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Eco-Friendly Synthesis of Solid-Support *Bis*-Dihydropyrimidines and their Antimicrobial Studies

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This article is aimed at the synthesis of new *bis*-dihydropyrimidines using solid-support. The reactions were carried out using microwave energy. Presented protocol is associated with higher yields of desired products, easier work-up conditions, less reaction times, higher reproducibility and maximum efficiency of method in comparison to conventional heating. The structures of prepared moieties were confirmed by spectroscopic techniques. Further, these *bis*-heterocycles were also examined for their biological behaviours. Agar well diffusion method was used to carry out *in-vitro* antimicrobial evaluations. Compound **6j** came forward as most active antibacterial and **6b** proved itself to be the most potent antifungal agent.

Keywords: *Bis*-Dihydropyrimidines, Solid-support, Microwave-irradiations, Antimicrobial studies.

INTRODUCTION

In environmentally conscious days, incorporation of pollution-prevention techniques is gaining popularity. It involves preparation of chemicals and their utilization in such a way so as to eliminate or reduce their unpleasant influence on the environment. One of such practices involve the use of microwave-irradiations as source of energy. Microwave energy is engaged in carrying out chemical transformations in greener way. Microwave-assisted reactions occur more rapidly in ecofriendly manner giving higher yields of desired products. Additional advantageous facts are associated with these reactions, when carried out by using solid-support in the absence of any solvent. These days, this technology has attracted much attention as it involves higher selectivity of reactions, easy separation of desired products from reaction-mixture, easy handling as well as re-usability of solid-support.

Across the world, wide range of heterocyclic compounds are being synthesized with desire to develop medicinally significant moieties. Pyrimidine is an important scaffold from pharmaceutical point of view. This nitrogen containing heterocyclic motif is associated with broad spectrum of biological activities such as antimicrobial [1-3], antitumor [4,5], antioxidant [6], antitubercular [7,8] sedative [9], anti-inflammatory [10,11]. Further, dihydropyrimidines have proved to possess coronary-vasodilating activity [12]. Dihydropyrimidines are the bio-isosters of dihydropyridines; which show remarkable Ca-channel blocking activity.

In order to carry out organic synthesis in such a way so as to cause least or no harm to ecology, we had prepared and reported the various biologically significant heterocycles using solid-support in our laboratory in previous years [13,14].

Keeping in mind the above mentioned facts and in continuity of our research towards development of new pyrimidine derivatives by green approach, we herein report new *bis*-heterocycles using suitable linker. These new *bis* moieties have been developed using silica-gel as solid-support. No costly catalyst and toxic solvent has been used to carry out these reactions.

The prepared compounds have also been evaluated for their antimicrobial behaviours.

EXPERIMENTAL

Melting points were determined on open end capillary M.P. apparatus and are uncorrected. All synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, ESI-MS, elemental analyses. IR spectra were recorded as potassium bromide pellets on Perkin Elmer spectrum RX IFT-IR System. ^{1}H-NMR and ^{13}C-NMR spectra were determined on BRUKER AVANCE II 400 NMR spectrometer. Chemical shifts were expressed as

parts per million; (δ values, ppm) relative tetetramethylsilane as internal standard. The mass spectra were obtained on Q-TOF MICROMASS (LC-MS) and the elemental analyses were recorded on VARIO MICRO CHNS Analyzer. The synthesized compounds gave satisfactory results within ± 0.4 % of theoretical values. Analytical thin layer chromatography (TLC) was employed to follow course of reaction and to check the purity of products, on silica gel (60, GF254, Merck) using glass plates. The spots were visualized by exposure to iodine vapours. The biological evaluation of the synthesized compounds was conducted at Biogenic Research and Training Centre in Biotechnology, Hubli, Karnatka.

Synthesis: The entitled *bis*-pyrimidine derivatives were prepared by the following two steps:

Step-1: Preparation of 6-methyl-4-(substituted phenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-carboxylic acid ethyl ester (4a-j) [15]**:** An equimolar mixture of ethylacetoacetate (0.01 mol), suitable aromatic aldehyde (0.01 mol) and thiourea (0.01 mol) was irradiated under microwave-irradiations using absolute alcohol in presence of conc. HCl as catalyst. The progress of reaction was followed by thin layer chromatography. On assurance of completion of reaction, the reaction mixture was kept undisturbed for 24-36 h. Thereafter, the solid thus separated was isolated using absolute alcohol and purified using suitable solvent.

