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

ISSN: 2278-1862

Journal of Applicable Chemistry

2019, 8 (3): 1146-1154

(International Peer Reviewed Journal)

Synthesis, Characterization and *in vitro* Antimicrobial Activity of Newly Synthesized 4-(2'-cyanobiphenylic-2-yl)-3, 4-dihydropyrimidines

Atul H Makwana* and Alimamad H Malani

St. Xavier's College (Autonomous) (Affiliated to Gujarat University), Navarangpura, Ahmedabad-380009, **INDIA**

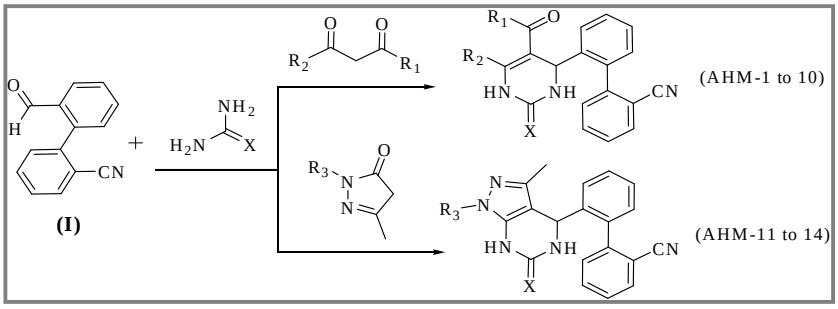
Email: makwana_atul@yahoo.com

Accepted on 1st May, 2019

ABSTRACT

A series of fourteen novel cyano group containing 3, 4-dihydropyrimidine derivatives was synthesized through Biginelli reaction. It has used different seven β-Keto esters i.e. ethylacetoacetate, acetoacetanilide, 2,6-dimethylaceto acetanilide, methyl acetoacetate, ethylcyanoacetate, 3-methyl-5-pyrazolone, 1-phenyl, 3-methyl-5-pyrazolone etc and urea or thiourea along with 2'-formylbiphenyl-2-carbonitrile (I) in dimethyl sulphoxide 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-microbial activity by using broth dilution method to determine their minimum inhibitory concentration.

Graphical Abstract



Keywords: Cyano biphenyl dihydropyrimidine, Biginelli product, Suzuki reaction, Anti-microbial activity, Multicomponent reactions (MCRs).

INTRODUCTION

3,4-dihydropyrimidines are multifunctionalized nitrogen containing heterocyclic molecules which can be easily synthesized by one pot multi-component reaction of variable aldehydes, β-keto esters and urea or thiourea. It was first reported by P. Biginelli in the year 1891 [1]. Dihydropyrimidine derivatives have attracted considerable attention of scientists due to their wide range of therapeutics importance [2]. In the context of therapeutic compounds heterocyclic moieties are very important to

study for the development of medicinally important lead molecules. Among the heterocyclic compounds, 3,4-dihydropyrimidines have their own importance as biologically active compounds [**3**]. The 3,4-dihydropyrimidines (DHPMs) possess broad range of pharmaceutical activities like anti-depressant [**4**], calcium channel blockers, anti-hypertensive [**5**], anti-cancer [**6**] anti-tumour [**7**], antibacterial [**8**], anti-fungal [**9**] anti-mitotic [**10**] etc.

Looking to the importance of 3,4-dihydropyrimidines, we have synthesized novel derivatives of 4-(2'-cyanobiphenylic-2-yl)-3, 4-dihydropyrimidines by Biginelli reaction and evaluated the newly synthesized compounds for their anti-microbial activity by broth dilution method to determine minimum inhibitory concentration (MIC) in this scope of work.

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 2'-formylbiphenyl-2-carbonitrile (I): For the preparation of intermediate-(I) a mixture of 2-Bromobenzaldehyde (0.5405 mol), 2-cyanobenzenboronicacid 1,3-propanediol ester (0.6486 mol) were taken in round bottom flask in toluene (500 ml), followed by the addition of K_2CO_3 (0.8108 mol) in water (200 ml). Tetrakis Pd (0) (5%) was used as catalyst. The reaction mass was heated at 120°C for 3-4 hours. The reaction mass was cooled to room temperature after completion of the reaction. 500 ml water was added to it. The reaction mass was allowed to separate. The organic residue was distilled off under vacuum at 50°C-60°C to give intermediate-I. The progress and completion of the reaction was confirmed by TLC (mobile phase: 3:7 Ethyl acetate: Cyclohexane, 2-3 drops of ammonia). IR (cm^{-1}): 2222 (-CN), 1726.29 (-CHO) ^{1}H NMR: 7.60(2H, m), 7.75 (3H, m), 7.95 (1H, d), 8.53(1H, d), 10.36 (1H, s), Mass: (m/z) 206, M. P. 121°C, Yield= 72%.

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 hours 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 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 hours 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 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) ethylacetoacetate (0.0768M) and hydrazine (0.0768M) 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. Finally the reaction mass was filtered, washed with MTBE and recrystalized from ethanol to form white product 5-methyl-2, 4-dihydro-3*H*-pyrazol-3-one (IV). M.P. 110°C, Yield: 90%.

