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Microwave assisted one-pot catalyst free green synthesis of new methyl-7-amino-4-oxo-5-phenyl-2-thioxo-2,3,4,5-tetrahydro-*1H*-pyrano[2,3-d]pyrimidine-6-carboxylates as potent *in vitro* antibacterial and antifungal activity

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A B S T R A C T

An efficiently simple protocol for the synthesis of methyl 7 amino-4-oxo-5-phenyl-2-thioxo-2, 3, 4,5-tetrahydro-*1H*-pyrano[2,3-d]pyrimidine-6-carboxylates via one-pot three component condensation pathway is established via microwave irradiation using varied benzaldehyde derivatives, methylcyanoacetate and thio-barbituric acid in water as a green solvent. A variety of functionalized substrates were found to react under this methodology due to its easy operability and offers several advantages like, high yields (**78–94%**), short reaction time (3–6 min), safety and environment friendly without used any catalyst. The synthesized compounds (**4a–4k**) showed comparatively good *in vitro* antimicrobial and antifungal activities against different strains. The Compounds **4a, 4b, 4c, 4d 4e and 4f** showed maximum antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus* (gram-positive bacteria), *Escherichia coli*, *Klebshiella pneumonia*, *Pseudomonas aeruginosa* (gram-negative bacteria). The synthesized compound **4f** showed maximum antifungal activity against *Aspergillus Niger* and *Penicillium chrysogenum* strains. *Streptomycin* is used as standard for bacterial studies and *Mycostatin* as standards for fungal studies. Structure of all newly synthesized products was characterized on the basis of IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis.

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Introduction

Nitrogen and oxygen-containing heterocycles serve both as a biomimetic and reactive pharmacophores due to their diverse therapeutic property thus, plays vital role in natural and synthetic organic chemistry [1,2]. Certain annulated uracils have received considerable attention in medicinal chemistry as their wide biological activities such as, antibacterial,

antifungal, antileishmanial agents, antimalarial, antimetabolite, antitumor, antiviral, antihypertensive activity and emerged as an integral backbone of several medicinal drugs [3–8]. The assorted medicinal agents are composed of several uracil rings in which Pyranopyrimidines create a significant status. Hence, these multifaceted uracils, fascinated large efforts toward their synthetic manipulation of annulated Pyrano[2,3-d]pyrimidine derivatives.

The development of environmentally benign and clean protocol has become the goal of synthetic methodology in aqueous conditions as water plays a vital role in life processes, ambient reaction medium, unique reactivity and selectivity in organic synthesis [9–11]. Thus, there is a need for developing multicomponent reactions (MCRs) paths in water without using any harmful organic solvents and catalysts.

Green chemistry has now become a subject of demanding research emerged in the early 1990s [12], which is now widely adopted to meet the fundamental scientific challenges so as to protect the humans and environment, to achieve commercial viability and to reduce hazardous wastes as well as eliminate the use of conventional volatile organic solvents [13–15]. Thus microwave-irradiated multi-component reactions showed attractive synthetic strategy for rapid-efficient library generation and provided these potential green chemistry techniques in present scenario for various heterocyclic syntheses [16]. Here, microwaves irradiations couples directly with colliding molecules of the entire reaction mixture, leads to rapid temperature rise at the moment of fruitful collision. As a result mere a reaction contents get heated and not the vessel; gives better homogeneity and selective heating of polar molecules to impart advantages viz: environmentally friendly, improved bond forming efficiency (BFE), time saving, experimental simplicity, and atom economy [17–19]. In recent years, synthesis of Pyrano[2,3-d]pyrimidine derivatives were reported using plethora of reagents under traditional thermal condition [20], microwave irradiation [21], ultrasonic irradiation [22], solvent and catalyst free condition [23,24], using different catalysts such as, Zn[(L)PROLINE]$_2$ [25], diammonium hydrogen phosphate (DAHP) [26], L-proline [27], ionic liquids [28] and DABCO [29]. Reported methods appearing in the literature usually require forcing conditions, prolonged reaction time, effluent pollution, high cost of catalyst; create wastes, complex synthetic pathway, low yields, and involved organic solvents as well high energy to proceed. Thus, investigation has been carried out under microwave-organic reaction enhancement (MORE) techniques for synthesis of targeted products. Moreover, to the best of our knowledge there is

no report on the use of methylcyanoacetate as reactant for the synthesis of annulated pyrano[2,3-d]pyrimidines. Therefore we report here, to explore the catalyst free efficient, simple and fast green pathway synthesis of highly functionalized methyl 7 amino-4-oxo-5-phenyl-2-thioxo-2, 3, 4, 5-tetrahydro-1H-Pyrano [2, 3-d] pyrimidine-6-carboxylate derivatives via one-pot three-component domino Knoevenagel-Michael addition reaction under microwave irradiation (Scheme 1).

