Available online at **www.joac.info**

ISSN: 2278-1862

Journal of Applicable Chemistry

2019, 8 (3): 1279-1288

(International Peer Reviewed Journal)

Novel Biphenylic 3, 4-dihydropyrimidine Derivatives as Anti-Microbial Agents: Synthesis, Characterization and *in vitro* Antimicrobial Activity

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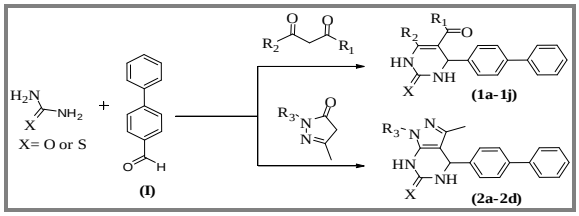
Accepted on 28th April, 2019

ABSTRACT

In the present scope of work we have prepared a series of dihydropyrimidine derivatives via Biginelli reaction by using seven different β-Keto esters i.e. ethylacetoacetate, acetoacetanilide, 2,6-dimethylacetoacetanilide, methyl acetoacetate, ethylcyanoacetate, 3-methyl-5-pyrazolone, 1-phenyl, 3-methyl-5-pyrazolone etc and urea or thiourea along with synthesized biphenyl 4-carbaldehyde in DMSO solvent. The structures of newly synthesized compounds were established by IR, ^{1}H NMR, and Mass spectrometry. The synthesized compounds were evaluated for their in-vitro anti bacterial activity against S. aureus and B. megaterium (Gram+ve bacteria) and P. fluorescens, S. marcensus (Gram-ve bacteria) with reference standard drug ciprofloxacin and anti fungal activity against A. niger fungus with reference standard drug carbendazim.

Graphical Abstract



General scheme for the synthesis of final compounds.

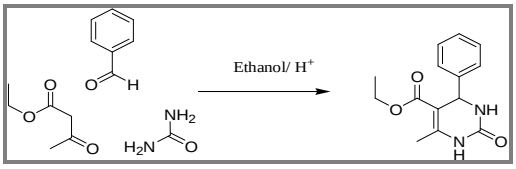
Keywords: Biphenyl dihydropyrimidine, Biginelli Reaction, Suzuki reaction, Antimicrobial activity, Antifungal activity, Multicomponent reaction.

INTRODUCTION

Multi component reactions (MCR's) are attracting attention of scientist as one of the most powerful synthetic tools for the development of the molecular diversity. Various heterocyclic compounds are synthesized by multicomponent reaction. These reactions are more competent, cheap and cost-effective than conventional methods [1]. Multi component reactions are of increasing importance in organic and medicinal chemistry. In such reactions three or more components are allowed to react together in a single reaction vessel to form a new product that contains portions of all the reacting

components. The building blocks of such a reaction may be commercially available or synthesized in the laboratory [2]. MCRs offer significant profits over conventional methods by virtue of their economy, environment-friendly, efficiency and percentage yields [3]. The nitrogen containing heterocyclic compounds are of great interest because many natural and manmade heterocyclic compounds exhibited compatible pharmacological activities [4].

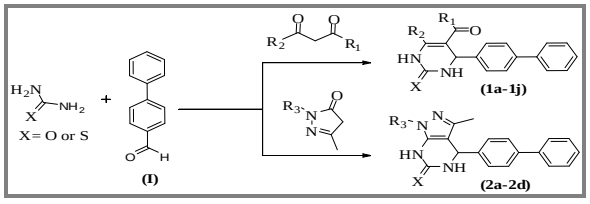
One such multi-component reaction leading to production of heterocyclic compounds is Biginelli Reaction. It is found to be useful for the production of dihydropyrimidine derivatives. An Italian chemist, P. Biginelli, reported in 1893 an acid catalyzed reaction of Benzaldehyde, ethylacetoacetate (β-keto ester) and urea [5]. The reaction was carried out by simply heating a mixture of the three reagents dissolved in ethanol with catalytic amount of hydrochloric acid at reflux temperature. The novel one pot three component synthesis which on cooling produced precipitates of 3, 4-dihydropyrimidine-2(1*H*)-one or Biginelli product (Scheme 1). 3, 4-dihydro-2-pyrimdone and it's sulphur analogue can be synthesize by MCRs like Biginelli reaction [6]. Many scientists have made variation in the original reaction scheme-1 to synthesize more biologically active compounds in the decades of 1970 and 1980 [7].



Scheme 1. 3, 4-dihydropyrimidine-2(1*H*)-one.