Preparation of 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (V): For the preparation of intermediate-(V) a mixture of ethylacetoacetate (0.0768M) and phenyhydrazine (0.0768M) was heated at 120°C-130°C in oil bath for7-8 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 product 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (V). M.P. 127°C, Yield: 85%.

Preparation of final compounds AHM-1 to AHM-14: In the final step of the present work 2'-formylbiphenyl-2-carbonitrile (I) (0.0241 mol), β-Keto esters (II to IV or EAA or MAA or ECA) (00.0241 mol) and urea or thiourea (0.0241 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 hours. 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 **AHM-1** to **AHM-14**. The progress and completion of the reaction was confirmed by TLC (3:7 Ethyl acetate: Cyclohexane).

Spectral data of final products (AHM-1 to AHM-14):

Ethyl4-(2'-cyanobiphenyl-2-yl)-6-methyl-3,4-dihydropyrimidine-2(1*H*)-one-5-carboxylate(AHM-1): IR (cm^{-1}): 756 (C-H banding, 1,2-substituted benzene ring), 1311 (C-N bending, Ar 2°-Amine), 1458 (C-H bending, -CH_2, Methylene), 1636 (C=C Stretching, aromatic ring), 1690 (C=O Stretching, 2° Amide), 2222 (C-N Stretching, -CN), 2970 (C-H Stretching, Alkyl, -CH_3), 3109 (C-H Stretching, Aromatic ring), 3325 (N-H Stretching, 2°-Amine); NMR (DMSO, 400 MHz) (δ ppm): 0.88 (3H, t, J=13.6, 6.8), 2.10 (3H, s), 3.69 (2H, q, J= 17.6, 7.2), 5.03 (1H, s), 7.19 (1H, d, J= 7.2), 7.38, (2H, m) 7.50 (2H, m), 7.58 (2H, m), 7.92 (2H, m), 8.96 (1H, s); Mass: (m/z) 361, Yield= 72%.

4-(2'-cyanobiphenyl-2-yl)-*N*-(2,6-dimethylphenyl)-6-methyl-2-oxo-3,4-dihydropyrimidine-5-carboxamide (AHM-2): IR (cm^{-1}): 1658 (C=C Stretching, aromatic ring), 1683 (C=O Stretching, 2° Amide), 2226 (C-N Stretching, -CN), 3009 (C-H Stretching, Alkyl, -CH_3), 3130 (C-H Stretching, Aromatic ring); NMR (DMSO, 400 MHz) (δ ppm): 2.18 (6H, s), 2.29 (3H, s), 5.13 (1H, s), 7.19 (3H, m), 7.33, (3H, m) 7.70 (4H,m), 7.98 (1H, s, -NH), 9.10 (1H, s, -NH), 10.40 (1H, s, -NH); Mass: (m/z) 436, Yield= 28%.

4-(2'-cyanobiphenyl-2-yl)-*N*-phenyl-6-methyl-2-oxo-3,4-dihydopyrimidine-5-carboxamide(AHM-3): IR (cm^{-1}): 1664 (C=C Stretching, aromatic ring), 1683 (C=O Stretching, 2° Amide), 2223 (C-N Stretching, -CN), 3010 (C-H Stretching, Alkyl, -CH_3), 3133 (C-H Stretching, Aromatic ring); NMR (DMSO, 400 MHz) (δ ppm): 2.29 (3H, s), 5.13 (1H, s), 7.19 (4H, m), 7.43, (2H, d, J=7.2) 7.71 (7H, m), 7.97 (1H, s, -NH), 9.10 (1H, s, -NH), 10.64 (1H, s, -NH); Mass: (m/z) 408, Yield= 26%.

Methyl4-(2'-cyanobiphenyl-2-yl)-6-methyl-3,4-dihydropyrimidine-2(1*H*)-one-5-carboxylate (AHM-4): IR (cm^{-1}): 756 (C-H banding, 1,2-substituted benzene ring), 1311 (C-N bending, Ar 2°-Amine), 1636 (C=C Stretching, aromatic ring), 1697 (C=O Stretching, 2° Amide), 2222 (C-N Stretching, -CN), 2970 (C-H Stretching, Alkyl, -CH_3), 3117 (C-H Stretching, Aromatic ring), 3364 (N-H Stretching, 2°-Amine); NMR (DMSO, 400 MHz) (δ ppm): 2.23 (3H, s), 3.26 (3H, s), 4.96 (1H, s), 7.19 (1H, d, J= 7.2), 7.28, (1H, d, 7.6), 7.40 (1H,m), 7.45 (2H,m), 7.58 (1H,m), 7.75 (1H, m), 7.92 (1H,m), 9.31 (1H, s); Mass: (m/z) 347, Yield= 81%.