Experimental

Instruments and analysis

Melting points were determined by open capillary method and were uncorrected. IR spectra were recorded on a Perkin–Elmer 298 spectrophotometer using KBr pellet. ^{1}H NMR spectra were obtained on a Bruker instrument (400 MHz) and ^{13}C NMR spectra were (100 MHz) recorded in DMSO-d6 as solvent with TMS as internal standard. Chemical shifts are reported in ppm. Mass spectra were measured using high resolution GC–MS (DFS) thermo spectrometers with EI (70 EV). Molecular ion peak was observed in agreement with molecular weight of respective compound. Reactions have been monitored by thin layer chromatography on 0.2-mm pre-coated plates of silica gel G60 F254 (Merck). Microwave irradiation was carried out in a Microwave Oven, Model No. NNK571MF (2450 M*Hz*, 1000 W) equipped with a 35 mL vessel. The *in vitro* antimicrobial and antifungal activity of synthesized compounds has studied in pharmacy department, Kashmir University.

General procedure for the preparation of methyl 7-amino-4-oxo-5-phenyl-2-thioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d] pyrimidine-6-carboxylate derivatives (4a–k).

Conventional heating

Benzaldehyde derivatives 1 (1 mmol), methylcyanoacetate 2, (1.2 mmol), thio-barbituric acid 3 (1 mmol) and water (8–10 mL) as solvent were taken in an RB flask and stirred at 48 °C, 60 °C and at room temperature without using catalyst. The reaction was monitored by thin layer chromatography using eluent petroleum ether and ethyl acetate (7:3 ratio). The solid compound was filtered, washed with cold water and recrystallization from 95% ethanol to obtain pure product methyl 7 amino-4-oxo-5-phenyl-2-thioxo-2,3,4,5-tetrahydro-1H-Pyrano[2,3-d]pyramidine-6-carboxylate derivatives.
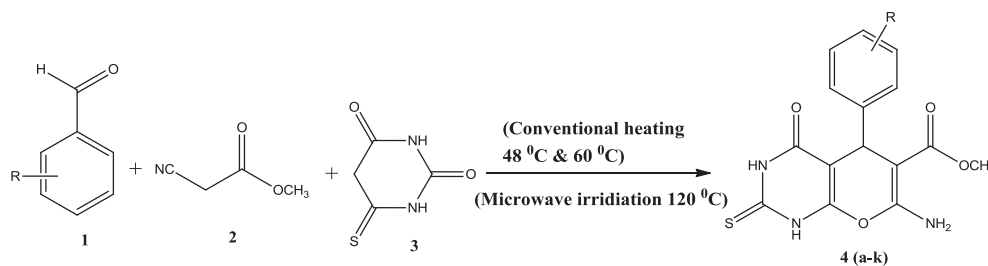
Scheme 1 Microwave and conventional synthesis of methyl 7-amino-4-oxo-5-phenyl-2-thioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carboxylate derivatives (4a–k).

A mixture of benzaldehyde derivatives **1** (1 mmol), methylcyanoacetate **2**, (1.2 mmol), thio-barbituric acid **3**, (1 mmol) and water (3.0 mL) was placed into Teflon vessel and subjected to microwave irradiation under catalyst free conditions for a given time at power of 250 W and 120 °C. After completion of the reaction as followed by TLC examination at an interval of 30 s using eluent petroleum ether:ethylacetate (7:3 ratio). The reaction mixture was cooled to room temperature and poured into cold water, causing the precipitation of the product. The solid product was filtered under vacuum, washed with water and subsequently recrystallized from 95% ethanol to yield the pure product in excellent yield **(78–94%).**

Selected spectral data

Methyl 7-amino-4-oxo-5-phenyl-2-thioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carboxylate **4a**

