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Green, Efficient Microwave-Assisted Synthesis, Antimicrobial Activity And Molecular Docking Studies of 1,2,3,6-Tetrahydropyrimidine-4-Carboxylate

B. Chellakili, and M. Gopalakrishnan*

*Department of Chemistry, Annamalai University, Annamalainagar – 608 002, Tamil Nadu, **INDIA**

Email: profmgk61@gmail.com

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ABSTRACT

A simple and efficient microwave assisted three-component condensation of β-keto ester, aldehyde and urea or thio-urea catalyzed by $NaHSO_4.SiO_2$using microwave irradiation technique. A series of novel diethyl 6,6'-(1,4-phenylene)bis(2-imino-5-methyl-1,2,3,6-tetrahydropyrimidine-4-carboxylate) has been synthesized, confirmed by analytical and spectral data and evaluated for their antimicrobial activity and in silicoantitubercular activity. The catalyst used for this process is eco-friendly, easy to handle, non-toxic for environment and recyclable at least up to 5 cycles with good to excellent yield.

Keywords: Microwave, Heterogeneous catalyst, Biginelli reaction, Multicomponent reactions (MCRs), 1,2,3,4-tetrahydropyrimidine-4-carboxylates.

INTRODUCTION

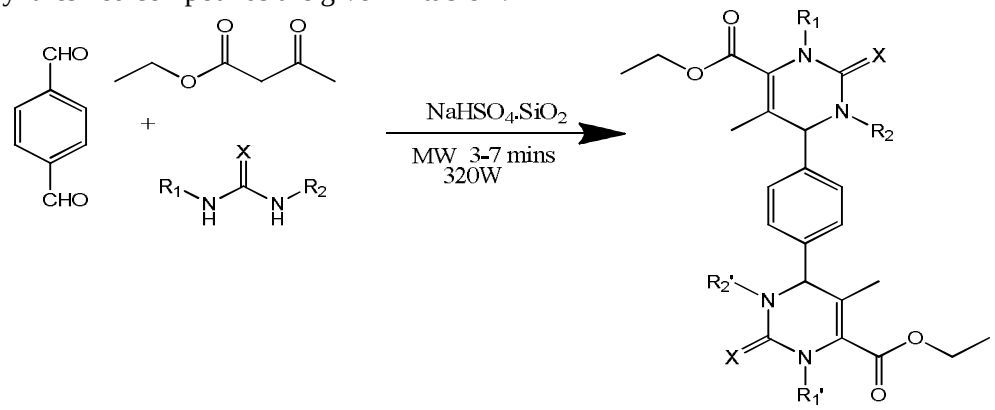
Multi-component reactions (MCRs) constitute a highly valuable synthesis implement for the construction of polyfunctionalized heterocyclic compounds required for drug revelation programs. [1,2] this methodology sanction molecular intricacy and diversity to be engendered by the facile formation of several incipient covalent bonds in a one pot transformation quite proximately approaching the concept of an ideal synthesis and is particularly well acclimated for combinational chemistry. Astronomically immense number of antimicrobial agents has prompted studies on the development of incipient potential antimicrobial compounds. The molecular manipulation of promising lead compounds is still a major line of approach to develop incipient drugs. So, the revelation of novel and potent antimicrobial agents is the best way to surmount microbial resistance and develop efficacious therapies. The nitrogen containing heterocyclic compounds is of interest because they constitute a consequential class of natural and non-natural products, many of which exhibit utilizable biological activities and Clinical applications [3] Pyrimidine derivatives and heterocyclic annulled pyrimidines exhibits a wide variety of fascinating pharmacological properties. Recently, many tetrahydropyrimidine compounds have been synthesized and evaluated biologically in an effort to develop clinically subsidiary medicines. Tetrahydropyrimidine derivatives with a variety of structures have been reported in a wide range of biological activities, such as antihypertensive [4], antimicrobial [5], anticancer [6], melanogenesis inhibitory [7], neutrophil chemotaxis inhibitive [8], HIV-1 protease inhibitors [9], free radical scavenging [10] and muscarinic receptor agonists

[11] effects. Antiproliferative, [12] antiviral, [13] antitumor,[14] anti-inflammatory, [15] antibacterial, [16] antifungal, [17] and antitubercular activity [18].