Similar groups or similar structure often exhibit similar biological activities. However, they usually exhibit different potency. The traditional structure activity relationship (SAR) is a useful tool in the search for new drugs. However, SAR is usually determined by making small changes to the structure of the existing moiety and assessing the effect on its biological activity. In the same way structural analogy played a vital role in designing compounds of higher potency. One of the structural analogies is seen in 3, 4-dihydropyrimidine [8]. From 1980s a tremendous increment has seen in the publications and patent of dihydropyrimidine-2 (1H)-one. It is well know that the different derivatives of dihydropyrimidine scaffold (DHPMs or Biginelli compound) show remarkable biological activities [9]. These derivatives and related fused heterocyclic compounds are important classes of heterocyclic compounds that exhibit a broad range of biological activities such as anticancer, antiviral, antibacterial, antioxidant and anti-inflammatory [10, 11], analgesic, sedative, antipyretic [12]. The moiety dihydropyrimidines are calcium channel blocker used in the treatment of hypertension [13]. DHPMs are also used for the treatment of cardiovascular diseases [14].

In the synthesis of dihydropyrimidine most of the researchers have focus on using different catalyst to increase rate of reaction or to increase percentage of yield by using $NiCl_2$ $5H_2O$ [15], Na_2HPO_4, $ZrCl_4$, $SnCl_2$ etc [16]. Also the use of microware irradiation, polymer supported catalyst have also been reported [17]. Some researchers have concentrated to prove the mechanism of the reaction [18]. Here, we have synthesized novel aldehyde (Scheme 1) by using Suzuki reaction. We have used this novel biphenylic aldehyde (I) for the synthesis of different dihydropyrimidines by using different β-Keto esters with urea or thiourea as shown in scheme 2.



Scheme 2. General scheme for the synthesis of final compounds.

MATERIALS AND METHODS

General: All the chemicals required are obtained from Sigma Aldrich and used as it is without purification. Merck Kieselgel 60 F254 plates were used for TLC. The ^{1}H NMR spectra were recorded in DMSO solution in 5 mm tubes at room temperature, on a BRUKER 400 MHz FT-NMR, with TMS as internal standard. IR Spectra were recorded on SHIMADZU FT-IR 8400 using potassium bromide pallets. Mass spectra were recorded on SHIMADZU QP-2010. The antimicrobial activity was carried out using broth dilution method to determine minimum inhibitory concentration (MIC).

Preparation of 4'-formylbiphenyl (I): 4-Bromobenzaldehyde (0.0540 mol) and Phenyl Boronic acid (0.0540 mol) were taken in a round bottom flask in Isopropyl alcohol (100 mL), followed by the addition of K_2CO_3 (0.0811 mol) dissolved in water (20 ml). Catalyst Pd (0) (5%) was added to it. The reaction mass was heated at 80°C-90°C for 3-4 h. The reaction mixture was poured into ice cold water and charges 100 mL ethyl acetate to it. It was stirred for 30 min and allowed to separate layer. The organic layer was filtered through hyflowbed. Organic layer was subject to distillation under reduced pressure at 50°C to 60°C temperature to remove solvents. The product obtained after distillation was purified with the treatment of methanol, activated carbon at reflux temperature followed by the filtration through hyflowbed. The filtrate was distilled off to remove half of the methanol under reduced pressure at 50°C to 60°C. Stir the solid in remaining methanol for 1 h at 25-35°C. Filter the solid and wash twice with 50 mL methanol to yield 4'-formylbiphenyl (I).

The progress and completion of the reaction was confirmed by TLC (mobile phase; 5:5 Ethyl acetate: Cyclohexane). IR (cm^{-1}): 1726 (-CHO); ^{1}H NMR (DMSO, 400 MHz) (δ ppm): 5H (m, 7.55), 2H (m, 7.82), 2H (d, 8.09), 1H (s, 10.02 CHO) Mass: (m/z) 181, M. P. 41°C, Yield= 91%.

Preparation of *N*-(2, 6-dimethylphenyl)-3-oxobutanamide (2, 6-dimethyl Acetoacetanilide) (II): For the preparation of intermediate-(II) a mixture of Ethylacetoacetate (0.0768M) and 2,6-dimethyl aniline (0.0768M) in toluene solvent were heated for 12 h using Tetramethylethylenediamine (TMEDA) as catalyst. The mixture was cooled to room temperature and then washed with aqueous sodium bisulphite solution. The layers were separated. The organic layer was further washed with water. The organic layer was distilled off to form product *N*-(2, 6-dimethylphenyl)-3-oxobutanamide (2, 6-dimethyl Acetoacetanilide) (II). Yield: 40%.

