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

SUMEET KAUR BHATIA, VARSHA SAMDHIAN and BALBIR KAUR*

Department of Chemistry, Punjabi University, Patiala-147002, India

*Corresponding author: E-mail: drbalbirkaur@gmail.com

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 a eco-friendly 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-isoster 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, ¹H-NMR, ¹³C-NMR, ESI-MS, elemental analyses. IR spectra were recorded as potassium bromide pellets on Perkin Elmer spectrum RX IFT-IR System. ¹H-NMR and ¹³C-NMR spectra were determined on BRUKER AVANCE II 400 NMR spectrometer. Chemical shifts were expressed as...
parts per million; (δ values, ppm) relative tetramethysilane 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, Karnataka.

**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-diylbis(sulfanediyl))bis(6-methyl-4-(3’-nitrophenyl)-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-yn 5 (0.01 mol); thoroughly mixed with pre-conditioned 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 open system.

The synthetic methodology has been shown in Scheme-I.

**Spectral data**

Diethyl-2,2’-(but-2-yne-1,4-diylbis(sulfanediyl))bis(6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate) (6a): Yellow solid (MeOH + CHCl3); yield (0.76 g, 76 %); m.p. 156-158 °C. IR (KBr, νmax, cm-1): 3300 (N-H), 3065 (aromatic C-H), 2962, 2823 (methyl C-H), 2148 (C≡C), 1682 (C≡O), 1682 (C=C), 1600 (C=C), 1523 (antisymmetric NO2), 1461 (C=C skeletal vibr.), 1346 (symmetric NO2). 1H-NMR (400 MHz, DMSO-d6) (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, -CH3), 4.68 (2H, d, S-CH2), 4.59 (2H, d, S-CH2), 4.08 (4H, q, O-CH2-CH3), 2.32 (6H, s, CH3-6), 1.08 (6H, t, O-CH2-). 13C-NMR (100 MHz, DMSO-d6) (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=O), 60.04 (O-CH2-CH3), 55.23 (C-4’), 34.97 (S-CH2-), 16.75 (-CH3), 14.78 (O-CH2-CH3). ESI-MS: (m/z): 602 [M+] . Anal. calcd. (%) for C32H34N4O8S2: C, 55.48; H, 4.66; N, 12.13; O, 18.48; S, 9.26, Found (%): C, 55.88; H, 4.26; N, 12.3; O, 18.84, S, 9.66.

Diethyl-2,2’-(but-2-yne-1,4-diylbis(sulfanediyl))bis(6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate) (6b): Yellow solid (MeOH + CHCl3); yield (0.76 g, 76 %); m.p. 156-158 °C. IR (KBr, νmax, cm-1): 3300 (N-H), 3065 (aromatic C-H), 2962, 2823 (methyl C-H), 2148 (C≡C), 1682 (C≡O), 1682 (C=C), 1600 (C=C), 1523 (antisymmetric NO2), 1461 (C=C skeletal vibr.), 1346 (symmetric NO2). 1H-NMR (400 MHz, DMSO-d6) (δ, ppm): 9.58 (2H, brs, N-H), 7.62 (2H, d, H-4’, Jp = 8.02 Hz), 7.55 (2H, d, H-2’, Jp = 2.02 Hz), 7.32 (2H, dd, H-6’, Jm = 7.68 Hz, 3.20 Hz), 7.06 (2H, d, H-5’, Jp = 0.82 Hz), 4.75 (2H, s, H-4), 4.69 (2H, d, S-CH2-), 4.50 (2H, d, S-CH2-), 4.24 (4H, q, O-CH2-CH2), 2.16 (6H, s, CH3-6), 1.04 (6H, t, O-CH2-CH3). 13C-NMR (100 MHz, DMSO-d6) (δ, ppm): 165.90 (C=O), 164.80 (C-2), 147.35 (C-6), 145.54 (C-3’), 141.04 (C-1’), 135.24 (C-2’, 6’), 134.93 (C=O), 132.01 (C-6’, 129.89 (C-5’), 101.06 (C-5), 81.03 (C=C), 61.01 (O-CH2-CH3), 55.04 (C-4’), 34.29 (S-CH2-), 16.18 (CH3), 14.15 (O-CH2-CH3). ESI-MS: (m/z): 692 [M+] . Anal. calcd. (%) for C32H32N6O8S2: C, 55.48; H, 4.66; N, 12.13; O, 18.48; S, 9.26, Found (%): C, 55.88; H, 4.26; N, 12.3; O, 18.84; S, 9.66.