Step-2: Preparation of diethyl 2,2'-(but-2-yne-1,4-diyl*bis*(sulfanediyl))*bis*(6-methyl-4-(substitutedphenyl)-1,4-dihydropyrimidine-5-carboxylate): The mixture containing 1,2,3,4-tetrahydropyrimidines **4(a-j)** (0.02 mol) and 1,4-dichloro-but-2-yne **5** (0.01 mol); thoroughly mixed with preconditioned silica gel as solid-support was irradiated under microwave-irradiations in an open borosil beaker. Thin layer chromatography was employed to follow the course of reaction. Ethyl acetate and benzene in specific ratios were used as eluting solvent. The spots were visualized using iodine vapours in an iodine chamber. After the completion of reaction, the desired product was isolated by extraction using ethyl acetate and alcohol. The solid was then recrystallized using suitable solvent.

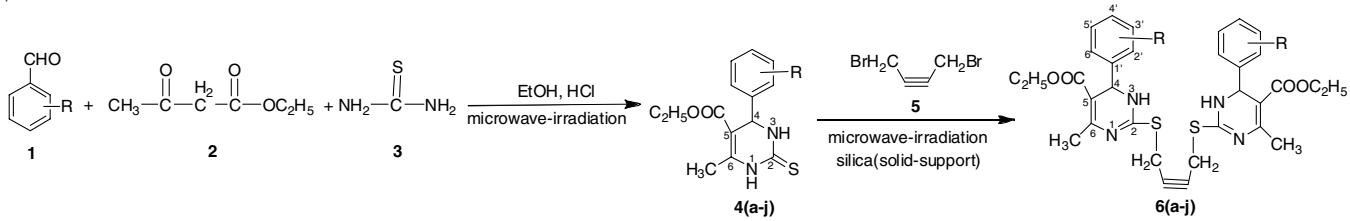
The synthetic methodology has been shown in **Scheme-I**.

Spectral data

Diethyl-2,2'-(but-2-yne-1,4-diyl*bis*(sulfanediyl))*bis*(6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate) (6a): Yellow solid (MeOH + $CHCl_3$); yield (0.70 g, 70 %); m.p. 184-186 °C. IR (KBr, ν_{max}, cm^{-1}): 3324 (N-H), 3069 (aromatic C-H), 2989, 2872 (methyl C-H), 2153 (C≡C), 1689 (C=O), 1681 (C=N), 1602 (C=C), 1467 (C=C skeletal vibr.). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ, ppm): 10.18 (2H, brs, N-H), 7.83-7.69 (10, m, H- 2', 3', 4', 5', 6'), 5.02 (2H, s, H-4), -C$\boldsymbol{H}_3$), 4.68 (2H, d, S-C$\boldsymbol{H}_a$-), 4.59 (2H, d, S-C$\boldsymbol{H}_b$-), 4.08 (4H, q, O-C$\boldsymbol{H}_2$-CH_3), 2.32 (6H, s, C$\boldsymbol{H}_3$-6), 1.08 (6H, t, O-CH_2-). ^{13}C-NMR (100 MHz, DMSO-d_6) (δ, ppm): 169.02 (C=O), 153.65 (C-2), 147.02 (C-6), 139.65 (C-4'), 131.23 (C-1'), 129.82 (C-3',5'), 127.65 (C-2',6'), 101.03 (C-5), 81.02 (C≡C), 60.04 (O-$\boldsymbol{C}H_2$-CH_3), 55.23 (C-4), 34.97 (S-$\boldsymbol{C}H_2$-), 16.75 (-$\boldsymbol{C}H_3$), 14.78 (O-CH_2-$\boldsymbol{C}H_3$). ESI-MS: (*m/z*): 602 [M^+]. Anal. calcd. (%) for $C_{32}H_{34}N_4O_4S_2$: C, 63.76; H, 5.69; N, 9.29; O, 10.62; S, 10.64, Found (%): C, 64.16; H, 6.09; N, 8.89; O, 10.22; S, 10.24.

