Synthesis of some new conjugative derivatives of 2-[1-(3ˈ,4ˈ-substituted-biphenyl-4-carbonyl)- 2-butyl-4-methyl-6-oxo-1,6-dihydro-pyrimidin-5-yl]-N,N-dimethyl acetamides and their evaluation of antimicrobial and *in vitro* anticancer activities

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Abstract

A series of ten new conjugative derivatives of 2-[1-(3',4'-substituted-biphenyl-4-carbonyl)-2-butyl-4-methyl-6-oxo-1,6-dihydro-pyrimidin-5-yl]-N,N-dimethyl acetamide derivatives (**3a-3j**) starting from 2-(2-butyl-1,6-dihydro-4-methyl-6-oxopyrimidin-5yl)-N,N-dimethylacetamide (**1**) with 3`,4`-substituted-biphenyl-4-carbonyl chloride (**2a-j**) in dimethylformamide with good yields. The compounds were screened for antimicrobial and *in vitro* anticancer activities. The synthesized pyrimidine-biphenyl derivatives (**3a-3j**) possess superior antimicrobial activity against selected pathogens. All derivatives **3a-3j** were tested against three cancer cell lines, HEPG-2, MCF-7 and HCT116 cancer cell lines were investigated by MTT assay. Compound **3b** showed highest activity against HEPG2 cancer cell line. The compound **3e** showed more potent activity against MCF-7 and HCT116 cancer cell lines.

Keywords: 2-(2-butyl-1,6-dihydro-4-methyl-6-oxopyrimidin-5yl)-N,N-dimethyl acetamide, 3`,4`-substituted-biphenyl-4-carbonyl chloride, antimicrobial activity, *in vitro* anticancer activity.

1. INTRODUCTION

Biphenyl is a neutral molecule and fairly non-reactive due to lack of functional group. However, biphenyl participates in many of the reactions that are typical for benzene, for example, substitution reactions upon treatment with halogens in the presence of a Lewis acid. Also, it is required to convert biphenyls into the structural analogs containing the active groups in order for it to be able to use for synthetic intermediate for the production of a host of other organic compounds such as emulsifiers, optical brighteners, crop protection products, plastics and pharmaceuticals.[1] For this, it is important to consider the o, p-directing and/or m directing effect, especially when substitution at a specific position is desired i.e. mono, di-, tri- or tetra- substitutions. It is possible to acetylate the carboxylic part, also various other

biphenyl derivative synthesis is possible by carrying out the amination, halogenation, sulphonation, alkylation, hydroxylation, metal complexation etc.

The biphenyl group is one of the most important substructures in a number of bioactive and functional molecules. It's derivatives posses a wide range of biological activities like antimicrobial[1,2] (antifungal and antibacterial), anti-inflammatory,[3] antihypertensive,[4] antiviral,[5] anticancer,[6] diuretic[7] and antidiabetic[8] activities. Moreover, the benzene–benzene bond is present in numerous natural products as well as in biologically active agrochemicals.[9]

The hypertensive class of drugs such as Losartan, Candesartan, Valsartan, Irbesartan, Telmisartan, and Olmesartan (**Figure 1**) are having biphenyl fragment bearing an acidic moiety in common and differ in the nature of pendent heterocyclic system connected to the para position of the distal phenyl by means of a methylene group.[10-15] In fact, in the design of new nonpeptide angiotensin II receptor blockers, the strategy followed by most medicinal chemists is the modification of the amino moiety of above drugs.

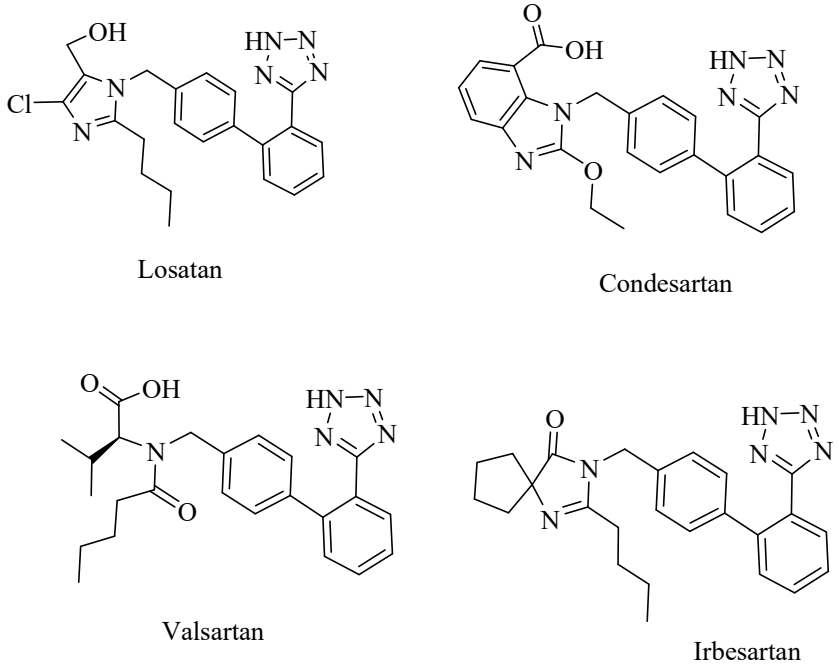


Figure 1: Chemical drugs with biphenyl group nucleus.

In continuation to it, authors have explored antimicrobial activities of pyrimidine-biphenyl derivatives along with anticancer activity of interest.

2. EXPERIMENTAL

The reaction progress was monitored by TLC Merk silica gel plates. The synthesized new pyrimidine-biphenyl derivatives were characterized by their ^{1}H NMR, ^{13}C NMR and mass spectral characterizations.