Ethyl 6-(2'-cyanobiphenyl-2-yl)-4-amino-1,2-dihydropyrimidine-2(1*H*)-one-5-carboxylate (AHM-5): IR (cm^{-1}): 1430 (C-H bending, -CH_2, Methylene), 1636 (C=C Stretching, aromatic ring), 1730 (C=O, Stretching, ester), 2228 (C-N Stretching, -CN), 2992 (C-H Stretching, Alkyl, -CH_3), 3106 (C-H Stretching, Aromatic ring), NMR (DMSO, 400 MHz) (δ ppm): 1.26 (3H, t, 15.2, 7.6), 4.26 (2H, q, J= 18.2, 7.6), 6.66 (2H, s, -NH_2), 7.29 (3H, m), 7.58, (1H, t, J=15.4, 7.2), 7.70 (3H, m), 7.95 (1H, s, -NH), 8.18 (1H, d, J=6.8); Mass: (m/z) 362, Yield= 40%.

Ethyl4-(2'-cyanobiphenyl-2-yl)-6-methyl-3,4-dihydropyrimidine-2(1*H*)-thione-5-carboxylate (AHM-6): IR (cm^{-1}): 762 (C-H banding, 1,2-substituted benzene ring), 1436 (C-H bending, -CH_2, Methylene), 1648 (C=C Stretching, aromatic ring), 2224 (C-N Stretching, -CN), 3012 (C-H Stretching, Alkyl, -CH_3), 3126 (C-H Stretching, Aromatic ring), NMR (DMSO, 400 MHz) (δ ppm): 0.89 (3H, t, J=12.8, 6.4), 2.20 (3H, s), 3.70 (2H, q, J= 16.4, 9.2), 5.03 (1H, s), 7.17-7.98 (9H, m), 9.22 (1H,s); Mass: (m/z) 477, Yield= 50%.

4-(2'-cyanobiphenyl-2-yl)-*N*-(2,6-dimethylphenyl)-6-methyl-2-thio-3,4-dihydropyrimid-ine-5-carboxamide (AHM-7): IR (cm^{-1}): 1659 (C=C Stretching, aromatic ring), 1682 (C=O Stretching, 2° Amide), 2222 (C-N Stretching, -CN), 3010 (C-H Stretching, Alkyl, -CH_3), 3130 (C-H Stretching, Aromatic ring); NMR (DMSO, 400 MHz) (δ ppm): 2.19 (6H, s), 2.30 (3H, s), 5.03 (1H, s), 7.17 (3H, m), 7.33, (3H, m) 7.72 (4H,m), 7.97 (1H, s, -NH), 9.12 (1H, s, -NH), 10.42 (1H, s, -NH); Mass: (m/z) 452, Yield= 26%.

4-(2'-cyanobiphenyl-2-yl)-*N*-(phenyl)-6-methyl-2-thio-3,4-dihydopyrimidine-5-carboxamide (AHM-8): IR (cm^{-1}): 1664 (C=C Stretching, aromatic ring), 1684 (C=O Stretching, 2° Amide), 2226 (C-N Stretching, -CN), 3012 (C-H Stretching, Alkyl, -CH_3), 3132 (C-H Stretching, Aromatic ring); NMR (DMSO, 400 MHz) (δ ppm): 2.29 (3H, s), 5.13 (1H, s), 7.17 (4H, m), 7.42, (2H, d, J=7.4) 7.72 (7H,m), 7.97 (1H, s, -NH), 9.11 (1H, s, -NH), 10.60 (1H, s, -NH); Mass: (m/z) 424, Yield= 32%.

Methyl 4-(2'-cyanobiphenyl-2-yl)-6-methyl-3, 4-dihydropyrimidine-2 (1*H*)-thione-5-carboxylate (AHM-9): IR (cm^{-1}): 758 (C-H banding, 1,2-substituted benzene ring), 1636 (C=C Stretching, aromatic ring), 1700 (C=O Stretching, 2° Amide), 2226 (C-N Stretching, -CN), 2990 (C-H Stretching, Alkyl, -CH_3), 3118 (C-H Stretching, Aromatic ring); NMR (DMSO, 400 MHz) (δ ppm): 2.23 (3H, s), 3.26 (3H, s), 5.03 (1H, s), 7.17 (1H, d, J= 7.4), 7.26, (1H, d, 7.4), 7.40 (1H, m), 7.45 (2H, m), 7.58 (1H, m), 7.75 (1H, m), 7.92 (1H,m), 9.32 (1H, s); Mass: (m/z) 363, Yield= 42%.

Ethyl 6-(2'-cyanobiphenyl-2-yl)-4-amino-1, 2-dihydropyrimidine-2 (1*H*)-thione-5-carboxylate (AHM-10): IR (cm^{-1}): 1430 (C-H bending, -CH_2, Methylene), 1638 (C=C Stretching, aromatic ring), 1732 (C=O, Stretching, ester), 2222 (C-N Stretching, -CN), 2990 (C-H Stretching, Alkyl, -CH_3), 3116 (C-H Stretching, Aromatic ring), NMR (DMSO, 400 MHz) (δ ppm): 1.26 (3H, t, 15.2, 7.4), 4.26 (2H, q, J= 16.2, 7.4), 6.67 (2H, s, -NH_2), 7.29 (3H, m), 7.57, (1H, t, J=15.2, 7.2), 7.70 (3H, m), 7.97 (1H, s, -NH), 8.20 (1H, d, J=7.2); Mass: (m/z) 376, Yield= 31%.