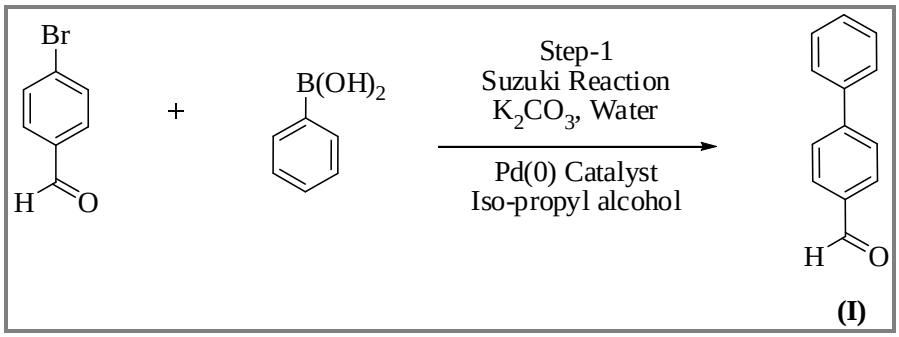
Preparation of *N*-phenyl-3-oxobutanamide (Acetoacetanilide) (III): For the preparation of intermediate-(III) a mixture of Ethylacetoacetate (0.0768M) and aniline (0.0768M) in toluene solvent were heated for 12 h using Tetramethylethylenediamine (TMEDA) as catalyst. The mixture was cooled to room temperature and then washed with aqueous sodium bisulphite solution. The layers were separated. The organic layer was further washed with water. The organic layer was distilled off to form product N-phenyl-3-oxobutanamide (Acetoacetanilide) (III); Yield: 60%.

Preparation of 5-methyl-2, 4-dihydro-3*H*-pyrazol-3-one (IV): For the preparation of intermediate-(IV) a mixture of ethylacetoacetate (0.0768 M) and hydrazine (0.0768 M) was mixed in a round bottom flask with constant stirring. The reaction mixture was cooled and Methyl tertiary butyl ether (MTBE) was added. The mixture was stirred for half an hour. The reaction mass was filtered, washed with MTBE and recrystalized from ethanol to form white product 5-methyl-2, 4-dihydro-3H-pyrazol-3-one (IV). M.P. 110°C, Yield: 90%.

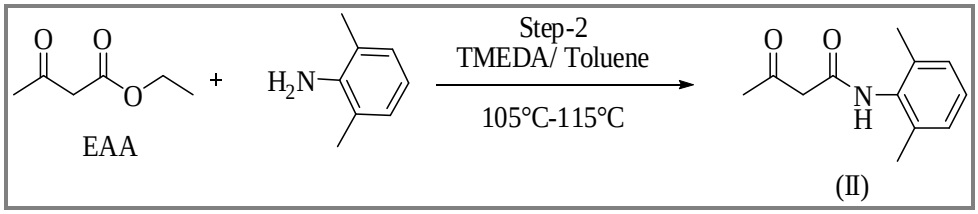
Preparation of 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (V): For the preparation of intermediate-(V) a mixture of ethylacetoacetate (0.0768 M) and phenyhydrazine (0.0768 M) was heated at 120°C-130°C in oil bath for 7-8 h. The reaction mixture was cooled and Methyl tertiary butyl ether (MTBE) was added. The mixture was stirred for one hour at 25°C to 35°C. The reaction mass was filtered, washed with MTBE and recrystalized from ethanol to form white product 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (V). M.P. 127°C, Yield: 85%.

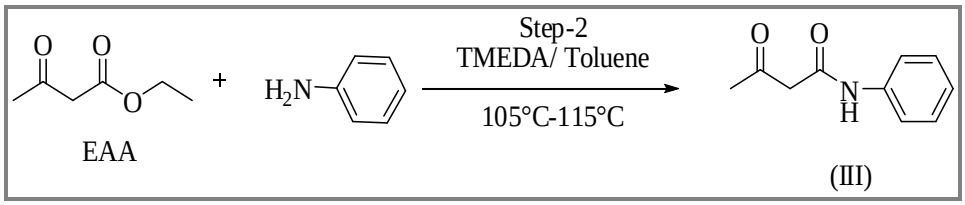
Preparation of final compounds 1a to 1n: In the final step of the present work 4'-formylbiphenyl (I) (0.0055 mol), β-Keto esters (II to IV or EAA or MAA or ECA) (0.0055 mol) and urea or thiourea (0.0055 mol) were taken in a round bottom flask with 25 mL DMSO and 0.25 mL conc. HCl. The reaction mass was stirred at 130°C-140°C for 3-4 h. After completion of reaction, it was cooled to room temperature and poured in to crushed ice. The products obtained were recrystalized from methanol or acetone to form final product **1a** to **1n**. Completion of the reaction was confirmed by TLC (3:7 Ethyl acetate: Cyclohexane).