General experimental procedure for synthesis of pyrimidine-biphenyl derivatives (3a-j)

2-(2-butyl-1,6-dihydro-4-methyl-6-oxopyrimidin-5yl)-N,N-dimethylacetamide (0.01mmol) (**1**) was dissolved in 15 ml of dry dimethylformamide and cool the mixture to 0-5 ^{o}C. Add sodium hydride (0.5 mmol) in to the reaction mixture at 0-5 ^{o}C. Dissolve 3`,4`-substituted-biphenyl-4-carbonyl chloride (0.9 mmol) (**2a-j**) in dimethylformamide (5 mL) in a separate flask and add this solution to the reaction mixture at 0-5 ^{o}C slowly for 30 minutes. Once addition is completed raise the temperature of the mixture to 25-30 ^{o}C. Stir the Reaction mixture at 25-30 ^{o}C 18 to 30 hours. Quench the reaction mixture with water (30 mL) and extract product with ethyl acetate (3 x60 mL). Dry the organic layer over anhydrous sodium sulfate and concentrate to give crude residue of 2-[1-(3',4'-Substituted-biphenyl-4-carbonyl)-2-butyl-4-methyl-6-oxo-1,6-dihydro-pyrimidin-5-yl]-N,N-dimethyl-acetamides (pyrimidine-biphenyl derivatives). Purify the crude material with flash chromatography to give **3a-j** compounds were obtained with a yield from 70 to 80 %.

2-(1-([1,1'-biphenyl]-4-carbonyl)-2-butyl-4-methyl-6-oxo-1,6-dihydro-pyrimidin-5-yl)-N,N-dimethylacetamide (3a): Yield: 48 %; M.P: 175-177 ^{o}C; ^{1}H NMR (DMSO-d_6) δ: 0.93-0.96 (3H, t, -CH_3), 1.38-1.43 (2H, m, -CH_2), 1.51-1.60 (2H, m, -CH_2), 2.32-2.36 (2H, t, -CH_2), 2.85 (2H, s, -CH_2), 2.98 (6H, s, -CH_3), 3.22 (3H, s, -CH_3), 7.42-7.50 (3H, m, -Ar-H), 7.71-7.79 (4H, m, -Ar-H), 8.00-8.06 (2H, dd, -Ar-H); ^{13}C NMR (DMSO-d_6) δ: 13.6, 20.2, 21.5, 22.4, 23.4, 25.8, 38.4, 123.6, 127.3., 127.7, 128, 129.4, 130.5, 130.9, 140.7, 144.3, 153.5, 156.3, 164.5, 166.2, 170.1; FAB Mass: m/z 432.22 (M^{+1}); CHN Analysis: Found: C (72.38 %), H (6.80 %), N (9.71 %), Calc: C (72.37 %), H (6.77 %), N (9.74 %).

2-(2-butyl-1-(4'-chloro-[1,1'-biphenyl]-4-cabonyl)-4-methyl-6-oxo-1,6-dihydro-pyrimidin-5-yl)-N,N-dimethylacetamide (3b): Yield: 40 %, M.P: 145-148 ^{o}C, ^{1}H NMR (DMSO-d_6) δ: 0.93-0.96 (3H, t, -CH_3), 1.34-1.41 (2H, m, -CH_2), 1.52-1.61 (2H, m, -CH_2), 2.30-2.34 (2H, t, -CH_2), 2.85 (2H, s, -CH_2), 2.97 (6H, s, -CH_3), 3.20 (3H, s, -CH_3), 7.64-7.69 (2H, dd, -Ar-H), 7.73-7.77 (2H, dd, -Ar-H), 8.01-8.05 (2H, dd, -Ar-H), 8.12-8.16 (2H, dd, -Ar-H); ^{13}C NMR (DMSO-d_6) δ: 13.5, 20.7, 21.6, 22.8, 23.6, 25.8, 38.2, 123.1, 128, 129.5, 130.3, 130.5, 133.0, 138.7, 144.3, 153.6, 156.7, 164.8, 166.1, 170.5; FAB Mass: m/z 466.18

(M^{+1}); CHN Analysis: Found: C (67.06%), H (6.03 %), N (9.12 %), Calc: C (67.02 %), H (6.06 %), N (9.02 %).

2-(1-(4'-bromo-[1,1'-biphenyl]-4-carbonyl)-2-butyl-4-methyl-6-oxo-1,6-dihydro-pyrimidin-5-yl)-N,N-dimethylacetamide (3c): Yield: 42 %, M.P: 172-175 ^{o}C, ^{1}H NMR (DMSO-d_6) δ: 0.94-0.97 (3H, t, -CH_3), 1.36-1.42 (2H, m, -CH_2), 1.54-1.60 (2H, m, -CH_2), 2.33-2.37 (2H, t, -CH_2), 2.83 (2H, s, -CH_2), 2.99 (6H, s, -CH_3), 3.20 (3H, s, -CH_3), 7.66-7.72 (4H, m, -Ar-H), 7.75-7.80 (2H, dd, -Ar-H), 8.00-8.06 (2H, dd, -Ar-H); ^{13}C NMR (DMSO-d_6) δ: 13.7, 20.8, 21.7, 22.4, 23.2, 25.8, 38.2, 121.5, 122.2, 123.6, 128.1, 129.4, 130.2, 130.4, 130.6, 139.5, 144.3, 153.6, 156.2, 164.5, 166.2, 170.4; FAB Mass: m/z 510.13 (M^{+1}); CHN Analysis: Found: C (61.22 %), H (5.50 %), N (8.26 %), Calc: C (61.18 %), H (5.53 %), N (8.23 %).