MATERIALS AND METHODS

All the reagents and solvents used were of high grade and purchased from Fluka and Merck and were distilled prior to use unless otherwise stated. TLC was carried out to monitor the course of the reaction and the purity of the product. The melting points were recorded in open capillaries and are uncorrected. IR spectra were recorded in AVATAR-330 FT-IR spectrophotometer (Thermo Nicolet) and only noteworthy absorption levels (reciprocal centimeters) are listed. ^{1}H-NMR spectra were recorded at 400 MHz on BRUKER AMX 400 MHz spectrophotometer using DMSO as solvent and TMS as an internal standard. ^{13}C-NMR spectra were recorded at 100 MHz on BRUKER AMX 400 MHz spectrometer in DMSO and tetramethylsilane (TMS) as internal standard. Results are presented in the following format; chemical shift (in ppm, multiplicity, number of protons, proton's position. Multiplicities are shown as the abbreviations: s (singlet), and m (multiplet). Microanalyses were performed on Heraeus Carlo Erba 1108 CHN analyzer.

Experimental: A mixture containing ethyl acetoacetate, (0.01 mol), guanidine hydrochloride, urea/ thiourea (0.01 mol), terephthaldehyde (0.01 mol) in the ratio of 2:2:1 and NaHSO4.SiO2 (20 mg) was mixed properly and then irradiated in microwave oven for 120 s at P=320W. After completion of the reaction, the reaction mixture was extracted with methanol. The catalyst was removed by filtration. The filtrate was concentrated to furnish the product and recrystallized in methanol. The physical and analytical data of newly synthesized compounds are given in table 1.



Compound	X	R1, R1’	R2, R2’
1	**NH**	**H**	**H**
2	**O**	**H**	**H**
3	**O**	**CH_3**	**CH_3**
4	**S**	**H**	**H**
5	**S**	**CH_3**	**H**
6	**S**	**C_6H_5**	**C_6H_5**

Scheme 1. Schematic diagram showing the synthesis of 1,2,3,6-tetrahydropyrimidines (**1-6**)

RESULTS AND DISCUSSION

IR spectrum of compound 1 showed an absorption band in the region of 2848-2993 cm^{-1} which is due to aliphatic and aromatic C-H stretching. A sharp and intense absorption band around 3314 cm^{-1} is assigned to NH stretching. The absorption band appeared in 1730, 1691cm^{-1} is due to C=O and C=N stretching frequency.

^{1}H NMR spectrum of compound 1 the triplet at 1.28 ppm is due to methyl protons of ester moiety at C-4 and 2.43 ppm is assigned to methyl protons at C-5 carbon of pyrimidine ring. The methylene protons appeared as quartet at 4.33 ppm and a singlet at 7.47 ppm is assigned to aromatic protons. A singlet at 1.66 and 9.54 ppm which is due to NH protons of compound 1.^{13}C NMR spectrum 1,2,3,6-tetrahydropyrimidine-4-carboxylate compounds formation was confirmed by the presence of carbon signals at 167.52, 135.58, 135.07, 60.03 ppm which are assigned to C-2, C-4 C-5 and C-6 respectively. The aromatic carbon signal appeared at 129.86 ppm. The signal at 194.39 ppm due to carbonyl carbon. The signal at 26.74 ppm is due to the presence of methyl carbons of ester and 13.91 ppm methyl at C-5 carbon. The methylene carbon appeared at 61.98ppm.