Diethyl-2,2'-(but-2-yne-1,4-diyl*bis*(sulfanediyl))*bis*(6-methyl-4-(3'-nitrophenyl)-1,4-dihydropyrimidine-5-carboxylate) (6b): Yellow solid (MeOH + $CHCl_3$); yield (0.76 g, 76 %); m.p. 156-158 °C. IR (KBr, ν_{max}, cm^{-1}): 3300 (N-H), 3065 (aromatic C-H), 2962, 2823 (methyl C-H), 2148 (C≡C), 1685 (C=O), 1682 (C=N), 1600 (C=C), 1523 (antisymmetric NO_2), 1461 (C=C skeletal vibr.), 1346 (symmetric NO_2). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ, ppm): 9.58 (2H, brs, N-H), 7.62 (2H, d, H-4', J_o = 8.02 Hz), 7.55 (2H, d, H-2', J_m = 2.02 Hz), 7.32 (2H, dd, H-6', $J_{o,m}$ = 7.68 Hz, 3.20 Hz), 7.06 (2H, d, H-5', J_p = 0.82 Hz), 4.75 (2H, s, H-4), 4.69 (2H, d, S-C$\boldsymbol{H}_a$-), 4.50 (2H, d, S-C$\boldsymbol{H}_b$-), 4.24 (4H, q, O-C$\boldsymbol{H}_2$-CH_3), 2.16 (6H, s, C$\boldsymbol{H}_3$-6), 1.04 (6H, t, O-CH_2-C$\boldsymbol{H}_3$). ^{13}C-NMR (100 MHz, DMSO-d_6) (δ, ppm): 165.90 (C=O), 164.80 (C-2), 147.35 (C-6), 145.54 (C-3'), 141.04 (C-1'), 135.23 (C-4'), 134.93 (C-2'), 132.01 (C-6'), 129.89 (C-5'), 101.06 (C-5), 81.03 (C≡C), 61.01 (O-$\boldsymbol{C}H_2$-CH_3), 55.04 (C-4), 34.29 (S-$\boldsymbol{C}H_2$-), 16.18 (-$\boldsymbol{C}H_3$), 14.15 (O-CH_2-$\boldsymbol{C}H_3$). ESI-MS: (*m/z*): 692 [M^+]. Anal. calcd. (%) for $C_{32}H_{32}N_6O_8S_2$: C, 55.48; H, 4.66; N, 12.13; O, 18.48; S, 9.26, Found (%): C, 55.88; H, 4.26; N, 12.53; O, 18.08; S, 9.66.

Diethyl-2,2'-(but-2-yne-1,4-diyl*bis*(sulfanediyl))*bis*(6-methyl-4-(4'-nitrophenyl)-1,4-dihydropyrimidine-5-carboxylate) (6c): Brown solid (MeOH + $CHCl_3$); yield (0.67 g, 67 %); m.p. 140-142 °C. IR (KBr, ν_{max}, cm^{-1}): 3299 (N-H), 3072 (aromatic C-H), 2965, 2821 (methyl C-H), 2163 (C≡C), 1690 (C=N), 1685 (C=O), 1600 (C=C), 1526 (antisymmetric NO_2), 1469 (C=C skeletal vibr.), 1365 (symmetric NO_2). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ, ppm): 9.62 (2H, brs, N-H), 7.84 (4H, d, H-3',5', J_o = 7.62 Hz), 7.35 (4H, d, H-2',6', J_o = 7.62 Hz), 5.51 (2H, s, H-4), 4.82 (2H, d, S-C$\boldsymbol{H}_a$-), 4.52 (2H, d, S-C$\boldsymbol{H}_b$-), 4.08 (4H, q, O-C$\boldsymbol{H}_2$-CH_3), 2.32 (6H, s, C$\boldsymbol{H}_3$-6), 1.06 (6H, t, O-CH_2-C$\boldsymbol{H}_3$).^{13}C-NMR (100 MHz, DMSO-d_6) (δ, ppm): 167.09 (C=O), 165.12 (C-2), 163.62 (C-6), 155.55 (C-4'), 149.02 (C-1'), 135.79 (C-3',5'), 129.62 (C-2',6'), 102.08 (C-5), 83.05 (C≡C), 60.42 (O-$\boldsymbol{C}H_2$-CH_3), 55.26 (C-4), 34.23 (S-$\boldsymbol{C}H_2$-), 18.64 (-$\boldsymbol{C}H_3$), 14.65 (O-CH_2-$\boldsymbol{C}H_3$). ESI-MS: (*m/z*): 693 [M + 1]. Anal. calcd. (%) for $C_{32}H_{32}N_6O_8S_2$: C, 55.48; H, 4.66;



where R= a.H, b. 3-NO_2, c.4-NO_2, d. .2,4-Cl, e.2-OH, f.4-OH, g.4-OH,3-OCH_3, h.4-OCH_3, i.3,4-OCH_3 j.2,3-OCH_2O

Scheme-I

N, 12.13; O, 18.48; S, 9.26, Found (%): C, 55.88; H, 4.26; N, 12.53; O, 18.08; S, 8.86.