M.p. 221–223 °C; –IR (KBr) (v_{max}): 3387 (NH_2), 3328, 3103 (NH), 3072 (C—H), 2159 (C≡N), 1768 (C=O), 1654 (C=C) cm^{-1}; –^{1}H NMR (400 MHz, DMSO-$d6$) δ 10.98 (s, 1H, NH), 10.80 (s, 1H, NH), 7.27–7.11 (m, 4H, Ar—H), 7.08 (s, 1H, Ar—H), 6.82 (s, 2H, NH_2), 3.94 (s, 1H, Ar—H), 3.61 (s, 3H, OCH_3); –^{13}C NMR (100 MHz, DMSO-$d6$) δ 179.44 (>C=S), 170.24 (≡COCH_3), 159.49 (>C=O), 155.08 (>C—NH_2), 151.65 (C-4), 140.52 (C-11), 128.59 (C-16), 127.61 (C-14), 93.41 (C-5), 84.22 (C-9), 52.03 (CH_3), 39.43 (C-10); –EI–MS, m/z ($C_{15}H_{13}N_3O_4S$): 331 (M +), 315, 303, 300, 253, 239.

Methyl 7-amino-4-oxo-2-thioxo-5-(p-tolyl)-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carboxylate **4b**

M.p. 286–287 °C; –IR (KBr) (v_{max}): 3304 (NH_2), 3312, 3196 (NH), 3032 (C—H), 2107 (C≡N), 1734 (C=O), 1629 (C=C) cm^{-1}; –^{1}H NMR (400 MHz, DMSO-$d6$) δ 10.98 (s, 1H, NH), 10.80 (s, 1H, NH), 7.04 (d, J = 6.7 Hz, 2H, Ar—H), 6.95 (s, 2H, Ar—H), 6.82 (s, 2H, NH_2), 3.94 (s, 1H Ar—H), 3.61 (s, 3H, OCH_3), 2.19 (s, 3H, CH_3); –^{13}C NMR (100 MHz, DMSO-$d6$) δ 179.44 (>C=S), 170.24 (≡COCH_3), 159.50 (>C=O), 155.08 (>C—NH_2), 151.66 (C-4), 138.94 (C-11), 137.99 (C-14), 129.82 (C-13), 128.92 (C-16), 93.42 (C-5), 84.22 (C-9), 52.03 (CH_3), 39.43 (C-10), 21.13 (CH_3); –EI–MS, m/z ($C_{16}H_{15}N_3O_4S$): 345 (M +), 330, 329, 317, 314, 253.

Methyl 7-amino-5-(4-hydroxyphenyl)-4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H- pyrano[2,3-d]pyrimidine-6-carboxylate **4f**

M.p. 182–182 °C; –IR (KBr) (v_{max}): 3634 (OH), 3510 (NH_2), 3415, 3309 (NH), 3137 (C—H), 2204 (C≡N), 1654 (C=O), 1431 (C=C) cm^{-1}; –^{1}H NMR (400 MHz, DMSO-$d6$) δ 10.98 (s, 1H, NH), 10.80 (s, 1H, NH), 6.98 (d, J = 6.8 Hz, 2H, Ar—H), 6.82 (s, 2H, NH_2), 6.61 (d, J = 7.0 Hz, 2H, Ar—H), 6.05 (s, 1H, OH), 3.94 (s, 1H, Ar—H), 3.61 (s, 3H, OCH_3); –^{13}C NMR (100 MHz, DMSO-$d6$) δ 179.43 (>C=S), 170.23 (≡COCH_3), 159.49 (>C=O), 157.19 (>C—OH), 155.08 (>C—NH_2), 151.65 (C-4), 129.31 (C-16), 129.20 (C-120), 115.15 (C-13), 93.41 (C-5), 84.21 (C-9), 52.03 (CH_3), 39.43

(C-10). –EI–MS, m/z ($C_{15}H_{13}N_3O_5S$): 347 (M +), 331, 319, 316, 253.

Biological evaluation

Synthesized compounds (**4a–4k**) were screened for their *in vitro* antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus* (gram-positive bacteria), *Escherichia coli*, *Klebshiella pneumonia*, *Pseudomonas aeruginosa* (gram-negative bacteria) and also tested for their *in vitro* antifungal activity against *Aspergillus Niger* and *Penicillium chrysogenum* strains. The minimum inhibitory concentration (MIC) of µg/mL values is carried out by the disk-diffusion technique [30,31] to assess the activity of the chosen compounds. Samples were dissolved in dimethyl sulfoxide (DMSO) for dilution to prepare stock of 1 mg mL^{-1} and Whatman filter paper disks (No. 1) were impregnated with the solutions. The impregnated disks were placed on the surface of solidified nutrient agar dishes seeded by the test bacteria and sabourauds dextrose agar dishes seeded by the test fungi. The medium in the plates was allowed to stand at room temperature for 10 min and was set to solidify in a refrigerator for 30 min. The minimum inhibitory concentrations (MICs) were measured in millimeters by the end of the incubation period 48 h at 37 °C (for bacteria) and 72–91 h at 28 °C (for fungi). *Streptomycin* (25 µg mL^{-1}) is used as standard for bacterial studies and *Mycostatin* (25 µg mL^{-1}) as standards for fungal studies. The results are described in Table 4.