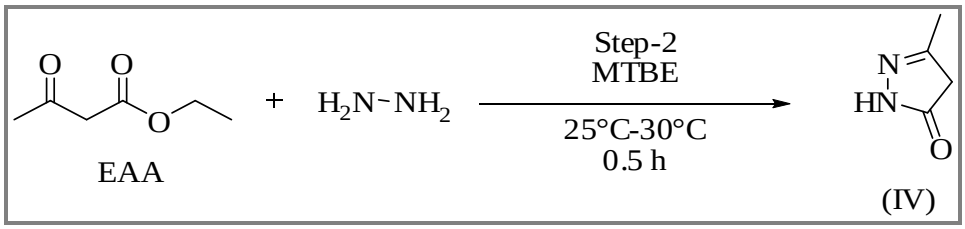
Step 1. Scheme for synthesis of intermediate 4'-formylbiphenyl (I):

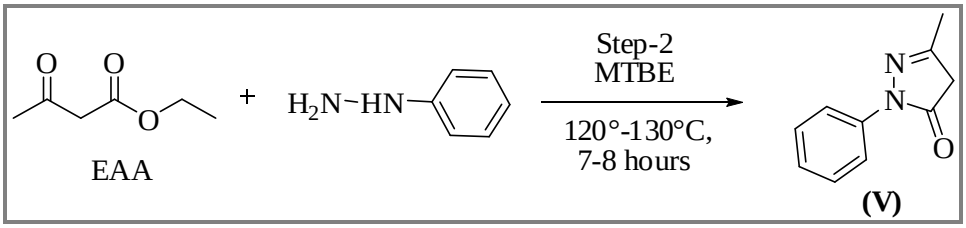


Step 2. Scheme for synthesis of intermediate β-keto esters (II, III, IV and V):

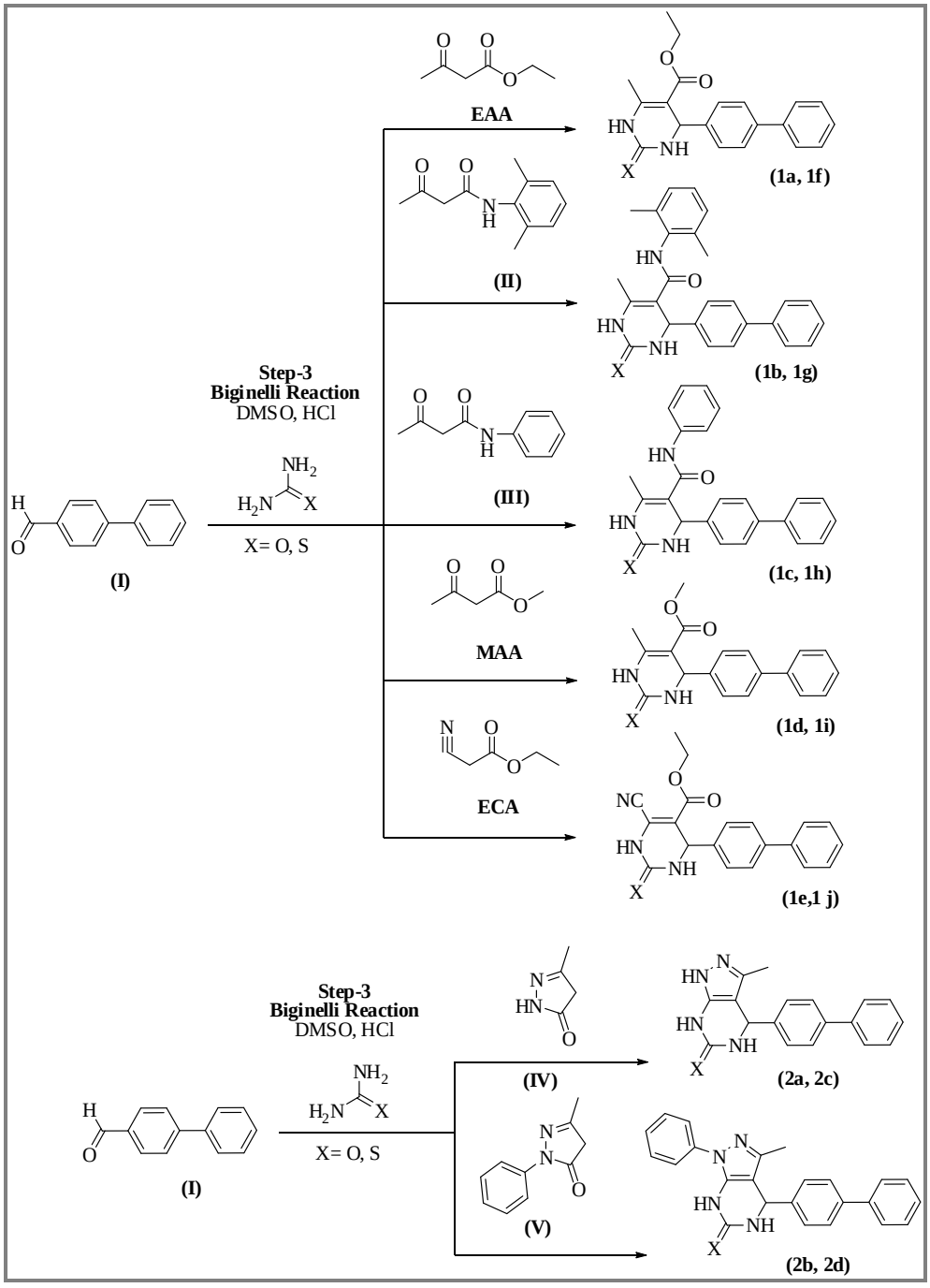








Step 3: Scheme for synthesis of final products (1a to 1n):



