EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 04
735-743
Year: 2017

A WORK ON SYNTHESIS AND CHARACTERIZATION OF NOVEL PURINE AMINO ACID DERIVATIVES WITH ANTIMICROBIAL EVALUATION.

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Article Received on 19/02/2017 **Article Revised on 12/03/2017** **Article Accepted on 02/04/2017**

ABSTACT
A series of novel Purine coupled with different amino acids derivatives were synthesized, characterized and their antimicrobial properties were evaluated. These compounds were synthesized with 2-ACP react with n-propyl bromide coupling with various amino acids to get Purine propyl amino acids. The synthesized Purine propyl amino acids act as cobalt chloride to get intermediate compound of [(2-chloro-9-propyl-9*H*-purin-6-yl) amino] acetic acid. Finally intermediate compound condensed with morpholin, piperidine, methyl piperidine, Piperazin to get desired compound. Final compound characterized using IR, ^{1}H and ^{13}C-NMR The synthesized compounds were screened for their *in vitro* antimicrobial activity against *S. aurous, E. coli, P. aeruginosa, S. typhimurium, F. oxysporum and A. alternate*. Some of these compounds exhibited moderate to good activity, where as some were inactive.

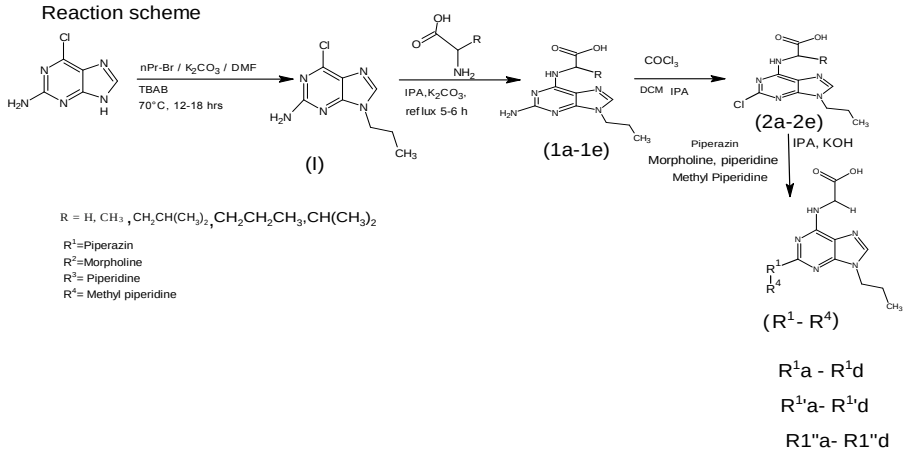
KEYWORDS: 2- ACP**,** Amino acids, antimicrobial activity, coupling**,** Intermediate.

INTRODUCTION

Purine derivatives derived from 2-amino chloro Purine and amino acids form an important group of compounds in synthetic chemistry due to their useful chemical and physical properties. It is also useful as preventives and therapeutic agents. It has been found derivatives show very good antibacterial, antiviral[1–2], anti-inflammatory/analgesic[3], anti-tuberculosis[4], anticancer[5], activities and offer an emerging Amino acids play very important role in nutrition, metabolic processes, and translation of information, so they have been an important target in the design of ant metabolites. Currently there is a tendency to use amino acid/peptide residues during the process. The literature reports that bioactive compounds shows enhanced activity when linked to amino acids.[8–10] The presence of natural amino acid has stimulated interest in new synthetic methodology and strategies to obtain a target structure.

The objective of the present work was to synthesize Purine derivatives bearing amino acids at position 6 and an amine at position 2.

Scheme: Synthesis of Purine derivatives with amino acids.