4'-(3-methyl-4,5,-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6(7*H*)-one-2-yl)biphenyl-2-carbonitrile (AHM-11): IR (cm^{-1}): 756(1,2-substituted C-H banding), 1425 (C-H bending, -CH_2, Methylene), 1640 (C=C Stretching, Aromatic rig), 2223 (C-N Stretching, -CN), 2998 (C-H Stretching, Alkyl,-CH_3), 3120 (C-H Stretching, Aromatic ring), NMR(DMSO, 400 MHz) (δppm): 1.59 (3H, s), 4.76 (1H, s), 7.03 (1H, d, J=7.2), 7.19 (1H, d, J=7.6), 7.26 (1H, t, J= 14.8, 7.6), 7.35 (1H, t, J= 15.2, 7.6), 7.54 (2H, m), 7.66 (1H, t, J= 15.2, 7.6), 7.87 (1H, d, J= 7.6), 10.98 (3H, s, -NH); Mass: (m/z) 329, Yield = 36%.

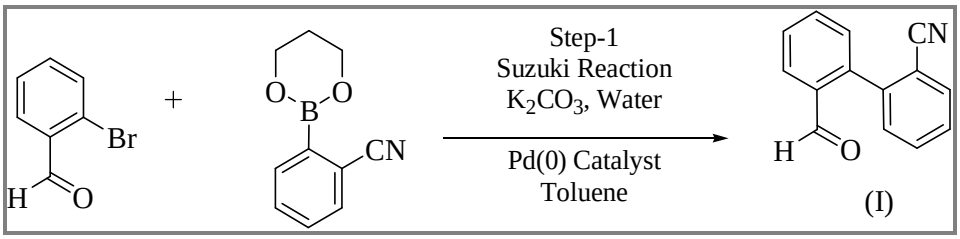
4'-(3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-d]pyrimidin-6(7*H*)-one-2-yl)biphenyl-2-carbonitrile (AHM-12): IR (cm^{-1}): 760(1,2-substituted C-H banding), 1330 (C-N bending, 2°-Amine), 1455 (C-H bending, -CH_2, Methylene), 1636 (C=C Stretching, Aromatic rig), 1690 (C=O Stretching, 2°-Amide), 2220 (C-N Stretching, -CN), 2980 (C-H Stretching, Alkyl,-CH_3), 3110 (C-H Stretching, Aromatic ring), 3345 (N-H Stretching, 2°-Amine); NMR(DMSO, 400 MHz) (δppm): 1.65 (3H, s), 4.84 (1H, s), 7.07 (1H, d, J=7.6), 7.24 (2H, s), 7.32 (1H, t), 7.42 (1H, m), 7.61 (4H, m), 7.73 (1H, m), 7.90 (1H, d, J= 7.2), 12.32 (1H, s), 13.30 (1H, s); Mass: (m/z) 405, Yield = 30%.

4'-(3-methyl-4,5,-dihydro-1*H*-pyrazolo[3,4-d]pyrimidin-6(7*H*)-thione-2-yl)biphenyl-2carbo-nitrile(AHM-13): IR (cm^{-1}): 762(1,2-substituted C-H banding), 1432 (C-H bending, -CH_2, Methylene), 1640 (C=C Stretching, Aromatic rig), 2222 (C-N Stretching, -CN), 3000 (C-H

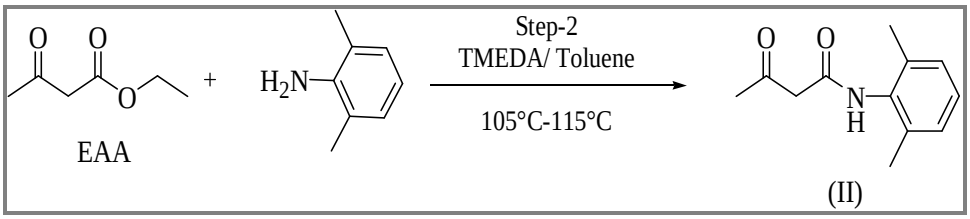
Stretching, Alkyl,-CH_3), 3112 (C-H Stretching, Aromatic ring), NMR(DMSO, 400 MHz) (δppm): 1.59 (3H, s), 4.76 (1H, s), 7.04 (1H, d, J=7.2), 7.20 (1H, d, J=7.6), 7.25 (1H, t, J= 14.8, 7.2), 7.36 (1H, t, J= 15.4, 7.2), 7.57 (3H, m), 7.90 (1H, d, J= 7.2), 11.03 (3H, s, -NH); Mass: (m/z) 345, Yield = 32%.

