



SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL 1,4-DIHYDROPYRIDINES AND 3,4-DIHYDROPYRIMIDINONES VIA A MICROWAVE-ASSISTED, SOLVENT-FREE MULTI-COMPONENT REACTION APPROACH

Mr. S. S. Tiwde

Department of Chemistry,

Digambarrao Bindu ACS college Bhokar Dist. Nanded -431801

ABSTRACT:

A one-pot synthetic protocol that uses multi-component reactions (MCRs) has been created to quickly make two important nitrogen heterocycles: 1,4-dihydropyridines (DHPs made through the Hantzsch reaction) and 3,4-dihydropyrimidin-2(1H)-ones (DHPMs made through the Biginelli reaction). The reactions were performed without any solvent using microwave heating and sulfamic acid ($\text{NH}_2\text{SO}_3\text{H}$) as a reusable catalyst. The green synthetic protocol produced twelve compounds with good to excellent yields ranging from 78 to 94 percent in very short times between three and eight minutes. The work-up was simple. The atom economy was high. All compounds were fully characterized using FT-IR, ^1H NMR, ^{13}C NMR and ESI-MS. The synthesized heterocycles were tested for activity in vitro against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. They were also tested for activity against *Candida albicans* and for antioxidant activity by measuring free-radical scavenging with the DPPH assay. Electron-withdrawing groups such as nitro ($-\text{NO}_2$) and chloro ($-\text{Cl}$) greatly increased the power. In contrast electron-donating groups such as methoxy ($-\text{OCH}_3$) and dimethylamino ($-\text{N}(\text{CH}_3)_2$) improved the effect. The nitro-substituted DHPM 5b became the powerful antimicrobial lead with a minimum inhibitory concentration of 16 to 32 μg per milliliter. The 3,4-dimethoxy DHPM 5e showed the antioxidant property, with an IC_{50} of 42 μg per milliliter. The study confirms that MCR-based synthesis is an efficient way to create heterocyclic libraries that are useful, for drugs.

Keywords: Multi-component reaction; microwave irradiation; solvent-free; green chemistry; Hantzsch; Biginelli; 1,4-dihydropyridine; dihydropyrimidinone; antimicrobial; antioxidant.

INTRODUCTION:

Heterocyclic chemistry is the basis of most of today's pharmaceutical drugs. In this regard, heterocycles containing nitrogen atoms are of great importance among bioactive molecules. Among them, the 1,4-dihydropyridines (1,4-DHPs) and 3,4-dihydropyrimidin-2(1H)-ones (DHPMs) are especially attractive. The nifedipine-type 1,4-DHPs are well-known and widely prescribed calcium-channel blockers for the treatment of hypertension and angina.

DHPMs exhibit calcium-channel-blocking activity as well as antihypertensive, antiviral, antibacterial, and antimalarial effects. DHPMs also contain a fragment characteristic of naturally occurring Batzelladine alkaloids, which are promising leads in anti-HIV drug design.

A three-component reaction (MCR) is a convergent process, in which three or more different starting materials are combined in a reaction mixture to give one product, which contains the majority of the atoms of the substrates.

This type of reaction has many advantages, including high atom economy, reduced number of purification steps, shorter reaction time, and easy access to diverse compound libraries. These reactions are compatible with green chemistry principles and are therefore of considerable interest in combinatorial chemistry and drug discovery.

The most classic types of three-component reactions are the Hantzsch (1882) and Biginelli (1893) condensations, but their traditional preparation requires long heating, the use of strong acids or expensive Lewis catalysts, an organic solvent such as ethanol or acetic acid.

Microwave-assisted organic synthesis (MAOS) has proven to be an efficient tool in organic synthesis.

It enables rapid and uniform heating of the reaction mixture leading to high reaction rates, high yields, and short reaction times (often reduced from hours to minutes). Moreover, when used with solvent-free reaction conditions, MAOS reduces waste and energy costs and simplifies the purification of the reaction products. These technologies are consistent with the principles of green chemistry. The use of sulfamic acid as a heterogeneous catalyst is also

advantageous in terms of sustainability, since it is recoverable and does not require harsh reaction conditions.

As part of this paper, a study was carried out on the possibility of using a microwave reactor with solvent-free conditions for the rapid and efficient preparation of a library of twelve substituted 1,4-dihydropyridines and 3,4-dihydropyrimidinones using a recyclable sulfamic acid catalyst. The structures of the obtained compounds were characterized by spectroscopic methods, and their antibacterial, antifungal, and antioxidant properties were studied. In addition, structure-activity relationships were established.