2-(1-(3'-bromo-4'-chloro-[1,1'-biphenyl]-4-carbonyl)-2-butyl-4-methyl-6-oxo-1,6-dihydro-pyrimidin-5-yl)-N,N-dimethylacetamide (3d): Yield: 37 %, M.P: 170-173 ^{o}C, ^{1}H NMR (DMSO-d_6) δ: 0.94-0.97 (3H, t, -CH_3), 1.36-1.42 (2H, m, -CH_2), 1.53-1.60 (2H, m, -CH_2), 2.32-2.36 (2H, t, -CH_2), 2.84 (2H, s, -CH_2), 2.97(6H, s, -CH_3), 3.20 (3H, s, -CH_3), 7.35 (1H, s, -Ar-H), 7.52-7.54 (1H, d, -Ar-H), 7.72-7.79 (3H, m, -Ar-H), 8.00-8.06 (2H, dd, -Ar-H); ^{13}C NMR (DMSO-d_6) δ: 13.5, 20.8, 21.5, 22.6, 23.2, 25.6, 38.5, 118.7, 123.4, 128.2, 128.4, 130.1, 130.5, 130.7, 131.4, 134.6, 136.1, 144.4, 153.7, 156.4, 164.2, 166.2, 170.3; FAB Mass: m/z 544.09 (M^{+1}); CHN Analysis: Found: C (57.33 %), H (4.97 %), N (7.75 %), Calc: C (57.31 %), H (4.99 %), N (7.71 %).

2-(2-butyl-4-methyl-1-(4'-methyl-[1,1'-biphenyl]-4-carbonyl)-6-oxo-1,6-dihydro-pyrimidin-5-yl)-N,N-dimethylacetamide (3e): Yield: 38 %, M.P: 162-165 ^{o}C, ^{1}H NMR (DMSO-d_6) δ: 0.92-0.95 (3H, t, -CH_3), 1.34-1.39 (2H, m, -CH_2), 1.53-1.60 (2H, m, -CH_2), 2.30-2.36 (2H, t, -CH_2), 2.33 (3H, s, -CH_3), 2.84 (2H, s, -CH_2), 2.97 (6H, s, -CH_3), 3.21 (3H, s, -CH_3), 7.14-7.17 (2H, dd, -Ar-H), 7.31-7.34 (2H, dd, -Ar-H), 7.71-7.76 (2H, dd, -Ar-H), 8.01-8.07 (2H, dd, -Ar-H); ^{13}C NMR (DMSO-d_6) δ: 13.6, 20.5, 21.2, 21.7, 22.3, 23.1, 25.2, 38.0, 123.2, 127.6, 128, 129.6, 130.1, 130.4, 130.6, 137.4, 144.1, 153.6, 156.2, 164.3, 166.2, 170.2; FAB Mass: m/z 446.24 (M^{+1}); CHN Analysis: Found: C (72.77 %), H (6.98 %), N (9.46 %), Calc: C (72.78 %), H (7.01 %), N (9.43 %).

2-(2-butyl-1-(3'-fluoro-[1,1'-biphenyl]-4-carbonyl)-4-methyl-6-oxo-1,6-dihydro-pyrimidin-5-yl)-N,N-dimethylacetamide (3f): Yield: 32 %, M.P: 175-176 ^{o}C, ^{1}H NMR (DMSO-d_6) δ: 0.92-0.95 (3H, t, -CH_3), 1.38-1.44 (2H, m, -CH_2), 1.52-1.57 (2H, m, -CH_2), 2.32-2.35 (2H, t, -CH_2), 2.87 (2H, s, -CH_2), 2.97 (6H, s, -CH_3), 3.24 (3H, s, -CH_3), 7.24-7.29 (2H, m, -Ar-H), 7.51-7.57 (2H, m, -Ar-H), 7.73-7.76 (2H, dd, -Ar-H), 8.02-8.08 (2H, dd, -Ar-

H); ^{13}C NMR (DMSO-d_6) δ: 13.6, 20.6, 21.7, 22.3, 23.6, 25.3, 38.2, 114.5, 116.2, 123.4, 127.9, 128.2, 130.1, 130.3, 141.7, 144.3, 153.6, 156.3, 162.1, 164.5, 166.3, 170.2; FAB Mass: m/z 450.21 (M^{+1}); CHN Analysis: Found: C (69.44 %), H (6.30 %), N (9.37 %), Calc: C (69.47 %), H (6.28 %), N (9.35 %).

2-(2-butyl-1-(3'-methoxy-[1,1'-biphenyl]-4-carbonyl)-4-methyl-6-oxo-1,6-dihydro-pyrimidin-5-yl)-N,N-dimethylacetamide (3g): Yield: 43 %, M.P: 192-195 ^{o}C, ^{1}H NMR (DMSO-d_6) δ: 0.92-0.95 (3H, t, -CH_3), 1.35-1.41 (2H, m, -CH_2), 1.50-1.56 (2H, m, -CH_2), 2.32-2.35 (2H, t, -CH_2), 2.84 (2H, s, -CH_2), 2.99 (6H, s, -CH_3), 3.20 (3H, s, -CH_3), 3.83 (3H, s, -OCH_3), 7.02-7.04 (1H, d, -Ar-H), 7.17-7.20 (1H, d, -Ar-H), 7.27 (1H, s, -Ar-H), 7.41-7.44 (1H, t, -Ar-H), 7.74-7.79 (2H, dd, -Ar-H), 8.00-8.05 (2H, dd, -Ar-H); ^{13}C NMR (DMSO-d_6) δ: 13.7, 20.6, 21.8, 22.3, 23.4, 25.8, 38.2, 55.6, 113.1, 120.3, 123.7, 128.2, 130.4, 130.7, 133.5, 144.4, 146.9, 153.7, 156.2, 161.2, 164.5, 166.2, 170.2; FAB Mass: m/z 462.23 (M^{+1}); CHN Analysis: Found: C (70.23 %), H (6.73 %), N (9.12 %), Calc: C (70.26 %), H (6.77 %), N (9.10 %).

