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Synthesis and evaluation of 7,8,9,10-tetrahydro-*1H*-pyrimido[1,2-a]quinoline-2,5-dicarbonitrile derivatives as antimicrobial agents

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ABSTRACT

The present communication describes the efficient synthesis of some new 7,8,9,10-tetra- hydro-1H-pyrimido[1,2-a]quinoline-2,5-dicarbonitrile derivatives via two step reaction by using a green catalytic system i.e PEG-400 and Bleaching earth clay. The structure assignments of the newly synthesized compounds based on chemical and spectroscopic evidences. All the synthesized compounds were screened for their antimicrobial activity. Most of the compounds showed significant antimicrobial activity compared with standard drugs.

Keywords: 7,8,9,10-tetra-hydro-1H-pyrimido[1,2-a]quinoline-2,5-dicarbonitrile, PEG-400, Bleaching earth clay, Antimicrobial activity

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INTRODUCTION

Over the years, increasing prevalence's of infection induced by the rapid progress of bacterial resistance to most of the known antibiotics is a severe health issue. This has turned up into rises in morbidity and mortality, and has become a worldwide health problem. As a consequence, the development of new antimicrobial agents is in continual demand. As multidrug-resistant bacterial strains prapogate, the necessity for competent remedy has stimulated research into the design and synthesis of novel anti-microbial molecules

The chemistry of pyrimido[1,2-a] quinoline compounds have been studied effectively and is the subject of several scrutiny. The pyrimido[1,2-a]quinoline scaffold has implemented the support for the design of biologic compatible molecules with immense biomedical value as therapeutics. For example their derivatives possess significant anticancer [1], antimalarial [2, 3], antimicrobial [4-8] activity. Furthermore, some of these derivatives possess antitumor [9-11], anti-inflammatory [12-14], analgesic [15,16] activity.

Accordingly above cited facts and as a continuity of our studies on the development of adequate and environmental friendly synthetic methodologies [17,18], Here we report the synthesis and biological evaluation of some 7,8,9,10-tetrahydro-1H-pyrimido[1,2-a]quinoline-2,5-dicarbonitrile derivatives, via two step reaction in presence Bleaching earth clay (pH12.5) and PEG-400 as a green reaction medium. Bleaching earth clay (pH12.5) has been rising as one of such alternative green and heterogeneous catalyst [19-23].

EXPERIMENTAL SECTION

Melting points were found out in an open capillary tube and are uncorrected. The chemical solvents utilized were distilled prior to practice. The end point of the reaction was observed by thin layer chromatography on a precoated sheet of silica gel-G uses iodine vapors for detection. IR spectra were recorded in the Perkin Elmer spectrometer. ^{1}H-NMR spectra were confirmed in dimethyl sulphoxide (DMSO)-d_6 using an Advance spectrometer at a frequency of 400 MHz using tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on an EI-Shimadzu QP2010PLUS GC–MS.

*General procedure for the preparation of 2-amino-8-(2/3/4-substituted benzylidene)-4-(2/3/4-substituted phenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (**3a-i**)*

A mixture of 2,6-bis(2/3/4-substituted benzylidene)cyclohexanone (1.00 mmol), malononitrile (1.00 mmol), ammonium acetate (1.00 mmol) and Bleaching earth clay (pH12.5, 10 wt%) was stirred at 70-80^{o}C in PEG-400 for 1hour. After complete conversion as indicated by TLC, the catalyst was filtered out by simple filtration and the mother liquor poured into ice-cold water, solid separated out. The separated solid was filtered; the crude product was recrystallized from ethanol to afford the pure product.

*2-amino-8-benzylidene-4-phenyl-5,6,7,8-tetrahydroquinoline-3-carbonitrile (**3a**)*

M.p. 140–145^{o}C; IR (KBr): 3350 (NH_2 stretching), 3040 (Ar, C–H stretching), 2232 (C≡N), 1626 (C=N) cm-1; ^{1}H NMR (300 MHz, DMSO-d6, TMS):δ 8.80 (s,1H, –C=CH), 7.68–8.21 (m, 12H, 10Ar–H + 2NH_2), 1.53–2.55 ppm (m, 6H, CH_2 protons of tetrahydroquinoline ring); Mass (m/z):337 [M^+] (100); Elem. anal. calculated (found) for $C_{23}H_{19}N_3$: C, 81.87 (81.84); H, 5.68 (5.71); N, 12.45 (12.42).