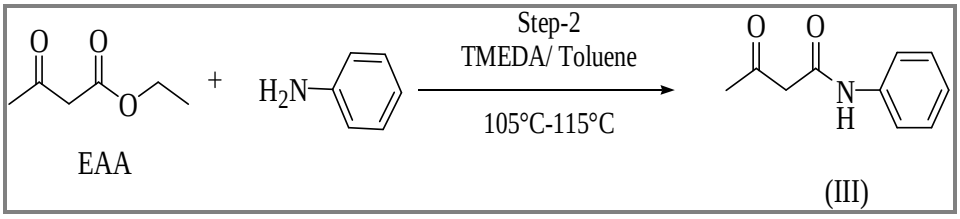
4'-(3-methyl-1-phenyl-4,5,-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6(7*H*)-thione-2-yl)bip-henyl-2-carbonitrile (AHM-14): IR (cm^{-1}): 763(1,2-substituted C-H banding), 1433 (C-H bending, -CH_2, Methylene), 1636 (C=C Stretching, Aromatic rig), 2221 (C-N Stretching, -CN), 2985 (C-H Stretching, Alkyl,-CH_3), 3112 (C-H Stretching, Aromatic ring), NMR(DMSO, 400 MHz) (δppm): 1.65 (3H, s), 4.84 (1H, s), 7.10 (1H, d, J=7.4), 7.24 (2H, s), 7.34 (1H, t, J=15.2, 7.6), 7.42 (2H, m), 7.66 (3H, m), 7.83 (2H, m), 12.40 (1H, s), 13.38 (1H, s); Mass: (m/z) 421, Yield = 44%.

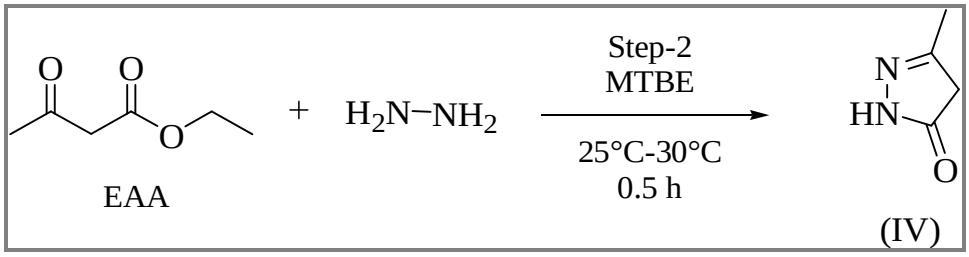
Step 1. Scheme for synthesis of intermediate 2'-formylbiphenyl-2-carbonitrile (I)

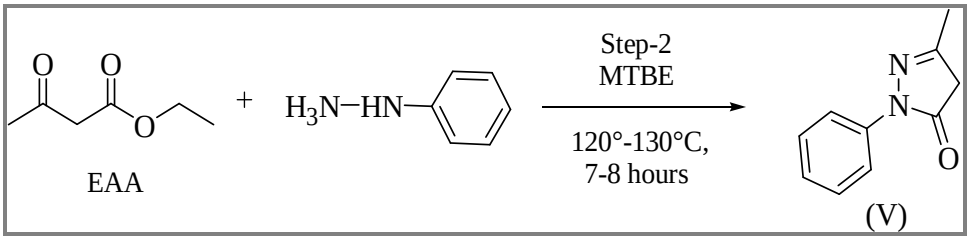


Step 2. Scheme for synthesis of intermediate β-keto esters (II, III, IV and V)