Spectral data of final products (1a to 1n):

Ethyl 4-(biphenyl-4-yl)-6-methyl 2-oxo-3, 4-dihydropyrimidine-5-carboxylate (1a): IR (cm^{-1}): 1707 (COOR), 682-869 (Ar C-H bending), 3049.46 (Ar C-H Stretching), 1529-1761 (Ar C=C); ^1H NMR (DMSO, 400 MHz) (δ ppm): 1.13 (3H, t), 2.29 (3H, s), 4.02 (2H, q), 5.22 (1H, s), 7.35 (3H, d), 7.45 (2H, d), 7.63 (4H, s), 7.84 (1H, s), 9.29 (1H, s), Mass: (m/z) 336, Yield= 88%.

4-(biphenyl-4-yl)-N-(2,6-dimethylphenyl)-6-methyl-2-oxo-3,4-dihydropyrimidine-5-carboxamide (1b): IR (cm^{-1}): 1658 (CONH), 1707 (COOR), 682-869 (Ar C-H bending), 3049.46 (Ar C-H Stretching), 1529-1761 (Ar C=C) ^1H NMR (DMSO, 400 MHz) (δ ppm): 2.12 (6H, s), 2.26 (3H, s),

5.13 (1H, s), 7.21 (3H, d), 7.33 (4H, m), 7.57 (6H, m), 9.10 (1H, s), 10.4 (1H, s) Mass: (m/z) 411, Yield= 24%.

4-(biphenyl-4-yl)-6-methyl-2-oxo-N-phenyl-3,4-dihydropyrimidine-5-carboxami-de (1c): IR (cm^{-1}): 1658 (CONH), 1707 (COOR), 682-869 (Ar C-H bending), 3049.46 (Ar C-H Stretching), 1529-1761 (Ar C=C) ^{1}H NMR (DMSO, 400 MHz) (δ ppm): 2.26 (3H, s), 5.19 (1H, s), 7.19 (1H, t), 7.34 (4H, m), 7.46 (7H, m), 7.63 (2H, d), 9.09 (1H, s), 9.63 (1H, s) Mass: (m/z) 383, Yield= 23%.

Methyl 4-(biphenyl-4-yl)-6-methyl-2-oxo-3,4-dihydropyrimidine-5-carboxylate (1d): IR (cm^{-1}): 1707 (COOR), 680-870 (Ar C-H bending), 3049 (Ar C-H Stretching), 1529-1761 (Ar C=C); ^{1}H NMR (DMSO, 400 MHz) (δ ppm): 2.28 (3H, s), 3.56 (3H, s), 5.21 (1H, s), 7.34 (1H, d), 7.37 (2H, d), 7.57 (2H, t), 7.63 (4H, t), 7.84 (1H, s), 9.31 (1H, s), Mass: (m/z) 322, Yield= 86%.

Ethyl 6-(biphenyl-4-yl)-4-amino-1, 2-dihydropyrimidine-2 (1H)-one-5-carboxylate (1e): IR (cm^{-1}): 3350 (NH_2), 680-870 (Ar C-H bending), 3049 (Ar C-H Stretching), 1529-1761 (Ar C=C); ^{1}H NMR (DMSO, 400 MHz) (δ ppm): 1.33 (3H, t), 4.34 (2H, q), 7.46 (1H, m), 7.54 (2H, m), 7.81 (2H, d), 7.93 (2H, d), 8.18 (2H, d), 8.84 (1H, s), Mass: (m/z) 337, Yield= 56%.

Ethyl 4-(biphenyl-4-yl)-6-methyl-2-thio-3, 4-dihydropyrimidine-5-carboxylate (1f): IR (cm^{-1}): 1700 (COOR), 680-868 (Ar C-H bending), 3050 (Ar C-H Stretching), 1530-1760 (Ar C=C); ^{1}H NMR (DMSO, 400 MHz) (δ ppm): 1.13 (3H, t), 2.27 (3H, s), 4.02 (2H, q), 5.20 (1H, s), 7.37 (3H, m), 7.46 (2H, t), 7.64 (4H, t), 7.81 (1H, s), 9.35 (1H, s), Mass: (m/z) 352, Yield= 52%.