Diethyl-2,2’-(but-2-yne-1,4-diylbis(sulfanediyl))bis(6-methyl-4-(4’-nitrophenyl)-1,4-dihydropyrimidine-5-carboxylate) (6c): Brown solid (MeOH + CHCl3); yield (0.76 g, 76 %); m.p. 140-142 °C. IR (KBr, νmax, cm-1): 3300 (N-H), 3065 (aromatic C-H), 2965, 2821 (methyl C-H), 2148 (C≡C), 1682 (C≡O), 1682 (C=C), 1600 (C=C), 1523 (antisymmetric NO2), 1469 (C=C skeletal vibr.), 1365 (symmetric NO2). 1H-NMR (400 MHz, DMSO-d6) (δ, ppm): 9.62 (2H, brs, N-H), 7.84 (4H, d, H-3’, 5’, Jp = 7.62 Hz), 7.35 (4H, d, H-2’, 6’, Jp = 7.62 Hz), 5.51 (2H, s, H-4), 4.82 (2H, d, S-CH2-), 4.52 (2H, d, S-CH2-), 4.08 (4H, q, O-CH2-CH2), 2.32 (6H, s, CH3-6), 1.06 (6H, t, O-CH2-CH3). 13C-NMR (100 MHz, DMSO-d6) (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-CH2-CH3), 55.26 (C-4), 34.23 (S-CH2-), 18.64 (-CH3), 14.65 (O-CH2-CH3). ESI-MS: (m/z): 693 [M+1] . Anal. calcd. (%) for C32H32N6O8S2: C, 55.48; H, 4.66;
Diethyl-2,2'-(but-2-yne-1,4-diylbis(sulfanediyl))bis(4-(2'-dichlorophenyl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate) (6d): White solid (EtOH); yield (0.73 g, 79 %); m.p. 188-190 °C. IR (KBr, \( \nu_{\text{max}} \), cm\(^{-1} \)) = 3304 (N-H), 3292 (amide I), 2938 (methyl C-H), 1677 (N-H). 1H-NMR (400 MHz, DMSO-\( d_6 \)) (\( \delta \) ppm): 12.95 (2H, brs, N-H), 7.68 (2H, d, H-6', \( J = 8.26 \) Hz), 7.63 (2H, d, H-5', \( J = 8.16 \) Hz), 6.79 (2H, d, H-2', \( J = 5.47 \) Hz), 4.63 (2H, d, S-CH_{2}-), 4.41 (2H, d, S-CH_{2}-). 13C-NMR (100 MHz, DMSO-\( d_6 \)) (\( \delta \) ppm): 165.87 (C=O), 159.96 (C-2), 159.29 (C-6), 156.47 (C-3'), 153.70 (C-4'), 147.82 (C-1'), 147.45 (C-6'), 147.18 (C-5'), 129.02 (C-2',6'), 127.64 (C-3',5'), 104.18 (C-5), 83.24 (C), 34.99 (S-CH_{2}-), 21.09 (S, -OCH_{2}-). ESI-MS: (\( m/z \)) = 740 \([\text{M} + \text{H}]^+\). Anal. calcd. (%) for C_{32}H_{30}N_{4}O_{4}S_{4}: C, 51.50; H, 4.48; N, 9.23; O, 18.08; S, 8.86. Found (%): C, 51.50; H, 4.48; N, 9.23; O, 18.08; S, 8.86.

Diethyl-2,2'-(but-2-yne-1,4-diylbis(sulfanediyl))bis(4-(3'-4'-dimethoxyphenyl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate) (6e): White solid (EtOH); yield (0.72 g, 72 %); m.p. 164-166 °C. IR (KBr, \( \nu_{\text{max}} \), cm\(^{-1} \)) = 3309 (N-H), 3292 (amide I), 2938 (methyl C-H), 1677 (N-H). 1H-NMR (400 MHz, DMSO-\( d_6 \)) (\( \delta \) ppm): 12.95 (2H, brs, N-H), 7.68 (2H, d, H-6', \( J = 7.72 \) Hz), 7.63 (2H, d, H-5', \( J = 7.72 \) Hz), 6.79 (2H, d, H-2', \( J = 5.47 \) Hz), 4.63 (2H, d, S-CH_{2}-), 4.41 (2H, d, S-CH_{2}-). 13C-NMR (100 MHz, DMSO-\( d_6 \)) (\( \delta \) ppm): 165.87 (C=O), 159.96 (C-2), 159.29 (C-6), 156.47 (C-3'), 153.70 (C-4'), 147.82 (C-1'), 147.45 (C-6'), 147.18 (C-5'), 129.02 (C-2',6'), 127.64 (C-3',5'), 104.18 (C-5), 83.24 (C), 34.99 (S-CH_{2}-), 21.09 (S, -OCH_{2}-). ESI-MS: (\( m/z \)) = 740 \([\text{M} + \text{H}]^+\). Anal. calcd. (%) for C_{32}H_{30}N_{4}O_{4}S_{4}: C, 51.50; H, 4.48; N, 9.23; O, 18.08; S, 8.86. Found (%): C, 51.50; H, 4.48; N, 9.23; O, 18.08; S, 8.86.