*2-amino-8-(4-fluorobenzylidene)-4-(4-fluorophenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (**3b**)*

M.p. 150–155^{o}C; IR (KBr): 3343 (NH_2 stretching), 3036 (Ar, C–H stretching), 2228 (C≡N), 1619 (C=N) cm-1;1H NMR (300 MHz, DMSO-d6, TMS):δ 8.73 (s,1H, –C=CH), 7.65–8.11 (m, 10H, 8 Ar–H + 2NH_2), 1.40–2.48 ppm (m, 6H, CH_2 protons of tetrahydroquinoline ring); Mass (m/z):373 [M^+] (100); Elem. anal. calculated (found) for $C_{23}H_{17}F_2N_3$: C, 73.98 (73.96); H, 4.59 (4.61); N, 11.25 (11.22).

*2-amino-8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (**3c**)*

M.p. 152–157^{o}C; IR (KBr): 3340 (NH_2 stretching), 3042 (Ar, C–H stretching), 2237 (C≡N), 1629 (C=N) cm-1;1H NMR (300 MHz, DMSO-d6, TMS):δ 8.76 (s,1H, –C=CH), 7.63–8.17 (m, 10H, 8 Ar–H + 2NH_2), 1.31–2.38 ppm (m, 6H, CH_2 protons of tetrahydroquinoline ring); Mass (m/z):406 [M^+] (100); Elem. anal. calculated (found) for $C_{23}H_{17}Cl_2N_3$: C, 73.98 (73.96); H, 4.59 (4.61); N, 11.25 (11.22).

*2-amino-8-(4-nitrobenzylidene)-4-(4-nitrophenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (**3e**)*

M.p. 152–157^{o}C; IR (KBr): 3347 (NH_2 stretching), 3033 (Ar, C–H stretching), 2228 (C≡N), 1621 (C=N) cm-1;1H NMR (300 MHz, DMSO-d6, TMS):δ 8.70 (s,1H, –C=CH), 7.59–8.22 (m, 10H, 8 Ar–H + 2NH_2), 1.20–2.34 ppm (m, 6H, CH_2 protons of tetrahydroquinoline ring); Mass (m/z):427 [M^+] (100); Elem. anal. calculated (found) for $C_{23}H_{17}N_5O_4$: C, 64.63 (64.66); H, 4.01 (4.03); N, 16.39 (16.40).

*2-amino-8-(2-hydroxybenzylidene)-4-(2-hydroxyphenyl)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (**3g**)*

M.p. 152–157^{o}C; IR (KBr): 3415 (OH stretching), 3344 (NH_2 stretching), 3037 (Ar, C–H stretching), 2234 (C≡N), 1632 (C=N) cm-1;1H NMR (300 MHz, DMSO-d6, TMS):δ 11.11 (s, 1H, OH), 11.07 (s, 1H, OH), 8.74 (s,1H, –C=CH), 7.63–8.25 (m, 10H, 8 Ar–H + 2NH_2), 1.27–2.30 ppm (m, 6H, CH_2 protons of tetrahydroquinoline ring); Mass (m/z):369 [M^+] (100); Elem. anal. calculated (found) for $C_{23}H_{19}N_3O_2$: C, 74.78 (74.76); H, 5.18 (5.20); N, 11.37 (11.40).

*General procedure for the preparation of 10-(2/3/4-substituted benzylidene)-6-(2/3/4-substituted phenyl)-3-(methylthio)-1-oxo-7,8,9,10-tetrahydro-1H-pyrimido[1,2-a]quinoline-2,5-dicarbonitrile (**5a-i**)*

An equimolar mixture of **3a-i** (1 mmol) and ethyl 2-cyano-3,3-bis(methylthio)acrylate **4** (prepared by reported method) was stirred in PEG-400 in presence of Bleaching earth clay (pH12.5, 10 wt%) at 70-80^{o}C for 2-3hrs. After complete conversion as indicated by TLC, the catalyst was filtered out by simple filtration and the mother liquor poured into ice-cold water, solid separated out. The separated solid was filtered; the crude product was recrystallized from ethanol to afford the pure product **5a-i**.