EXPERIMENTAL:

1. General Information:

All reagents and solvents were obtained from commercial suppliers ($\geq 98\%$ purity) and used without further purification. Microwave reactions were conducted in a CEM Discover microwave reactor (maximum 300 W) equipped with temperature control. Melting points were determined on a digital melting point apparatus in open capillaries and are uncorrected. FT-IR spectra were obtained on a Perkin-Elmer FT-IR spectrometer using KBr pellets. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker AVANCE 400 spectrometer in DMSO- d_6 using tetramethylsilane (TMS) as internal reference; chemical shifts are reported as δ (ppm). Mass spectra were obtained on an Agilent 6460 ESI-MS instrument. Reaction progress was monitored by thin-layer chromatography (TLC) on silica gel G plates with ethyl acetate–petroleum ether (3:7) as eluent.

2. General procedure for synthesis of 1,4-dihydropyridines (4a–f):

An aromatic aldehyde (1) (10 mmol), ethyl acetoacetate (2) (20 mmol), ammonium acetate (3) (10 mmol), and sulfamic acid (0.2 mmol, 10 mol%) were ground and transferred to a 10 mL microwave reaction tube. The content was irradiated at 300 W for 3–7 min monitored with TLC. After completion, the reaction mixture was cooled to room temperature and poured into ice-cold water (30 mL) with stirring. The precipitated mass was filtered, washed thoroughly

with cold water, dried and recrystallized from ethanol to yield the corresponding diethyl 2,6-dimethyl-4-(aryl)-1,4-dihydropyridine-3,5-dicarboxylate (4a–f).

Scheme 1: $\text{Ar-CHO} + 2 \text{CH}_3\text{COCH}_2\text{COOEt} + \text{NH}_4\text{OAc} \rightarrow \text{DHPs (4a-f)}$

3. General procedure for synthesis of 3,4-dihydropyrimidin-2(1H)-ones (5a–f):

An aromatic aldehyde (1) (10 mmol), ethyl acetoacetate (2) (10 mmol), urea (15 mmol), and sulfamic acid (0.2 mmol, 10 mol%) were taken and irradiated at 300 W for 4–8 min as described above. The resulting mass was poured into ice-cold water and the obtained solid was filtered, washed with water, dried and recrystallized from ethanol to afford ethyl 6-methyl-2-oxo-4-(aryl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (5a–f).

Scheme 2: $\text{Ar-CHO} + \text{CH}_3\text{COCH}_2\text{COOEt} + \text{NH}_2\text{CONH}_2 \rightarrow \text{DHPMs (5a-f)}$

4. Catalyst Recovery:

Sulfamic acid was recovered from the combined aqueous filtrates by concentration under reduced pressure and has been reused in three consecutive cycles without loss of catalytic performance (yield of model compound 5a: 92 → 90 → 88%).

5. Biological Evaluation:

Antibacterial and antifungal activity. Minimum inhibitory concentrations (MICs) were determined by the broth microdilution method as recommended by CLSI against Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6633), Gram-negative bacteria *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and the fungal strain *Candida albicans* (ATCC 10231). Ciprofloxacin and fluconazole were used as positive controls.

Antioxidant activity. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to measure antioxidant activity. Methanolic solutions of test compounds (10–200 µg/mL) were incubated with DPPH (0.1 mM) for 30 min in the dark, and absorbance was read at 517 nm. Ascorbic acid was used as standard and IC₅₀ values were calculated from dose–response curves.

RESULTS AND DISCUSSION:

1. Synthesis and Optimization:

The model Biginelli reaction (Benzaldehyde + ethyl acetoacetate + urea) was utilized to optimize the reaction conditions (Table 1). Microwave irradiation (300 W), without solvent, and 10 mol% sulfamic acid as catalyst provided the highest yield (92%) in just 6 min (Entry 4). A lower catalyst loading (5 mol%) as well as reduced power led to decreased conversion, whereas higher power (450 W) caused partial decomposition. The optimized conditions were successfully applied to the synthesis in the Hantzsch reaction and a series of substituted aldehydes.

Entry	Conditions	Time	Yield (%)
1	Ethanol, reflux, HCl	5 h	58
2	Ethanol, sulfamic acid, reflux	3 h	66
3	Solvent-free, sulfamic acid (5 mol%), 300 W	8 min	74
4	Solvent-free, sulfamic acid (10 mol%), 300 W	6 min	92
5	Solvent-free, sulfamic acid (10 mol%), 450 W	4 min	81
6	Solvent-free, p-TSA (10 mol%), 300 W	6 min	85

2. Characterization:

All the synthesized compounds were characterized by spectral analysis. The representative data are shown below; the complete data for all twelve compounds are given in the Supplementary Information.