Diethyl 6,6'-(1,4-phenylene)bis(2-imino-5-methyl-1,2,3,6-tetrahydropyrimidine-4-carboxylate) 1 : IR (cm^{-1}): 3314, (NH stretching); 2993-2948 (CH stretching); 1730, 1691 (O=C, C-N stretching); 1541, 1492, 1459, 1362,1325, 1238, 1073, 1017, 828, 786, 666, 465: ^{1}H NMR (δ ppm), DMSO-D_6;1.28 (t,6H, CH_3-ester); 4.33 (q,4H, CH_2-ester); 2.43 (s, 6H, CH_3 at C5)5.30 (s, 2H, H-6 proton); 7.47 (s, 4H, aryl protons); 1.66, 9.54 (s, 6H, H-1,H-2,H-3 protons of NH): ^{13}C NMR (δ ppm) DMSO-D_6: 26.74 (CH3- ester); 61.98 (CH2- ester); 13.91 (CH3 at C-5); 60.03 (C-6); 194.39 (ester C=O); 167.52 (C-2, C=N); 139.72 (C-5); 129.86 (aryl carbons); 135.07-135.58 (ipso carbons)

Diethyl 6,6'-(1,4-phenylene)bis(5-methyl-2-oxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate)2 : IR (KBr): 3333, 2848,2919, 2985, 1701, 1667, 1371, 801, 650: ^{1}H NMR (δ ppm), DMSO-D_6;1.09 (t,6H, CH_3-ester); 3.98 (q,4H, CH_2-ester); 2.23 (s, 6H, CH_3 at C5)5.17 (s, 2H, H-6 proton); 7.17 (s, 4H, aryl protons); 8.51, 9.18 (s, 4H, H-1,H-3 protons of NH): ^{13}C NMR (δ ppm) DMSO-D_6: 17.74 (CH3- ester); 59.26 (CH2-ester); 14.03 (CH3 at C-5); 59.18 (C-6); 189.77 (ester C=O); 165.30 (C-2, C=O); 139.72 (C-5); 126.26 (aryl carbons); 147.84-155.37 (ipso carbons).

Diethyl 6,6'-(1,4-phenylene)bis(1,3,5-trimethyl-2-oxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate)3 : IR (KBr): 3361, 2849, 2920, 2965, 1726, 1306, 911, 729, 650: ^{1}H NMR (δ ppm), DMSO-D_6;1.38 (t,6H, CH_3- ester); 4.30 (q,4H, CH_2-ester); 2.20 (s, 6H, CH_3 at C5); 2.68, 2.70 (s, 12H, N-CH_3 at H-1,H-3); 5.74 (s, 2H, H-6 proton); 7.51-7.53 (d, 4H, aryl protons): ^{13}C NMR (δ ppm) DMSO-D_6: 16.51 (CH3- ester); 61.10 (CH2- ester); 13.98 (CH3 at C-5); 60.03 (C-6); 165.39 (ester C=O); 161.52 (C-2, C=N); 123.72 (C-5); 128.86 (aryl carbons); 124.07-135.58 (ipso carbons).

Table 1. Analytical data for compounds **1-6**

Entry	Molecular Formulae	Yield (%)	m.p (°C)	Elemental Analysis (%)					
				Calculated			Found		
				C	H	N	C	H	N
1	$C_{22}H_{28}N_6O_4$	63	155	61.97	5.41	11.56	61.95	5.43	11.58
2	$C_{22}H_{26}N_4O_6$	74	97	59.45	5.54	10.27	59.45	5.56	10.28
3	$C_{26}H_{34}N_4O_6$	75	185	62.36	5.07	8.81	62.36	5.05	8.80
4	$C_{22}H_{26}N_4O_4S_2$	70	122	58.03	5.06	10.83	58.01	5.07	10.83
5	$C_{24}H_{30}N_4O_4S_2$	68	94	58.02	5.04	10.86	58.02	5.06	10.87
6	$C_{46}H_{42}N_4O_4S_2$	62	138	69.02	7.19	11.10	69.03	7.17	11.10

Diethyl 6,6'-(1,4-phenylene)bis(5-methyl-2-thioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate*)* 4
IR (KBr): 3308, 2834, 2901, 2985, 1720, 1667, 1391, 951, 801, 765, 651: ^{1}H NMR (δ ppm), DMSO-D_6;1.09 (t,6H, CH_3- ester); 4.01 (q,4H, CH_2-ester); 2.27 (s, 6H, CH_3 at C5)5.13 (s, 2H, H-6 proton); 7.18 (s, 4H, aryl protons); 10.33, 9.62 (s, 6H, H-1,H-2,H-3 protons of NH): ^{13}C NMR (δ ppm) DMSO-D_6: 17.13 (CH3- ester); 59.59 (CH2- ester); 13.97 (CH3 at C-5); 71.02 (C-6); 196.19 (ester C=O); 175.21 (C-2, C=S);129.86 (aryl carbons); 127.18-149.37 (ipso carbons).