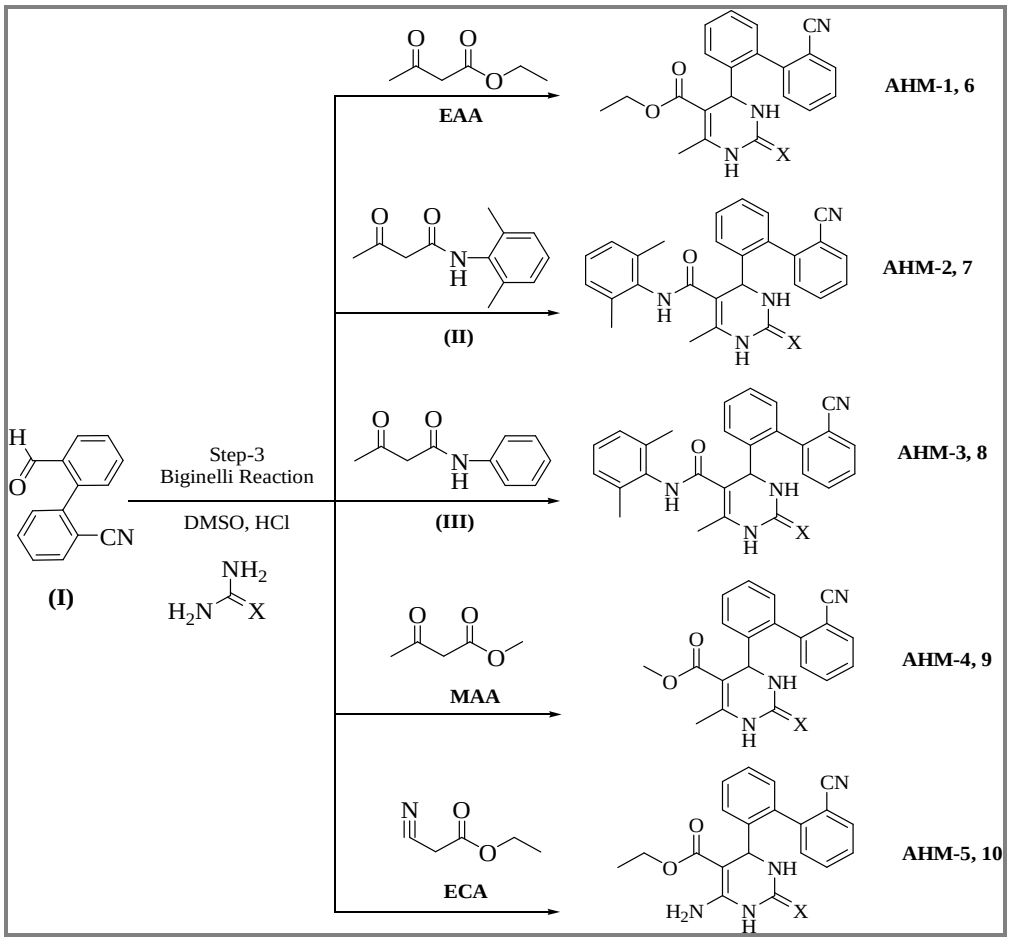


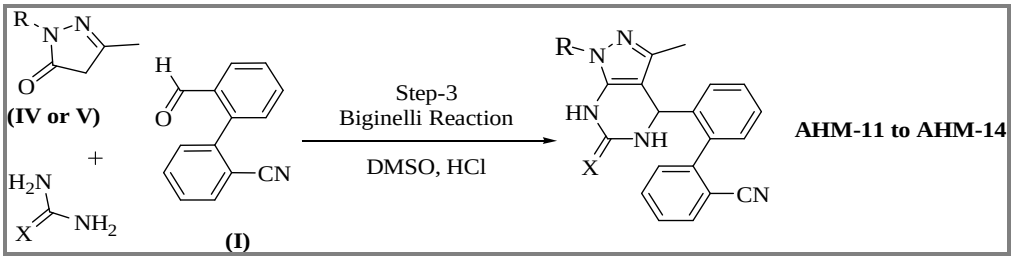






Step 3. Scheme for synthesis of final products (AHM-1 to AHM-14)





Where X = O or S, Where R = -H, or C_6H_5

RESULTS AND DISCUSSION

In the present work, synthesized fourteen novel derivatives of dihydropyrimidine by using seven different β-Keto esters i.e. ethylacetoacetate, acetoacetanilide, 2,6-dimethylacetoacetanilide, methyl acetoacetate, ethyl cyanoacetate, 3-methyl-5-pyrazolone and 1-phenyl, 3-methyl-5-pyrazolone with urea or thiourea and biphenyl 2'-formylbiphenyl-2-carbonitrile in DMSO solvent in a acidic medium. 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 culture activity against *A.*

niger fungal with reference standard drug Carbendazim. Results of microbial activities are recorded in given following table 2.

From the *in-vitro* analysis data, it was found that compounds AHM-1 and AHM-4 were highly active against Gram +ve bacteria *S. aureus* and *B. Megaterium* respectively. While compounds AHM-6 and AHM-14 were also found highly active against Gram -ve bacteria *P. fluorescens* and *S. marcensus* respectively. The compounds AHM-7, AHM-9, AHM-11 and AHM-12 were found moderately active against Gram +ve bacteria *S. Aureus*. While the compounds AHM-3, AHM-5 were also found moderately active against Gram +ve bacteria *B. Megaterium*. Additionally the compounds AHM-8**,** AHM-6 and AHM-10were found moderately active against Gram -ve bacteria *S. Marcensus* when compared to standard drug Ciprofloxacin.

Out of the fourteen newly synthesized compounds AHM-9 and AHM-10 were found highly potent towards fungal culture *A. niger*. While compounds AHM-4 and AHM-8 seems to be moderately active against fungal culture *A. niger* compared to standard drug Carbendazim.

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	AHM-1	-$COOC_2H_5$	-CH_3	-	O	$C_{21}H_{19}N_3O_3$	361.39	72	210
2	AHM-2	-$CONHC_6H_3$2,6-$(CH_3)_2$	-CH_3	-	O	$C_{27}H_{24}N_4O_2$	436.51	28	95
3	AHM-3	-$CONHC_6H_{5s}$	-CH_3	-	O	$C_{25}H_{20}N_4O_2$	408.45	26	112
4	AHM-4	-$COOCH_3$	-CH_3	-	O	$C_{20}H_{17}N_3O_3$	347.37	81	229
5	AHM-5	-$COOC_2H_5$	-NH_2	-	O	$C_{20}H_{16}N_4O_3$	360.37	40	120
6	AHM-6	-$COOC_2H_5$	-CH_3	-	S	$C_{21}H_{19}N_3O_2S$	377.46	50	211
7	AHM-7	-$CONHC_6H_3$2,6-$(CH_3)_2$	-CH_3	-	S	$C_{27}H_{24}N_4O$ S	452.57	26	128
8	AHM-8	-$CONHC_6H_5$	-CH_3	-	S	$C_{25}H_{20}N_4O$ S	424.52	32	144
9	AHM-9	-$COOCH_3$	-CH_3	-	S	$C_{20}H_{17}N_3O_2$ S	463.43	42	231
10	AHM-10	-$COOC_2H_5$	-NH_2	-	S	$C_{20}H_{16}N_4O_2S$	376.43	31	138
11	AHM-11	-	-	-H	O	$C_{19}H_{15}N_5O$	329.36	36	185
12	AHM-12	-	-	-C_6H_5	O	$C_{25}H_{19}N_5O$	405.45	30	150
13	AHM-13	-	-	-H	S	$C_{19}H_{15}N_5S$	345.42	32	192
14	AHM-14	-	-	-C_6H_5	S	$C_{25}H_{19}N_5S$	421.52	44	168