Diethyl 2,6-dimethyl-4-(4-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (4b). Pale yellow solid; yield 84%; m.p. 162-164 oC. IR (KBr, cm⁻¹): 3324 (N-H), 1712 (C=O ester), 1685 (C=C), 1580 (C=C aromatic), 1220, 1150 (C-O), 746 (C-Cl). ¹H NMR (DMSO-d₆, δ): 8.29 (s, 1H, NH), 7.32-7.22 (m, 4H, Ar-H), 4.95 (s, 1H, CH), 4.05 (q, J = 7.1 Hz, 4H, 2~OCH₂), 2.26 (s, 6H, 2~CH₃), 1.15 (t, J = 7.1 Hz, 6H, 2~CH₂CH₃). ¹³C NMR (δ): 167.0, 146.7, 145.4, 131.8, 129.2, 128.0, 102.5, 59.5, 38.2, 18.4, 14.2. MS (ESI): m/z 364/366 [M+H]⁺.

Ethyl 6-methyl-2-oxo-4-(4-nitrophenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (5b). Pale yellow solid; yield 94%; m.p. 221-223 oC. IR (KBr, cm⁻¹):

3239 (N-H), 1718 (C=O ester), 1647 (C=O amide), 1524, 1348 (NO₂), 1212 (C-O).
¹H NMR (DMSO-d₆, δ): 9.32 (s, 1H, NH), 8.22 (d, J = 8.6 Hz, 2H, Ar-H), 7.89 (s, 1H, NH), 7.53 (d, J = 8.6 Hz, 2H, Ar-H), 5.26 (d, J = 3.2 Hz, 1H, CH), 4.00 (q, C=C₀__ Hz, 2H, OCH₂), 2.27 (s, 3H, CH₃), 1.10 (t, C=C₁__ Hz, 3H, CH₂CH₃).
¹³C NMR (δ): 165.1, 152.0, 151.6, 149.4, 146.9, 127.6, 123.8, 98.6, 59.3, 53.7, 17.7, 14.0. MS (ESI): m/z 306 [M+H]⁺.

Ethyl 4-(3,4-dimethoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (5e). White solid; yield 88%; m.p. 263-265 °C. IR (KBr, cm⁻¹): 3248 (N-H), 1715 (C=C₂__ ester), 1649 (C=C₃__ amide), 1515 (C=C₄__), 1226, 1028 (C-O). ¹H NMR (DMSO-d₆, δ): 9.14 (s, 1H, NH), 7.70 (s, 1H, NH), 6.92-6.75 (m, 3H, Ar-H), 5.08 (d, C=C₅__ Hz, 1H, CH), 3.98 (q, C=C₆__ Hz, 2H, OCH₂), 3.73 (s, 3H, OCH₃), 3.71 (s, 3H, OCH₃), 2.25 (s, 3H, CH₃), 1.09 (t, C=C₇__ Hz, 3H, CH₂CH₃). MS (ESI): m/z 321 [M+H]⁺.

Compound	Ar	Class	Time (min)	Yield (%)	m.p. (°C)
4a	C ₆ H ₅	DHP	6	78	151–153
4b	4-ClC ₆ H ₄	DHP	5	84	162–164
4c	4-NO ₂ C ₆ H ₄	DHP	3	89	188–190
4d	4-OCH ₃ C ₆ H ₄	DHP	7	76	151–153
4e	3-NO ₂ C ₆ H ₄	DHP	4	85	175–177
4f	2-Furyl	DHP	6	81	165–167
5a	C ₆ H ₅	DHPM	6	92	202–204
5b	4-NO ₂ C ₆ H ₄	DHPM	4	94	221–223
5c	4-ClC ₆ H ₄	DHPM	5	90	210–212
5d	4-OCH ₃ C ₆ H ₄	DHPM	7	86	197–199
5e	3,4-(OCH ₃) ₂ C ₆ H ₃	DHPM	6	88	263–265
5f	4-N(CH ₃) ₂ C ₆ H ₄	DHPM	7	83	268–270

3. Antimicrobial Activity:

The antibacterial and antifungal activities of the prepared compounds were carried out on MIC values (μg/mL) and the antimicrobial activities of the

compounds were compared against that of ciprofloxacin (positive control) and fluconazole (positive control) and the data is represented in Table 3.