*10-benzylidene-3-(methylthio)-1-oxo-6-phenyl-7,8,9,10-tetrahydro-1H-pyrimido[1,2-a]quinoline-2,5-dicarbonitrile (**5a**)*
M.p. 150–155^{o}C; Yield 89%; IR (KBr): 3054 (Ar, C–H stretching), 2244 (C≡N), 1698 (C=O), 1639 (C=N) cm-1;1H NMR (300 MHz, DMSO-d6, TMS):δ 8.79 (s,1H, –C=CH), 7.62–8.41 (m, 10H, Ar–H), 2.60 (s, 3H, CH_3), 1.21–2.42 ppm (m, 6H, CH_2 protons of tetrahydroquinoline ring); ^{13}C NMR (100 MHz, DMSO-d6, TMS):δ 170.56, 168.00, 160.67, 158.88, 140.54, 135.21, 132.53, 129.34, 128.59, 128.25, 127.18, 127.11, 124.14, 118.70, 115.65, 86.80, 30.57, 25.21, 24.16, 17.24; Mass (m/z):460 [M^+] (100); Elem. anal. calculated (found) for $C_{28}H_{20}N_4OS$: C, 73.02 (73.06); H, 4.38 (4.40); N, 11.37 (11.40).

*10-(4-fluorobenzylidene)-6-(4-fluorophenyl)-3-(methylthio)-1-oxo-7,8,9,10-tetrahydro-1H-pyrimido[1,2-a] quinoline- 2,5-dicarbonitrile (**5b**)*
M.p. 160–165^{o}C; Yield 87%; IR (KBr): 3044 (Ar, C–H stretching), 2251 (C≡N), 1689 (C=O), 1636 (C=N) cm-1;1H NMR (300 MHz, DMSO-d6, TMS):δ 8.87 (s,1H, –C=CH), 7.49–8.35 (m, 8H, Ar–H), 2.48 (s, 3H, CH_3), 1.19–2.37 ppm (m, 6H, CH_2 protons of tetrahydroquinoline ring); ^{13}C NMR (100 MHz, DMSO-d6, TMS):δ 170.65, 166.55, 160.19, 157.45, 140.21, 136.08, 132.48, 130.04, 129.13, 128.55, 127.64, 127.46, 123.18, 118.96, 114.71, 88.24, 30.35, 25.19, 24.10, 18.00; Mass (m/z):496 [M^+] (100); Elem. anal. calculated (found) for $C_{28}H_{18}F_2N_4OS$: C, 67.73 (67.75); H, 3.65 (3.67); N, 11.28 (11.30).

*10-(4-chlorobenzylidene)-6-(4-chlorophenyl)-3-(methylthio)-1-oxo-7,8,9,10-tetrahydro-1H-pyrimido[1,2-a] quinoline-2,5-dicarbonitrile (**5c**)*
M.p. 162–167^{o}C; Yield 85%; IR (KBr): 3046 (Ar, C–H stretching), 2233 (C≡N), 1694 (C=O), 1624 (C=N) cm-1;1H NMR (300 MHz, DMSO-d6, TMS):δ 8.90 (s,1H, –C=CH), 7.57–8.41 (m, 8H, Ar–H), 2.51 (s, 3H, CH_3), 1.26–2.31 ppm (m, 6H, CH_2 protons of tetrahydroquinoline ring); ^{13}C NMR (100 MHz, DMSO-d6, TMS):δ 171.16, 167.10, 161.32, 158.65, 141.12, 136.08, 132.48, 130.04, 129.13, 128.55, 127.64, 127.46, 123.18, 118.96, 114.71, 88.24, 30.35, 25.19, 24.10, 18.00; Mass (m/z):529 [M^+] (100); Elem. anal. calculated (found) for $C_{28}H_{18}Cl_2N_4OS$: C, 63.52 (63.55); H, 3.43 (3.41); N, 10.58 (10.55).

*10-(2-chlorobenzylidene)-6-(2-chlorophenyl)-3-(methylthio)-1-oxo-7,8,9,10-tetrahydro-1H-pyrimido[1,2-a] quinoline-2,5-dicarbonitrile (**5d**)*
M.p. 165–170^{o}C; Yield 87%; IR (KBr): 3050 (Ar, C–H stretching), 2238 (C≡N), 1687 (C=O), 1616 (C=N) cm-1;1H NMR (300 MHz, DMSO-d6, TMS):δ 8.71 (s,1H, –C=CH), 7.49–8.34 (m, 8H, Ar–H), 2.46 (s, 3H, CH_3), 1.18–2.29 ppm (m, 6H, CH_2 protons of tetrahydroquinoline ring); ^{13}C NMR (100 MHz, DMSO-d6, TMS):δ 170.22, 168.34, 161.61, 157.11, 140.21, 136.20, 132.19, 130.38, 129.44, 128.16, 127.77, 127.25, 123.13, 117.00, 114.29, 86.87, 30.46, 25.33, 24.19, 18.32; Mass (m/z):529 [M^+] (100); Elem. anal. calculated (found) for $C_{28}H_{18}Cl_2N_4OS$: C, 63.52 (63.51); H, 3.43 (3.44); N, 10.58 (10.54).