EXPERIMENTAL CHEMICAL PART

All chemicals were purchased from commercial suppliers and used without further purification. Melting points (m.p.) were determined using a Veego VMP-PM melting point apparatus and are uncorrected. Mass spectra (MS) were recorded on a Waters Q-TOF instrument in only positive ion detection mode. The ^{1}H-NMR spectra were recorded on a Bruker Avance II 500 (500 MHz) instrument using either $CDCl_3$ / DMSO-*d*6 as solvent and TMS as internal reference. Chemical shifts are expressed as δ values (ppm). IR spectra were recorded on Perkin Elmer spectrum 100 FT-IR spectrophotometer. The course of reactions was monitored and the purity of synthesized compounds checked by TLC using silica gel 60 F 254 Al-plates (Merck, Germany) in Ethyl acetate -Hexane (8:2) solvent system and the spots were visualized under UV illumination.

Synthesis of 6-chloro-9-propyl-9H-purin-2-amine

2-ACP (10 mmol) is suspended in DMF as a solvent (10 vol) at 25 to 30°C. N-propyl bromide (12 mmol) is suspended in DMF & added slowly to below 25-30°C & catalytic amount of TBAB and potassium carbonate into the reaction mass under stirring. Raised the temperature of reaction mass up to 70 to 75°C .Once temperature achieved stirring is continued to till completion of reaction. Reaction monitored by TLC. After completion of reaction, add water into the reaction mass and cool reaction mass at 25 to 30°C. Add ethyl acetate and stir for 30 min separate organic layer and wash the organic layer with water. Organic layer is distilled off and resulting mass dissolved in ethanol and distilled off to remove the traces of water. Add acetone (4 vol) and distill out the solvent under vacuum at below 50°C to get product, *6-chloro-9-propyl-9H-purin-2-amine* is used as such for next step.

Synthesis of 6-chloro-9-(propan-2-yl)-9*H*-purin-2-amine

2-ACP (10 mmol) suspended in DMF as a solvent (10 vol) at 25 to 30°C. Iso propyl bromide (12 mmol) suspended in DMF & added slowly to below 25-30°C. Add catalytic amount of TBAB and potassium carbonate into the reaction mass under stirring. Raised the temperature of reaction mass up to 70 to 75°C.Once temperature achieved to till completion of reaction. Reaction is monitored by TLC. After completion of reaction, add water into the reaction mass and cool reaction mass at 25 to 30°C. Add ethyl acetate and stir for 30 min separate organic layer and wash the organic layer with water. Organic layer is distilled off and resulting mass is dissolved in ethanol and distilled off to remove the traces of water. Add acetone (4 vol) and distill out the solvent under vacuum at below 50°C to get product & crystallize the product with ethanol and filter and dry it. 6-chloro-9-(propan-2-yl)-9H-purin-2-amine *is* used as such for next step.

Synthesis of propyl purine amino acids derivatives. (1a-1e)

6-chloro-9-propyl-9H-purin-2-amine is react with amino acids in presence of potassium carbonate as a base with IPA as a solvent. Reflux the reaction mass for 5-6 hrs till completion of reaction. Monitor the reaction by TLC (80:20) ethyl acetate: hexane. After completion of reaction filter reaction mass and distilled out the solvent under reduce pressure to get oil mass. This oil mass crystallize with ethanol water (90:10) to get coupled amino acid Purine compounds.(1a-1e).

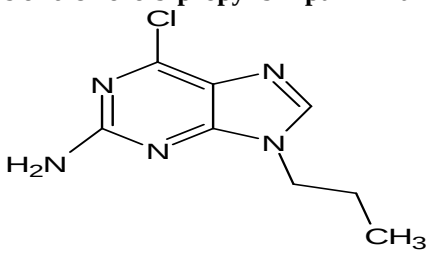
Synthesis of chloro propyl Purine amino acids derivatives (2a-2e)

Propyl purine amino acids are react with cobalt chloride in presence of DCM/ IPA at 35 to 40 °C. Maintain reaction at 35 to 40°C for 5-6 hrs. Monitor the reaction by TLC (80: 20) ethyl acetate: hexane. After completion of reaction filter reaction mass and distilled out the solvent under reduce pressure to get oil mass. This oil mass crystallize with ethanol water (80:20) to get chloro propyl Purine amino acids derivatives.(2a-2e).