2-(2-butyl-1-(3',4'-dichloro-[1,1'-biphenyl]-4-carbonyl)-4-methyl-6-oxo-1,6-dihydro-pyrimidin-5-yl)-N,N-dimethylacetamide (3h): Yield: 41 %, M.P: 173-175 ^{o}C, ^{1}H NMR (DMSO-d_6) δ: 0.92-0.95 (3H, t, -CH_3), 1.37-1.42 (2H, m, -CH_2), 1.51-1.57 (2H, m, -CH_2), 2.31-2.35 (2H, t, -CH_2), 2.86 (2H, s, -CH_2), 2.97 (6H, s, -CH_3), 3.21 (3H, s, -CH_3), 7.53-7.56 (1H, d, -Ar-H), 7.62-7.65 (1H, d, -Ar-H), 7.67-7.70 (1H, d, -Ar-H), 7.73-7.78 (2H, dd, -Ar-H), 7.92 (1H, s, -Ar-H), 8.02-8.08 (2H, dd, -Ar-H); ^{13}C NMR (DMSO-d_6) δ: 13.6, 20.7, 21.5, 22.3, 23.2, 25.6, 38.5, 123.4, 127.5, 128.2, 129.4, 130.4, 130.7, 130.9, 132.1, 132.5, 153.7, 156.3, 161.2, 164.7, 166.2, 170.3; FAB Mass: m/z 500.14 (M^{+1}); CHN Analysis: Found: C (62.37 %), H (5.40 %), N (8.42 %), Calc: C (62.40 %), H (5.44 %), N (8.40 %).

2-(2-butyl-4-methyl-1-(3'-methyl-[1,1'-biphenyl]-4-carbonyl)-6-oxo-1,6-Dihydro-pyrimidin-5-yl)-N,N-dimethylacetamide (3i): Yield: 42 %, M.P: 195-198 ^{o}C, ^{1}H NMR (DMSO-d_6) δ: 0.92-0.95 (3H, t, -CH_3), 1.38-1.44 (2H, m, -CH_2), 1.51-1.57 (2H, m, -CH_2), 2.32-2.36 (2H, t, -CH_2), 2.45 (3H,s,-CH_3), 2.84 (2H, s, -CH_2), 2.97 (6H, s, -CH_3), 3.21 (3H, s, -CH_3), 7.12-7.15 (1H, d, -Ar-H), 7.42-7.48 (1H, dd, -Ar-H), 7.56-7.59 (1H, t, -Ar-H), 7.75--7.82 (3H, m, -Ar-H), 8.01-8.07 (2H, dd, -Ar-H); ^{13}C NMR (DMSO-d_6) δ: 13.6, 20.7, 21.5, 21.4, 22.2, 23.6, 38.1, 123.7, 124.9, 127.5, 128.2, 128.5, 129.3, 130.1, 130.3, 138.7, 141.5, 144.4, 153.7, 156.8, 164.1, 166.4, 170.2; FAB Mass: m/z 446.24 (M^{+1}); CHN Analysis: Found: C (72.75 %), H (7.00 %), N (9.41 %), Calc: C (72.78 %), H (7.01 %), N (9.43 %).

2-(2-butyl-1-(4'-methoxy-[1,1'-biphenyl]-4-carbonyl)-4-methyl-6-oxo-1,6-dihydro-pyrimidin-5-yl)-N,N-dimethylacetamide (3j): Yield: 34 %, M.P: 156-158 ^{o}C, ^{1}H NMR

(DMSO-d_6) δ: 0.94-0.97 (3H, t, -CH_3), 1.36-1.42 (2H, m, -CH_2), 1.51-1.57 (2H, m, -CH_2), 2.32-2.36 (2H, t, -CH_2), 2.84 (2H, s, -CH_2), 2.97 (6H, s, -CH_3), 3.21 (3H, s, -CH_3), 3.80 (3H,s,-OCH_3), 6.97-7.01 (2H, dd, -Ar-H), 7.52-7.57 (2H, dd, -Ar-H), 7.72-7.76 (1H, dd, -Ar-H), 7.77-8.01 (2H, dd, -Ar-H), 8.02-8.06 (2H, dd, -Ar-H); ^{13}C NMR (DMSO-d_6) δ: 13.7, 20.7, 21.9, 22.6, 23.3, 25.8, 38.5, 55.7, 114.5, 123.4, 128.2, 130.2, 130.4, 130.6, 133.2, 144.4, 153.7, 156.5, 159.4, 164.2, 166.4, 170.2; FAB Mass: m/z 462.23 (M^{+1}); CHN Analysis: Found: C (70.24 %), H (6.74 %), N (9.10 %), Calc: C (70.26 %), H (6.77 %), N (9.10 %).

