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Synthesis, Characterisation and Biological Evaluation of Some New Aryl Substituted Purine Derivatives

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ABSTRACT

The Reaction of 4, 6-dichloropyrimidine-2, 5-diamine hydro chloride with different substituted anilines in presence of alcohol results in the formation of aryl substituted pyrimidine derivatives (1-5). Aryl substituted pyrimidine derivatives react with Tri ethyl ortho formate in presence of acid form respective 6-chloro purine derivatives (6-10). Further 6-chloro purine derivatives react with cyclopropyl amine results in the formation of substituted purinyl derivatives (11-15). The structures of these compounds have been established by ^{1}H NMR studies, IR studies and Mass data. All the substituted purinyl derivatives were screened for their antimicrobial activity against four bacteria and three fungi activities. Some of the compounds found to have fairly good activity.

KEY WORDS: Pyrimidine, Aniline, 6-Chloro Purines, Substituted Purinyl Derivatives, Anti-Bacterial Activity, Anti-Fungal Activity.

1. INTRODUCTION

Purinyl derivatives have been used as effective anti virals (Nair, 2003; Ogilvie, 1984; Holy, 1999; Yahyazadeh, 2007; Murti, 2011). Some purinyl derivatives are used as potassium channel modulating agents which are useful for the treatment or alleviation of diseases of disorders associated with the activity of potassium channels. Few purinyl derivatives showed promising activity against some species of fungi (Ge, 2010; Hu, 2010). Some purinyl derivatives are used as antitumor agents (Shalaby, 1998; Raic, 1999) which are useful for the treatment of tumor. This article describes the preparation of 9- (3-chloro-4-fluorophenyl)-N^6-cyclopropyl-9H-purine-2,6-diamine,N^6-cyclopropyl-9-(3, 4-dichlo -rophenyl)-9H-purine-2, 6-diamine, 9-(3-chlorophenyl)-N^6-cyclopropyl-9H-purine-2, 6 - diamine, 4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl] benzonitrile, 3-[2-amino-6-(cyclopropyl amino)-9H-purin-9-yl] benzonitrile. The structures of these compounds have been established by ^{1}H NMR studies and synthesis.

2. MATERIALS AND METHODS

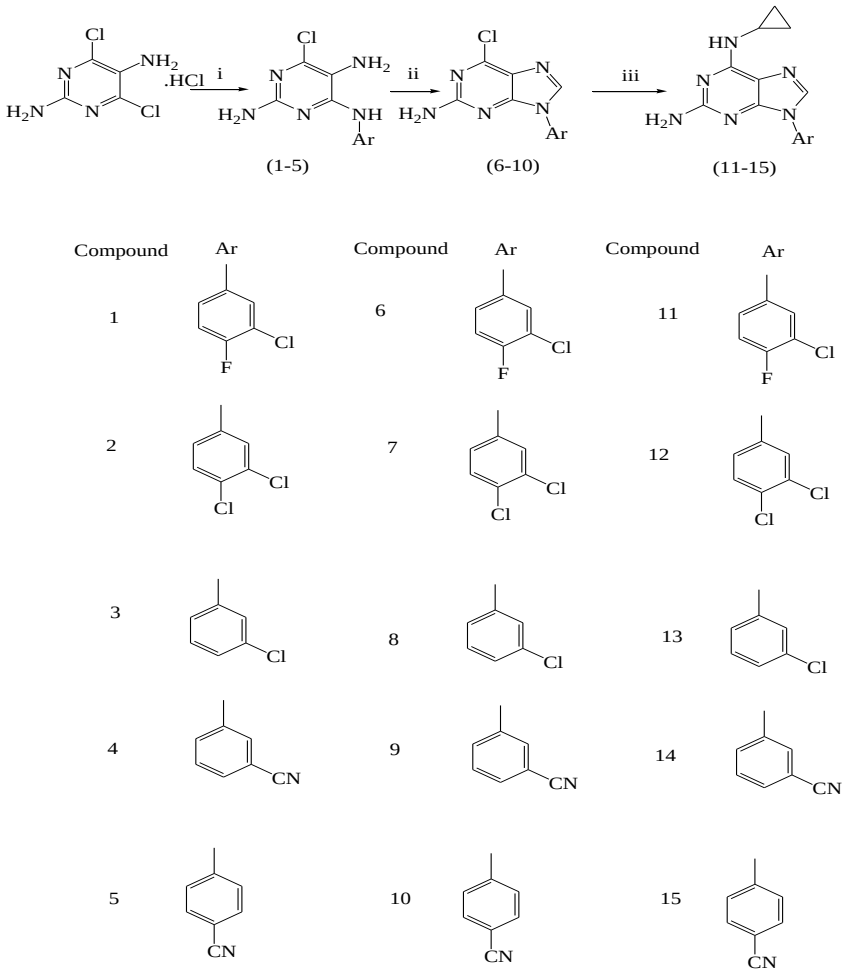
Melting points were determined on Mel-temp apparatus, laboratory devices, Cambridge, MA, USA and are uncorrected. IR spectra were recorded using KBr pellets on Perkin-Elmer SPECTRUM 100 FT-IR spectrophotometer. ^{1}H NMR spectra were recorded at 300 MHz on a BRUKER avance-11 FT-NMR spectrometer. All chemical shifts are reported in (ppm) using tetra methyl silane (TMS) as an internal standard. The electron spray ionisation MS (ESIMS) studies were performed on triple quadrupole mass spectrometer waters Quattro Micro API. Starting material and all chemicals used are received from Laurus labs limited, Hyderabad, Telangana, INDIA.