Synthesis of N-ring purine amino acids derivatives (R^1-R^4) (General Method)

Chloro propyl Purine amino acids are reacting with N-ring compound like Piperazin, N-methyl piperidine, Piperidine, Morpholin etc. in presence of potassium hydroxide base and IPA as a solvent. Reflux reaction mixture at 80-82 °C for 5-8 hrs. Reaction monitor by TLC (70:30) ethyl acetate and hexane. Once reaction complies filter the reaction mass, collect filtrate mother liquor and add ethyl acetate and water. Adjust pH of reaction mass towards acidic with hydrochloric acid at 20 -30 °C & stir the reaction mass for 20 min. Separate organic layer & re-extract aqueous layer with ethyl acetate. Combine both organic layers and wash with purified water. Again separate organic layer and dried with sodium sulphate.Distilled out the organic layer under reduced pressure to get crude oily mass. This crude oily mass crystallizes with ethanol water/ methanol water at lower temprature to get desired product.

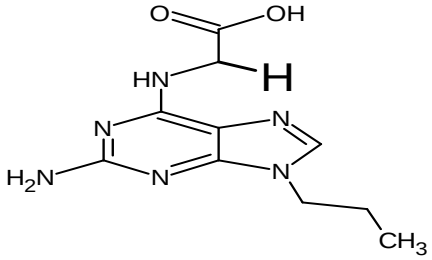
Synthesis of 6-chloro-9-propyl-9*H*-purin-2-amine



Molecular Formula: $C_8H_{10}ClN_5$
M.W. 211.65

Yield (40.5%); MF $C_8H_{10}Cl\ N_5$; M.W. 211.65; IR (KBr, cm^{-1}) : 3453 (N-H), 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.02 (t,1H, CH), 6.87 (s,2H, NH_2), 4.13 (t, 2H, -CH_2-), 1.52 (s, 2H, -CH_2-), 0.93 (t, 3H, -CH_3),; MS, (*m/z*): 234.6 (M + Na).

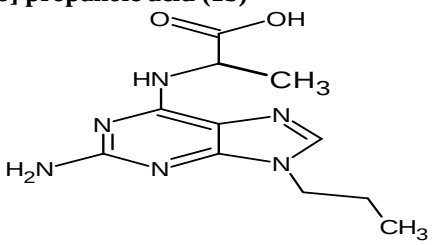
Synthesis of [(2-amino-9-propyl-9*H*-purin-6-yl)amino]acetic acid (1a)



Molecular Formula: $C_{10}H_{14}N_6O_2$
M.W. 250.25

Yield (43.5%); M.P., 214°C; MF $C_{10}H_{14}N_6O_2$; M.W. 250.25; IR (KBr, cm^{-1}) : 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.18 (t, 1H, -CH), 7.4 (s, 2H, NH_2), 7.4 (s, 1H, NH), 7.4 (s, 1H, -COOH), 4.58 (s, 2H, -CH2) 4.13 (t, 2H, CH_2), 1.53(s, 2H, -CH_2), 0.93 (t, -CH_3); MS, (*m/z*): 273.2 (M + Na).

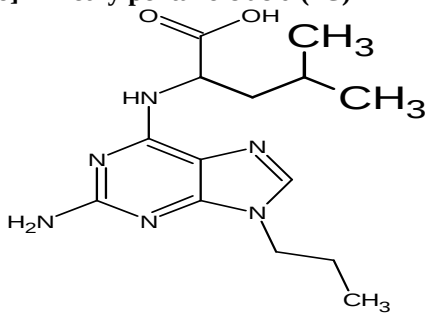
Synthesis of 2-[(2-amino-9-propyl-9*H*-purin-6-yl)amino] propanoic acid (1b)



Molecular Formula: $C_{11}H_{16}N_6O_2$
M.W.264.28

Yield (44.5%); M.P., 208°C; MF $C_{11}H_{16}N_6O_2$; M.W. 264.28. IR (KBr, cm^{-1}) : 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.22 (t, 2H, -CH), 7.4 (s, 2H, NH_2), 7.4 (s, 1H, NH), 7.4 (s, 1H, -COOH), 5.15 (q, 1H, -CH), 4.13 (t, 2H, -CH_2), 1.52 (m, 2H, -CH_2), 1.46 (s, 3H, -CH_3); 0.94(t, 3H, -CH_3) MS, (*m/z*): 287.2 (M + Na).