4-(biphenyl-4-yl)-N-(2,6-dimethylphenyl)-6-methyl-2-thio-3,4-dihydropyrimidine-5-Carbox-amide (1g): IR (cm^{-1}): 1665 (CONH), 1700 (COOR), 680-860 (Ar C-H bending), 3060 (Ar C-H Stretching), 1530-1760 (Ar C=C) ^{1}H NMR (DMSO, 400 MHz) (δ ppm): 2.13 (6H, s), 2.27 (3H, s), 5.19 (1H, s), 7.71 (3H, d), 7.37 (4H, m), 7.60 (5H, m), 9.30 (1H, s), 9.70 (1H, s), 10.42 (1H, s) Mass: (m/z) 427, Yield= 18%.

4-(biphenyl-4-yl)-6-methyl-2-thio-N-phenyl-3,4-dihydropyrimidine-5-carboxamide (1h): IR (cm^{-1}): 1660 (CONH), 1710 (COOR), 680-868 (Ar C-H bending), 3055.46 (Ar C-H Stretching), 1525-1760 (Ar C=C) ^{1}H NMR (DMSO, 400 MHz) (δ ppm): 2.27 (3H, s), 5.28 (1H, s), 7.21 (1H, t), 7.30 (4H, m), 7.45 (3H, t), 7.51 (4H, m), 7.63 (2H, d), 9.27 (1H, s), 9.64 (2H, s) Mass: (m/z) 399, Yield= 21%.

methyl 4-(biphenyl-4-yl)-6-methyl-2-thio-3,4-dihydropyrimidine-5-carboxylate (1i): IR (cm^{-1}): 1707 (COOR), 680-870 (Ar C-H bending), 3049 (Ar C-H Stretching), 1529-1761 (Ar C=C); ^{1}H NMR (DMSO, 400 MHz) (δ ppm): 2.28 (3H, s), 3.56 (3H, s), 5.20 (1H, s), 7.37 (2H, m), 7.43 (1H, m), 7.53 (2H, m), 7.63 (4H, t), 7.80 (1H, m), 7.83 (1H, s), 9.35 (1H, s), Mass: (m/z) 388, Yield= 76%.

Ethyl 6-(biphenyl-4-yl)-4-amino-1, 2-dihydropyrimidine-2 (1H)-thione-5-carboxylate (1j): IR (cm^{-1}): 3351 (NH_2), 680-870 (Ar C-H bending), 3050 (Ar C-H Stretching), 1530-1760 (Ar C=C); ^{1}H NMR (DMSO, 400 MHz) (δ ppm): 2.0 (3H, t), 4.8 (2H, q), 7.41 (1H, t), 7.44 (2H, d), 7.51 (4H, d), 7.63 (2H, d), 12.44 (1H, s), Mass: (m/z) 353, Yield= 28%.

4-(biphenyl-4-yl)-3-methyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(7H)-one (1k): IR (cm^{-1}): 1710 (COOR), 682-875 (Ar C-H bending), 3050 (Ar C-H Stretching), 1530-1760 (Ar C=C); ^{1}H NMR (DMSO, 400 MHz) (δ ppm): 2.00 (3H, s), 5.22 (1H, s), 7.27 (1H, s), 7.36 (4H, m), 7.45(1H, t), 7.53 (4H, m), 7.63 (1H, s), 13.00 (1H, s), Mass: (m/z) 304, Yield= 36%.

4-(biphenyl-4-yl)-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6(7H)-one (1m): IR(cm^{-1}): 1707 (COOR), 680-870 (Ar C-H bending), 3049 (Ar C-H Stretching), 1529-1761 (Ar C=C);

^{1}H NMR (DMSO, 400 MHz) (δ ppm): 2.35 (3H, s), 5.02 (1H, s), 7.27 (1H, t), 7.36 (2H, m), 7.54(4H, m), 7.57 (2H, d), 7.63 (2H, d), 14.05 (1H, s), Mass: (m/z) 380, Yield= 42%.

4-(biphenyl-4-yl)-3-methyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidine-6(7H)-thione (1l): IR (cm^{-1}): 1712 (COOR), 680-880 (Ar C-H bending), 3052 (Ar C-H Stretching), 1535-1755 (Ar C=C); ^{1}H NMR (DMSO, 400 MHz) (δ ppm): 1.93 (3H, s), 5.21 (1H, s), 7.33 (4H, m), 7.43(1H, t), 7.51 (4H, m), 7.81 (1H, s), 9.35 (1H, s), Mass: (m/z) 320, Yield= 33%.

4-(biphenyl-4-yl)-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidine-6(7H)-thione (1n): IR (cm^{-1}): 1710 (COOR), 682-870 (Ar C-H bending), 3050 (Ar C-H Stretching), 1530-1761 (Ar C=C); ^{1}H NMR (DMSO, 400 MHz) (δ ppm): 2.00 (3H, s), 5.20 (1H, s), 7.28 (1H, s), 7.34 (4H, m), 7.43 (1H, t), 7.51(4H, m), 7.63 (4H, m), 7.81 (1H, s), 13.85 (1H, s), Mass: (m/z) 396, Yield= 38%.