General procedure for the preparation of Pyrimidine derivatives (1-5): A solution of 4, 6-dichloropyrimidine-2,5-diamine hydro chloride (0.0190 moles) and substituted anilines (0.0209 moles) in n-Butanol (41 mL) was refluxed for 3-10 hrs .After the full conversion of the starting material was detected by TLC, the reaction mass cooled to room temperature. The reaction mixture stirred at room temperature for 1-2 h. The solid is filtered off, washed with n-Butanol (5 mL) and dried under suction.

General procedure for the preparation of Purine derivatives (6-10): A solution of the corresponding substituted pyrimidines (2.5gms) and conc. Hydrochloric acid (0.0226 moles) in of triethyl ortho formate (25 mL, 0.1498 moles) was stirred for 3-10 hrs at room temperature .After the full conversion of the starting material was detected by TLC, The solid is filtered off, washed with methanol (5 mL) and dried under suction.

General procedure for the preparation of Substituted Purine derivatives (11-15): A solution of 6-chloropurine derivatives (0.003 moles) and cyclo propyl amine(60.018 mole) in Isopropyl alcohol (5 mL) was refluxed for 8-10 hrs .After the full conversion of the starting material was detected by TLC, the reaction mass cooled to room temperature. The reaction mixture stirred at room temperature for 1-2 h. The solid is filtered off, washed with Isopropyl alcohol (5 mL) and dried under suction.

Scheme-1



Scheme 1: Reagents and conditions: - i) Different substituted anilines/n-Butanol, Reflux ii) Triethyl ortho formate/Conc. HCl, RT iii) Cyclo propyl amine/Iso propyl alcohol, 80-85°C

6-chloro-N4-(3-chloro-4-fluorophenyl) pyrimidine-2, 4, 5-triamine (1): The solid is purified in isopropyl alcohol to yield 5.1g (93.06%) of compound (4). IR (KBr pellet), ν, cm^{-1}:3367, 3299, 3166, 1498, 1345, 1300, 1058. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 4.17 (2H, S, NH_2), 5.98 (2H, S, NH_2), 7.26-8.06 (3H, m, ArH), 8.46 (1H, S, NH), MS, m/z (%) =288(M+1,100%), m.p 203-205 °C.

6-chloro-*N*^{4}-(3, 4-dichlorophenyl) pyrimidine-2, 4, 5-triamine (2): The solid is purified in isopropyl alcohol to yield 5.21g (89.98%) of compound (2). IR (KBr pellet), ν, cm^{-1}:3371, 3298, 3169, 1508, 1346, 1296, 1046. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 4.21 (2H, S, NH_2), 6.02 (2H, S, NH_2), 7.74-7.78 (1H, m, ArH), 7.45 (1H, db, ArH), 8.12 (1H,db, ArH),8.55 (1H, S, NH), MS, m/z (%) =304 (M+1,100%), m.p 202-203°C.