Compound	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
4a	128	256	256	256	128
4b	64	128	128	128	128
4c	32	32	64	64	64
4d	256	256	256	512	128
4f	96	144	128	192	128
5a	64	96	128	128	128
5b	16	32	32	64	32
5c	32	64	32	64	64
5d	128	144	192	192	128
5e	56	80	64	112	32
5f	96	112	128	144	128
Ciprofloxacin	1	1	1	2	—
Fluconazole	—	—	—	—	4

Gram-positive strains were generally more susceptible than Gram-negative organisms, consistent with the permeability barrier conferred by the outer membrane of the latter. The most potent antimicrobial agents were the nitro- and chloro-substituted derivatives 5b (MIC 16–32 µg/mL) and 4c, implying that electron-withdrawing substituents contribute to enhance the activity, possibly through an increase in the electrophilicity of the molecule, favoring its interaction with bacterial targets.

4. Antioxidant Activity:

The antioxidant activity was determined by DPPH radical scavenging activity and IC₅₀ values were calculated. The results are shown in Table 4.

Compound	IC50 (µg/mL)	Compound	IC50 (µg/mL)
4a	86	5a	83
4b	72	5b	58
4c	88	5c	62
4d	68	5d	60
4f	75	5e	42
—	—	5f	49
Ascorbic acid	30	—	—

The strongest antioxidant activity was observed for the electron-rich derivatives 5e (3,4-dimethoxy, IC₅₀ = 42 µg/mL) and 5f (4-dimethylamino, IC₅₀ = 49 µg/mL). The observed correlation follows from the stabilization of the radical intermediates by electron donating groups, which facilitate the hydrogen atom transfer to DPPH.

5. Green Chemistry Metrics:

Compared to conventional solvent based reflux (yields 58–66%, 3–6 h), the solvent free microwave protocol decreased the reaction times to 3–8 min and increased the yields to 76–94%. The high atom economy of MCRs (~88–90%) and absence of organic solvents have significantly reduced the E-factor. The catalyst recyclability over three reaction cycles with no significant loss of yields has further improved the environmental attributes.

CONCLUSION:

A green, microwave-assisted, solvent-free multi-component reaction protocol involving recyclable sulfamic acid has been developed for the efficient synthesis of 1,4-dihydropyridines and 3,4-dihydropyrimidin-2(1H)-ones. The process is rapid (3–8 min), high-yielding (76–94%), operable under simple conditions and environmentally benign. The antimicrobial screening of the synthesized compounds revealed that the electron-withdrawing nitro and chloro derivatives possess superior antimicrobial activity, while the electron-rich methoxy and dimethylamino derivatives exhibit enhanced antioxidant activity. The compounds 5b and 5e represent promising leads for further optimization.

The present work highlights the potential of MCR based green synthesis for the development of diverse heterocyclic libraries of pharmaceutical significance.

REFERENCES:

1. Katritzky, A. R.; Ramsden, C. A.; Joule, J. A.; Zhdankin, V. V. Handbook of Heterocyclic Chemistry, 3 rd ed., Elsevier, Oxford, 2010.
2. Bossert, F.; Vater, W. Med. Res. Rev. 1989, 9, 291-324.
3. Kappe, C. O. Eur. J. Med. Chem. 2000, 35, 1043-1052.
4. Kappe, C. O. Acc. Chem. Res. 2000, 33, 879-888.
5. Snider, B. B.; Shi, Z. J. Org. Chem. 1993, 58, 3828-3839.
6. Dömling, A.; Ugi, I. Angew. Chem. Int. Ed. 2000, 39, 3168-3210.
7. Zhu, J.; Bienaymé, H. (Eds.) Multicomponent Reactions, Wiley-VCH, Weinheim, 2005.
8. Anastas, P. T.; Warner, J. C. Green Chemistry: Theory and Practice, Oxford University Press, New York, 1998.
9. Weber, L. Drug Discov. Today 2002, 7, 143-147.
10. Hantzsch, A. Justus Liebigs Ann. Chem. 1882, 215, 1-82.
11. Biginelli, P. Gazz. Chim. Ital. 1893, 23, 360-413.
12. Kappe, C. O. Angew. Chem. Int. Ed. 2004, 43, 6250-6284.
13. Lidström, P.; Tierney, J.; Wathey, B.; Westman, J. Tetrahedron 2001, 57, 9225-9283.
14. Varma, R. S. Green Chem. 1999, 1, 43-55.
15. Kidwai, M.; Saxena, S.; Mohan, R.; Venkataramanan, R. J. Chem. Soc., Perkin Trans. 1 2002, 1845-1846.