Diethyl-2,2'-(but-2-yne-1,4-diyl*bis*(sulfanediyl))*bis*(4-(2',4'-dichlorophenyl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate) (6d): White solid (EtOH); yield (0.73 g, 73 %); m.p. 152-154 °C. IR (KBr, ν_{max}, cm^{-1}): 3305 (N-H), 3087 (aromatic C-H), 2923, 2853 (methyl C-H), 2149 (C≡C), 1705 (C=O), 1687 (C=N), 1599 (C=C), 1465 (C---C skeletal vibr.). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ, ppm): 9.12 (2H, brs, N-H), 7.80 (2H, d, H-6', J_o = 7.82 Hz), 7.53 (2H, d, H-5', J_m = 2.21 Hz), 7.29 (2H, d, H-3', J_p = 0.76 Hz), 5.02 (2H, s, H-4), 4.50 (2H, d, S-CH_a-), 4.46 (2H, d, S-CH_b-), 4.04 (4H, q, O-CH_2-CH_3), 2.41 (6H, s, CH_3-6), 1.10 (6H, t, O-CH_2-CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) (δ, ppm): 164.98 (C=O), 154.31 (C-2), 145.99 (C-6), 139.32 (C-1'), 138.85 (C-6'), 137.63 (C-5'), 135.04 (C-3'), 131.92 (C-4'), 128.72 (C-2'), 101.05 (C-5), 82.96 (C≡C), 61.08 (O-*C*H_2-CH_3), 54.91 (C-4), 35.35 (S-*C*H_2-), 18.97 (-*C*H_3), 13.95 (O-CH_2-*C*H_3). ESI-MS: (*m/z*): 740 [M^+]. Anal. calcd. (%) for $C_{32}H_{30}N_4O_4S_2Cl_4$: C, 51.90; H, 4.08; Cl, 19.15; N, 7.57; O, 8.64; S, 8.66, Found (%): C, 51.50; H, 4.48; Cl, 19.55; N, 7.97; O, 8.24; S, 8.26.

Diethyl-2,2'-(but-2-yne-1,4-diyl*bis*(sulfanediyl))bis(4-(2'-hydroxyphenyl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate) (6e): White solid (EtOH); yield (0.79 g, 79 %); m.p. 188-190 °C. IR (KBr, ν_{max}, cm^{-1}): 3340 (O-H), 3300 (N-H), 3065 (aromatic C-H), 2962, 2823 (methyl C-H), 2225 (C≡C), 1685 (C=O), 1682 (C=N), 1600 (C=C), 1461 (C---C skeletal vibr.). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ, ppm): 12.83 (2H, brs, O-H), 12.83 (2H, brs, N-H), 7.40 (4H, dd, H-4',6', J_o = 7.56 Hz, 3.12 Hz), 6.83 (2H, t, H-5', $J_{o,o}$ = 7.76 Hz, 7.68 Hz), 6.75 (2H, dd, H-3', $J_{o,m}$ = 7.68 Hz, 2.00 Hz,), 7.06 (2H, d, H-5', J_p = 0.82 Hz), 5.54 (2H, s, H-4), 5.18 (2H, d, S-CH_a-), 4.51 (2H, d, S-CH_b-), 4.07 (4H, q, O-CH_2-CH_3), 2.29 (6H, s, CH_3-6), 1.11 (6H, t, O-CH_2-CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) (δ, ppm): 163.93 (C=O), 159.87 (C-2), 147.53 (C-6), 143.83 (C-2'), 138.54 (C-1'), 134.94 (C-4'), 133.78 (C-6'), 129.37 (C-5'), 126.67 (C-3'), 101.18 (C-5), 81.32 (C≡C), 60.48 (O-*C*H_2-CH_3), 53.76 (C-4), 34.86 (S-*C*H_2-), 16.83 (-*C*H_3), 14.15 (O-CH_2-*C*H_3). ESI-MS: (*m/z*): 634 [M^+]. Anal. calcd. (%) for $C_{32}H_{34}N_4O_6S_2$: C, 60.55; H, 5.40; N, 8.83; O, 15.12; S, 10.10, Found (%): C, 60.95; H, 5.80; N, 9.23; O, 15.52; S, 10.50.

Diethyl-2,2'-(but-2-yne-1,4-diyl*bis*(sulfanediyl))*bis*(4-(4'-hydroxyphenyl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate) (6f): Light yellow solid (EtOH); yield (0.75 g, 75 %); m.p. 202-204 °C. IR (KBr, ν_{max}, cm^{-1}): 3491 (O-H), 3301 (N-H), 3066 (aromatic C-H), 2938, 2875 (methyl C-H), 2162 (C≡C), 1684 (C=O), 1691 (C=N), 1598 (C=C), 1455 (C---C skeletal vibr.). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ, ppm): 12.89 (2H, brs, O-H), 12.25 (2H, brs, N-H), 7.62 (4H, d, H-2',6', J_o = 8.12 Hz), 6.60 (4H, d, H-3',5', J_o = 8.12 Hz), 4.82 (2H, s, H-4), 4.54 (2H, d, S-CH_a-), 4.49 (2H, d, S-CH_b-), 4.10 (4H, q, O-CH_2-CH_3), 2.24 (6H, s, CH_3-6), 1.12 (6H, t, O-CH_2-CH_3).^{13}C-NMR (100 MHz, DMSO-d_6) (δ, ppm): 164.09 (C=O), 159.70 (C-2), 147.75 (C-6), 147.24 (C-4'), 143.57 (C-1'), 130.70 (C-2',6'), 129.47 (C-3',5'), 104.57 (C-5), 83.23 (C≡C), 60.46 (O-*C*H_2-CH_3), 55.54 (C-4), 34.97 (S-*C*H_2-), 16.81 (-*C*H_3), 13.91 (O-CH_2-*C*H_3). ESI-MS: (*m/z*): 636 [M + 2]. Anal. calcd. (%) for $C_{32}H_{34}N_4O_6S_2$: C, 60.55; H, 5.40; N, 8.83; O, 15.12; S, 10.10, Found (%): C, 60.95; H, 5.00; N, 8.43; O, 15.52; S, 10.50.