Diethyl 6,6'-(1,4-phenylene)bis(3,5-dimethyl-2-thioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate) 5 IR (KBr): 3319, 2925, 2979, 1705, 1631, 1378, 931, 810, 629: ^{1}H NMR (δ ppm), DMSO-D_6;1.15 (t,6H, CH_3- ester); 4.09 (q,4H, CH_2-ester); 3.42 (s, 6H, CH_3 at C5); 3.47 (s,6H, N-CH_3)5.16 (s, 2H, H-6 proton); 7.15 (s, 4H, aryl protons); 9.82 (s, 2H, H-3, H-3' protons of NH): ^{13}C NMR (δ ppm) DMSO-D_6: 16.22 (CH3- ester); 60.11 (CH2- ester); 13.98 (CH3 at C-5); 59.57 (C-6); 165.21 (ester C=O); 177.99 (C-2, C=S); 126.33 (aryl carbons); 141.64-147.93 (ipso carbons).

Diethyl6,6'-(1,4-phenylene)bis(5-methyl-1,3-diphenyl-2-thioxo-1,2,3,6-tetrahydropyrimidine-4-arboxylate) 6 IR (KBr): 3349, 2849, 2918, 2981, 1726, 16697, 1368, 970, 823, 752, 687: ^{1}H NMR (δ ppm), DMSO-D_6;1.03 (t,6H, CH_3- ester); 3.67, 3.94 (q,4H, CH_2-ester); 1.91 (s, 6H, CH_3 at C5);5.05 (s, 2H, H-6 proton); 7.05-7.43 (s, 24H, aryl protons): ^{13}C NMR (δ ppm) DMSO-D_6: 25.74 (CH3- ester); 62.10 (CH2- ester); 13.41 (CH3 at C-5); 60.03 (C-6); 168.28 (ester C=O); 179.54 (C-2, C=S); 121.74-129.88 (aryl carbons); 136.56-152.53 (ipso carbons).

APPLICATIONS

Antibacterial activity: All newly synthesized compounds (1-6) were screened for their antibacterial activity against gram-positive and gram- negative pathogenic bacterial strains and the MIC values compared with ciprofloxacin as standard. The MIC values are given in table 2. Among the tested compounds, the compound 2 has emerged as active against all the tested microorganisms. Compound 1 showed excellent activity against *Bacillus subtilis, Staphylococcus aureus, Vibreo cholera, Escherichia coli, Klebsiella*pneumonia at MIC values of (6.25-12.5 µg mL^{-1}). The oxopyrimidine, compound 2 showed incredible activities against *Bacillus subtilis, Staphylococcus aureus, Vibreo cholera, Salmonella typhii ,Klebsiella pneumonia, Pseudomonas* at MIC values of (6.25-12.5 µg mL^{-1}) and moderate activity against *Escherichia coli.* The introduction of*a methyl group* at 1,3 position of pyrimidine ring the compound 3 showed exerted good activity against only *Bacillus subtilis.* The thiopyrimidine compound 4 exerted strong activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas* at MIC values of (6.25-12.5 µg mL^{-1}). The introduction of methyl and phenyl in compound 4 in 1,3 positions of pyrimidine ring, the compound 5,6 showed moderate activity against all the tested pathogens at MIC values of (50-200 µg mL^{-1}).

