RESEARCH PAPER

Facile, Efficient, Microwave Assisted One-Pot Green Synthesis of 3, 4-Dihydropyrimidin-2-(1-H)-One/Thione and Evaluation of its Antimicrobial Activities

SACHIN S. FAWADE* and SURENDRA N. TAKALE
*Department of Chemistry, Sir Sayyed College of Arts, Commerce & Science,
Chhatrapati Sambhajinagar-431001, Maharashtra, India
***Corresponding Author**
Email: sachinsf18@gmail.com
Received:1stOctober,2026; **Revised:**5thOctober,2026; **Accepted:**8thOctober,2026; **Available Online:**20thOctober, 2026

Abstract
A simple, rapid and environmentally favourable microwave-aided one-pot green protocol was developed in order to synthesise a novel cluster of 3,4-dihydropyrimidin-2(1H)-one frameworks and related thione derivatives spanning 1a to 1h through a modified Biginelli reaction. The combination of aromatic aldehydes that have been substituted and ethyl acetoacetate, along with urea or corresponding thiourea analog, with citric acid as a mild Bronsted-acid catalyst. Microwave irradiation at 360 W completed the reactions within three to six minutes and the intended compounds were obtained in isolated yields that vary between 68% and 89%. Products underwent purification through recrystallization from ethanol and characterised using melting-point determination, TLC, ^{1}H NMR, FTIR, LC–MS, ^{13}C NMR and elemental examination. Synthesised substances were examined for their capacity to stop the development of two bacterial species: Gram (+) as well as Gram (-), along with two varieties of fungus by means of the agar-well diffusion techniques. The research demonstrates that citric-acid-mediated microwave synthesis provides a simple and efficient route to biologically promising dihydropyrimidine derivatives.
KEYWORDS: Biginelli reaction; Dihydropyrimidinones; Microwave-assisted synthesis; Citric acid; Antimicrobial activity
How to cite this article: SACHIN S. FAWADE* and SURENDRA N. TAKALE. Facile, Efficient, Microwave Assisted One-Pot Green Synthesis of 3, 4-Dihydropyrimidin-2-(1-H)-One/Thione and Evaluation of its Antimicrobial Activities. Int J Drug Deliv Technol. 2026;16(89s):2244-2246. DOI: 10.25258/ijddt.16.89s.216.

Introduction

Heterocyclic compounds constitute a significant group of organic molecules as a result of their widespread presence in biologically active natural products, pharmaceutical agents and functional materials. Among nitrogen-containing heterocycles, pyrimidine and its partially reduced derivatives have received significant interest in medicinal and synthetic chemistry. Their structural adaptability, favourable hydrogen-bonding characteristics and capacity to accommodate diverse substituents make them useful scaffolds for the advancement of substances with varied biological characteristics. In particular, 3,4-dihydropyrimidin-2(1H)-ones and its respective thione counterparts constitutes pharmacologically important class of compounds with heterocyclic rings that have biological activity can be modified through substitution at different positions of the dihydropyrimidine ring.

One of the most direct approaches for synthesizing 3, 4-dihydropyrimidin-2(1H)-one, Biginelli reaction is utilised. First documented by Pietro Biginelli in 1891, this multicomponent transformation involves the acid-catalysed cyclocondensation involving urea, aldehyde and β-ketoester to manage a functionalised dihydropyrimidinone in a single reaction vessel[i,ii]. Thiourea may be employed instead of urea to yield relevant 3, 4-dihydropyrimidin-2(1H)-thiones. The response is particularly valuable because it combines three readily available starting materials, rapidly generates molecular complexity and permits structural variation through modification of each reaction component.

A mechanistic investigation indicates acid-mediated Biginelli reactions generally advances through the preliminary combining the aldehyde with thiourea or urea for condensation, resulting in the development of an iminium or N-acyliminium-type intermediate. Subsequent nucleophilic addition of the enolised β-ketoester, intramolecular cyclisation and dehydration produce the desired dihydropyrimidine ring[iii]. The efficiency of these individual steps is significantly affected by nature and concentration of the acid catalyst, reaction temperature, solvent, energy source and electronic characteristics of the aldehyde substituents. Consequently, extensive research has been directed towards developing catalysts and reaction conditions that can improve conversion, product yield, selectivity and operational simplicity.

The medicinal importance of the dihydropyrimidine framework has further stimulated interest in the Biginelli reaction. Substituted dihydropyrimidines have been explored as modulators of calcium channels and as agents for hypertension management and adenosine-receptor ligands[iv,v]. Monastrol a well-known

dihydropyrimidinethione derivative, was identified as EG5 mitotic kinesin inhibitor and demonstrated potential of this scaffold in the development of biologically active small molecules[vi]. The dihydropyrimidin-2-one exhibit different properties including antibacterial[vii,viii], anti-inflammatory[viii], antiviral[ix], antitumor[x], antimalarial agents[xi], hypnotic, anticonvulsants, anti-thyroid agents, antihistamines and antibiotics[xii]. These findings show that relatively small changes in the aryl substituent, ester group or carbonyl/thioxo functionality can significantly alter the physicochemical and biological behaviour of dihydropyrimidine derivatives. The presence of nitrogen, oxygen or sulphur donor atoms, together with an aromatic substituent and ester functionality, also provides several potential sites for interaction with biological macromolecules.