Results and discussion

Chemistry

Herein, we wish to report the synthesis of methyl 7 amino-4-oxo-5-phenyl-2-thioxo-2, 3,4, 5-tetrahydro-*1H*-Pyrano[2,3-d]pyrimidine-6-carboxylate derivatives from aromatic aldehydes 1 (**a–k**) (1 mmol), methylcyanoacetate 2 (1.2 mmol), thio-barbituric acid 3 (1 mmol) using water (3.0 mL) as solvent under microwave irradiation. Initially, the same reaction has also monitored under conventional heating (48 °C and 60 °C). The result showed that reaction completed in 3–6 min with excellent yield (78–94%) under microwave irradiation as compared to conventional heating were obtained moderate yields (69–86%) in 2–6 h at 48 °C and (71–87%) in 1–4 h at 60 °C respectively. Further the yields (67–82%) of targeted compounds were obtained in 2–7 h under room temperature (Table 1). Therefore, microwave irradiation reducing the reaction time and improving the reaction yields. The nature of different substituents containing electron-withdrawing groups (such as nitro group, halide) or electron-donating groups (such as hydroxyl group, alkoxyl group) did not showed strongly obvious effects in terms of reaction time and yield of products. In order to optimize the reaction condition of different solvents for the model product **4f**, using reaction mixture of 4-hydroxy benzaldehyde 1 (1 mmol), methylcyanoacetate **2** (1.2 mmol) and thio-barbituric acid **3** (1 mmol) under conventional heating (48 °C and 60 °C), room temperature and microwave irradiation 120 °C (Scheme 2). Results are summarized in Table 2, showed that best conversion was obtained using water as solvent in reaction medium. Mechanistically, the formation

Table 1 Synthesis of **4a–k** compounds under conventional heating (48 °C and 60 °C), room temperature and microwave irradiation at 120 °C.

Product	Room temperature		Conventional heating				MW irradiation		M.P (°C)
	Time (h)	Yield (%)[a]	Time (h) 48 °C	Yield (%)[a]	Time (h) 60 °C	Yield (%)[a]	Time (min)	Yield (%)[a]	Found. Reported[Lit.]
4a	2	82	2	73	1	71	4	82	221–223 224–225 [28]
4b	3	78	3	78	2	83	6	87	286–287 296–298 [25]
4c	4	76	3	79	2	81	5	91	238–240 230 [29]
4d	4	81	3	83	3	81	4	84	213–214 206–210 [25]
4e	7	79	6	82	4	82	6	77	211–212 210–212 [28]
4f	4	82	3	86	1	87	5	94	182–182 163–167 [25]
4g	6	78	5	83	2	86	3	83	253–255 242–244 [28]
4h	6	72	4	81	3	76	4	89	223–225 215–216 [28]
4i	3	67	2	78	1	73	5	86	239–243 237–240 [25]
4j	4	78	3	69	2	75	4	78	231–234 227–229 [20]
4k	3	81	2	83	1	79	5	82	302–304 289–293 [25]

[a] Isolated yields.

of the product is a sequence of reactions involving Knoevenagel condensation of methylcyanoacetate with aromatic aldehydes by loss of water molecule, followed by Michael addition of thio-barbituric acid on electron deficient C-atom and an intra molecular heterocyclization that leads to the formation of the pyrano[2,3-d]pyrimidine derivatives [29]. A reasonable mechanism for the formation of targeted products via three component reaction is outlined in (Scheme 3).