*3-(methylthio)-10-(4-nitrobenzylidene)-6-(4-nitrophenyl)-1-oxo-7,8,9,10-tetrahydro-1H-pyrimido[1,2-a]quinoline-2,5-dicarbonitrile (**5e**)*
M.p. 175–180^{o}C; Yield 86%; IR (KBr): 3037 (Ar, C–H stretching), 2244 (C≡N), 1697 (C=O), 1622 (C=N) cm-1;1H NMR (300 MHz, DMSO-d6, TMS):δ 8.82 (s,1H, –C=CH), 7.40–8.40 (m, 8H, Ar–H), 2.44 (s, 3H, CH_3), 1.20–2.34 ppm (m, 6H, CH_2 protons of tetrahydroquinoline ring); ^{13}C NMR (100 MHz, DMSO-d6, TMS):δ 171.04, 168.84, 161.75, 157.41, 140.13, 136.24, 132.26, 130.16, 129.12, 128.47, 127.56, 127.46, 123.15, 117.33, 114.52, 87.20, 30.25, 25.07, 24.48, 17.66; Mass (m/z):550 [M^+] (100); Elem. anal. calculated (found) for $C_{28}H_{18}Cl_2N_6O_5S$: C, 61.08 (61.10); H, 3.30 (3.32); N, 15.26 (15.24).

*3-(methylthio)-10-(3-nitrobenzylidene)-6-(3-nitrophenyl)-1-oxo-7,8,9,10-tetrahydro-1H-pyrimido[1,2-a]quinoline-2,5-dicarbonitrile (**5f**)*
M.p. 175–180^{o}C; Yield 80%; IR (KBr): 3026 (Ar, C–H stretching), 2237 (C≡N), 1695 (C=O), 1625 (C=N) cm-1;1H NMR (300 MHz, DMSO-d6, TMS):δ 8.79 (s,1H, –C=CH), 7.33–8.24 (m, 8H, Ar–H), 2.37 (s, 3H, CH_3), 1.26–2.30 ppm (m, 6H, CH_2 protons of tetrahydroquinoline ring); ^{13}C NMR (100 MHz, DMSO-d6, TMS):δ 171.10, 168.80, 161.79, 157.40, 140.18, 136.20, 132.29, 130.17, 129.11, 128.51, 127.55, 127.40, 123.25, 117.30, 114.60, 87.25, 30.23, 25.11, 24.52, 17.74; Mass (m/z):550 [M^+] (100); Elem. anal. calculated (found) for $C_{28}H_{18}Cl_2N_6O_5S$: C, 61.08 (61.10); H, 3.30 (3.33); N, 15.26 (15.27).

*10-(2-hydroxybenzylidene)-6-(2-hydroxyphenyl)-3-(methylthio)-1-oxo-7,8,9,10-tetrahydro-1H-pyrimido[1,2-a] quinoline-2,5-dicarbonitrile (**5g**)*
M.p. 155–160^{o}C; Yield 85%; IR (KBr): 3412 (OH stretching), 3021 (Ar, C–H stretching), 2233 (C≡N), 1684 (C=O), 1619 (C=N) cm-1;1H NMR (300 MHz, DMSO-d6, TMS):δ 11.23 (s, 1H, OH), 11.20 (s, 1H, OH), 8.77 (s,1H, –C=CH), 7.30–8.20 (m, 8H, Ar–H), 2.40 (s, 3H, CH_3), 1.29–2.38 ppm (m, 6H, CH_2 protons of tetrahydroquinoline ring); ^{13}C NMR (100 MHz, DMSO-d6, TMS):δ 170.23, 167.07, 162.60, 158.48, 140.43, 136.22, 132.29, 130.10, 129.34, 128.22, 127.66, 127.13, 123.68, 118.65, 114.56, 87.55, 30.12, 25.32, 24.78, 17.44; Mass (m/z):492 [M^+] (100); Elem. anal. calculated (found) for $C_{28}H_{20}N_4O_3S$: C, 68.28 (68.30); H, 4.09 (4.10); N, 11.37 (11.38).