Despite its synthetic utility, the classical Biginelli reaction frequently requires prolonged heating, strongly acidic conditions with large quantities of organic solvent along with various hazardous catalysts. Such conditions may lead to moderate yields, difficult product isolation, increased energy consumption and the generation of hazardous chemical waste. Several modified procedures involving Lewis acids, Bronsted acids, ionic liquids, supported catalysts and solvent-free conditions have therefore been investigated. Although many of these methods improve the reaction some depend on expensive catalysts, toxic reagents, moisture-sensitive materials or complicated catalyst-recovery procedures. The development of a simple protocol employing an inexpensive, readily available and comparatively environmentally acceptable catalyst therefore remains desirable.

Microwave-assisted organic synthesis offers an effective means of improving multicomponent reactions. Microwave irradiation provides rapid dielectric heating of polar molecules and ionic species within the reaction mixture, allowing the target temperature for the reaction to be achieved more rapidly than with conventional surface heating. As a result, microwave-assisted reactions can substantially shorten reaction time and in suitable systems, improve product yield, minimise side reactions and reduce energy consumption[xiii,xiv]. These advantages make microwave irradiation especially suitable for one-pot cyclocondensation reactions such as the Biginelli synthesis.

Citric acid is a readily available polycarboxylic acid that can function as a mild Bronsted-acid catalyst. Its acidity can facilitate carbonyl activation, iminium-intermediate formation and subsequent cyclisation during the Biginelli reaction. The application of citric acid is advantageous because it is inexpensive, easy to handle, highly accessible and avoids the requirement for many corrosive mineral acids or metal-based catalysts. Combining citric acid catalysis with microwave irradiation may therefore provide a practical approach for obtaining dihydropyrimidinone and dihydropyrimidinethione derivatives within short reaction periods and under comparatively mild conditions with good yield.

In the present study, sequence of ethyl-6-methyl-4-aryl-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives was synthesised through a modified protocol of one-pot Biginelli reaction. Aromatic aldehydes bearing hydrogen, methoxy, chloro and nitro substituents conducted reaction utilizing thiourea or urea, along with ethyl acetoacetate in conjunction with citric acid subjected to microwave irradiation. The selected substituents represent electron-donating and electron-accepting groups and were included to examine their influence on reaction behaviour and biological response. The synthesised compounds were characterised using appropriate physicochemical and spectroscopic techniques and subsequently evaluated for antimicrobial activity. The study was undertaken to establish a Green, facile, rapid and efficient synthetic method while simultaneously exploring the antimicrobial potential of resulting 3,4-dihydropyrimidin-2(1H)-one and thione analogues.

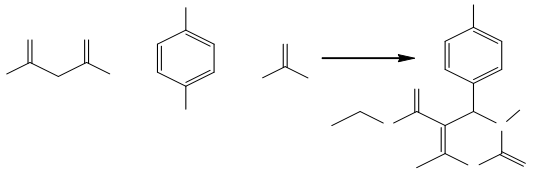
EXPERIMENTAL SECTION:

MATERIAL AND METHODS:

All compounds and reagents utilised in the current investigation were procured from recognised commercial vendors and utilised without additional refinement. The progress of reaction as well as the purity of synthesised products were monitored through TLC employing silica gel plates and a suitable mobile phase while the spots were illuminated through ultraviolet light. Melting points were ascertained utilising a digital melting-point device from Systronics (model no. EQ-730). Infrared spectra were obtained, utilising an FTIR spectrophotometer by Bruker, between 4000-666 cm^{-1} ranges, in order to determine characteristic functional groups. Both the ^{13}C NMR as well as ^{1}H NMR spectra were captured utilising spectrometers by Bruker, utilising tetramethylsilane as internal standard with $CDCl_3$ as solvent with chemical shifts articulated in δ ppm. Mass spectra obtained, utilising the Mass spectrometer from Shimadzu, the GCMS-QP 1000, run at 70 eV, to confirm that, the molecular mass of the synthesised products and a Perkin-Elmer elemental 240C micro-analyser was utilised to elemental analysis of hydrogen, carbon and nitrogen. The experimentally obtained values were compared with the theoretically calculated composition to establish the purity and molecular formula of the final compounds.

MODIFIED PROTOCOL FOR SYNTHESIZING 3, 4-DIHYDROPYRIMIDIN-2-(1-H)-ONE/THIONE (1a-1h):

Aromatic aldehydes (2 mmol), ethyl acetoacetate (1 mmol) and of thiourea or urea (2 mmol) were combined within a microwave vial, along with concentrated solution of citric acid was incorporated into the reactive mixture. This combination was subjected to heat at 360 W within a microwave for three to six minutes, while TLC tracked the reaction's development. The finished product underwent a filtration, followed by rinsing with H_2O and was subsequently recrystallized utilizing ethanol to get the pure substance 1a. Then, it was dried. In similar way, the Compounds 1b-1h was prepared.