Biological activities

All the synthesized pyrimidine-biphenyl derivatives (**3a-3j**) were studied for their antimicrobial activities by disk diffusion method[17,18] and *in vitro* anticancer activity by MTT assay method. [19]

Procedure for antimicrobial activity

The antimicrobial activity of the synthesized compounds was evaluated by disk diffusion method[17] against Gram positive bacteria *Staphylococcus aureus* (*S. aures*) and *Bacillus subtilis* (*B. subtilis*) and Gram negative bacteria screened were *Escherichia coli* (*E. Coli*) and *Pseudomonas aeruginosa* (*P. aerugonisa*). The synthesized compounds were used at the concentration of 250 µg/mL using DMSO as a solvent. The suspension of bacterial strain was added sterile nutrient agar at 45 ºC. The mixture was transferred to sterile petri dishes to give a depth of 3 to 4 mm and allowed to solidify. Precautions were observed to reduce uniform layer of medium on the plate. Sterile discs 5 mm in diameter (made from Whatman Filter paper) were immersed in the solutions of synthesized compounds (250 µg/mL) and maintain an untreated control sample for comparison. Leave the plates to stand for 1 hour at room temperature as a period of preincubation diffusion to minimize the effects of variations in different time. Then the plates were incubated at 37 ºC for 24 hours and observed for antibacterial activity. The diameter of the zone of inhibition was measured for each plate in which the zone of inhibition was observed. The average zone of inhibition was calculated and compared with that of standard as Ciprofloxacine. A similar procedure was carried out for studying the antifungal activity the synthesized compounds against *Candida albicans* (*C. albicans*).[18]

Procedure for *in vitro* anticancer activity

The *In vitro* anticancer activity of the newly synthesized pyrimidine-biphenyl derivatives were investigated for their activity with respect to three cell lines, i.e. HEPG-2 (Human liver carcinoma), MCF-7 (Human breast cancer) and HCT116 (human colorectal carcinoma) cell lines was investigated by means of 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay[19], which is founded on the incision of the tetrazolium salt by mitochondrial dehydrogenases in viable cells. The cells were distributed in a 96-well sterile microplate (5 × 104 cells/well), and incubated with each of synthesized compound or Doxorubicin® (positive control), prepared in a set of various concentrations in DMSO, at 37 °C for 48 hours in a serum-free medium prior to the assay. The media were cautiously isolated after incubation, and then MTT (2.5 mg/mL, 40 µL) was provided to each well and then incubated for further four hours. The crystals of purple formazan dye were solubilized by providing dimethyl sulfoxide (200 µL). At 590 nm (SpectraMax® Paradigm®Multi-Mode microplate reader), the absorbance was determined. The average percentage of viable cells with respect to the untreated control cells expresses the relative cell viability.

