZrCl_2

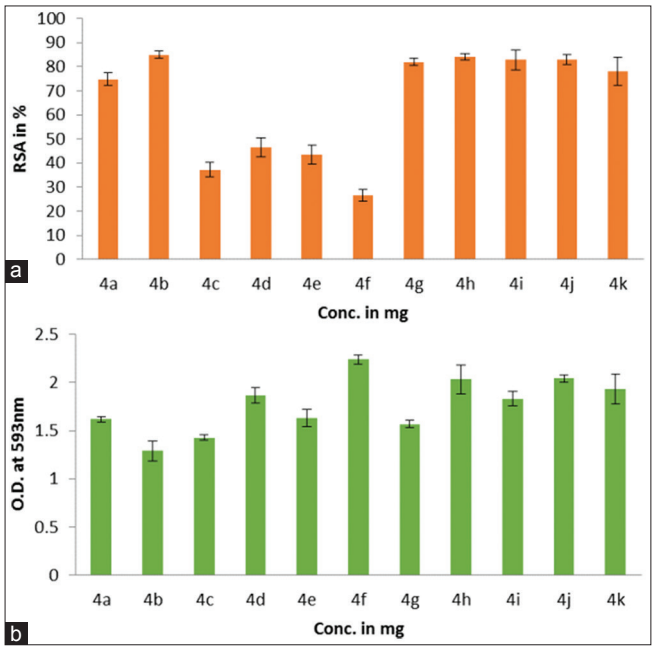


Fig 1: Radical scavenging activity (RSA) of pyrano[2,3-d] pyrimidinediones (a) 1,1-diphenyl-2-picrylhydrazyl RSA in (b) ferric reducing antioxidant power expressed in O. D. at 593 nm

determined using simple, rapid, and inexpensive assays. The synthesized derivatives show strong DPPH, RSA, and FRAP activity.

The synthesized pyrano[2,3-d]pyrimidinediones (4a-k) were tested for antimicrobial activity against bacterial strains, namely *B. subtilis* (NCIM-2635) and *E. coli* (NCIM-2832) as per earlier methodology [33] and zone of clearance around the wells inoculated with the pyrano[2,3-d]pyrimidinediones. The antifungal potential of pyrano[2,3-d]pyrimidinedione derivatives (4 a-k) was examined by the agar gel diffusion method. These results indicate that pyrano[2,3-d] pyrimidinediones derivative (4 a-k) also shows decent antibacterial activity against *E. coli* (NCIM-2832) and *B. subtilis* (NCIM-2635) (Table 5).

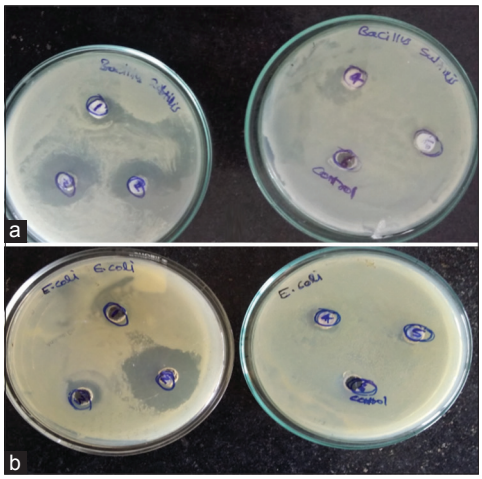


Fig. 2: Plate containing (a) *Bacillus subtilis* (NCIM-2635) and (b) *Escherichia coli* (NCIM-2832) showing the clear zone around the well inoculated with the pyrano[2,3-d]pyrimidinediones

Antifungal testing was carried out against *C. albicans* (NCIM-3466) and *S. cerevisiae* (NCIM-3495) (Table 6). These results suggest that pyrano[2,3-d]pyrimidinediones derivative (4 a-k) also depicts good antifungal activity against *C. albicans* (NCIM-3466) and *S. cerevisiae* (NCIM-3495).