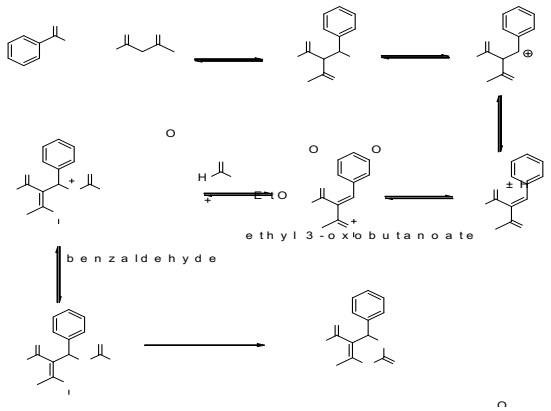


Scheme 1: Synthesis of Pyrimidine Derivatives - 1a-1h

Table 1: Substrate Scope for preparing 3, 4-Dihydropyrimidin-2-(1-H)-one/thione (1a-1h) utilizing Microwave Irradiation

Compound	R	X	Time (mins.)	Yield (%)
1a	H	O	4-5	71
1b	4-OCH_3	O	5-6	85
1c	4-Cl	O	4-5	70
1d	4-NO_2	O	3-4	87
1e	H	S	4-5	89
1f	4-OCH_3	S	4-5	68
1g	4-Cl	S	4-5	89
1h	4-NO_2	S	3-4	78

REACTION MECHANISM:



Scheme 2: Plausible Mechanism for the synthesis of 3, 4-Dihydropyrimidin-2-(1-H)-one/thione (1a-1h)

SPECTRAL CHARACTERIZATIONS:

ethyl-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1a):

mp: 202-204 ºC; FTIR (KBr, cm-1): 3744.28, 3309.30, 3064.03, 2923.48, 2853.54, 2631.30, 2571.77, 2376.30, 2309.57, 1646.00, 1575.32, 1506.85, 1436.16, 1338.71, 1245.22, 1159.62, 1089.76; ^{1}H NMR ($CDCl_3$, 400 MHz,): δ 1.34-1.46 (t, 3H, CH_3), 2.45 (s, 3H, CH_3), 4.19-4.37 (m, 2H, CH_2), 5.91 (s, 1H, CH), 6.97 (s, 1H, NH), 7.20 (s, 1H, NH), 7.32-7.71 (m, 5H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$,) δ 169.14, 155.56, 152.53, 140.75, 128.53 (4C), 100.95, 61.50, 53.34, 18.97, 14.70; LCMS m/z $[M]^+$ 261.2; Anal. Calcd. for $C_{14}H_{16}N_2O_3$ (261.29): C, 64.60%; H, 6.20%; N, 10.76%. Found: C, 64.64%; H, 6.50%; N, 10.53%

ethyl-4-(4-methoxyphenyl)-6-methyl-2-oxo 1,2,3,4-tetrahydropyrimidine-5-carboxylate (1b):

mp: 204-206 ºC; FTIR (KBr, cm-1): 3308.74, 3065.18, 2922.75, 2853.72, 2568.14, 2309.06, 1646.14, 1574.54, 1505.40, 1445.43, 1212.54, 1151.67; ^{1}H NMR ($CDCl_3$, 400 MHz,): δ 1.32-1.44 (t, 3H, CH_3), 2.30 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 4.16-4.37 (m, 2H, CH_2), 6.16 (s, 1H, CH), 6.83 (s, 1H, NH), 6.91-6.99 (d, 2H, Ar-H), 7.26-7.37 (d, 2H, Ar-H), 8.39 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$,) δ 169.14, 159.20, 155.56, 152.53, 131.12, 129.49 (2C), 113.50 (2C), 100.95, 61.50, 56.03, 53.34, 18.97, 14.70; LCMS m/z $[M]^+$ 291.1; Anal. Calcd. for $C_{15}H_{18}N_2O_4$ (290.32): C, 62.05%; H, 6.25%; N, 9.36%. Found: C, 62.09%; H, 6.12%; N, 9.34%

ethyl-4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1c):

mp: 212-214 ºC; FTIR (KBr, cm-1): 3308.52, 3063.92, 2915.35, 2854.25, 2635.86, 2566.87, 2309.88, 1645.69, 1573.98, 1504.54, 1444.16, 1328.43, 1223.84, 1150.27, 981.79; ^{1}H NMR ($CDCl_3$, 400 MHz,): δ 1.37-1.48 (t, 3H, CH_3), 2.34 (s, 3H, CH_3), 4.22-4.40 (m, 2H, CH_2), 5.76 (s, 1H, CH), 6.56 (s, 1H, NH), 7.26-7.33 (d, 2H, Ar-H), 7.37-7.45 (d, 2H, Ar-H), 7.77 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$,) δ 169.14, 155.56, 152.53, 138.87, 133.38, 130.63 (2C), 128.55 (2C), 100.95, 61.50, 53.34, 18.97, 14.70; LCMS m/z $[M]^+$ 295.6. Anal. Calcd. for $C_{14}H_{15}ClN_2O_3$ (294.74): C, 57.05%; H, 5.13%; N, 9.51%. Found: C, 56.94%; H, 5.21%; N, 9.51%