6-chloro-N4-(3-chlorophenyl) pyrimidine-2, 4, 5-triamine (3): The solid is purified in isopropyl alcohol to yield 4.7g (91.61%) of compound (3). IR (KBr pellet), ν, cm^{-1}: 3428, 3296, 3176, 1559, 1376, 1261, 1075. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 4.21 (2H, S, NH_2), 5.97 (2H, S, NH_2), 6.97-7.90 (4H, m, ArH), 8.47 (1H, S, NH), MS, m/z (%) =270(M+1,100%), m.p 183-185 °C.

4-[(2, 5-diamino-6-chloropyrimidin-4-yl) amino] benzonitrile (4): The solid is purified in isopropyl alcohol to yield 4.51g (91.11%) of compound (4). IR (KBr pellet), ν, cm^{-1}: 3428, 3362, 3194, 1514, 1357, 1321, 1097. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 4.22 (2H, S, NH_2), 6.06 (2H, S, NH_2), 7.37-8.32 (4H, m, ArH), 8.60 (1H, S, NH), MS, m/z (%) =261(M+1,100%), m.p 172-173°C.

3-[(2, 5-diamino-6-chloropyrimidin-4-yl) amino] benzonitrile (5): The solid is purified in isopropyl alcohol to yield 4.2g (84.84%) of compound (5). IR (KBr pellet), ν, cm^{-1}: 3407, 3315, 3180, 1508, 1313, 1264, 1036. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 4.36 (2H, S, NH_2), 6.07 (2H, S, NH_2), 7.66-7.70 (2H, m, ArH), 8.73 (1H, S, NH), MS, m/z (%) =261(M+1,100%), m.p 238-240 °C.

6-chloro-9-(3-chloro-4-fluorophenyl)-9H-purin-2-amine (6): The solid is purified in methanol to yield 2.15g (74.13%) of compound (6). IR (KBr pellet), ν, cm^{-1}: 3398, 3313, 2926, 1503, 1360, 1266, 1043. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 7.09 (2H, S, NH_2), 7.61-8.15 (3H, m, ArH), 8.51 (1H, S, CH), MS, m/z (%) =298(M+1,100%), m.p 294-296 °C.

6-chloro-9-(3, 4-dichlorophenyl)-9H-purin-2-amine (7): The solid is purified in methanol to yield 2.21g (76.73%) of compound (7). IR (KBr pellet), ν, cm^{-1}: 3308, 3198, 3094, 1503, 1332, 1285, 1032. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 7.12 (2H, S, NH_2), 7.83-7.92 (2H, m, ArH), 802 (H, db, ArH), 8.58 (1H, S, CH), MS, m/z (%) =315(M+1,100%), m.p 265-268 ºC.

6-chloro-9-(3-chlorophenyl)-9H-purin-2-amine (8): The solid is purified in methanol to yield 2.05g (69.96%) of compound (8). IR (KBr pellet), ν, cm^{-1}: 3483, 3304, 3097, 1519, 1329, 1307, 1040. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 7.09 (2H, S, NH_2), 7.48-8.00 (4H, m, ArH), 8.55 (1H, S, CH), MS, m/z (%) =280 (M+1,100%), m.p 246-248ºC.

4-(2-amino-6-chloro-9*H*-purin-9-yl) benzonitrile (9): The solid is purified in methanol to yield 2.31g (78.57%) of compound (9). IR (KBr pellet), ν, cm^{-1}: 3492, 3309, 3100, 2231, 1517, 1332, 1302, 1047. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 7.11 (2H, S, NH_2), 7.75-8.39 (4H, m, ArH), 8.61 (1H, S, CH), MS, m/z (%) =271 (M+1,100%), m.p 282-281ºC.

3-(2-amino-6-chloro-9*H*-purin-9-yl) benzonitrile (10): The solid is purified in methanol to yield 2.01g (68.36%) of compound (10). IR (KBr pellet), ν, cm^{-1}: 3436, 3317, 3099, 2231, 1505, 1333, 1306, 1032. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 7.14 (2H, S, NH_2), 8.05-8.17 (4H, m, ArH), 8.66 (1H, S, CH), MS, m/z (%) =271(M+1,100%), m.p 269-270ºC.

9-(3-chloro-4-fluorophenyl)-N^6-cyclopropyl-9*H*-purine-2, 6 diamine (11): The solid is purified in Iso propyl alcohol to yield 0.60g (63.02%) of compound (11). IR (KBr pellet), ν, cm^{-1}: 3338, 3276, 3207, 1502, 1357, 1311, 1057,672. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 0.56-0.66 (4H, m, CH_2-CH_2), 3.02 (1H, S, CH), 6.64 (2H, S, NH_2), 7.48 (1H, S, NH), 7.56-8.19 (3H, m, ArH), 8.16 (1H, S, CH), MS, m/z (%) =319 (M+1,100%), m.p 201-203ºC.