*10-(4-hydroxybenzylidene)-6-(4-hydroxyphenyl)-3-(methylthio)-1-oxo-7,8,9,10-tetrahydro-1H-pyrimido[1,2-a] quinoline-2,5-dicarbonitrile (**5h**)*
M.p. 157–162^{o}C; Yield 88%; IR (KBr): 3423 (OH stretching), 3016 (Ar, C–H stretching), 2243 (C≡N), 1688 (C=O), 1628 (C=N) cm-1;1H NMR (300 MHz, DMSO-d6, TMS):δ 11.26 (s, 1H, OH), 11.24 (s, 1H, OH), 8.86 (s,1H, –C=CH), 7.21–8.55 (m, 8H, Ar–H), 2.46 (s, 3H, CH_3), 1.33–2.43 ppm (m, 6H, CH_2 protons of tetrahydroquinoline ring); ^{13}C NMR (100 MHz, DMSO-d6, TMS):δ 171.12, 167.15, 162.65, 158.56, 140.49, 136.34, 132.45, 130.17, 129.65, 128.28, 127.60, 127.15, 123.56, 118.16, 114.45, 87.44, 30.31, 25.00, 24.55, 17.37; Mass (m/z):492 [M^+] (100); Elem. anal. calculated (found) for $C_{28}H_{20}N_4O_3S$: C, 68.28 (68.29); H, 4.09 (4.11); N, 11.37 (11.41).

*10-(3,4-dichlorobenzylidene)-6-(3,4-dichlorophenyl)-3-(methylthio)-1-oxo-7,8,9,10-tetrahydro-1H-pyrimido[1,2-a] quinoline-2,5-dicarbonitrile (**5i**)*
M.p. 160–165^{o}C; Yield 90%; IR (KBr): 3027 (Ar, C–H stretching), 2241 (C≡N), 1687 (C=O), 1627 (C=N) cm-1;1H NMR (300 MHz, DMSO-d6, TMS):δ 8.75 (s,1H, –C=CH), 7.26–8.22 (m, 8H, Ar–H), 2.44 (s, 3H, CH_3), 1.20–2.34 ppm (m, 6H, CH_2 protons of tetrahydroquinoline ring); ^{13}C NMR (100 MHz, DMSO-d6, TMS):δ 170.42, 167.34, 160.55, 156.22, 141.10, 136.45, 132.83, 130.34, 129.12, 128.56, 127.11, 127.68, 123.43, 117.55, 114.19, 87.42, 30.11, 25.00, 24.34, 17.53; Mass (m/z):550 [M^+] (100); Elem. anal. calculated (found) for $C_{28}H_{16}Cl_4N_4OS$: C, 56.21 (56.22); H, 2.70 (2.72); N, 9.36 (9.36).

BIOLOGY

Antibacterial activity

The antimicrobial activity of the synthesized compounds **5a-i** were calculated by the agar diffusion mode [24,25], the compounds were evaluated for antibacterial activity against *Escherichia coli* (MTCC 443), *Staphylococcus aureus* (MTCC 96). The antibiotic Ampicillin (25µg/mL) was used as a standard for antibacterial activity.

The culture strains of the bacteria were cultivated on nutrient agar slants at 37 ± 0.5 ^{o}C for 24 h. The antibacterial activity was evaluated using nutrient agar plates seeded with 0.1 ml of the respective bacterial culture strain suspension prepared in sterile saline (0.85 %) at 10^5 CFU/mL dilutions. The stock solutions were made by diluting compounds in DMSO to final concentrations ranging from 25 to 100 µg/mL. The wells, of 6 mm diameter, were filled with 0.1 ml of the compound solution separately for each bacterial strain. All the plates were incubated at 37 ± 0.5 ^{o}C for 24 h. Zones of inhibition of compounds in mm and MIC were noted. The results of antibacterial activity given in **Table-2**.