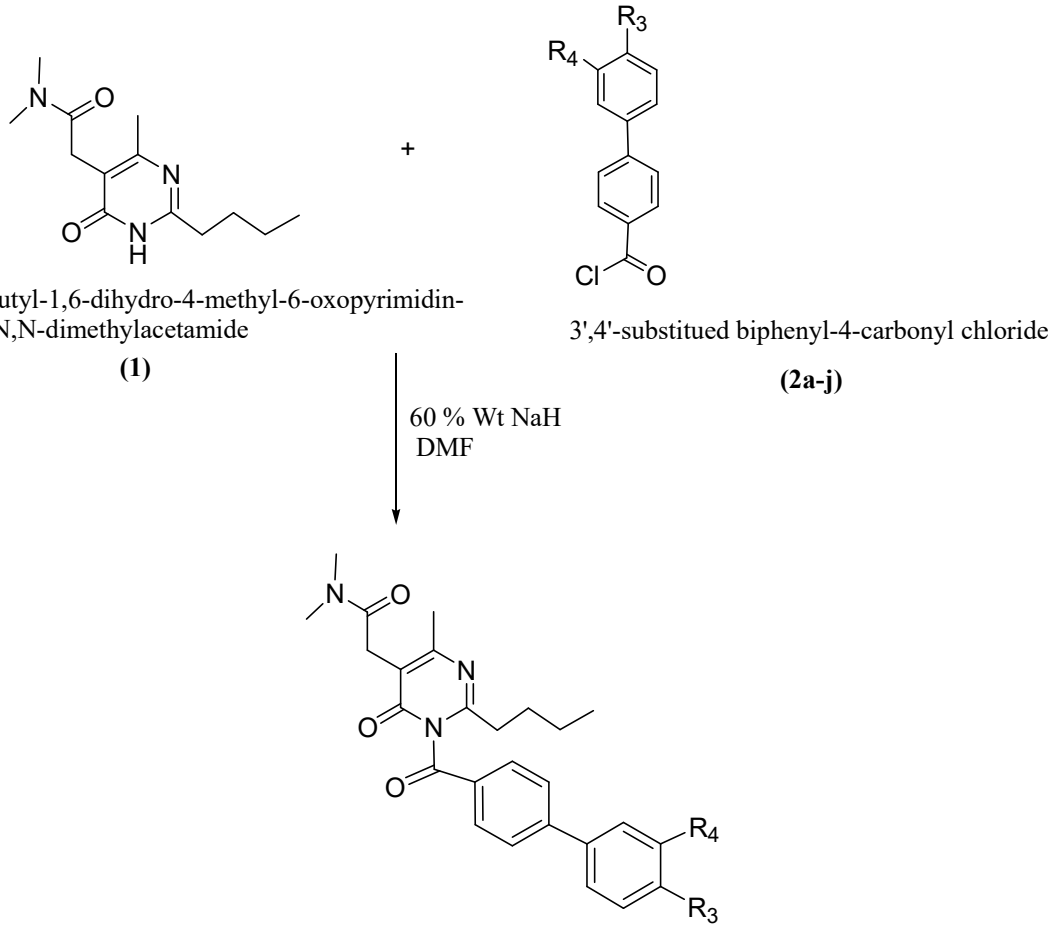
3. RESULTS AND DISCUSSION

Chemistry

In the present study, the authors described the synthesis of pyrimidine-biphenyl derivatives as a new conjugative derivatives of 2-[1-(3',4'-Substituted-biphenyl-4-carbonyl)-2-butyl-4-carbonyl-2-butyl-4-methyl-6-oxo-1,6-dihydro-pyrimidin-5-yl]-N,N-dimethyl-acetamides as shown in **Scheme 1**. Initially, 2-(2-butyl-1,6-dihydro-4-methyl-6-oxopyrimidin-5-yl)-N,N-dimethylacetamide (**1**) is treated with 3',4'-substituted biphenyl-4-carbonyl chlorides (**2a-j)** in presence sodium hydride in DMF at 25-30 ^{o}C 18 to 30 hours. Purify the crude material with flash chromatography to give conjugative derivatives of 2-[1-(3',4'-Substituted-biphenyl-4-carbonyl)-2-butyl-4-carbonyl-2-butyl-4-methyl-6-oxo-1,6-dihydro-pyrimidin-5-yl]-N,N-dimethyl-acetamides (**3a-j**) as shown in **Scheme 1**. The yields of the products obtained were good and the details of pyrimidine-biphenyl derivatives are provided in **Table 1**.

The compound **3a** formed from 2-(2-butyl-1,6-dihydro-4-methyl-6-oxopyrimidin-5-yl)-N,N-dimethylacetamide with simple biphenyl-4-carbonyl chloride was obtained in the highest yield (48 %) followed by compounds **3c** and **3g** with 3-bromo substitution and 4-methoxy substitution along with **3i**, **3h** and **3c** compounds respectively.

All the synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR and mass spectral analysis. In the ^{1}H NMR spectrum of the compounds, the three protons appeared as a triplet at δ 0.93-0.96 due to $-CH_3$ group. A multiplet was observed at δ 1.38-2.36 is due to the presence of three $-CH_2$ group of butyl moiety. The singlet of two protons at δ 2.85 is due to $-CH_2$ attached to carbonyl functional group. The singlet of six protons at δ 2.98 is due to $-N(CH_3)_2$ group. The singlet of three protons at δ 3.22 is due to $-CH_3$ of pyrimidine. The multiplet of nine protons at δ 7.42-8.06 is due to aromatic protons of biphenyl. In the ^{13}C NMR spectrum of the compounds showed peaks at chemical shifts δ 13.6, 20.2, 21.5, and 23.4 (n-butyl carbons), 22.4 (carbon of $-CH_3$ attached with pyrimidine), 25.8 (carbon of $-CH_2$ attached with carbonyl), 38.4 (carbons of two $-CH_3$ groups attached with Nitrogen of amide), 123.6, 127.3, 127.7, 128, 129.4, 130.5, 130.9, 140.7, 144.3, 153.5, 156.3 and 164.5 (aromatic carbons of biphenyl and pyrimidine ring), 166.2 (carbonyl carbon of biphenyl), 170.1 (carbonyl carbon attached with $-N(CH_3)_2$).



2-(2-butyl-1,6-dihydro-4-methyl-6-oxopyrimidin-5-yl)-N,N-dimethylacetamide

(1)

3',4'-substitued biphenyl-4-carbonyl chloride

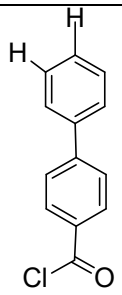
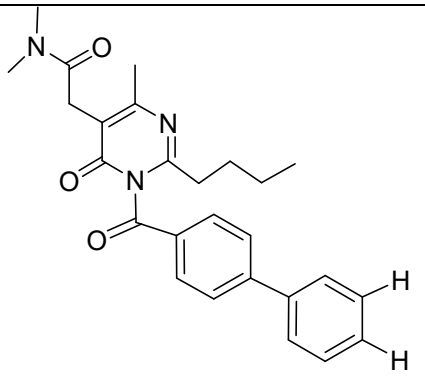
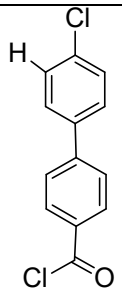
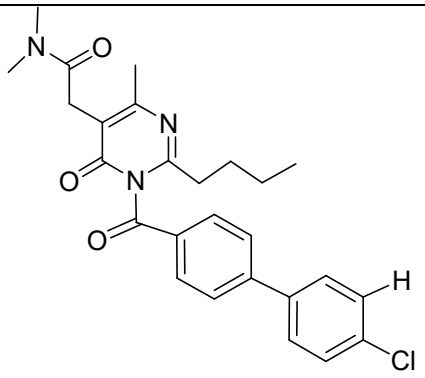
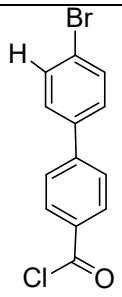
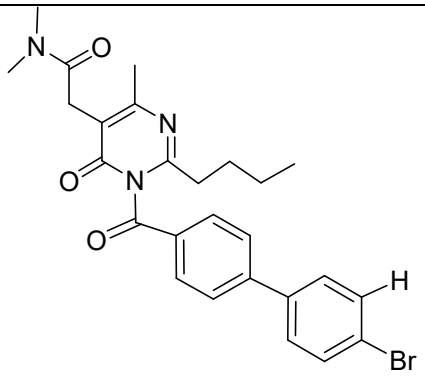
(2a-j)