ethyl-6-methyl-4-(4-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate(1d):

mp: 206-208 ºC; FTIR (KBr, cm-1): 3145.42, 3023.20, 1648.49, 1557.29, 1522.05, 1340.96, 1221.86, 1165.95, 969.53, 825.02, 725.33; ^{1}H NMR ($CDCl_3$, 400 MHz,): δ 1.32-1.44 (t, 3H, CH_3), 2.33 (s, 3H, CH_3), 4.15-4.33 (m, 2H, CH_2), 5.99 (s, 1H, CH), 6.66 (s, 1H, NH), 7.50-7.57 (d, 2H, Ar-H), 8.20-8.27 (d, 2H, Ar-H), 8.79 (s, 1H,

NH); ^{13}C NMR (100 MHz, $CDCl_3$,) δ 169.14, 155.56, 152.53, 149.75, 148.95, 128.10 (2C), 123.25 (2C), 100.95, 61.50, 53.34, 18.97, 14.74; LCMS m/z $[M]^+$ 306.2; Anal. Calcd. for $C_{14}H_{15}N_3O_5$ (305.29): C, 55.08%; H, 4.95%; N, 13.77%. Found: C, 55.35%; H, 4.68%; N, 13.79%.

ethyl-6-methyl-4-phenyl-2-thioxo-1,2,3,4 tetrahydropyrimidine-5-carboxylate(1e):

mp: 200-202 ºC; FTIR (KBr, cm-1): 3308.98, 2926.00, 2856.34, 1646.62, 1575.05, 1506.46, 1436.58, 1346.56, 1223.29, 1157.24, 1094.69, 1019.23, 889.64, 836.12, 776.20, 744.59; ^{1}H NMR ($CDCl_3$, 400 MHz,): δ 1.38-1.50 (t, 3H, CH_3), 2.40 (s, 3H, CH_3), 4.21-4.39 (m, 2H, CH_2), 6.01 (s, 1H, CH), 7.16 (s, 1H, NH), 7.33-7.96 (m, 5H, Ar-H), 8.57 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$,) δ 180.66, 169.14, 156.11, 140.75, 128.47 (5C), 104.82, 61.50, 55.68, 18.97, 14.68; LCMS m/z $[M]^+$ 277.6; Anal. Calcd. for $C_{14}H_{16}N_2O_2S$ (276.36): C, 60.84%; H, 5.84%; N, 10.14%. Found: C, 60.79%; H, 5.94%; N, 10.43%.

ethyl-4-(4-methoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate(1f):

mp: 196-198 ºC; FTIR (KBr, cm-1): 3308.56, 2914.54, 2571.03, 2315.69, 1645.67, 1574.83, 1506.57, 1436.37, 1254.46, 1160.36, 888.61, 775.96, 751.84; ^{1}H NMR ($CDCl_3$, 400 MHz,): δ 1.31-1.45 (t, 3H, CH_3), 2.36 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 4.20-4.37 (m, 2H, CH_2), 5.54 (s, 1H, CH), 6.91-6.98 (d, 2H, Ar-H), 7.26-7.35 (d, 2H, Ar-H), 8.64 (s, 1H, NH), 9.93 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.66, 169.14, 159.20, 156.11, 131.12, 129.49 (2C), 113.50 (2C), 104.82, 61.50, 56.02, 55.68, 18.97, 14.71; LCMS m/z $[M]^+$ 307.7; Anal. Calcd. for $C_{15}H_{18}N_2O_3S$ (306.39): C, 58.80%; H, 5.92%; N, 9.15%. Found: C, 58.81%; H, 5.55%; N, 9.32%.

ethyl-4-(4-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1g):

mp: 200-202 ºC; FTIR (KBr, cm-1): 3326.87, 3063.84, 2974.86, 2917.57, 1919.16, 1660.20, 1576.02, 1495.28, 1346.57, 1160.27, 1092.79, 1067.91, 1015.51, 991.35, 917.46, 901.07, 882.33, 731.97; ^{1}H NMR ($CDCl_3$, 400 MHz,): δ 1.36-1.48 (t, 3H, CH_3), 2.26 (s, 3H, CH_3), 4.21-4.39 (m, 2H, CH_2), 5.46 (s, 1H, CH), 7.26-7.38 (d, 2H, Ar-H), 7.44-7.46 (d, 2H, Ar-H), 8.14 (s, 1H, NH), 9.34 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$,) δ 180.66, 169.14, 156.11, 138.87, 133.38, 130.63 (2C), 128.55 (2C), 104.82, 61.50, 55.68, 18.97, 14.73; LCMS m/z $[M]^+$ 311.6; Anal. Calcd. for $C_{14}H_{15}ClN_2O_2S$ (310.81): C, 54.10%; H, 4.86%; N, 9.02%. Found: C, 54.42%; H, 4.78%; N, 9.14%

ethyl-6-methyl-4-(4-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1h):