Molecular docking

Molecular docking studies have been found useful to understand interactions between enzymes and ligands [34-37]. Thus, molecular docking studies were carried out between carotenoid dehydrosqualene synthase (PDB ID: 3ACX) and pyrano[2,3-d]pyrimidinediones using Patch dock server [38,39]. The carotenoid dehydrosqualene synthase has been used as a target for pyrano[2,3-d]pyrimidinediones due to its important role in bacterial cell for the production of the C30 carotenoid backbone, that is, dehydrosqualene [40].

Three-dimensional models of pyrano[2,3-d]pyrimidinediones were constructed using SPARTAN versus 6.0.1 Software [41]. Then, energy minimization was carried out by Hartree–Fock method [42]. Further,

Table 4: Comparison of catalytic activity of Cp_2ZrCl_2 with reported catalyst

Entry	Catalyst	Solvent	Quantity	Temp (°C)	Time	Yield (%)	References
1	L-Proline	EtOH	17 mol%	Reflux	30 min	80	[18]
2	$H_{14}[NaP_5W_{30}O_{110}]$	EtOH	1 mol%	Reflux	30–60 min	85	[22]
3	SBA-$PrSO_3H$	Solvent Free	0.02 g	140	45 min	91	[20]
4	[BMIM] BF_4	[BMIM] BF4	1.5 g	90	5 h	82	[21]
5	DAHP	EtOH	(10 mol%)	R.T.	2 h	71	[17]
6	Cp_2ZrCl_2	EtOH	5 mol%	R.T.	30 min	95	This work

Table 5: Zone of clearance around the wells inoculated with the pyrano[2,3-d] pyrimidinediones in the (b) *Escherichia coli* (NCIM-2832) plates and *Bacillus subtilis* (NCIM-2635)

Entry	Compound	Zone of Clearance in mm *Escherichia coli* (NCIM-2832)	Zone of clearance in mm *Bacillus subtilis* (NCIM-2635)
1	4a	4	3
2	4b	13	9
3	4c	9	11
4	4d	5	5
5	4e	1	1
6	4f	8	9
7	4g	7	8
8	4h	5	9
9	4i	7	7
10	4j	8	9
11	4k	8	5
12	Ciprofloxacin	16	17

Table 6: Zone of clearance around the wells inoculated with the pyrano[2,3-d] pyrimidinediones in the *Candida albicans* (NCIM-3466) and *Saccharomyces cerevisiae* (NCIM-3495)

Entry	Compound	Zone of clearance in mm *Candida albicans* (NCIM-3466)	Zone of clearance in mm *Saccharomyces cerevisiae* (NCIM-3495)
1	4a	5	7
2	4b	14	11
3	4c	12	13
4	4d	7	9
5	4e	8	10
6	4f	7	12
7	4g	10	9
8	4h	13	11
9	4i	9	14
10	4j	12	8
11	4k	7	11