The structure of model compound **4f** was confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectrometric analysis. The IR spectrum showed absorptions at 3634, 3510, 3415, 3309, 3137, 2204, 1654, 1431 cm^{-1} due to the OH, NH$_2$, two NH, C—H, C≡N, C=O, C=C groups respectively. The ^{1}H NMR spectrum showed the presence of two amido protons (NH) as singlet at δ 10.98–10.80 and other peaks at δ 6.98 (d, $J = 6.8$ Hz, 2H, Ar—H), 6.82 (s, 2H, NH$_2$), 6.61 (d, $J = 7.0$ Hz, 2H, Ar—H), 6.05 (s, 1H, OH), 3.94 (s, 1H, Ar—H), 3.61 (s, 3H, OCH$_3$) (Fig. 1). The ^{13}C NMR spectrum showed 13 peaks at δ 179.43 (>C=S), 170.23 (≡COCH$_3$), 159.49 (>C=O), 157.19 (>C—OH), 155.08 (>C—NH$_2$), 151.65 (C-4), 129.31 (C-16), 129.20 (C-12), 115.15 (C-13), 93.41 (C-5), 84.21 (C-9), 52.03 (CH$_3$), 39.43 (C-10) (Fig. 2). The mass spectrum of **4f** revealed a strong molecular ion peak at m/z 347 (M$^+$) in agreement with molecular weight of compound.

Further, we have worked on systematic evaluation of different catalysts for the model product **4f**, by reacting a mixture of 4-hydroxy benzaldehyde (1 mmol), methylcyanoacetate (1.2 mmol) and thio-barbituric acid (1 mmol) using water (3.0 mL) as solvent under microwave irradiation (Table 3). We found that yield of model product **4f** is 94% without using catalyst. These results indicated that time taken for the

synthesis of model product **4f** using different catalysts is 5–20 min with poor yield 57–82% (Table 3). We observed that due to more addition of catalysts the product formation is very low and the removal of catalysts by simple washing is difficult.

The structural assignment of **4(a–k)** was confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectrometric analysis. The IR spectra exhibited sharp bands regions at 3634 cm^{-1} (OH), 3304–3510 cm^{-1} (NH$_2$), 3103–3329 cm^{-1} (NH), 2107–2201 cm^{-1} (CN) and 1676–1768 cm^{-1} (C=O) groups. ^{1}H NMR spectra of the synthesized products exhibited the following characteristic signals: protons of two amido groups (NH) on pyrimidine ring are directly attached to electro negative nitrogen atoms showed deshield the protons toward downfield region at 10.80–10.98 δ ppm. Protons of primary amine (NH$_2$) are directly attached to the electronegative nitrogen atom observed broad singlet in the region of 6.80–3.85 δ ppm downfield and protons of phenyl ring showed doublet, triplet and multiplet signals in the aromatic region 3.94–8.38 ppm. Hydroxyl proton observed at 6.05 ppm and methoxy protons observed broad singlet at 3.81–3.84 δ ppm. Upon studying the ^{1}H NMR spectrum a characteristic sharp singlet is observed toward up-field region at 2.19 δ ppm. This signal is assigned to the three equivalent methyl protons at Para position of phenyl ring. Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the J values for each of the proton it can be clearly suggested the proposed structure for synthesized compounds has been confirmed. The ^{13}C NMR spectrum of synthesized compounds showed 12–14 peaks at different δ values. The significant peaks observed at δ 179.44 (>C=S), 170.24 (≡C—OCH$_3$), 159.50 (>C=O), 157.19 (>C—OH), 155.08 (>C—NH$_2$), 149.31 (>C—NO$_2$),

Table 2 Optimization of different solvents for the synthesis of **4f** product under conventional heating (48 °C and 60 °C), room temperature and microwave irradiation at 120 °C.

Solvent	Room temperature		Conventional method				Microwave irradiation	
	Time (h)	Yield (%)[a]	Time (h) 48 °C	Yield (%)[a]	Time (h) 60 °C	Yield (%)[a]	Time (min)	Yield (%)[a]
Ethanol	3	72	3	69	2	71	4	76
Water	4	82	3	78	2	83	5	94
DMF	2	62	2	71	1	62	4	61
DMSO	2	57	2	61	2	58	10	42
CH_2Cl_2	3	61	3	62	2	56	4	67
EtOH:H_2O	1	78	1	67	1	64	5	72
Solvent less	8	63	6	56	5	59	12	58

[a] Isolated yields.

Table 3 Optimization of catalysts for the synthesis of **4f** product under microwave irradiation[b].