Diethyl-2,2'-(but-2-yne-1,4-diyl*bis*(sulfanediyl))*bis*(4-(4'-hydroxy-3'-methoxyphenyl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate) (6g): Light yellow solid (EtOH); yield (0.76 g, 76 %); m.p. 198-200 °C. IR (KBr, ν_{max}, cm^{-1}): 3408 (O-H), 3321 (N-H), 3073 (aromatic C-H), 2923, 2856 (methyl C-H), 2125 (C≡C), 1698 (C=O), 1682 (C=N), 1598 (C=C), 1459 (C---C skeletal vibr.). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ, ppm): 12.82 (2H, brs, O-H), 12.47 (2H, brs, N-H), 7.34 (2H, d, H-5', J_p = 0.62 Hz), 7.27 (2H, d, H-6', J_o = 7.72 Hz), 6.77 (2H, d, H-2', J_m = 2.26 Hz), 5.05 (2H, s, H-4), 4.65 (2H, d, S-CH_a-), 4.50 (2H, d, S-CH_b-), 4.06 (4H, q, O-CH_2-CH_3), 3.60 (6H, s, -OCH_3), 2.41 (6H, s, CH_3-6), 1.09 (6H, t, O-CH_2-CH_3).^{13}C-NMR (100 MHz, DMSO-d_6) (δ, ppm): 163.89 (C=O), 159.98 (C-2), 158.29 (C-6), 143.38 (C-4'), 142.90 (C-3'), 134.79 (C-1'), 131.89 (C-5'), 129.35 (C-6'), 128.16 (C-2'), 104.85 (C-5), 82.35 (C≡C), 61.35 (O-*C*H_2-CH_3), 60.42 (-O-*C*H_3), 54.76 (C-4), 34.76 (S-*C*H_2-), 16.78 (-*C*H_3), 13.82 (O-CH_2-*C*H_3). ESI-MS: (*m/z*): 694 [M^+]. Anal. calcd. (%) for $C_{34}H_{38}N_4O_8S_2$: C, 58.77; H, 5.51; N, 8.06; O, 18.42; S, 9.23, Found (%): C, 58.37; H, 5.11; N, 8.46; O, 18.02; S, 9.63.

Diethyl-2,2'-(but-2-yne-1,4-diyl*bis*(sulfanediyl))*bis*(4-(4'-methoxyphenyl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate) (6h): White solid (EtOH); yield (0.72 g, 72 %); m.p. 164-166 °C. IR (KBr, ν_{max}, cm^{-1}): 3299 (N-H), 3089 (aromatic C-H), 2925, 2885 (methyl C-H), 2139 (C≡C), 1695 (C=O), 1682 (C=N), 1603 (C=C), 1462 (C---C skeletal vibr.). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ, ppm): 9.05 (2H, s, N-H), 7.35 (4H, d, H-2',6', J_o = 8.28 Hz), 7.01 (4H, d, H-3',5', J_o = 8.28 Hz), 5.31 (2H, s, H-4), 4.48 (2H, d, S-CH_a-), 4.35 (2H, d, S-CH_b-), 4.28 (4H, q, O-CH_2-CH_3), 3.68 (6H, s, -O-*C*H_3), 2.31 (6H, s, CH_3-6), 1.12 (6H, t, O-CH_2-CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) (δ, ppm): 165.99 (C=O), 165.30 (C-2), 159.23 (C-6), 145.23 (C-4'), 138.29 (C-1'), 129.48 (C-2',6'), 127.65 (C-3',5'), 104.23 (C-5), 83.02 (C≡C), 59.95 (O-*C*H_2-CH_3), 60.04 (-O-*C*H_3), 55.40 (C-4), 35.48 (S-*C*H_2-), 16.92 (-*C*H_3), 14.08 (O-CH_2-*C*H_3). ESI-MS: (*m/z*): 662 [M^+]. Anal. calcd. (%) for $C_{34}H_{38}N_4O_6S_2$: C, 61.61; H, 5.78; N, 8.45; O, 14.48; S, 9.68, Found (%): C, 62.01; H, 5.38; N, 8.05; O, 14.08; S, 9.28.