the minimized structures of pyrano[2,3-d]pyrimidinediones were used to dock with carotenoid dehydrosqualene synthase (PDB ID: 3ACX) [38]. All the docked complexes were analyzed with the help of CHIMERA [43]. The residues of carotenoid dehydrosqualene synthase (PDB ID: 3ACX) forming hydrogen bonding interactions with pyrano[2,3-d]pyrimidinediones were studied by CHIMERA [43]. Similarly, the earlier reports suggested that chemically synthesized molecules can be further used for their activity against several enzymes [44,45]. Docking energy values for all the synthesized compounds along with standard ciprofloxacin are shown in Table 7. The negative docking energy indicates the proper binding mode of compounds within the active site of dehydrosqualene synthase (Table 7). The binding mode of the most potent compounds 4b, 4f, 4i, and 4k is shown in Fig. 3. These compounds are exhibit in hydrogen bonding interactions with the active site residues of carotenoid dehydrosqualene synthase. The H-bond interactions of compound 4b with the active site residues of dehydrosqualene synthase are shown in Fig. 3a. The compound 4b interacts with oxygen atom of TYR 248 and NH2 group of ASN 168 with bond distances 2.397 and 2.183 Å, respectively (Table 8, entry 4a and 4b). The compound 4f has hydrogen bonding interactions with the oxygen atom of ASP 176 (Fig. 3b) with a bond distance 3.083 Å (Table 8, entry 4c and 4d). The compound 4i forms hydrogen bonding interactions with the oxygen atoms of ASP 176 and ASP 48 (Fig. 3c) with the interatomic distances 1.993 and 1.745 Å, respectively (Table 8, entry 4e and 4f), indicating strong hydrogen bonding interactions with the active site residues of dehydrosqualene synthase. The compound 4i interacts with dehydrosqualene synthase by hydrogen bonding interactions with hydroxyl group of TYR 248 and NH2 group of ARG 265 (Fig. 3d) with the bond distances 2.051 and 2.936 Å (Table 8, entry 4g, 4h, and 4i). The docking studies revealed

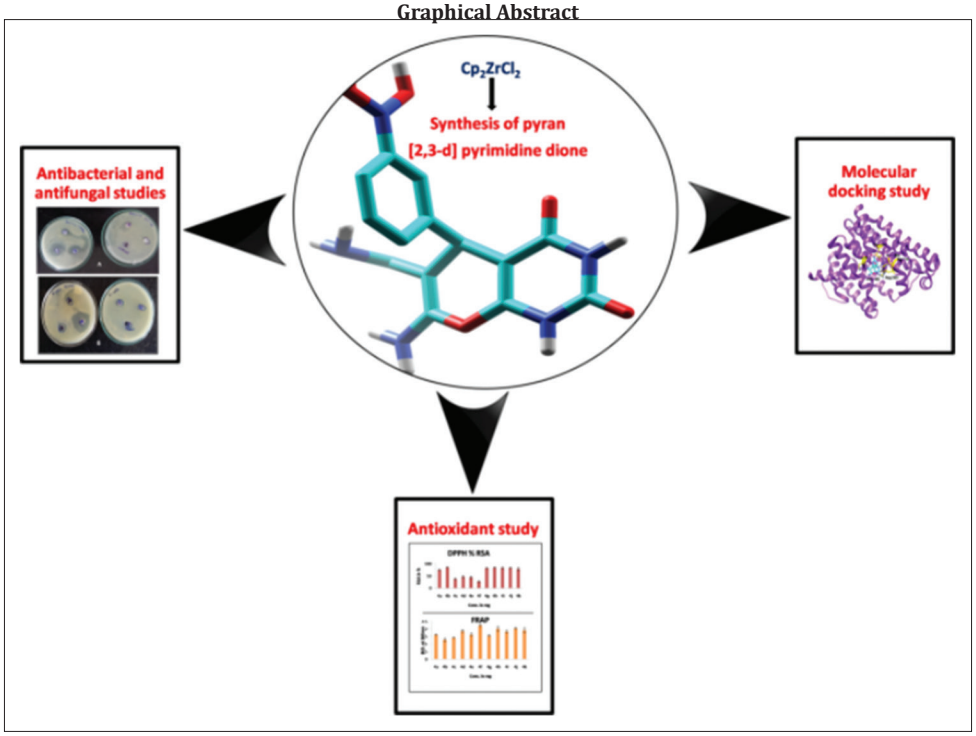
Table 7: Molecular docking between pyrano[2,3-d] pyrimidinediones and carotenoid dehydrosqualene synthase

Entry	Compound	Energy (in kcal/mol)	PATCHDOCK score
1	4a	−1178.6541620	470.00
2	4b	1178.6614029	491.70
3	4c	−1178.6943076	471.40
4	4d	1049.6561871	473.80
5	4e	−1049.6517024	437.70
6	4f	−3534.9100575	452.90
7	4g	−975.2209925	458.10
8	4h	−1088.4677932	456.60
9	4i	1088.4640411	491.10
10	4j	−1432.0428743	440.90
11	4k	−3534.9103618	459.60
12	Ciprofloxacin	−1135.2195612	524.00