RESULTS AND DISCUSSION

We have synthesized fourteen novel derivatives of dihydropyrimidine by using seven different β-Keto esters i.e. ethylacetoacetate, acetoacetanilide, 2,6-dimethylacetoacetanilide, methyl acetoacetate, ethylcyanoacetate, 3-methyl-5-pyrazolone and 1-phenyl, 3-methyl-5-pyrazolone with urea or thiourea and biphenyl 4-carbaldehyde in DMSO solvent. They are listed in table 1. The structures of synthesized compounds were confirmed by ^{1}H NMR, IR and Mass spectrometry. The synthesized compounds were evaluated for their *in-vitro* anti-bacterial activity against *S. Aureus*, *B. megaterium* (gram+ve bacteria) and *P. fluorescens*, *S. marcensus* (gram-ve bacteria) with reference standard drug Ciprofloxacin and anti fungal activity against *A. niger* fungus with reference standard drug Carbendazim. Results of microbial activity are recorded in given following table 2.

From the *in-vitro* analysis data, It is found that compound **1j** is highly active against *P. fluorescens* (Gram-ve bacteria). Compound **1b** is moderately active against *S. aureus, B. Megaterium* (Gram+ve bacteria) and *P. fluorescens* (Gram-ve bacteria). Compound **1i** is moderately active against *P. fluorescens* (Gram-ve bacteria). Compounds **2b** and **2d** are moderately active against *S. aureus, B. megaterium* (Gram+ve bacteria) and *P. fluorescens, S. marcensus* (Gram-ve bacteria).

Compound **1j** is more active as compare to standard antifungal drug Carbendazim against *A. niger*. Compounds **1h**, **1i**, **2b** and **2d** are moderately active against *A.niger*.

Table 1. Physical properties of synthesized novel biphenylic compounds

S. No.	Id	-R_1	-R_2	-R_3	X	M. F.	M.W. (g mole^{-1})	Yield (%)	M.P. (°C)
1	1a	-$COOC_2H_5$	-CH_3	-	O	$C_{20}H_{20}N_2O_3$	336.38	88	236
2	1b	-$CONHC_6H_3$2,6-$(CH_3)_2$	-CH_3	-	O	$C_{26}H_{25}N_3O_2$	411.50	24	110
3	1c	-$CONHC_6H_5$	-CH_3	-	O	$C_{24}H_{21}N_3O_2$	383.44	23	132
4	1d	-$COOCH_3$	-CH_3	-	O	$C_{19}H_{18}N_2O_3$	322.36	86	265
5	1e	-$COOC_2H_5$	-NH_2	-	O	$C_{19}H_{17}N_3O_3$	345.35	56	117
6	1f	-$COOC_2H_5$	-CH_3	-	S	$C_{20}H_{20}N_2O_2$ S	352.45	52	198
7	1g	-$CONHC_6H_3$2,6-$(CH_3)_2$	-CH_3	-	S	$C_{26}H_{25}N_3O$ S	427.56	18	220
8	1h	-$CONHC_6H_5$	-CH_3	-	S	$C_{24}H_{21}N_3O$ S	399.51	21	198
9	1i	-$COOCH_3$	-CH_3	-	S	$C_{19}H_{18}N_2O_2$ S	338.42	76	185
10	1j	-$COOC_2H_5$	-NH_2	-	S	$C_{19}H_{17}N_3O_2S$	361.42	28	210
11	2a	-	-	-H	O	$C_{18}H_{16}N_4O$	304.35	36	205
12	2b	-	-	-C_6H_5	O	$C_{24}H_{20}N_4O$	380.44	42	202
13	2c	-	-	-H	S	$C_{18}H_{16}N_4S$	320.41	33	236
14	2d	-	-	-C_6H_5	S	$C_{24}H_{20}N_4S$	396.51	38	258

M.F. = Molecular Formula, M. W. = Molecular Weight

Table-2 Antimicrobial activity of synthesized compounds

S. No	Compound Id	Minimum Bacterial Concentration (MBC) (µg mL^{-1})				MFC (µg mL^{-1})
		Gram +ve Bacteria		Gram -ve Bacteria		
		S. aureus MTCC-96	*B.megaterium* MTCC-453	*P. fluorescens* HQ907732	*S. marcescen* MTCC-8708	*A.niger* KY964055
1	1a	>1000	>1000	>1000	>1000	>1000
2	1b	**100**	**100**	**100**	>1000	>1000
3	1c	>1000	>1000	>1000	>1000	>1000
4	1d	**100**	>1000	>1000	>1000	>1000
5	1e	>1000	>1000	>1000	>1000	>1000
6	1f	>1000	>1000	>1000	>1000	>1000
7	1g	>1000	>1000	>1000	>1000	>1000
8	1h	>1000	>1000	>1000	>1000	**100**
9	1i	>1000	>1000	**100**	>1000	**100**
10	1j	>1000	>1000	**10**	>1000	**10**
11	2a	>1000	>1000	>1000	>1000	>1000
12	2b	**100**	**100**	**100**	**100**	**100**
13	2c	500	**250**	500	500	**250**
14	2d	**100**	**100**	**100**	**100**	**100**
15	Ciprofloxacin	**50**	**10**	**10**	**10**	-
16	Carbendazim	-	-	-	-	**100**