Diethyl-2,2'-(but-2-yne-1,4-diyl*bis*(sulfanediyl))*bis*(4-(3',4'-dimethoxyphenyl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate) (6i): Dark yellow solid (EtOH); yield (0.73 g, 73 %); m.p. 114-116 °C. IR (KBr, ν_{max}, cm^{-1}): 3323 (N-H), 3086 (aromatic C-H), 2955, 2872 (methyl C-H), 2145 (C≡C), 1700 (C=O), 1681 (C=N), 1598 (C=C), 1467 (C---C skeletal vibr.). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ, ppm): 12.47 (2H, brs, N-H), 7.53 (2H, d, H-5', J_o = 8.28 Hz), 7.25 (2H, d, H-6', J_m = 2.96 Hz), 7.11 (2H, d, H-2', J_m = 2.40 Hz), 4.82 (2H, s, H-4), 4.54 (2H, d, S-CH_a-), 4.49 (2H, d, S-CH_b-), 4.08 (4H, q, O-CH_2-CH_3), 3.60 (6H, s, -O-CH_3), 2.24 (6H, s, CH_3-6), 1.12 (6H, t, O-CH_2-CH_3).^{13}C-NMR (100 MHz, DMSO-d_6) (δ, ppm): 164.06 (C=O), 159.73 (C-2), 157.94 (C-6), 143.46 (C-3'), 139.44 (C-4'), 136.89 (C-1'), 130.37 (C-5'), 129.47 (C-6'), 127.99 (C-2'), 104.79 (C-5), 81.21 (C≡C), 61.00 (O-*C*H_2-CH_3), 60.44 (-O-*C*H_3), 53.65 (C-4), 34.95 (S-*C*H_2-), 16.81 (-*C*H_3), 13.87 (O-CH_2-*C*H_3). ESI-MS: (*m/z*): 772 [M^+]. Anal. calcd.

(%) for $C_{36}H_{42}N_4O_8S_2$: C, 59.82; H, 5.86; N, 7.75; O, 17.71; S, 8.87, Found (%): C, 59.42; H, 5.46; N, 7.35; O, 18.11; S, 9.27.

Diethyl-2,2'-(but-2-yne-1,4-diyl*bis*(sulfanediyl))*bis*(4-(benzo[d][1,3]dioxol-4-yl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate) (6j): Yellow solid (EtOH); yield (0.76 g, 76 %); m.p. 158-160 °C. IR (KBr, ν_{max}, cm^{-1}): 3325 (N-H), 3085 (aromatic C-H), 2925, 2859 (methyl C-H), 2167 (C≡C), 1704 (C=O), 1690 (C=N), 1601 (C=C), 1462 (C⁼⁼⁼C skeletal vibr.). ^{1}H-NMR (400 MHz, DMSO-d_6) (δ, ppm): 12.46 (2H, brs, N-H), 7.37 (2H, d, H-5', J_o = 8.28 Hz), 7.22 (2H, d, H-6', J_o = 2.20 Hz), 7.13 (2H, d, H-4', J_m = 3.68 Hz), 5.97 (4H, s, -O-C***H***$_2$-O), 4.82 (2H, s, H-4), 4.54 (2H, d, S-C***H***$_a$-), 4.46 (2H, d, S-C***H***$_b$-), 4.00 (4H, q, O-C***H***$_2$-CH$_3$), 2.42 (6H, s, C***H***$_3$-6), 1.04 (6H, t, O-CH$_2$-C***H***$_3$). ^{13}C-NMR (100 MHz, DMSO-d_6) (δ, ppm): 163.61 (C=O), 144.81 (C-2), 139.30 (C-6), 138.68 (C-3'), 137.01 (C-2'), 133.98 (C-1'), 133.11 (C-5'), 129.48 (C-6'), 127.98 (C-4'), 104.48 (C-5), 101.35 (-O-***C***H$_2$-O), 82.05 (C≡C), 60.39 (O-***C***H$_2$-CH$_3$), 51.68 (C-4), 34.70 (S-***C***H$_2$-), 16.78 (-***C***H$_3$), 13.73 (O-CH$_2$-***C***H$_3$). ESI-MS: (*m/z*): 609 [M$^+$]. Anal. calcd. (%) for $C_{34}H_{34}N_4O_8S_2$: C, 59.12; H, 4.96; N, 8.11; O, 18.53; S, 9.28, Found (%): C, 59.52; H, 4.56; N, 8.51; O, 18.13; S, 9.68.

The physical data of prepared samples **6(a-j)** has been given in Table-1.