Antifungal activity

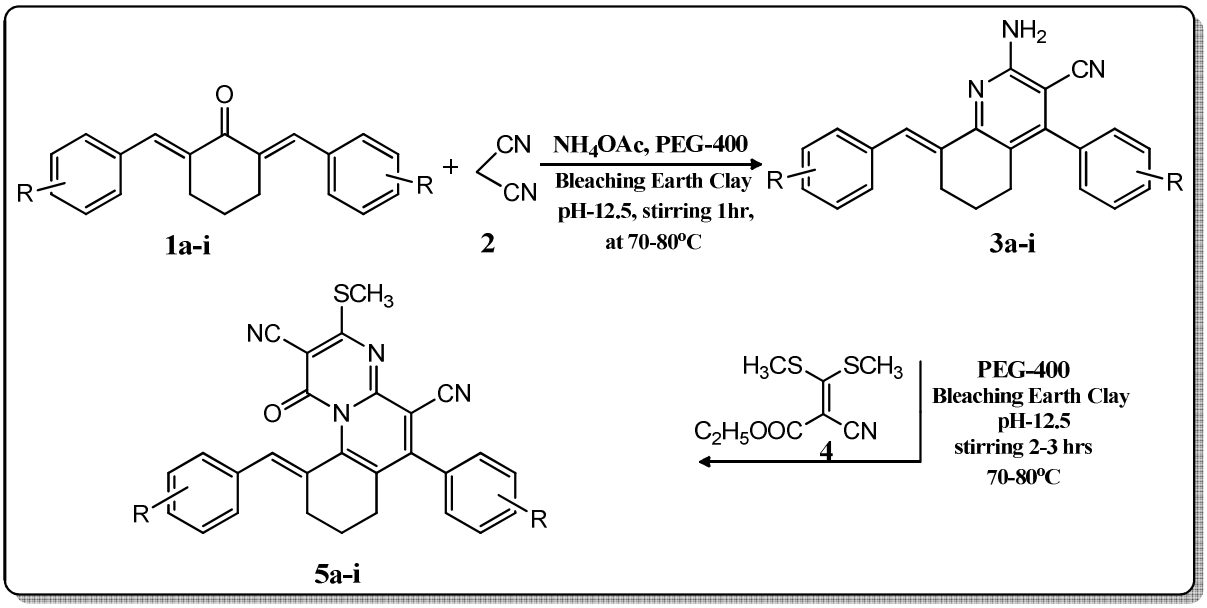
The synthesized compounds were assessed for antifungal activity by disc diffusion method [26]. The fungal (48 h) cultures from the slants were diluted with sterile water and mixed carefully to formulate a fair homogeneous suspension. These suspensions were dispersed on solidified agar, potato dextrose agar for fungi. The filter paper disks prepared by only DMSO (as a negative control) and with a solution of 50 µg/ml concentrations of test compounds (**5a-i)** as well as standard compounds Nystatin as positive control were precisely arranged over the spread cultures of fungi and incubated at 28-30 ^{o}C for 48 h. After the incubation duration, the plates were tested for the zone of inhibition. The diameter for the zones of inhibition was measured, with the diameter of the disk too. All the concentrations were made in triplicate for each of the compounds and the average value was taken. The antifungal activity was checked against A. *Niger, C. Albicans*, (fungal strains) using Nystatin as the standard drug.

RESULTS AND DISCUSSION

Chemistry

Encouraged by the varied biological activities of pyrimido[1,2-a]quinoline and as a part of our program aimed at developing new selective and environmentally friendly methodologies for the preparation of heterocyclic compounds [27-29], Herein, we report a green, effective, and clean procedure for synthesis of 7,8,9,10-tetrahydro-1H-pyrimido[1,2-a]quinoline-2,5-dicarbonitrile derivatives (**5a-i**) via two step reaction. The first step involves one pot synthesis of intermediate product (**3a-i**) by the reaction of 2,6-bis(2/3/4-substituted benzylidene)cyclohexanone (**1**), malononitrile (**2**), ammonium acetate and Bleaching earth clay (pH12.5, 10 wt%) in PEG-400 as green reaction media. Further step involves reaction of intermediate product (**3a-i**) and 2-cyano-3,3-bis(methylthio)acrylate (**4)** in the presence of Bleaching earth clay (pH12.5, 10 wt%) and PEG-400 gives the final products (**5a-i**). The 2-cyano-3,3-bis(methylthio)acrylate (**4**) was synthesized by earlier reported method. The reaction time, yield and melting point of these derivatives (**5a-i**) has been presented in **Table-1**. The reactions ensued rapidly and completed within 2-3 hrs.

The newly synthesized intermediate compounds (**3a-i**) and final compounds (**5a-i**) were established on the basis of spectroscopic methods (IR, ^{1}HNMR, ^{13}C-NMR and MASS). The compound **3b** were confirmed by IR spectra showed the presence of $-NH_2$ stretching at 3343 $cm^{-1}$and C≡N stretch at 2228 cm^{-1} indicating the formation of product. Furthermore, 1HNMR shows, a sharp singlet at δ 8.73 ppm for the proton of -C=CH, the NH_2 protons appear in the aromatic region i.e δ 8.11-7.65 ppm. A multiplate of six protons of tetrahydroquinoline ring appears at δ 1.40–2.48 ppm. On the other hand IR spectra of the final compound **5b** showed the absence of the characteristic band off $-NH_2$ in the range of 3200-3400 cm^{-1}, and C≡N stretch appear at 2251 cm^{-1}. In proton NMR spectra, a sharp singlet appears at δ 8.87 ppm due to the proton of –C=CH. Characteristic singlet of three protons of CH_3 appears at δ 2.48 ppm confirms the formation of cyclized final product. Mass spectrum revealed that the molecular weight of the compound was corresponds to the molecular ion peaks. The synthetic procedure and characterization data of compounds were presented in the experimental section.