Diethyl-2,2'-(but-2-yne-1,4-diylbis(sulfanediyl))bis(4-(4'-hydroxyl-3'-methoxyphenyl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate) (6f): Light yellow solid (EtOH); yield (0.73 g, 73 %); m.p. 114-116 °C. IR (KBr, \( \nu_{\text{max}} \), cm\(^{-1} \)) = 3323 (N-H), 3086 (aromatic C-H), 2955, 2875 (methyl C-H), 1700 (C=O), 1618 (C-N), 1598 (C=C), 1467 (C=C skeletal vibr.). 1H-NMR (400 MHz, DMSO-\( d_6 \)) (\( \delta \) ppm): 12.95 (2H, brs, N-H), 7.68 (2H, d, H-6', \( J = 8.26 \) Hz), 7.63 (2H, d, H-5', \( J = 8.26 \) Hz), 6.79 (2H, d, H-2', \( J = 5.47 \) Hz), 4.63 (2H, d, S-CH_{2}-), 4.41 (2H, d, S-CH_{2}-). 13C-NMR (100 MHz, DMSO-\( d_6 \)) (\( \delta \) ppm): 165.87 (C=O), 159.96 (C-2), 159.29 (C-6), 156.47 (C-3'), 153.70 (C-4'), 147.82 (C-1'), 147.45 (C-6'), 147.18 (C-5'), 129.02 (C-2',6'), 127.64 (C-3',5'), 104.18 (C-5), 83.24 (C), 34.99 (S-CH_{2}-), 21.09 (S, -OCH_{2}-). ESI-MS: (\( m/z \)) = 740 \([\text{M} + \text{H}]^+\). Anal. calcd. (%) for C_{32}H_{30}N_{4}O_{4}S_{4}: C, 51.50; H, 4.48; N, 9.23; O, 18.08; S, 8.86. Found (%): C, 51.50; H, 4.48; N, 9.23; O, 18.08; S, 8.86.
Diethyl-2,2'-[but-2-yne-1,4-diylbis(sulfanediyl)]bis(4-(benzo[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, 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.). ¹H-NMR (400 MHz, DMSO-d⁶) (λ, ppm): 12.46 (2H, brs, N-H), 7.37 (2H, d, H-5', J = 8.28 Hz), 7.22 (2H, d, H-6', J = 2.20 Hz), 7.13 (2H, d, H-4', J = 3.68 Hz), 5.97 (4H, s, -O-CH₂-CH₃), 51.68 (C-4), 34.70 (S-CH₂), 51.35 (C-2'), 133.98 (C-1'), 133.11 (C-5'), 129.48 (C-6'), 163.61 (C=O), 144.81 (C-2', 141.15 (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-CH₂-O), 82.05 (C≡C), 60.39 (O-CH₂-CH₃), 51.68 (C-4), 34.70 (S-CH₂), 16.78 (CH₃), 13.73 (O-CH₂-CH₃). ESI-MS: (m/z): 609 [M⁺]. Anal. calcd. (%) for C₃₆H₄₂N₄O₈S₂: C, 59.82; H, 5.86; N, 7.75; O, 17.71; S, 9.27.

6.039 (O-CH₂-CH₃), 4.82 (2H, s, -O-CH₂-CH₃), 4.46 (2H, d, S-CH₂), 4.00 (4H, q, O-C≡C), 3.73 (2H, t, O-CH₂-CH₃), 2.92 (2H, t, S-CH₂). Anal. calcd. (%) for C₃₄H₃₄N₄O₈S₂: C, 59.12; H, 4.96; N, 8.11; O, 18.53; S, 9.27.

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

**Biological studies**

Antimicrobial screenings: The newly 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, 10⁴ cfu) and spread evenly on the plate. After 20 min, the wells were filled with compound at different concentrations. 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**

| Compd. | R          | Time (MW irradi.) | Colour   | m.p. (°C) | m.f.   | Yield (%) |
|--------|------------|-------------------|----------|-----------|--------|-----------|
| 6a     | H          | 30 min            | Dark yellow | 184-186   | C₃₆H₄₂N₄O₈S₂ | 70        |
| 6b     | 3-NO₂      | 28 min            | Yellow   | 156-158   | C₃₆H₄₂N₄O₈S₂ | 76        |
| 6c     | 4-NO₂      | 30 min            | Dark brown | 140-142   | C₃₆H₄₂N₄O₈S₂ | 69        |
| 6d     | 2-Cl       | 18 min            | White   | 168-190   | C₃₆H₄₂N₄O₈S₂ | 73        |
| 6e     | 2-OH       | 30 min            | White   | 150-154   | C₃₆H₄₂N₄O₈S₂ | 79        |
| 6f     | 4-CH₃      | 31 min            | Yellow  | 195-204   | C₃₆H₄₂N₄O₈S₂ | 81        |
| 6g     | 3-OCH₃, 4-CH₃ | 20 min         | Yellow  | 198-200   | C₃₆H₄₂N₄O₈S₂ | 76        |
| 6h     | 4-OCH₃     | 20 min            | Yellow  | 154-156   | C₃₆H₄₂N₄O₈S₂ | 72        |
| 6i     | 3,4-OCH₃   | 15 min 20 s       | Yellow  | 114-116   | C₃₆H₄₂N₄O₈S₂ | 73        |
| 6j     | 2,3-OCH₃O  | 21 min            | Yellow  | 158-160   | C₃₆H₄₂N₄O₈S₂ | 76        |