mp: 208-210 ºC; FTIR (KBr, cm-1): 3312.33, 3033.52, 1686.46, 1639.65, 1574.49, 1461.61, 1367.20, 1303.17, 1224.29, 1147.94, 1114.47, 1064.25, 1024.83, 988.54, 904.28, 749.96, 693.24; ^{1}H NMR ($CDCl_3$, 400 MHz,): δ 1.35-1.45 (t, 3H, CH_3), 2.32 (s, 3H, CH_3), 4.15-4.33 (m, 2H, CH_2), 5.32 (s, 1H, CH), 7.91 (s, 1H, NH), 7.59-7.66 (d, 2H, Ar-H), 8.19-8.27 (d, 2H, Ar-H), 9.69 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$,) δ 180.66, 169.14, 156.11, 149.75, 148.95, 128.10 (2C), 123.25 (2C), 104.82, 61.50, 55.68, 18.97, 14.72. LCMS m/z $[M]^+$ 322.9; Anal. Calcd. for $C_{14}H_{15}N_3O_4S$ (321.36): C, 52.32%; H, 4.71%; N, 13.08%. Found: C, 52.49%; H, 4.51%; N, 13.33%.

ANTIMICROBIAL ACTIVITY:

The antimicrobial activity of the synthesized compounds 1a–1h estimation was led utilising agar-well diffusion technique, targeting 2 Gram+ bacterial species: *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus*, (ATCC 25923); 2 fungal strains, *Aspergillus brasiliensis* (ATCC 16404) formerly identified as *Aspergillus niger* and *Candida albicans* (ATCC 10231); along with 2 Gram- bacterial strains, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). The synthesized substances were solubilised in dimethyl sulfoxide to prepare solutions of 1 mg/mL. The bacterial cultures were grown in nutrient broth for 18–24 hrs, and their turbidity was adjusted to approximately 0.5 McFarland standards. The fungal inoculum of *C. albicans* was adjusted to 0.5 McFarland standards, whereas the spore suspension of *A. brasiliensis* was adjusted to approximately 10^6 spores/mL. Mueller–Hinton agar was employed for antibacterial screening, while Sabouraud dextrose agar was utilized for antifungal screening. The respective microbial suspensions were evenly distributed across the surface of sterile agar plates featuring wells with a diameter of about 6 mm were developed by employing a cork borer. A 100 µL volume of every test solution, equivalent to 100 µg per well, was incorporated into each well. *Ciprofloxacin* is the antimicrobial drug of choice (standard anti-bacterial drug), *amphotericin B* was employed as the benchmark anti-fungal medication (standard anti-fungal drug) and as the negative control DMSO employed. From 18 to 24 hours, bacterial plates were taken in an incubator set at 37±2°C, while the plates containing *C. albicans* and *A. brasiliensis* were preserved for a period of one to two days at a temperature of 28 ± 2 °C respectively. After incubation, by calculating the diameter in millimetres of the clear inhibition zone surrounding each well, antimicrobial activity was ascertained. Every experiment was repeated thrice and results were presented in mean inhibition zone ± SD. Agar-diffusion screening is based on the diffusion of the tested substance through the agar and the formation of a clear zone where microbial growth is inhibited. The synthesised dihydropyrimidinone and dihydropyrimidinethione

compounds tested in the study were designated as 1a–1h.

RESULTS AND DISCUSSION:

The focus is on the derivatives of 3,4-dihydropyrimidin-2(1H)-one and thione 1a–1h were synthesised by citric-acid-catalysed, microwave-assisted, green one-pot Biginelli reaction using aromatic aldehydes that have been substituted, ethyl acetoacetate, either thiourea or urea. Urea furnished oxo derivatives 1a–1d, whereas thiourea produced the corresponding thioxo derivatives 1e–1h. Citric acid facilitated carbonyl activation, condensation, cyclisation and dehydration to generate the dihydropyrimidine ring. Microwave irradiation at 360 W considerably shortened the reaction period with the reported reaction times ranging from 3 min. to 6 min. and isolated yields ranging from 68% to 89%. Compounds 1e and 1g gave the highest yields of 89%. The products were isolated by filtered, water rinsed, then subsequently refined using recrystallization of ethanol demonstrating the operational simplicity of the method. The synthesised compound's structures were supported by ^{1}H NMR, FTIR, LC–MS, ^{13}C NMR plus elemental testing. FTIR spectra showed characteristic N–H, ester C=O and C=S related absorptions. According to ^{1}H NMR data, the ethyl ester methyl and methylene protons appeared around δ 1.31–1.50 and 4.15–4.40 ppm respectively, while the diagnostic C-4 methine proton appeared at δ 5.32–6.16 ppm. The ^{13}C NMR spectra displayed the ester carbonyl near δ 169.14 ppm, whereas the thioxo derivatives showed a characteristic C=S signal near δ 180.66 ppm. Substituent-specific signals for OCH_3, Cl and NO_2 groups, together with the molecular-ion peaks as well as data from elemental analyses, were utilised to support suggested structures of compounds 1a–1h.