Scheme 1: Green synthesis of 7,8,9,10-tetrahydro-1H-pyrimido[1,2-a]quinoline-2,5-dicarbonitrile derivatives (5a-i)

Table 1: The physicochemical data of synthesized 7,8,9,10-tetrahydro-1H-pyrimido[1,2-a]quinoline-2,5-dicarbonitrile derivatives (5a-i)

Entry	R	M.P °C	[a]Yield %
5a	-H	150-155	89
5b	-4F	160-165	87
5c	-4Cl	162-167	85
5d	-2Cl	165-170	90
5e	-4NO_2	175-180	86
5f	-3NO_2	175-180	80
5g	-2OH	155-160	85
5h	-4OH	157-162	88
5i	-3,4-diCl	160-165	90

[a]Yields on isolated basis

BIOLOGY

The newly synthesized 7,8,9,10-tetrahydro-1H-pyrimido[1,2-a]quinoline-2,5-dicarbonitrile derivatives (**5a-i**) displayed a varying pattern of antimicrobial activity, results are shown in **Table 2**. A cursory look at the results of *in vitro* antibacterial activity reveals that most of the synthesized compounds **(5a-i)** exhibited equipotent activity in comparison with standard drug against *E. coli* and *S. aureus* (bacterial strain). Here ampicillin is employed as a reference drug for antibacterial activity. Hence, the present study is favorable for discovering the lead compounds against bacterial diseases.

The newly synthesized 7,8,9,10-tetrahydro-1H-pyrimido[1,2-a]quinoline-2,5-dicarbonitrile derivatives (**5a-i**) exhibited finest antifungal activity compared with the standard drug (Nystatin, 50 µg/ml concentration), the observed results have been presented in **Table 2**. The preliminary antifungal screening of synthesized derivatives (**5a-i)** acknowledged that maximum compounds in the series showed potent antifungal activity. Therefore, the present study is useful for finding the lead compounds against fungal diseases.

Table 2: Results of antibacterial and antifungal activity of 7,8,9,10-tetrahydro-1H-pyrimido[1,2-a]quinoline-2,5-dicarbonitrile derivatives (5a-i)

	Bacteria		Fungi	
Entry	***Escherichia coli*** *(MTCC443)*	***Staphylococcus aureus*** *(MTCC96)*	***Aspergillus niger*** *(MTCC 282)*	***Candida albicans*** *(MTCC227)*
5a	13(50)	16(50)	14(50)	14(50)
5b	14(25)	23(25)	18(25)	20(25)
5c	18(25)	24(25)	19(25)	22(25)
5d	21(25)	22(25)	20(25)	21(25)
5e	20(25)	21(25)	18(25)	19(25)
5f	19(25)	16(25)	17(25)	15(25)
5g	14(50)	19(50)	15(50)	14(50)
5h	15(50)	15(50)	14(50)	15(50)
5i	15(25)	17(25)	16(25)	16(25)
Ampicillin	20(25)	24(25)	-	-
Nystatin	-	-	20(25)	22(25)

Zones of inhibition measured in mm; MIC values (µg/ml) are given in parentheses

CONCLUSION

In the present study, a series of 7,8,9,10-tetrahydro-1H-pyrimido[1,2-a]quinoline-2,5 dicarbo- nitrile derivatives (**5a-i**) were synthesized by using a green catalytic system i.e PEG-400 and Bleaching earth clay (pH-12.5, 10 wt %) and screened for antibacterial and antifungal activities. The structures of the compounds were established on the basis of satisfactory spectral analysis. Most of the synthesized 7,8,9,10-tetrahydro-1H-pyrimido[1,2-a]quinoline-2,5 dicarbonitrile derivatives (**5a-i**) showed significant antibacterial and antifungal activity against *E.coli, S.aureus* (bacterial strain) and *Aspergillus niger, Candida albicans* (fungal strain) respectively, compared with standard drugs.