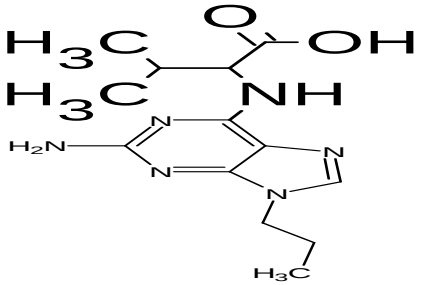
Synthesis of 2-[(2-amino-9-propyl-9*H*-purin-6-yl)amino]-4-methylpentanoic acid (1C)



Molecular Formula: $C_{14}H_{22}N_6O_2$
M.W.306.36

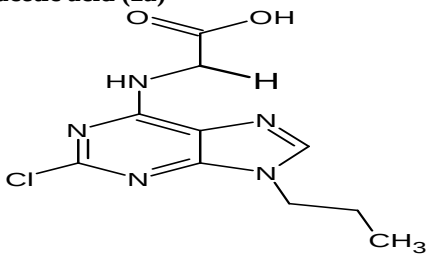
Yield (42.5%); M.P., 206°C; MF $C_{14}H_{22}N_6O_2$; M.W. 306.36. IR (KBr, cm^{-1}) : 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.21 (t, 1H, -CH), 7.76 (s, 2H, NH_2), 7.76 (s, 1H, NH), 7.6 (s, 1H, -COOH), 4.13 (t, 2H, -CH2), 3.76 (m, 1H, -NCH), 2.46(m, 1H, -CH), 1.85 – 1.65 (m, 2H, -CH_2), 1.52(m, 2H, CH_2), 0.93(t, 3H, CH_3), 0.84(dd, 6H, CH_3) ; MS, (*m/z*): 329.3 (M + Na).

Synthesis of 2-[(2-amino-9-propyl-9*H*-purin-6-yl)amino]-3-methylbutanoic acid (1e)



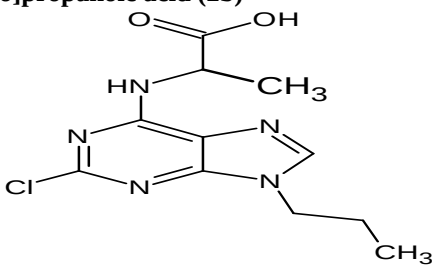
Molecular Formula: $C_{13}H_{20}N_6O_2$
M.W. 292.33
Yield (40.5%); M.P., 207°C; MF $C_{13}H_{20}N_6O_2$; M.W. 292.33. IR (KBr, cm^{-1}) : 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.22 (s, 1H, Ar-CH), 8.21 (s, 2H, NH_2), 8.21 (s, 1H, NH), 8.21 (s, 1H, -COOH), 4.13 (t, 2H, -NCH2),3.76 (d, 1H, -NCH), 2.13(m, 1H, -CH), 1.51 (m, 2H, -CH_2), 1.03 -1.00 (dd, 6H, CH_3), 0.93 (t, 3H, CH_3), ; MS, (*m/z*): 315.3 (M + Na).

Synthesis of [(2-chloro-9-propyl-9*H*-purin-6-yl) amino] acetic acid (2a)



Molecular Formula: $C_{10}H_{12}ClN_5O_2$
M.W. 269.68
Yield (37.5%); M.P., 201°C; MF $C_{10}H_{12}ClN_5O_2$; M.W. 269.68. IR (KBr, cm^{-1}) : 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 9.10 (s, 1H, Ar-NH), 9.10 (s, 1H, COOH), 8.52 (t, 1H, N-CH-N), 4.58 (s, 2H, -CH_2-NH), 4.13 (t, 2H, -CH2) 1.53 (sextet, 2H, -CH_2-), 0.93(t, 3H, -CH_3), MS, (*m/z*): 292.7 (M + Na).