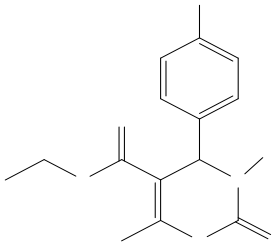
Table 2: An Assessment of the Catalytic Performance of Diverse Catalysts in 1a-1h Products

Citric acid as a mild Bronsted-acid catalyst, demonstrated to be a highly efficient Green catalyst that does not involve the use of metals, outperforming other documented catalytic systems in the synthesis of 1a-1h products (Table-2).

ANTIMICROBIAL ACTIVITY:

The antimicrobial findings suggested that synthesized compound's biological effect was influenced by both that which is substituted on aromatic rings and what is substituted for carbonyl oxygen by sulphur. Among the oxo derivatives, compound **1c** bearing a para-chloro substituent, exhibited the highest influence on various types of the Gram-+ve bacteria as well as fungi strain, whereas compound **1d** containing a para-nitro group showed comparatively greater activity against the Gram-negative bacterial strains. The unsubstituted compound **1a** exhibited the lowest antimicrobial activity while the para-methoxy-substituted compound **1b** demonstrated moderate activity. The thioxo derivatives **1e–1h** exhibited greater antimicrobial activity than their corresponding oxo analogues **1a–1d**. This improvement may hypothetically be attributed to the greater size, polarizability and lipophilic character of sulphur, which could enhance interaction with microbial cell membranes and biological targets. Compound **1g** containing both a thioxo group and a para-chloro substituent, displayed the highest resistance towards *S. aureus*, *B. subtilis*, *C. albicans* and *A. brasiliensis*. Compound **1h**, bearing a para-nitro group, demonstrated the greatest efficacy against *P. aeruginosa* as well as *E. coli*. The assumed overall activity order was: 1g > 1h > 1f > 1e for the thioxo derivatives and 1c ≈ 1d > 1b > 1a for the oxo derivatives

Although compounds **1g** and **1h** demonstrated comparatively promising activity, their inhibition zones remained lower than those of the antibacterial drug *ciprofloxacin* and the antifungal drug *amphotericin B* are both considered standard treatments.



Sr. No.	**Catalyst**	**Conditions**		**Time (hrs.)**	**Yield (%)**	**Reference**
1	$Sr(OTf)_2$	No Solvent	70^0 C	4	97	xv
2	$[Al(H_2O)_6](BF_4)_3$	CH_3CN	reflux	20	81	xvi
3	$Zr(H_2PO_4)_2$	No Solvent	90^0 C	1	88	xvii
4	LiBr	CH_3CN	reflux	3	92	xviii
5	$SbCl_3$	CH_3CN	reflux	18	90	xix
6	CaF_2	C_2H_5OH	reflux	2	98	xx
7	Chloroacetic acid	No Solvent	90^0 C	3	92	xxi
8	NH_4Cl	No Solvent	100^0 C	3	90	xxii
9	Triphenylphosphine	No Solvent	100^0 C	10	70	xxiii
10	$TiCl_4$-$MgCl_2$	No Solvent	100^0 C	3	90	xxiv
11	$Co(NO_3)_2.6H_2O$	No Solvent	80^0 C	0.6	90	xxv
12	$Al(H_2PO_4)_3$	Microwave	300 W	10-20 mins	85	xxvi
13	HCOOH	Microwave	120 W	3-8 mins	87	xxvii
14	**Citric Acid**	**Microwave**	**360 W**	**3-6 mins**	**89**	**Present Work**

Table 3: Antimicrobial Activity of Compounds 1a–1h

Compounds	R	X	*B. subtilis* Gram +ve	*S. aureus* Gram +ve	*P. aeruginosa* Gram −ve	*E. coli* Gram −ve	*C. albicans*	*A. brasiliensis*
1a	H	O	13.7 ± 0.19	14.3 ± 0.26	11.7 ± 0.23	12.3 ± 0.22	12.0 ± 0.23	11.3 ± 0.16
1b	4-OCH_3	O	16.0 ± 0.16	16.7 ± 0.34	13.7 ± 0.18	14.7 ± 0.16	15.3 ± 0.26	14.7 ± 0.13
1c	4-Cl	O	20.7 ± 0.24	21.3 ± 0.43	18.3 ± 0.26	19.7 ± 0.14	19.3 ± 0.24	18.7 ± 0.14
1d	4-NO_2	O	19.3 ± 0.22	20.0 ± 0.32	19.0 ± 0.25	20.3 ± 0.17	18.7 ± 0.11	18.0 ± 0.23
1e	H	S	15.3 ± 0.31	16.0 ± 0.21	13.0 ± 0.19	14.3 ± 0.22	14.7 ± 0.24	14.0 ± 0.17
1f	4-OCH_3	S	18.0 ± 0.12	18.7 ± 0.34	15.7 ± 0.24	16.7 ± 0.19	17.3 ± 0.14	16.7 ± 0.19
1g	4-Cl	S	23.7 ± 0.18	24.3 ± 0.22	21.3 ± 0.29	22.7 ± 0.18	22.3 ± 0.14	21.7 ± 0.24
1h	4-NO_2	S	22.3 ± 0.14	23.0 ± 0.18	22.0 ± 0.22	23.3 ± 0.18	21.7 ± 0.17	21.0 ± 0.25
Ciprofloxacin	-	-	31.7 ± 0.39	32.7 ± 0.46	30.3 ± 0.46	33.3 ± 0.51	-	-
Amphotericin B	-	-	-	-	-	-	28.7 ± 0.27	27.7 ± 0.39

Values are articulated as the mean zone of inhibition ± SD in millimetres, n = 3.