N^6-cyclopropyl-9-(3, 4-dichlorophenyl)-9*H*-purine-2, 6-diamine (12): The solid is purified in Iso propyl alcohol to yield 0.65g (68.13%) of compound (12). IR (KBr pellet), ν, cm^{-1}: 3386, 3305, 3190, 3088, 1518, 1356, 1313, 1073,681. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 0.60-0.66 (4H, m, CH_2-CH_2), 3.02 (1H, S, CH), 6.07 (2H, S, NH_2), 7.50 (1H, S, NH), 7.77-8.30 (3H, m, ArH), 8.23 (1H, S, CH), MS, m/z (%) =335 (M+1,100%), m.p 217-218ºC.

9-(3-chlorophenyl)-N^6-cyclopropyl-9*H*-purine-2, 6-diamine (13): The solid is purified in Iso propyl alcohol to yield 0.58g (61.11%) of compound (13). IR (KBr pellet), ν, cm^{-1}: 3408, 3310, 3198, 3106, 1524, 1344, 1307, 1046,689. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 0.56-0.69 (4H, m, CH_2-CH_2), 3.03 (1H, S, CH), 6.03 (2H, S, NH_2), 7.50 (1H, S, NH), 7.39-8.05 (3H, m, ArH), 8.19 (1H, S, CH), MS, m/z (%) =301 (M+1,100%), m.p 200-201ºC.

4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl] benzonitrile (14): The solid is purified in Iso propyl alcohol to yield 0.69g (72.78%) of compound (14). IR (KBr pellet), ν, cm^{-1}: 3446, 3337, 3212, 3129, 2232, 1508, 1353, 1323, 1033,682. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 0.56-0.69 (4H, m, CH_2-CH_2), 3.03 (1H, S, CH), 6.09 (2H, S, NH_2), 7.51 (1H, S, NH), 7.69-8.43 (3H, m, ArH), 8.25 (1H, S, CH), MS, m/z (%) =292 (M+1,100%), m.p 259-261ºC.

4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl] benzonitrile (15): The solid is purified in Iso propyl alcohol to yield 0.61g (64.34%) of compound (15). IR (KBr pellet), ν, cm^{-1}: 3473, 3381, 3190, 3106, 2223, 1507, 1357, 1308, 1073,666. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 0.56-0.69 (4H, m, CH_2-CH_2), 3.02 (1H, S, CH), 6.09 (2H, S, NH_2), 7.52 (1H, S, NH), 7.98-8.01 (2H, m, ArH),8.20-8.24(2H,m,ArH), 8.31 (1H, S, CH), MS, m/z (%) =292 (M+1,100%), m.p 213-215ºC.

3. RESULTS AND DISCUSSION

4, 6-dichloropyrimidine-2, 5-diamine Hydro chloride was prepared using *N*-(2-amino-4, 6-dichloro pyrimidin-5-yl) formamide in presence of ethanol and ethanolic HCl, at 50-55°C.

Aryl substituted derivatives were synthesized by reaction of different substituted anilines with 4, 6-dichloropyrimidine-2, 5-diamine Hydro chloride in refluxing n-Butanol for 3-10 hrs. (1 to 5). They were purified by using Iso propyl alcohol as a solvent (Scheme-1).

6-Chloro Purinyl derivatives were synthesized by reaction of aryl substituted pyrimidines with triethyl ortho formate in presence of Conc. HCl at room temperature for 8-12 hrs. (6 to 10). They were purified by using methanol as a solvent (Scheme-1).

Substituted purinyl derivatives were synthesized by reaction of different substituted 6-Chloro Purinyl derivatives with cyclo propyl amine in refluxing Isopropyl alcohol for 5-9 hrs (11 to 15).They were purified by using Isopropyl alcohol as a solvent (Scheme-1).1HNMR showed one singlet at δ 7.50 (1H, NH) and other characteristic signals were observed at 0.56-0.66 (4H, m, CH_2-CH_2) and 3.02 (1H, S, CH) confirming the presence of cyclo propyl group. The IR spectra of nitrile derivatives exhibited intense band at 2232 cm^{-1} confirming the presence of cyano group.