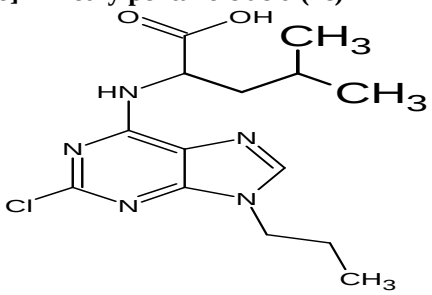
Synthesis of 2-[(2-chloro-9-propyl-9*H*-purin-6-yl)amino]propanoic acid (2b)



Molecular Formula: $C_{11}H_{14}ClN_5O_2$

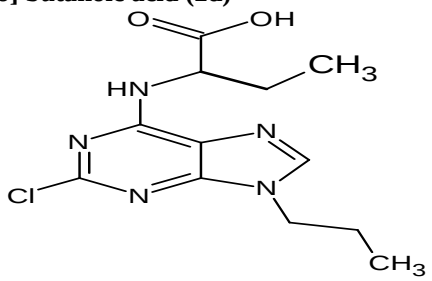
M.W. 283.71
Yield (39.5%); M.P., 209°C; MF $C_{11}H_{14}ClN_5O_2$; M.W. 283.71. IR (KBr, cm^{-1}) : 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 9.02 (s, 1H, -NH-), 9.02 (s, 1H, -COOH), 8.55 (t, 1H, N-CH-N), 5.14 (q, 1H, -CH-), 4.13 (t, 2H, -CH2),1.53 – 1.48 (m, 2H, $-CH_2-$), 1.46 (s, 3H, $-CH_3$), 0.93 (t, $-CH_3$); MS, (*m/z)*: 306.7 (M + Na).

Synthesis of 2-[(2-chloro-9-propyl-9*H*-purin-6-yl)amino]-4-methylpentanoic acid (2c)



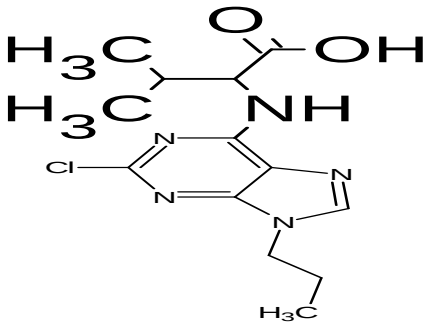
Molecular Formula: $C_{14}H_{20}ClN_5O_2$
M.W.325.79
Yield (39.7%); M.P., 203°C; MF $C_{14}H_{20}ClN_5O_2$ M.W. 325.79. IR (KBr, cm^{-1}) : 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 9.74 (s, 1H, -NH-), 9.74 (s, 1H, -COOH), 8.55 (t, 1H, N-CH-N), 4.13 (t, 2H, $-CH_2-$), 3.76 (m, 1H, N-CH-), 2.46 (m, 1H, -CH-), 1.85 – 1.65(m, 2H, $-CH_2-$), 1.51 (sextet, $-CH_2$), 0.93 (t, 2H, $-CH_3$), 0.84-0.85 (tt, 6H, $-CH_3$), MS, (*m/z)* 347.8 (M + Na).