Entry	Catalyst	Mole%	Solvent	Time (min)	Yield (%)[a]
1	DBU	20 mol	H_2O	15	82
2	DABCO	20 mol	H_2O	12	71
3	K_2CO_3	20 mol	H_2O	20	57
4	Et_3N	2–3 Drops	H_2O	11	64
5	No catalyst	–	H_2O	5	94

[a] Isolated yields.
[b] Reaction condition: 4-hydroxy benzaldehyde (1 mmol), methylcyanoacetate (1.2 mmol), thio-barbituric acid (1 mmol) and water (3.0 mL) as solvent.

Table 4 Antibacterial and antifungal activity methyl 7-amino-4-oxo-5-phenyl-2-thioxo-2, 3, 4, 5-tetrahydro-*1H*-pyrano [2, 3-*d*] pyrimidine-6-carboxylate derivatives (**4a–4k**).

Compd.	MIC (µg/mL)[a]					MIC (µg/mL)[b]	
	Gram positive			Gram negative		Fungi	
	S. aureus	*B. cereus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*A. Niger*	*P. chrysogenum*
4a	12	18	17	21	19	+ +	–
4b	15	12	16	19	21	+ + +	+ + +
4c	16	19	15	18	16	–	+ +
4d	17	21	17	17	20	+ + +	+ +
4e	13	15	16	14	12	+	+ +
4f	21	18	21	22	18	+ + +	+ + +
4g	–	10	7	3	13	–	+
4h	11	–	9	–	4	+	+
4i	10	12	8	11	9	+ +	–
4j	–	8	10	11	–	–	+
4k	13	11	13	12	10	+	+ +
Reference[c]	[28]	[28]	[28]	[28]	[28]	[29]	[29]

[a] Inhibition zone around the disks for antibacterial activity: 18–28 mm: very strong activity; 11–17 mm: strong activity; 6–16 mm: moderate weak activity; 0–5 mm weak activity; dash denotes no activity.
[b] Zone area for antifungal activity: + + + = 23–32 mm, + + = 12–22 mm, + = 0–11 mm, dash (–) = no activity.
[c] *Streptomycin* for antibacterial activity and *Mycostatin* for antifungal activity.

56.78 (—OCH_3), 52.03 (—OCH_3), 21.13 (CH_3). Systematic fragmentation pattern was observed in mass spectral analysis. Molecular ion peak was observed in agreement with molecular weight of compounds.