Antibacterial activity: The synthesized compounds were dissolved to prepare a stock solution of 500 mcg/ml and 50 mcg/ml using DMSO.All the compounds were tested for antibacterial activity against human and phytopathogenic gram positive bacteria (Staphylococcus aureus and Staphylococcus epidermidis) and gram negative (*E.coli*, *Salmonella abony*) using Mueller-Hinton broth and abacavir was used as positive control. The inhibition zones of microbial growth surrounding bacterial culture plates were measured in millimetres at end of an incubation period at 35-37°C for 18-24 hrs.

All tested compounds showed different activities towards the four species of. The results of the anti bacterial screening showed that all the compounds displayed good activity against gram negative (*E.coli, Salmonella abony*) at both low and high concentrations. Where as all the compounds showed no activity against gram positive bacteria (Staphylococcus aureus) at both high and low concentrations. Only two (11, 15) compounds showed activity against gram positive bacteria (Staphylococcus epidermidis) along with standard at both low and high concentrations. Where as three compounds (12, 13, 14) showed no activity against gram positive bacteria (Staphylococcus epidermidis) at both low and high concentrations. The compounds 11, 15, showed good activity when compared to the compounds 12, 13, 14 showed less activity.

Antifungal activity: The synthesized substituted purinyl derivatives were screened for their anti fungal activity against three species of fungi namely *Candida albicans, Saccharomyces cerevisiae, Aspergillus Niger* using Mueller-Hinton broth. The tested compounds were dissolved in DMSO to get required concentrations. Abacavir was taken as standard anti fungal reference. The inhibition zones of microbial growth surrounding fungal culture plates were measured in millimetres at end of an incubation period at 22-25°C for 18-24 hrs.

All tested compounds showed different activities towards the three species of fungi. The results of the anti fungal screening showed that all the compounds displayed good activity against Saccharomyces cerevisiae at both low and high concentrations. Where as all the compounds showed no activity against Candida arbicans and Aspergillus Niger at both low and high concentrations. The compounds 11, 12, 13, 14 showed good activity when compared to standard, where as the compound 15 showed less activity.

Table.1. Bacterial activity of Substituted purinyl derivatives. SW

Compound	Zone of inhibition in mm (MIC in mg/ml)			
	Echerichia coli	***Echerichia coli***	***Salmonella abony***	***Salmonella abony***
	High	Low	High	Low
11	14.1	9.9	14	9.9
12	13.8	10.1	14.1	9.6
13	14.1	10.2	13.9	9.9
14	14.2	10.4	14.1	10.1
15	14.1	10.1	14	9.9
Std	16.1	12.4	13.8	9.8
	Staphylococcus epidermidis	***Staphylococcus epidermidis***	***Staphylococcus aureus***	***Staphylococcus aureus***
	High	**Low**	**High**	**Low**
11	NG	NG	16.4	11.5
12	NG	NG	NG	NG
13	NG	NG	NG	NG
14	NG	NG	NG	NG
15	NG	NG	12.1	9.6
Std	NG	NG	15.5	11.8

Table.2. Fungicidal activity of Substituted purinyl derivatives

Compound	Zone of inhibition in mm (MIC in mg/ml)					
	Saccharomyces cerevisiae	**Saccharomyces cerevisiae**	**Candida arbicans**	**Candida arbicans**	**Aspergillus niger**	**Aspergillus niger**
	High	**Low**	**High**	**Low**	**High**	**Low**
11	12.4	9.4	NG	NG	NG	NG
12	12.4	9.2	NG	NG	NG	NG
13	12.6	9.4	NG	NG	NG	NG
14	12.5	9.3	NG	NG	NG	NG
15	11.9	9.1	NG	NG	NG	NG
Std	12.1	9.2	NG	NG	NG	NG

STD: Abacavir, NG: No Growth, High: 500mcg/ml, Low: 50mcg/ml

4. CONCLUSION

The successful synthesis of series of biologically active 6-substituted purines from commercially available N-(2-amino-4, 6-dichloropyrimidinyl-5-yl) formamide and evaluation of anti-bacterial activities, anti-fungal activities of the title compounds were reported. Fifteen derivatives were prepared and fourteen new ones were discovered. From the results of the anti-bacterial and anti-fungal screening, it can be concluded that the two compounds (11 and 15) having good activities have been synthesized. Therefore they me be used as lead compounds for further development.

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