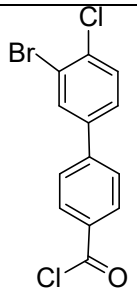
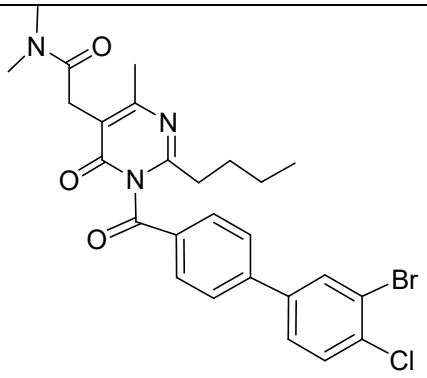
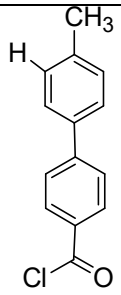
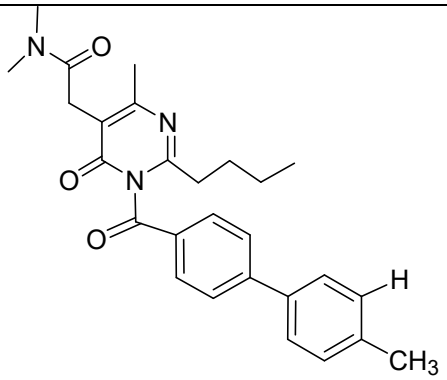
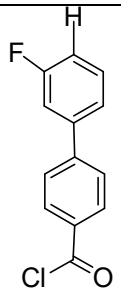
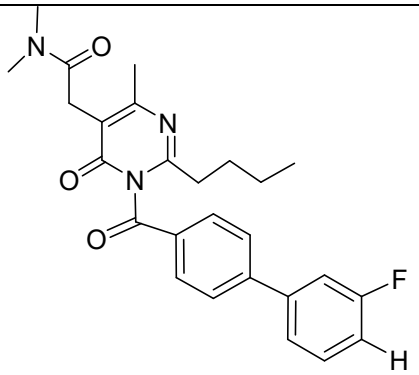
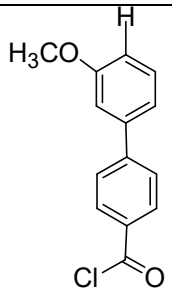
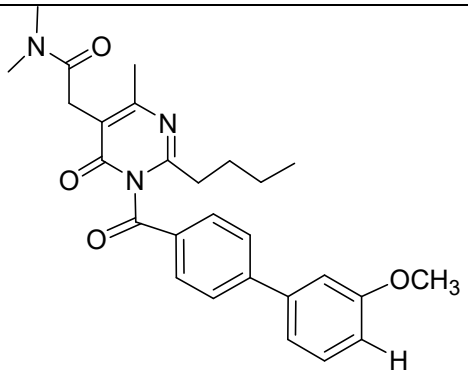
2-[1-(3' 4'-substituted-biphenyl-4-carbonyl)-2-butyl-4-methyl-6-oxo-1,6-dihydro-pyramidin-5-yl]-N, N-dimethylacetamide

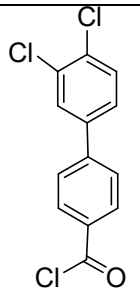
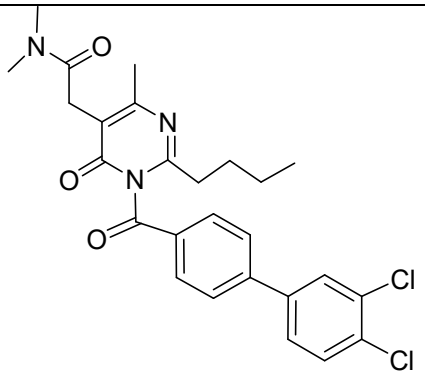
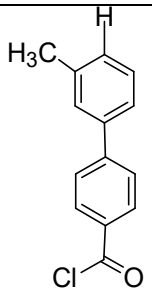
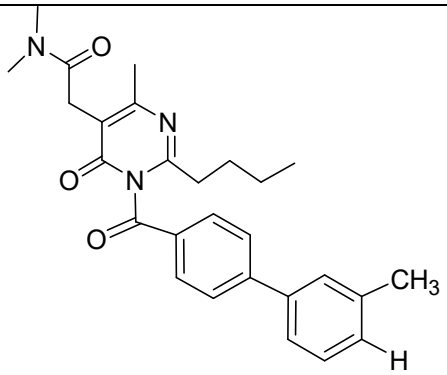
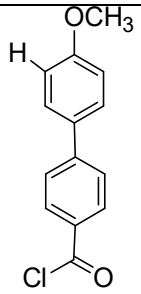
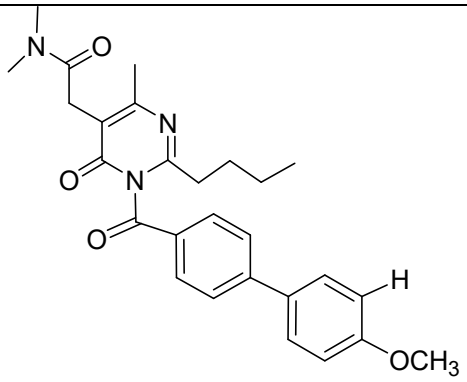
(3a-j)

Scheme 1. Synthesis of 2-[1-(3ˈ,4ˈ-substituted-biphenyl-4-carbonyl)-2-butyl-4-methyl-6-oxo-1,6-dihydro-pyrimidin-5-yl]-N,N-dimethyl-acetamides (**3a-j**).

Table 1: Optimisation of conjugative pyrimidine-biphenyl derivatives (**3a-3j**).