In vitro antibacterial and antifungal activity

Electron donating substituents viz; —OH, CH_3—OCH_3 and C_6H_5 on the annulated pyrano[2,3-*d*] pyrimidine skeleton increases solubility in the solvent showed high *in vitro* antimicrobial and antifungal activity. The synthesized compound **4f** showed maximum antibacterial activity against *S. aureus*, *B. cereus* (gram-positive bacteria), *E. coli*, *K. pneumonia*, *P. aeruginosa* (gram-negative bacteria) and also enhanced maximum antifungal activity against *A. Niger* and *P. chrysogenum* strains. The Compounds **4a, 4b, 4c, 4d** and **4e** showed maximum antibacterial activity against gram-positive and gram-negative bacteria like; *S. aureus, B. cereus, E. coli, K.*
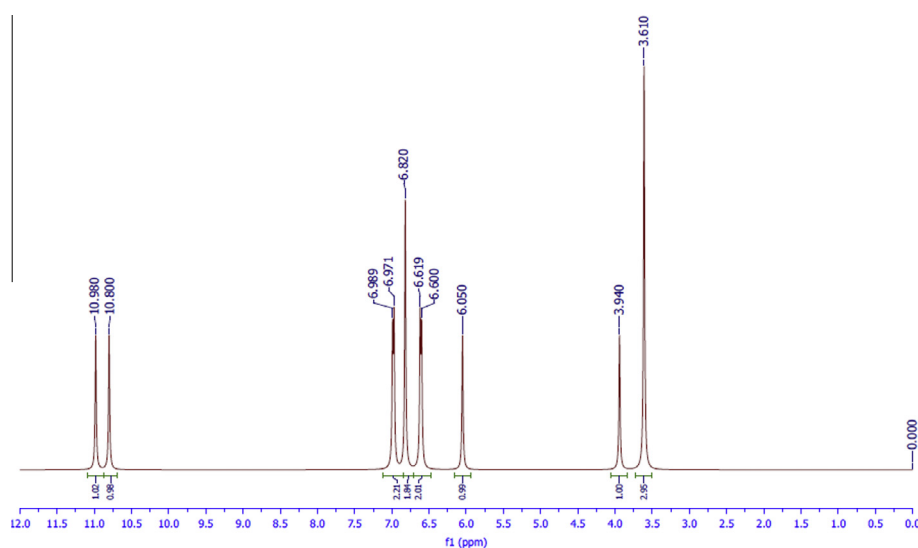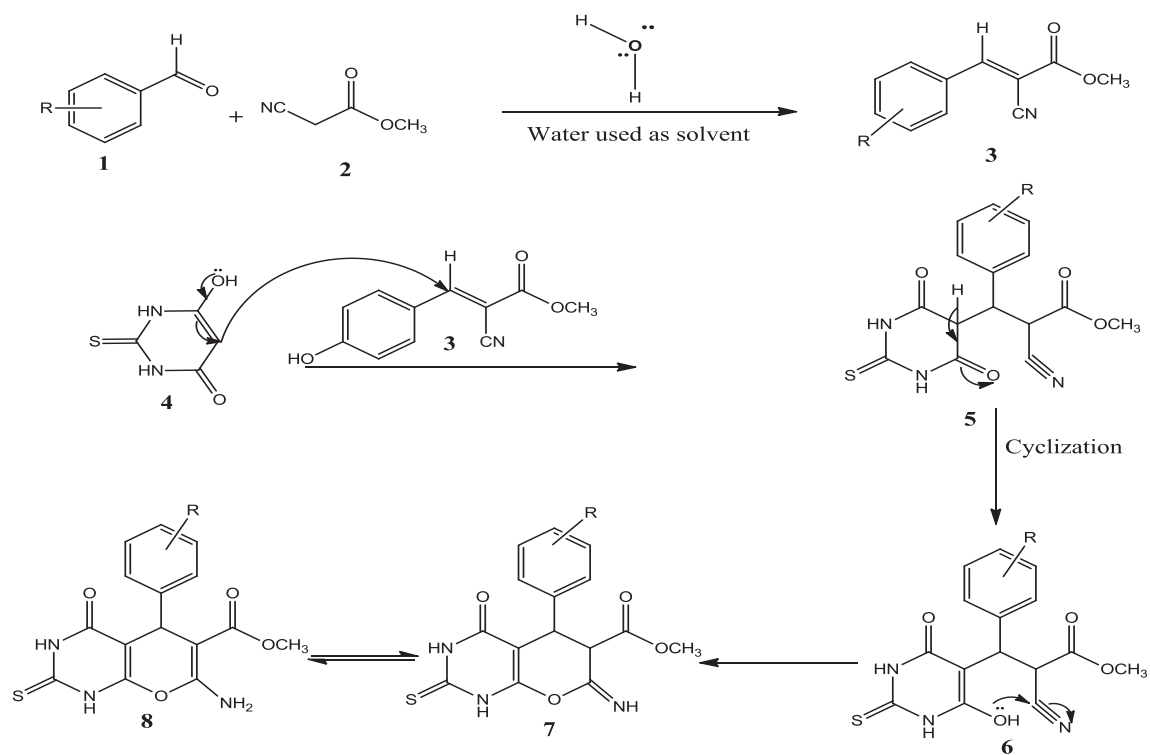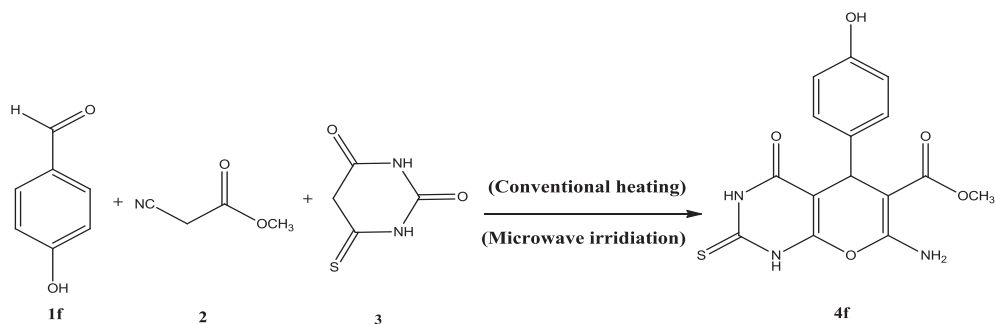
Scheme 2 Microwave and conventional synthesis of **4f** model compound.