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

Table 2 Antimicrobial activity of synthesized compounds

S. No	Compound Id	Minimum Inhibitory Concentration (MIC) (µg mL^{-1})				MIC (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	AHM-1	**10**	1000	250	500	1000
2	AHM-2	1000	1000	500	500	1000
3	AHM-3	500	**100**	1000	500	1000
4	AHM-4	500	**10**	500	1000	**250**
5	AHM-5	250	**100**	500	250	500
6	AHM-6	**100**	500	**10**	250	500
7	AHM-7	**100**	500	1000	250	500
8	AHM-8	1000	500	250	**100**	**250**
9	AHM-9	500	1000	250	**100**	**100**
10	AHM-10	500	1000	250	**100**	**100**
11	AHM-11	**100**	500	1000	1000	500
12	AHM-12	**100**	250	500	500	1000
13	AHM-13	1000	1000	1000	500	1000
14	AHM-14	1000	250	1000	**10**	1000
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.

In the present work we have prepared fourteen novel derivatives of 3,4-dihydropyrimidines by Biginelli reaction. The structures of the newly synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. Compound AHM-1 exhibited absorption at 2222 cm^{-1} due to C-N stretching (Nitrile group) in IR spectrum. The mass spectrum of compound AHM-1, the molecular ion peak at m/z = 361 corresponding to molecular formula $C_{21}H_{19}N_3O_3$..Some of the characteristics signals in ^{13}C NMR of the compound AHM-1 are165.3 δ ppm due to carbonyl carbon, 117.1 δ ppm due to nitrile group carbon. Some of the characteristics signals in ^{1}H NMR of the compound AHM-12 are1.39 δ ppm 3H singlet corresponds to methyl group, 4.84 δ ppm 1H due to one proton of 3,4-DHPM ring etc (Figures 1-4)

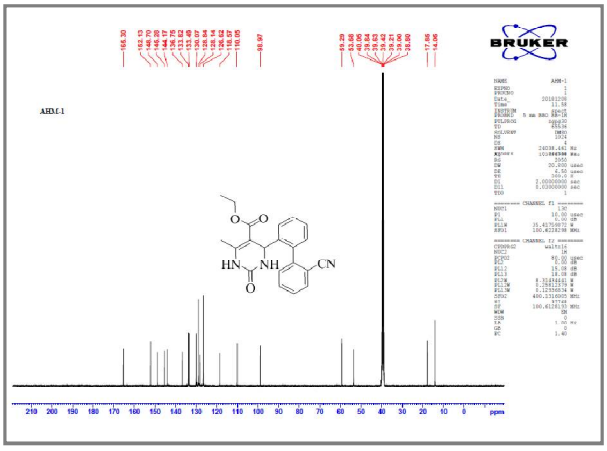


Figure 1. 13C NMR Spectra of Compound AHM-1.

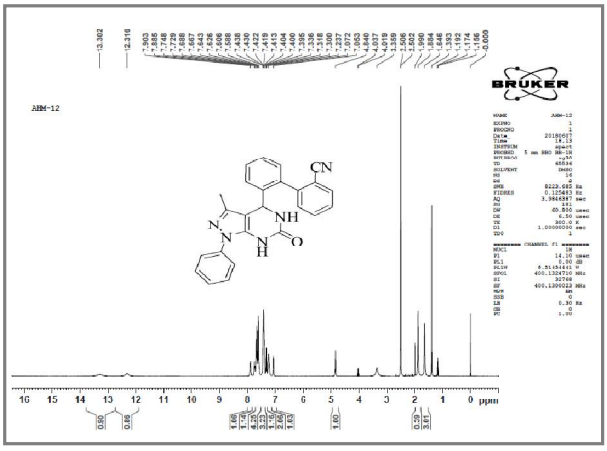


Figure 2. NMR Spectra of Compound AHM-12.

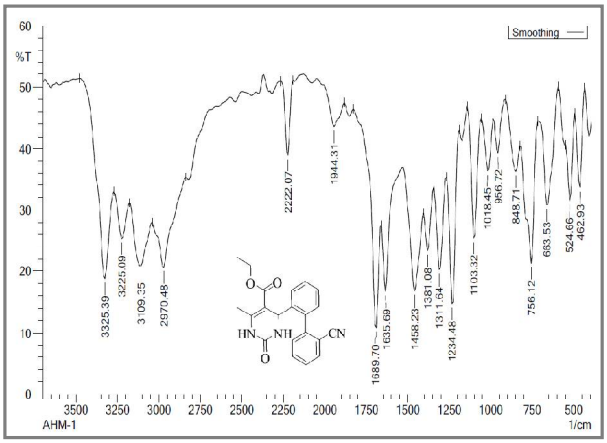


Figure 3. IR spectra of Compound AHM-1.