Table 8: Hydrogen bonding interactions between carotenoid dehydrosqualene synthase with pyrano[2,3-d] pyrimidinedione derivatives

Entry	Sr. No.	Interaction between active site residues of carotenoid dehydrosqualene synthase with pyrano[2,3-d] pyrimidinedione derivatives (4B, 4F, 4I, and 4K)	Distance in Å
1	4a	SER 21.A OG ------ 4B.het H:	0.839
2	4b	4B.het H ------ ASN 168.A ND2:	2.183
3	4c	4F.het H ------ ARG 265.A NH2:	1.592
4	4d	ASP 48.A OD1 ------ 4F.het Br:	2.254
5	4e	4I.het H ------ ASP 176.A OD1:	1.993
6	4f	ASP 48.A OD2 ------ 4I.het C:	1.177
7	4g	ASP 48.A OD2 ------ 4I.het C:	1.745
8	4h	4K.het H ------ TYR 248.A OH:	2.051
9	4i	ARG 265.A NH1 ------ 4K.het O:	2.936

Graphical Abstract



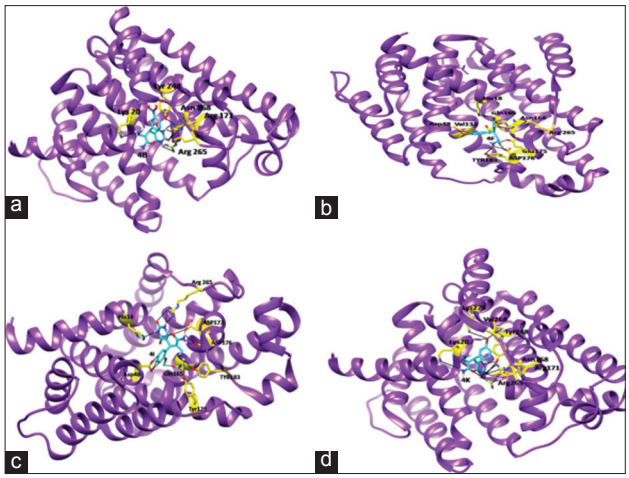


Fig. 3: Binding mode of (a) 4b, (b) 4f, (c) 4i, (d) 4k with carotenoid dehydrosqualene synthase

that the compounds 4b, 4k, 4f, and 4i interact strongly with the active site residues of dehydrosqualene synthase, indicating their proper binding. The studies suggest that compounds 4b, 4k, 4f, and 4i are likely to inhibit the carotenoid dehydrosqualene synthase enzyme present in the bacteria. The docking results are inconsistent with the observed antimicrobial activities.

CONCLUSION

We have reported one-pot multicomponent synthesis of pyrano[2,3-d] pyrimidinediones from aromatic aldehydes, malononitrile, and barbituric acid at ambient temperature using catalytic amount of Cp_2ZrCl_2. The present protocol grants advantages including high yields, effective simplicity, less reaction time, and smooth reaction conditions. In addition, the biological screening of pyrano[2,3-d]pyrimidinediones suggests good antimicrobial potential. Further, molecular docking studies of pyrano[2,3-d]pyrimidinediones reveal hydrogen bonding interactions of these compounds with the active site residues of carotenoid dehydrosqualene synthase enzyme present in the bacteria.

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AUTHORS' CONTRIBUTIONS

Babasaheb. D. Sonawane has done synthesis and experimental work; Maruti. J. Dhanvade has done antibacterial, antifungal, and molecular docking studies; Chetan. B. Aware has done antioxidant studies; Vikas. D. Sonawane and Sharad. K. Awate have done spectral data analysis; and Kailas. D. Sonawane and Suresh. V. Patil have contributed for manuscript preparation.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

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