Biological studies

Antimicrobial screenings: The newely synthesized *bis*-dihydropyrimidine derivatives **(6a-6j)** were examined for their *in-vitro* antibacterial behaviour using Gram-negative bacterial strains namely, *Escherichia coli, Pseudomonas aeruginosa* and Gram-positive bacteria; *Bacillus subtilis* and *Staphylococcus aureus* and in the similar manner, *Candida albicans* and *Cladosporium oxysporum* had been used as fungal strains to carry out antifungal activity. Agar well diffusion method [16], using DMSO (dimethyl sulfoxide) solvent as negative control and nutrient agar medium, was performed in inhibition assay to determine the average diameter of inhibition zones (mm) and MIC (minimum inhibition concentration) of bacterial and fungal growth. Ciprofloxacin and fluconazole had been used as standard drugs for screening of antibacterial and antifungal activities, respectively.

Methodology employed [16,17]**:** Initially, the stock cultures of bacteria and fungi were revived by inoculating in broth media and grown at 37 and 27 °C for 18 and 48 h, respectively. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated, with old cultures (100 µL, 10^{-4} cfu) and spread evenly on the plate. After 20 min, the wells were filled with compound at different volumes. All the plates were incubated at 37 °C for 24 h for bacterial and at 27 °C for 96 h, for fungal strains, respectively and the diameter of inhibition zone were noted in mm at various concentrations.

The results of antimicrobial studies carried out have been presented in Tables 2 and 3.

TABLE-1
PHYSICAL CHARACTERIZATION OF COMPOUNDS **6(a-j)**

Compd.	R	Time (MW irrad.)	Colour	m.p. (°C)	m.f.	Yield (%)
6a	H	30 min	Dark yellow	184-186	$C_{32}H_{34}N_4O_4S_2$	70
6b	3 -NO_2	28 min	Yellow	156-158	$C_{32}H_{32}N_6SO_2$	76
6c	4-NO_2	30 min	Dark brown	140-142	$C_{32}H_{32}N_6O_8S_2$	69
6d	2,4-Cl	18 min	White	152-154	$C_{32}H_{30}N_4O_4S_2Cl_4$	73
6e	2-OH	30 min	White	188-190	$C_{32}H_{34}N_4O_6S_2$	79
6f	4-OH	31 min	Yellow	202-204	$C_{32}H_{34}N_4O_6S_2$	75
6g	3-OCH_3, 4-OH	20 min	Yellow	198-200	$C_{34}H_{38}N_4O_8S_2$	76
6h	4-OCH_3	20 min	Yellow	154-156	$C_{34}H_{38}N_4O_6S_2$	72
6i	3,4-OCH_3	15 min 20 s	Yellow	114-116	$C_{36}H_{42}N_4O_8S_2$	73
6j	2,3-OCH_2O	21 min	Yellow	158-160	$C_{34}H_{34}N_4O_8S_2$	76

TABLE-2
in-vitro ANTIMICROBIAL SCREENING OF *BIS*-DIHYDROPYRIMIDINES **(6a-j)** USING AGAR WELL DIFFUSION METHOD

Compounds	Zone of inhibition (mm)																	
	Strains tested																	
	B. subtilis			*E. coli*			*P. aeruginosa*			*S. aureus*			*C. albicans*			*C. oxysporum*		
Conc. (µg/mL)	150	300	600	150	300	600	150	300	600	150	300	600	150	300	600	150	300	600
6a	–	–	–	–	–	13	–	–	–	–	–	14	–	–	–	–	–	–
6b	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8	–	–	9
6c	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	6
6d	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	7
6e	–	–	19	–	–	19	–	–	19	–	–	19	–	–	–	–	–	–
6f	–	–	17	–	–	16	–	–	16	–	–	17	–	–	–	–	–	–
6g	–	–	–	–	–	15	–	–	17	–	–	–	–	–	–	–	–	–
6h	–	–	13	–	–	–	–	–	–	–	–	15	–	–	5	–	–	7
6i	–	–	16	–	–	18	–	–	18	–	–	18	–	–	–	–	–	–
6j	–	–	20	–	–	21	–	–	19	–	–	20	–	–	–	–	–	–
Ciprofloxacin	24	26	28	26	28	30	23	25	27	25	27	29	–	–	–	–	–	–
Fluconazole	–	–	–	–	–	–	–	–	–	–	–	–	9	13	15	5	7	12

(–) Denotes activity not found.