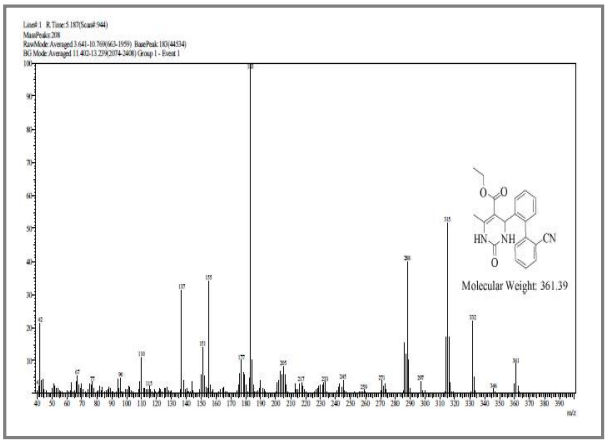


Figure 4. Mass spectra of Compound AHM-1.

APPLICATION

Out of fourteen compounds synthesized, compound **AHM-1** and **AHM-4** were found to possess high activity against *S. aureus* and *B.megaterium* (gram +ve bacteria) respectively. Similarly compound **AHM-6** and **AHM-14** exhibited high activity against *P. fluorescens* and *S. marcensus* gram (–ve bacteria) respectively. Compounds **AHM-9** and **AHM-10** showed good activity against *A. niger*. Further study of these compounds may help to identify more potent-lead molecule for anti-microbial activities in future.

CONCLUSION

Structural activity relationship study of final compounds (AHM-1 to AHM-14), it was observed that variation in the groups in the final compounds resulted into variation in anti-microbial activity. Over all it was observed that electron donating groups in some of the final structures have a little enhancement in anti-microbial activity.

ACKNOWLEDGEMENTS

The authors are thankful to Prof. N C Desai, Sr. Professor and Head, Department of Chemistry, M K Bhavnagar University, Bhavnagar, Gujarat, India for his guidance and excellent technical support throughout the research work. Authors are also thankful to Dr (Fr) Lancelot D'Cruz, Principal, St. Xavier`s College (Autonomous) (affiliated to Gujarat University) Ahmedabad for providing required research facilities.

REFERENCES

[1]. P. A. Ganie, A. Bhardwaj, S. K. Verma, A. Nabi, Microwave assisted synthesis and biological activity of dihydropyrimidine derivatives using DABCO: An efficient catalyst, *J. Applicable. Chem.*, **2019**, 8(1), 20-25.
[2]. N. C. Desai, A. R. Trivedi, H. V. Vaghani, H. C. Somani, K. A. Bhatt, Synthesis and biological evaluation of 1,3,4-oxadiazole bearing dihydropyrimidines as potential anti-tubercular agent, *Med. Chem. Res*., **2015**, 25 (2), 329-338.
[3]. R. Kaur, B. Kaur, Design, synthesis and study of fused 1,4-dihydropyrimidines of biological interest- A review, *J. Applicable. Chem.*, **2013**, 2(5), 1102-1115.
[4]. M. A. El-Borai, H. F. Rizk, I. Y. Al-Deeb, M. E. Sadek, A convenient synthesis of a series of pyrazolo[3,4-D]pyrimdines as potential anti-microbial and anti-oxidant agent, *J. Applicable. Chem.*, **2014**, 3(4), 1526-1537.
[5]. R. Gupta, Y. Madan, E. Menghani, Microwave assisted ZnO catalysed Biginelli synthesis of pyrazolopyrimidone derivatives and evaluation of their bioactivity, *J. Applicable. Chem.*, **2014**, 3(5), 1955-1966.
[6]. P. A. Ganie, A. Bhardwaj, A. R. Bhatt, $ZnCl_2$ supported with sand: An efficient synthetic protocol for synthesis of Biginelli product, *J. Applicable. Chem.*, **2018**, 7(1), 256-263.
[7]. K. S. Atwal, B. N. Swanson, S. E. Unger *et al*, Dihydropyrimidine calcium channel blockers. 3. 3-Carbamoyl-4-aryl-1,2,3,4-tetrahydro-6-methyl-5-pyrimidinecarboxylic acid esters as orally effective antihypertensive agents, *J. Med. Chem.*, **1991**, 34, 806-811.
[8]. L. M. Ramos, B. C. Guido, C. C. Nobrega *et al*, The Biginelli reaction with an imidazolium–tagged recyclable iron catalyst: kinetics, mechanism and anti-tumor Activity, *Chem. Eur. J.*, **2013**, 19(13), 4156-4168.
[9]. O. M. Singh, S. J. Singh, M. B. Devi *et al*, Synthesis and in vitro evaluation of the antifungal activities of dihydropyrimidinones, *Bio. Med. Chem. Let.*, **2008,** 18(24), 6462–6467.
[10]. H. Y. K. Kaan, V. Ulaganathan, C. O. Kappe *et al*, *J. Med. Chem.*, **2010**, 53, 5676–5683.