Table 2 Antibacterial activity of compounds **1-6** against some bacterial strains (MIC in µg mL^{-1})

Microorganism	Minimum inhibitory concentration (MIC) in µg mL^{-1}						Ciprofloxacin
	1	2	3	4	5	6	
Bacillus subtilis	6.25	6.25	12.5	12.5	25	50	12.5
Staphylococcus aureus	12.5	12.5	25	6.25	100	25	25
Salmonella typhii	25	12.5	25	25	100	50	25
Vibreocholerae	12.5	6.25	50	50	25	100	25
Escherichia coli	12.5	25	50	6.25	25	50	25
Klebsiella pneumonia	6.25	12.5	25	6.25	25	50	12.5
Pseudomonas	25	12.5	25	6.25	50	50	25

Antifungal activity: The synthesized compounds 1-6 were also screened for in vitro antifungal activity against clinically isolated pathogens and compare with standard. Here, Fluconazole was used as standard drug. The MIC values of testing compounds are given in table 3. The iminotetrahydropyrimidine compound 1 superior activity against *Aspergillusflavus*, *Candidaalbicans*, *Rhizopus*MIC values of (6.25-12.5 µg/mL). The oxo tetrahydropyrimidine-4-carboxylate, compound 2 exhibited well pronounced activity against *Aspergillusflavus*, *Aspergillusniger*, *Candida 6*, *Rhizopus*(6.25-12.5 µg/mL).The thio tetrahydropyrimidine-4-carboxylate excellent inhibition potency at minimum concentration (6.25-12.5 µg/mL). But the replacement of methyl and phenyl group at 1,3 position of pyrimidine ring registered minimum to moderate activity 25-100 µg mL^{-1}.

Table 3 Antifungal activity of compounds **1-6** against some fungal strains (MIC in µg mL^{-1})

Microorganism	Minimum inhibitory concentration (MIC) in µg mL^{-1}						Fluconazole
	1	2	3	4	5	6	
Aspergillusflavus	12.5	12.5	50	12.5	25	50	12.5
Aspergillusniger	25	12.5	25	25	50	100	12.5
Candidaalbicans	12.5	25	50	25	50	50	25
Mucor	100	100	200	6.25	100	100	25
Candida 6	25	12.5	25	25	50	100	25
Rhizopus	12.5	6.25	25	12.5	25	50	25

Molecular docking study: The docking analysis is performed on a series of imino,oxo/thio tetrahydropyrimidine-4-carboxylate with *Mycobacterium tuberculosis enoylreductase (InhA*) (PDB ID: 2H7I) by docking server using AutoDock 4.0. Figure 1 indicates docked ligand molecule **2** with the secondary structure of *enoylreductase* (*InhA*) in solid and ribbon model. The amino acids forming hydrogen bonds with target ligands has been labeled, where green dotted lines indicate hydrogen bonds and the atomic distance (Å) between the interacting residues with target ligands. THR196, SER94, GLY96, PHE41, TYR158, SER20, THR17 were the residues involved in hydrogen bonding interactions.The target molecule **2** at the active pocket of the protein(PDB ID 2H7I) showed in figure 2. The binding energy, docking energy and hydrogen bond counting with the distance of interacting residue were given in table 4. Figure 3 and 4 indicates 2D and hydrogen bond plot of interacted residues with targeted ligands. The other conserved residues within the active site of *enoylreductase (InhA)* are involved in hydrophobic interaction, that aid in the overall stability of the docked complex. The inhibitory activity depends on the substitution pattern on the pyrimidine ring. From that results all the docked molecules were found to exhibit binding affinity with different poses of nucleosides and the most potent against *Mycobacterium tuberculosis enoylreductase* (*InhA*) agent was compound 2.

Table 4 Molecular docking results of the target molecules with *Mycobacteriumtuberculosisenoylreductase* (*InhA*) (PDB ID 2H7I)

Molecules No	Binding energy	Docking energy	Inhibition constant	Imtermol energy	Hydrogen bond	Interaction moity
1	-6.37	-8.26	21.47	-8.29	9	ILE15, SER94, GLY96, MET98,THR196, ILE194, TYR158
2	-7.29	-9.15	4.56	-9.21	11	THR196, SER94, GLY96, PHE41, TYR158, SER20, THR17
3	-6.47	-8.59	18.00	-8.57	2	SER94, GLY96
4	-7.31	-9.39	4.36	-9.47	7	SER94, GLY96, ILE194, TYR158
5	-6.59	-9.14	14.81	-9.17	6	GLY96, TYR158, GLY96, TYR158, GLY96, TYR158
6	-9.77	-12.38	68.46	-12.37	2	LYS165, GLY96