TABLE-3
in-vitro MINIMUM INHIBITORY CONCENTRATION (MIC, μg/mL) OF
BIS-DIHYDROPYRIMIDINES **(6a-j)** DETERMINED BY AGAR WELL DIFFUSION METHOD

Compd. tested	*B. subtilis*	*E. coli*	*P. aeruginosa*	*S. aureus*	*C. albicans*	*C. oxysporum*
6a	Not found	800	Not found	800	Not found	Not found
6b	Not found	Not found	Not found	Not found	100	50
6c	Not found	Not found	Not found	Not found	800	400
6d	Not found	Not found	Not found	Not found	400	200
6e	200	200	200	200	Not found	Not found
6f	400	800	800	400	Not found	Not found
6g	Not found	800	400	Not found	Not found	Not found
6h	800	Not found	Not found	800	Not found	Not found
6i	400	800	800	800	Not found	Not found
6j	200	25	400	200	Not found	Not found
Ciprofloxacin	25	25	25	25	–	–
Fluconazole	–	–	–	–	50	50

(–) denotes activity not found

RESULTS AND DISCUSSION

In this article, we communicate the S-*bis*-alkylation of 1,2,3,4-tetrahydropyrimidine rings in the presence of solid-support. In this present study, the parent compounds **4(a-j)** had been obtained by traditional one-pot multicomponent Biginelli reaction. The two parent pyrimidine rings had been joined at sulphur atom present at C-2 of these rings *via* 1,4-dichloro-but-2-yne as linking agent. These reactions were carried out in presence of microwave-irradiations without using any solvent and costly catalyst.

All the synthesized compounds furnished **6(a-j)**, were characterized by IR, NMR and elemental analysis. In IR, the formation of *bis*-dihydropyrimidines was confirmed by presence of absorption band at around 1690 cm^{-1} corresponding to C=N bond. Absorption band at around 2145 cm^{-1} was attributed to C≡C bond. Among NMR spectra, the presence of resonating signals at around δ 5.10 and 4.50 ppm in ^{1}H-NMR assured that two pyrimidine rings had been joined by linker being employed. These two signals actually correspond to methylene protons attached to sulphur atom. These reason of presence of methylene protons at two separate positions could be ascribed to the fact that they are attached to carbon, which is further linked to heavy atom sulphur. This resulted in phenomenon that the rotation of atoms get slower down and NMR spectrometer experience these two protons in different environments.

Further, among ^{13}C-NMR spectra, acetylenic carbons C≡C were recorded at around δ 71.00-83.00 ppm. Carbon belonging to S-***C***H_2- appeared at δ 34-35 ppm. Rest of the characteristic absorption bands in IR and resonating signals in NMR were in close agreement with the proposed structures.

Biological evaluations

in-vitro **Antimicrobial activities:** In order to follow the influence of different electronic environment; thus created around the furnished molecules, by the (i) substituent (-R) attached to phenyl ring present at C-4 and (ii) effect of linker (**5**), that links two parent pyrimidine rings through the sulphur atom, in our present study, the synthesized organic moieties **6(a-j)** were evaluated for their antimicrobial activities. Agar well diffusion method was followed to determine the zone of inhibition at 150, 300, 600 μg/mL and MIC (minimum inhibitory concentration) using four bacterial strains namely *Bacillus subtilis*, *Staphylococcus aureus* (Gram-positive) & *Pseudomonas aeruginosa* and *Escherichia coli* (Gram-negative) and two fungal pathogens; *Candida albicans* and *Cladosporium oxysporum*. Ciprofloxacin and fluconazole were used as standard drugs to carry out these studies.

It was found that substituents present on phenyl ring affected the biological behaviours of *bis*-heterocycles in the following way:

From the above mentioned evaluations, it was found that the compounds having electron-releasing substituents on the phenyl ring at C-4 exhibited antibacterial activities. Whereas, presence of electron-withdrawing substituents resulted in antifungal behaviour.

From the results of antibacterial studies, it was observed that compound **6j** which carries 2,3-disubstituted phenyl ring was found to be the most active antibacterial agent. This compound has shown the most significant activity equivalent to that of standard drug employed. Compounds **6e** and **6i** showed excellent to moderate antibacterial behaviour against all the bacterial strains. Whereas, samples **6g**, **6h** exhibited antibacterial activity selectively against Gram-negative and Gram-positive strains, respectively.

The results of antifungal studies revealed that compound **6b** having nitro group at *meta* position was the most potent antifungal agent followed by nitro at *para* position and chloro substituents at *ortho* and *para* position.

Conclusion

This article was aimed at the synthesis of new *bis*-dihydropyrimidines in an environmentally benign manner. Reactions were performed using microwaves in presence of solid-support. Presented Synthetic protocol is associated with easier work-up conditions, higher reproducibility of reactions, production of no side products. Target *bis*-heterocycles were obtained in good yields. Further, synthesized compounds were also evaluated for their *in-vitro* antimicrobial properties. From these biological studies, we reached to the conclusion that nature as well as position of substituents present on the phenyl ring at C-4 of pyrimidine ring remarkably influenced the pharmacological behaviour.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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