Scheme 3 Proposed mechanism for the synthesis of new methyl 7-amino-4-oxo-5-phenyl-2-thioxo-2,3,4,5-tetrahydro-*1H*-pyrano [2,3-*d*]pyrimidine-6-carboxylate derivatives under microwave irradiation.
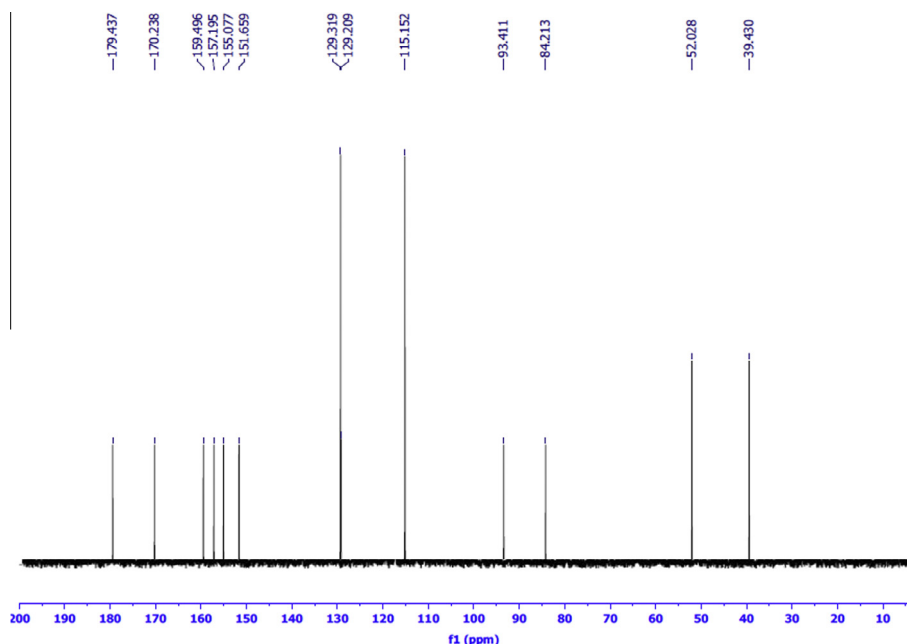

Fig. 1 ^{1}H NMR spectra of synthesized model compound **4f.**

Fig. 2 ^{13}C NMR spectra of synthesized model compound **4f.**

pneumonia, *P. aeruginosa*. The compound **4g** has antibacterial activity against *P. aeruginosa* (gram-negative bacteria). Whereas the compounds **4i** and **4k** have moderate antibacterial activity against *S. aureus*, *B. cereus* (gram-positive bacteria), *E. coli*, *K. pneumonia*, *P. aeruginosa* (gram-negative bacteria). The compounds **4g** and **4j** have least antibacterial activity against *B. cereus*, *E. coli* and *K. pneumonia* strains and showed no antibacterial activity against *S. aureus* (gram-positive). The compound **4h** has no antibacterial activity against *B. cereus* (gram-positive) and *K. pneumonia* (gram-negative).

The compounds **4d** and **4f** have maximum antifungal activity against *A. Niger* and *P. chrysogenum* strains. The compounds **4a, 4e, 4i, 4h** and **4k** have moderate antibacterial activity against *A. Niger* strain and compounds **4c, 4g** and **4j** have no antifungal activity against *A. Niger* strain. The compounds **4b, 4c, 4d, 4e, 4g, 4h, 4j** and **4k** have antifungal activity against *P. chrysogenum* strain. Whereas the compounds **4a** and **4i** have no antifungal activity against *P. chrysogenum* strain. These findings suggest that rather than disrupting cell membranes, the compounds acted outside the cell and became attached to surface groups of the bacterial cells enhanced its activity. The good activity is attributed in the presence of pharmacologically active benzaldehyde, ─OH, CH$_3$, ─OCH$_3$, ─Cl and ─NO$_2$ groups attached to phenyl ring on the pyran ring shows extensive effect on the membrane potential associated with bactericidal activity (Table 4).

Conclusions

Microwave-assisted methodology developed catalyst free, simple and green pathway for the synthesis of methyl 7 amino-4-oxo-5-phenyl-2-thioxo-2, 3, 4, 5-tetrahydro-*1H*-Pyrano [2, 3-d] pyrimidine-6-carboxylate derivatives. The advantages of this ecofriendly and safe procedure provide spectacular accelerations, higher yields under milder reaction conditions, short reaction time and simple work up. The relevant studies

showed that steric, electronic effects and polar parameters of the benzaldehyde substituents on pyrane ring were important for both *in vitro* antimicrobial and antifungal activities.

Conflict of Interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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