---

**Table 2**

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

(*) 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-ynie 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 ¹H-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 ¹³C-NMR spectra, acetylenic carbons C≡C were recorded at around δ 71.00-83.00 ppm. Carbon belonging to S-CH₂ 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 oxy sporum*. 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.

| 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
ACKNOWLEDGEMENTS

The authors are highly grateful to UGC-BSR for providing financial assistance and to Department of Chemistry, Punjabi University, Patiala, India for providing all the necessary research facilities.

CONFLICT OF INTEREST

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

REFERENCES

1. P. Vicini, A. Geronikaki, K. Anastasia, M. Incerti and F. Zani, *Bioorg. Med. Chem.*, 14, 3859 (2006); https://doi.org/10.1016/j.bmc.2006.01.043.
2. O. Bozdag-Dündar, Ö. Özgen, A. Mentese, N. Altanlar, O. Atli, E. Kendi and R. Ertan, *Bioorg. Med. Chem.*, 15, 6012 (2007); https://doi.org/10.1016/j.bmc.2007.06.049.
3. A. Cukurovali, I. Yilmaz, S. Gur and C. Kazaz, *Eur. J. Med. Chem.*, 41, 201 (2006); https://doi.org/10.1016/j.ejmech.2005.01.013.
4. M.M. Ramla, M.A. Omar, A.-M.M. El-Khamry and H.I. El-Diwani, *Bioorg. Med. Chem.*, 14, 7324 (2006); https://doi.org/10.1016/j.bmc.2006.06.033.
5. E. Gürsoy and N.U. Güzeldemirci, *Eur. J. Med. Chem.*, 42, 320 (2007); https://doi.org/10.1016/j.ejmech.2006.10.012.
6. M.-H. Shih and F.-Y. Ke, *Bioorg. Med. Chem.*, 12, 4633 (2004); https://doi.org/10.1016/j.bmc.2004.06.033.
7. M.R. Shiradkar, K.K. Murahari, H.R. Gangadasu, T. Suresh, C.A. Kalyan, D. Panchal, R. Kaur, P. Burange, J. Ghogare, V. Mokale and M. Raut, *Bioorg. Med. Chem.*, 15, 3997 (2007); https://doi.org/10.1016/j.bmc.2007.04.003.
8. G. Ariddos, S. Amirthaganesan, M.S. Kim, J.T. Kim and Y.T. Jeong, *Eur. J. Med. Chem.*, 44, 4199 (2009); https://doi.org/10.1016/j.ejmech.2009.05.015.
9. L. Louis, EU Patent 263,020 (1998); *Chem. Abstr.*, 109, 128995q (1998).
10. B.S. Holla, K.V. Malini, B.S. Rao, B.K. Sarojini and N.S. Kumari, *Eur. J. Med. Chem.*, 38, 313 (2003); https://doi.org/10.1016/S0223-5234(02)01447-2.
11. R.G. Kalkhambkar, G.M. Kulkarni, H. Shivkumar and N.R. Rao, *Eur. J. Med. Chem.*, 42, 1272 (2007); https://doi.org/10.1016/j.ejmech.2007.01.023.
12. D.R. Hannah and M.F.G. Stevens, *J. Chem. Res.*, 398 (2003); https://doi.org/10.3184/030823403103174533.
13. S. Kaur, R. Kaur, B. Kaur and M. Yusuf, *Int. J. Curr. Res.*, 5, 1589 (2013).
14. M. Bansal, R. Kaur and B. Kaur, *Heterocycl. Commun.*, 15, 417 (2009); https://doi.org/10.1515/HC.2009.15.6.417.
15. P. Biginelli, *Gazz. Chim. Ital.*, 23, 360 (1893).
16. J. Threlfall, I.S.T. Fisher, L.R. Ward, H. Tschäpe and P. Gerner-Smidt, *Microb. Drug Resist.*, 5, 195 (1999); https://doi.org/10.1089/mdr.1999.5.195.
17. S.K. Bhatia, V. Sandhian and B. Kaur, *J. Het. Chem.*, 55, 935 (2018); https://doi.org/10.1002/jhet.3121.