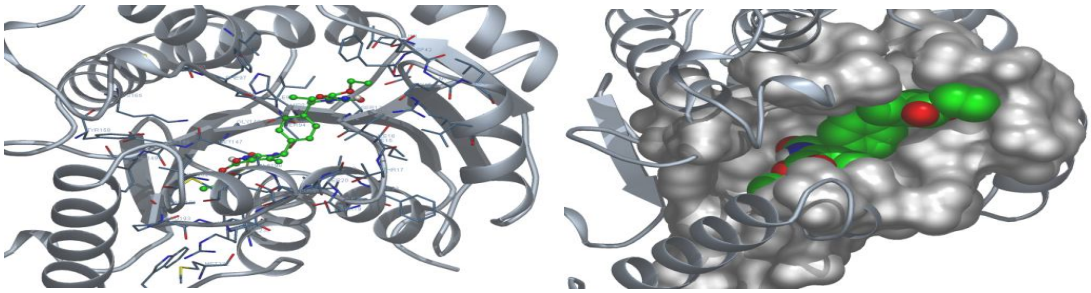


Figure 1. Docked ligand molecule **2** with the secondary structure of *enoylreductase* (*InhA*)insolid and ribbon model and

Figure 2. The surface cavity occupied with target molecule **2** at the active pocket of the protein (PDB ID 2H7I

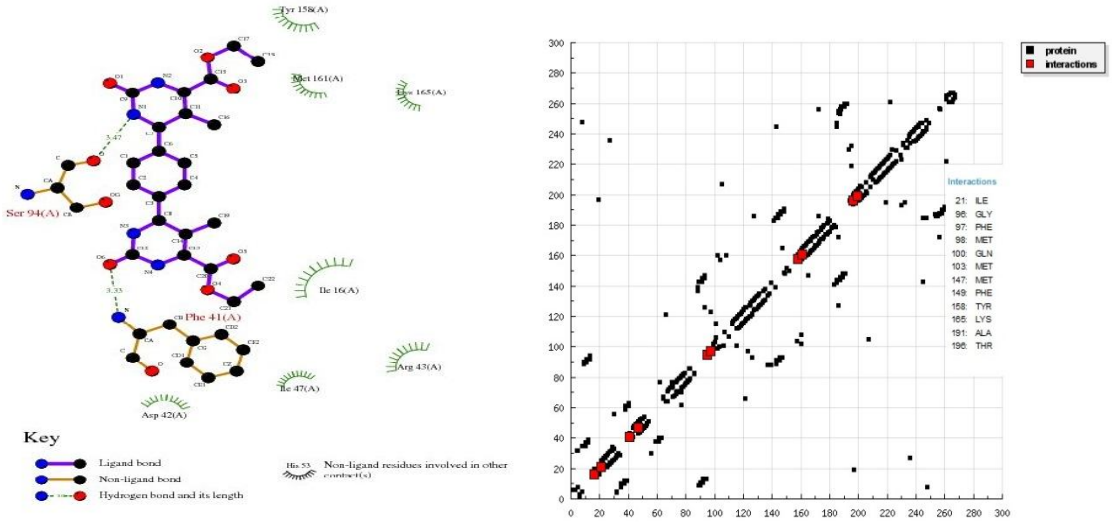


Figure 3.2D plot of hydrogen bond forming amino acids with target ligand. Dotted linesindicates hydrogen bond between amino acid and bonding atom of target molecule and

Figure 4. HB plot of interacted residues in protein of *M. tuberculosis* with compound **2**

CONCLUSIONS

The novel 1,2,3,6-tetrahydropyrimidines were synthesized, characterized and tested for antimicrobial activities. Among the tested compounds ,*2-oxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate* ***2*** has emerged as most potent against all tested microorganisms. The molecular docking study also revealed that compound **2** has minimum binding and docking energy and may be considered as a good inhibitor of *Mycobacterium tuberculosis enoylreductase (InhA*). Hence, this study has widened the scope of developing these tetrahydropyrimidine derivatives as promising antibacterial and antitubercular agents.

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