CONCLUSION:

A swift and effective approach utilising microwave assistance one-pot green method used to make 3,4-dihydropyrimidin-2(1H)-one and thione analogue 1a–1h via the citric-acid catalysed Biginelli reaction. The reactions were completed at 360 W within 3–6 minutes and the intended products in isolated yields ranging from 68% to 89%. Chemically, the synthesized products 1a-1h was supported through melting-point determination, TLC, ^{1}H NMR, FTIR, LC–MS, ^{13}C NMR and elemental analysis. This new green protocol offers benefits include rapid reaction time, straightforward work-up procedures, cost-effective catalysts, reduced solvent use and satisfactory product yields.

The reported antimicrobial screening indicated that the thioxo derivatives generally exhibited greater activity than their corresponding oxo analogues. The para-chloro thioxo derivative 1g demonstrated the most significant efficacy in combating the Gram+ve bacterial and fungal strains, whereas the para-nitro thioxo derivative 1h was comparatively more efficient in opposition to the Gram-negative bacteria. These findings suggest that both replacement of oxygen by sulphur and the presence of electron-withdrawing aromatic substituents may favour antimicrobial activity. Compounds 1g and 1h may therefore serve as useful lead structures for further optimisation and evaluation through the terms MBC, MIC, MFC and mechanistic studies.

ACKNOWLEDGEMENT:

The authors are gratefully acknowledged to Dr. Shamama Parveen, President of RECWS Chhatrapati Sambhajinagar and Dr. Shaikh Kabeer Ahmed, Principal Sir Sayyed College, Chhatrapati Sambhajinagar, for providing research facilities.

CONFLICT OF INTERESTS:

The authors declare that there is no conflict of interest.

REFERENCES:

i. Biginelli, P., Ueber Aldehyduramide des Acetessigäthers. Ber. Dtsch. Chem. Ges., 1891, 24, 1317-13191. https://doi.org/10.1002/cber.189102401228

ii. Biginelli, P., Aldehyde-Urea Derivatives of Aceto- and Oxaloacetic Acids, Gazz. Chim. Ital., 1893, 23, 360-413.

iii. Kappe C.O., A Reexamination of the mechanism of the Biginelli dihydropyrimidine synthesis: Support for an N-acyliminium ion intermediate (1), Journal of Organic Chemistry, 1997, 62, 7201–7204. https://doi:10.1021/jo971010u

iv. Rovnyak G.C., Atwal K.S., Hedberg A., et al, Dihydropyrimidine calcium-channel blockers: Basic 3-substituted-4-aryl-1,4-dihydropyrimidine-5-carboxylic acid esters as potent antihypertensive agents, Journal of Medicinal Chemistry, 1992, 35, 3254–3263. https://doi:10.1021/jm00095a023

v.Crespo A., Maatougui A.E., Biagini P., et al, Discovery of 3,4-dihydropyrimidin-2(1H)-ones as a novel class of potent and selective A_2B adenosine-receptor antagonists, ACS Medicinal Chemistry Letters, 2013, 4, 1031–1036. https://doi:10.1021/ml400185v

vi. Mayer T.U., Kapoor T.M., Haggarty S.J., King R.W., Schreiber S.L., Mitchison T.J., Small-molecule inhibitor of mitotic-spindle bipolarity identified in a phenotype-based screen, Science, 1999, 286, 971–974. https://doi:10.1126/science.286.5441.971

vii. Pansuriya A. M., Savant M. M., Bhuva C. V., Singh J. and Naliapara Y. T., One-pot synthesis of 5-carboxanilide-dihydropyrimidinones using etidronic Acid, ARKAT USA journal, 2009, 7, 79-85. https://doi.org/10.3998/ark.5550190.0010.707

viii. Hatamjafari F. and Nezhad F. G., An Efficient One-Pot Synthesis of Dihydropyrimidinones under Solvent-Free Conditions, Oriental journal of chemistry, 2014, 30, 355-357. https://dx.doi.org/10.13005/ojc/300148

ix. Hua K. M. and Le T. N., New Method for the Synthesis of 1-Methylimidazolium Trifluoroacetate and Its Application in Biginelli Reaction, Green and Sustainable Chemistry, 2013, 3, 14-17. http://dx.doi.org/10.4236/gsc.2013.34A003

x. Nevagi R. J. and Narkhede H. I., Novel dihydropyrimidine derivatives as antibacterial agents, Der Pharma Chemica, 2014, 6, 135-139.

xi. Hojati S. F.,Gholizadeh M., Haghdoust M. and Shafiezadeh F., 1,3-dichloro-5,5-dimethylhydantoin as a novel and efficient homogeneous catalyst in Biginelli reaction, Bulletin of the korean chemical society, 2010, 31, 3238-3239. http://dx.doi.org/10.5012/bkcs.2010.31.11.3238

xii. Aslam M. and Verma S., Biological activity of newly synthesized substituted dihydropyrimidinone and thione, International journal of Chemtech research, 2012, 4, 109-111.