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REFERENCES

[1] A Dlugosz; D Dus, *Farmaco*., **1996**, 51, 364-374.
[2] AA Joshi; CL Viswanathan, *Med Chem*., **2006**, 5, 105-122.
[3] AA Joshi; SS Narkhede; CLViswanathan, *Bioorg Med Chem Lett*., **2005**, 15, 73-76.
[4] ABA El-Gazzar; MM El-Enany; MN Mahmoud, *Bioorg Med Chem*., **2008**, 16, 3261-3273.
[5] T Suresh; RN Kumar; S Magesh; PS Mohan, *Ind J Chem B*., **2003**, 42, 2133–2135.
[6] ST Selvi; V Nadaraj; S Mohan; R Sasi; M Hema, *Bioorg Med Chem*., **2006**, 14, 3896-3903.
[7] OA El-Sayed; FM El-Bieh; BA Al-Bassam, *Boll. Chim. Farm*., **2002**, 141, 461-465.
[8] OA El-Sayed; BA Al-Bassam; ME Hussein, *Arch. Pharm. (Weinheim).*, **2002**, 335, 403-410.
[9] MS Al-Said; MM Ghorab; MS Al-Dosari; MM Hamed, *Eur J Med Chem*., **2011**, 46, 201-207.
[10] HAS Abbas; HN Hafez; A-RBA El-Gazzar, *Eur J Med Chem*., **2011**, 46, 21-30.
[11] MM Ghorab; FA Ragab; HI Heiba; RK Arafa; EM El-Hossary, *Eur J Med Chem*., **2010**, 45, 3677–3684.
[12] OA El-Sayed;TM Al-Turki; HM Al-Daffiri; BA Al-Bassam; ME Hussein, *Boll. Chim. Farm*., **2004**, 143, 227-238.
[13] ABA El-Gazzar; MM El-Enany; MN Mahmoud, *Bioorg Med Chem*., **2008**, 16, 3261-3273.
[14] OI Abd El-Salam; DA Abou El Ella; NSM Ismail; M Abdullah, *Pharmazie*., **2009**, 64,147–155.
[15] M Kidwai; N Negi, *Monatash Chem*., **1997**, 128, 85–89.
[16] ABA El-Gazzar; HN Hafez; AA Abu-Hashem; AS Aly, *Phosphorus Sulfur Silicon Relat Elem*., **2009a**, 184, 379–405.
[17] BS Dawane; SG Konda; GG Mandawad; BM Shaikh, *Eur. J. Med. Chem*., **2010,** 45, 387-392.
[18] SG Konda; BM Shaikh; SA Chavan; BS Dawane, *Chin. Chem. Letters*., **2011**, 22, 65-68.
[19] AP Acharya; RD Kamble; SD Patil; SV Hese; OS Yemul; SG Patil; SN Halale; BS Dawane, *Chem Papers*., **2014**, 68(5), 719.
[20] RD Kamble; GV Jawadwar; SD Patil; SV Hese; AP Acharya; BS Dawane; SS Pekamwar, *Org. Commun*., **2013**, 6(2), 95.
[21] AP Acharya; RD Kamble; SD Patil; SV Hese; OS Yemul; BS Dawane, *Res. Chem. Intermed*., **2013**, doi:10.1007/s11164-013-1403-x22.
[22] MV Gaikwad; RD Kamble; SV Hese; AP Acharya; PP Mogle; SN Kadam; BS Dawane, *Res.Chem. Intermed*., **2014**, doi:10.1007/s11164-014-1559-z.
[23] RD Kamble; BS Dawane; OS Yemul; AB Kale; SD Patil, *Res. Chem. Intermed*., **2013**, 39, 3859-3866.
[24] National Committee for Clinical Laboratory Standards, Methods for Determining Bactericidal Activity of Antimicrobial Agents: Tentative Guidelines,Villanova (PA), NCCLS; (Publication no NCCLS M 26-T), **1992** .
[25] National Committee for Clinical Laboratory Standards, Methods forAntimicrobial Susceptibility Testing Anaerobic Bacteria, Approved Standard-Fourth Edition, Villanova (PA), NCCLS; (Publication no NCCLS M 11–A4), **1997**.
[26] RD Biljana; SR Niko; SD Vidoslav; RD Vukicevic; RM Palic, *Molecules*., **2010,** 15, 2246-2256.
[27] SS Chobe; RD Kamble; SD Patil; AP Acharya; SV Hese; OS Yemul; BS Dawane, *Med.Chem. Res*., **2013**, doi:10.1007/s00044-013-0487-6.
[28] SS Chobe; BS Dawane; KM Tumbi; PP Nandekar; AT Saganwar, *Bioorg. Med. Chem. Lett*., **2012**, 22(24), 7566–7572.
[29] PP Mogle; RD Kamble; SV Hese; BS Dawane, *Res ChemIntermed*., **2014**, DOI 10.1007/s11164-014-1842-z.