S. aureus=Staphylococcus aureus, B. megaterium=Bacillus megaterium, P. fluorescens=Pseudomonas fluorescens, S. marcensus = Serratia marcescens, A. niger= Aspergillus niger, MFC= Minimum Fungal Concentration.

Out of fourteen compounds synthesized in the present work, a brief spectral discussion of compound 1a is as follows. Compound 1a exhibited absorption at 1707 cm^{-1} due to C=O ester carbonyl in IR spectrum. Some of the characteristics signals in ^{1}H NMR of the compound 1a are, 1.13 δ ppm 3H triplet corresponds to methyl group of ethyl ester, 2.29 δ ppm 3H singlet due to methyl group d attached at carbon of sixth position , 5.22 δ ppm 1H due to one proton of 3,4-DHPM ring etc. The mass spectrum of compound 1a, the molecular ion peak at m/z = 336 corresponding to molecular formula $C_{20}H_{20}N_2O_3$ (Figure 1-3).

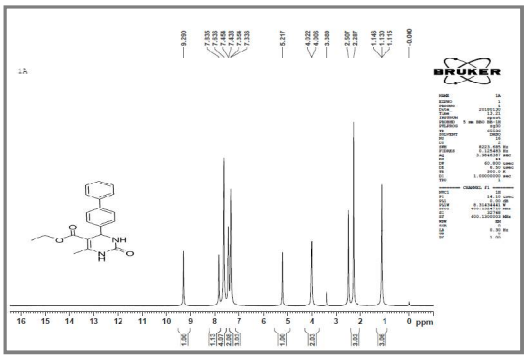


Figure 1. ^{1}H NMR of the compound 1a.

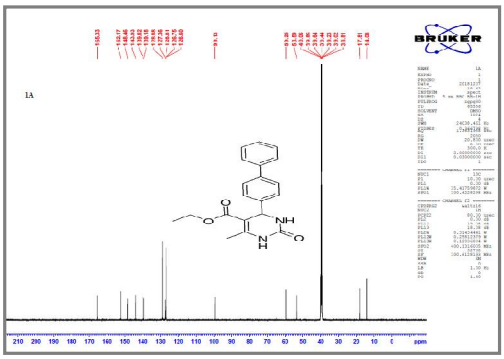


Figure 2. ^{13}C NMR of the compound 1a.

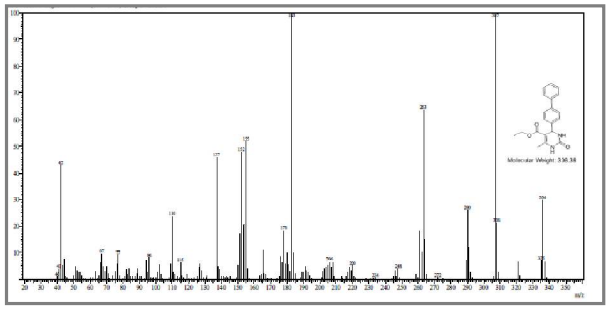


Figure 3. Mass spectra of compound 1a.

APPLICATION

Out of synthesized fourteen compounds, compound **1j** was found as active as standard drug Ciprofloxacin against *P. fluorescens* gram –ve bacteria and the same compound **1j** was found to be potent against against *A. niger* fungus as compare to standard drug Carbendazim. Further work on this compound may lead to invention of lead molecule in future.

CONCLUSION

The brief structure activity relationship study of final compounds (1a to 1n), it was observed that variety of groups resulted into variation in anti-microbial activity of the final structure. Over all it was found that electron donating groups like methyl, amino etc lead to improve anti-microbial activity in some of the final products.

ACKNOWLEDGEMENTS

The authors are thankful to Dr (Fr) Lancelot D' Cruz, Principal, St. Xavier`s College (Autonomous) (affiliated to Gujarat university) Ahmedabad for providing required research facilities. Authors are also thankful to Dr. Sudeshna Menon, and entire Department of Biochemistry-Biotechnology (In Xitu), St.Xavier's College, Ahmedabad (Gujarat) for carrying out antimicrobial activity of the newly synthesized compounds.

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