xiii. Gedye R., Smith F., Westaway K., et al., The use of microwave ovens for rapid organic synthesis. Tetrahedron Letters, 1986, 27, 279–282. https://doi.org/10.1016/S0040-4039(00)83996-9

xiv. Sharma S., Gangal S. and Rauf A., Green chemistry approach to the sustainable advancement to the synthesis of heterocyclic Chemistry, RASAYAN Journal of Chemistry, 2008, 1, 693-717.

xv. Su W. K., Li J. J., Zheng Z. G., Shen Y. C., One-pot synthesis of dihydropyrimidiones catalyzed by strontium (II) triflate, Tetrahedron Lett., 2005, 46, 6037-6040. https://doi.org/10.1016/j.tetlet.2005.07.021

xvi. Litvic M., Vecenaj I., Ladisic Z. M., Lovric M., Vinkovic V., Filipan- Litvic M., First Application of Hexaaquaaluminium(III) Tetrafluoroborate as a Mild, Recyclable, Non-hygroscopic Acid Catalyst in Organic Synthesis: A Simple and Efficient Protocol for the Multigram Scale Synthesis of 3,4-Dihydropyrimidinones by Biginelli Reaction, Tetrahedron, 2010, 66, 3463-3471. https://doi.org/10.1016/j.tet.2010.03.024

xvii. Besoluk S., Kukukislamoglu M., Zengin M., Arsalan M., Nebioglu M., Turk. J. Chem., An efficient one-pot synthesis of dihydropyrimidinones catalyzed by zirconium hydrogen phosphate under solvent-free conditions, 2010, 34, 411-416. https://doi.org/10.3906/kim-0912-357

xviii. Maiti G., Kundu P., Guin C., One Pot Synthesis of Dihydropyrimidinones Catalysed by Lithium Bromide: An Improved Procedure for the Biginelli Reaction, Tetrahedron Lett., 2003, 44, 2757-2758. https://doi.org/10.1016/S0040-4039(02)02859-9

xix. Cepanec I., Litvic M., Filipan-Litvic M., Grungold I., Antimony(III) chloride-catalysed Biginelli reaction: a versatile method for the synthesis of dihydropyrimidinones through a different reaction mechanism, Tetrahedron, 2007, 63, 11822-11827. https://doi.org/10.1016/j.tet.2007.09.045

xx. Chitra S., Pandiarajan K., Calcium fluoride: an efficient and reusable catalyst for the synthesis of 3,4-dihydropyrimidin-2(1H)-ones and their corresponding 2(1H)-thiones, Tetrahedron Lett., 2009, 50, 2222-2224. https://doi.org/10.1016/j.tetlet.2009.02.164

xxi. Yang Yu, Liu D., Liu C., Luo G., One-pot synthesis of 3,4-dihydropyrimidin-2(1H)-ones using chloroacetic acid as catalyst, Bioorg. Med. Chem. Lett., 2007, 17, 3508-3510. https://doi.org/10.1016/j.bmcl.2006.12.068

xxii. Shaabani A., Bazgir A., Teimouri F., Ammonium chloride-catalyzed one-pot synthesis of 3,4-dihydropyrimidin-2-(1H)-ones under solvent-free conditions, Tetrahedron Lett. 2003, 44, 857-859. https://doi.org/10.1016/S0040-4039(02)02612-6

xxiii. Debache A., Amimour M., Belfaitah A., Rhouati S., Carboni B., A one-pot synthesis of 3,4-dihydropyrimidin-2-(1H)-ones/thiones catalysed by triphenylphosphine as Lewis base, Tetrahedron Lett. 2008, 49, 6119-6121. https://doi.org/10.1016/j.tetlet.2008.08.013

xxiv. Kumar A., Maurya R. A., Synthesis of 3,4-dihydropyrimidin-2(1H)-ones using Ziegler–Natta catalyst system under solvent free conditions, J. Mol. Catalysis A: Chem., 2007, 272, 53-56. https://doi.org/10.1016/j.molcata.2007.03.026

xxv. Nasr-esfahani M., Montazerozohori M., Aghel-mirrezaee M., Kasha H., Efficient and green catalytic synthesis of dihydropyrimidinone (thione) derivatives using cobalt nitrate in solvent-free conditions, J. Chil. Chem. Soc., 2014, 59, 2311-2314. http://dx.doi.org/10.4067/S071797072014000100015

xxvi. Shaterian, H. R., Hosseinian, A.; Ghashang, M., Environmentally friendly preparation of 3,4-dihydropyrimidin-2(1H)-thiones catalyzed by $Al(H_2PO_4)_3$, Phosphorus, Sulfur, Silicon and the Related Elements, 2009, 184, 126-134. https://doi.org/10.1080/10426500802080576

xxvii. Jiang C.; You Q. D., An efficient and solvent-free one-pot synthesis of dihydropyrimidinones under microwave irradiation, Chinese Chem. Lett., 2007, 18, 647-650. https://doi.org/10.1016/j.cclet.2007.04.002