Entry	3',4'-substituted-biphenyl-4-carbonyl chloride (**2a-j**)	Product (**3a-j**)	Yield (%)
1	 **2a**	 **3a**	48
2	 **2b**	 **3b**	40
3	 **2c**	 **3c**	42

4	 **2d**	 **3d**	37
5	 **2e**	 **3e**	38
6	 **2f**	 **3f**	32
7	 **2g**	 **3g**	43

8	 **2h**	 **3h**	41
9	 **2i**	 **3i**	42
10	 **2j**	 **3j**	34

Biological activities

All the synthesized pyrimidine-biphenyl derivatives (**3a-j**) were screened for their antimicrobial activity using disk diffusion method[17,18] and *in vitro* anticancer activity MTT assay method as reported.[19]

Antimicrobial activities

The antimicrobial activity of ten pyrimidine-biphenyl derivatives against two gram positive and two gram negative pathogens and antifungal activity against one fungal species were evaluated by using disc diffusion method at 250 μg/mL and the results are presented in **Table 2.**

Table 2: Antimicrobial activity of pyrimidine-biphenyl derivatives at 250 μg/mL (**3a-3j**).

Sample Code	Antibacterial activity (Zone of inhibition in mm)				Antifungal activity (Zone of inhibition in mm)
	B. subtills	***S. aureus***	***E. coli***	***P. aeruginosa***	***C. albicans***
3a	19.5	8.9	15.2	14.5	8.6
3b	16.2	8.0	17.0	17.0	7.2
3c	19.5	9.0	16.0	16.5	8.5
3d	16.5	8.2	19.0	18.8	8.6
3e	17.5	8.5	16.2	14.2	7.0
3f	18.0	8.5	15.9	13.5	6.4
3g	19.0	9.0	14.0	14.0	7.0
3h	16.5	8.0	18.0	18.0	8.4
3i	17.3	8.5	16.0	14.0	6.9
3j	18.7	8.3	16.0	14.1	8.0
Ciprofloxacin	24.0	10.5	24.0	20.5	
Fluconazole					12.0

The compounds **3a**, **3c** and **3g** showed highest the highest zone of inhibition on *B. subtilis* with a zone of inhibition of 19.5 and 19.0 mm. the compounds **3b**, **3d** and **3h** showed good zone of inhibition on *P. aeruginosa* with a zone of inhibition of 17.0, 19.0, 18.0 mm and *E. coli* with a zone of inhibition of 17.0, 18.8 and 18.0 mm. Further, the compounds **3a-3j** also found to good activity with zone of inhibition of 8.0 to 9.00 mm on *S. aureus*.

The antifungal activity of 1,6-dihydro-pyrimidine derivatives (**3a-3j**) against one fungal species, i.e. *C. albicans* is at 250 μg/mL (**Table 2**) concentration in DMSO using *Fluconazole* as a standard. From these results, among the tested compounds **3a**, **3c**, **3d**, **3h** and **3j** showed good activity against selected strains at 250 μg/mL concentration as compared to *Fluconazole* as a standard. Further, the remaining compounds are good activity against *C. albicans.*

***In vitro* anticancer activity**

The newly synthesized pyrimidine-biphenyl derivatives (**3a-3j**) were studied *in vitro* anticancer activity against the cancer cell lines, HEPG2, HCT116 and MCF-7 cell lines and

the cell lines IC_{50} values were calculated using linear regression equation exhibited dose-dependent manner obtained at 100 μg/mL concentration. A comparative data of the IC_{50} values for the 1,6-dihydro-pyrimidine derivatives (**3a-3j**) are presented in **Table 3**.

Table 3: *In vitro* anticancer activity of pyrimidine-biphenyl derivatives (**3a-3j**)

Compound	**IC_{50}** in (μg/mL)		
	HEPG2	**MCF-7**	**HCT116**
3a	9.68	10.42	10.92
3b	7.35	10.60	10.40
3c	11.30	12.48	15.60
3d	11.40	12.50	14.70
3e	9.80	9.30	9.40
3f	16.54	18.30	18.50
3g	20.50	21.10	22.40
3h	15.40	13.40	14.70
3i	10.58	12.14	12.10
3j	11.15	14.80	15.23
Doxorubicin	4.62	8.50	8.32

Among, these synthesized compounds, **3b** showed highest activity (IC_{50} = 7.35 μg/mL) against HEPG2 cancer cell line. Similarly, the other compounds **3a** and **3c-3j** showed better activity against HEPG2 cancer cell line. Among these compounds **3a-3j**, except, **3g** all the remaining compounds were found to possess anticancer activity against MCF-7 cancer cell line. The compounds **3e**, **3a** and **3b** showed more potent activity followed by **3c**, **3d**, **3f**, **3h**, **3i** and **3j**.

Similarly, the compounds **3e** also showed better activity (IC_{50}= 9.40 μg/mL) against HCT116 cancer cell line and other compounds (except **3e**) showed the average results against the HCT116 cancer cell lines. From these results that are suggestive for the development of synthetic methodology useful for the synthesis of pyrimidine-biphenyl derivatives for development of anticancer properties.

4. CONCLUSION

In conclusion, nine new conjugative derivatives of 2-[1-(3',4'-substituted-biphenyl-4-carbonyl)-2-butyl-4-methyl-6-oxo-1,6-dihydro-pyrimidin-5-yl]-N,N-dimethyl-acetamides were synthesized with good yields. Further, the compounds were screened for antimicrobial and *in vitro* anticancer activities. The synthesized pyrimidine-biphenyl derivatives (**3a-3j**) possess superior antibacterial activity against selected pathogens and compounds **3a**, **3c**, **3d**, **3h** and **3j** showed good activity against selected fungal strains. All derivatives **3a-3j** were tested against three cancer cell lines, HEPG-2, MCF-7 and HCT116 cancer cell lines were investigated by MTT assay. Compound **3b** showed highest activity against HEPG2 cancer cell line. The compound **3e** showed more potent activity against MCF-7 and HCT116 cancer cell lines. Further, investigation in this research may help to create more potent drugs for the treatment of anticancer and antimicrobial activities.

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