Synthesis of 2-[(2-chloro-9-propyl-9*H*-purin-6-yl)amino] butanoic acid (2d)



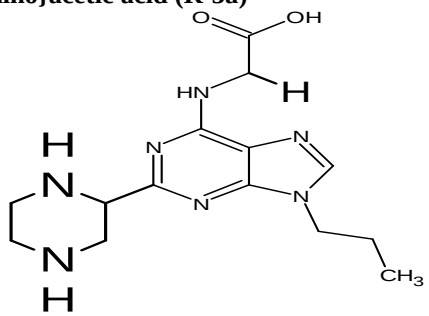
Molecular Formula: $C_{12}H_{16}ClN_5O_2$
M.W. 297.74
Yield (39.8%); M.P., 201°C; MF $C_{12}H_{16}ClN_5O_2$ M.W. 297.74. IR (KBr, cm^{-1}) : 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): -COOH) 2800, 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 10.01(s, 1H, -NH-), 10.01(s, 1H, -COOH), 8.66 (t, 1H, -N-CH-N), 4.13 (t, 2H, -N-CH-), 3.89 (q, 1H, -N-CH-), 1.78 – 1.86 (m, 2H, $-CH_2-$), 1.58 (sextet, 2H, $-CH_2-$), 1.17 (t, 3H, $-CH_3$); 0.93 (t, 3H, $-CH_3$) MS, (*m/z)*: 320.7 (M + Na).

Synthesis of 2-[(2-chloro-9-propyl-9*H*-purin-6-yl)amino]-3-methylbutanoic acid (2e)



Molecular Formula: $C_{13}H_{18}ClN_5O_2$
M.W. 311.71
Yield (39.8%); M.P., 201°C; MF $C_{12}H_{16}ClN_5O_2$ M.W. 311.71 IR (KBr, cm^{-1}) : 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 10.64 (s, 1H, -NH-), 10.64 (s, 1H, -COOH), 8.55 (t, 1H, -N-CH-N-), 4.12 (t, 2H, $-N-CH_2-$), 3.77 (d, 1H, -NH-CH-), 2.14 (m, 1H, -CH-), 1.53(sextet, 2H, $-CH_2-$), 1.00 – 1.03 (dd, 6H, $-CH_3$), 0.93 (t, 3H, $-CH_3$), MS, (*m/z)*: 334.7 (M + Na).

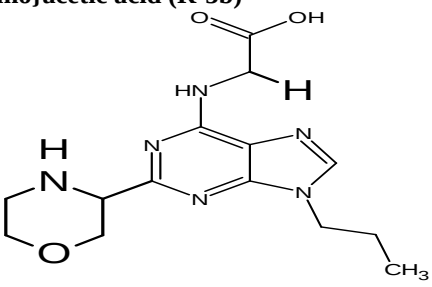
Synthesis of {[2-(piperazin-2-yl)-9-propyl-9*H*-purin-6-yl] amino}acetic acid ($R^1$3a)



Molecular Formula**:** $C_{14}H_{21}N_7O_2$
Formula Weight: 319.37

Yield (39.5%); M.P., 202°C; MF $C_{14}H_{21}N_7O_2$ M.W. 319.37. IR (KBr, cm^{-1}) : 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.17 (s, 4H,2-NH-, -NH-HH-, -COOH), 4.58 (s, 2H, $-N-CH_2-$), 4.13 (t, 2H,$-N-CH_2-$), 4.07-4.03 (mm, 1H, -N-CH-), 3.23-2.63 (mm, 2H, $-N-CH_2-$), 3.10 (m, 2H, $-N-CH_2-C-$), 2.75(m, 2H, $-N-CH_2-C-$), 1.53 (sextet, 2H, $-CH_2$), 0.93 (t, 3H, $-CH_3$), ; MS, (*m/z)*: 342.3 (M + Na).

Synthesis of {[2-(morpholin-3-yl)-9-propyl-9*H*-purin-6-yl]amino}acetic acid ($R^2$3b)

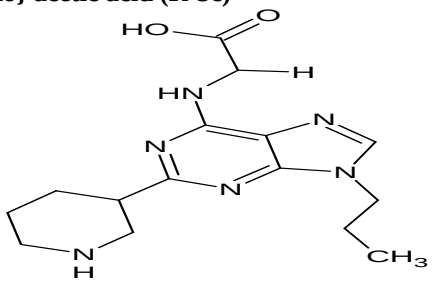


Molecular Formula: $C_{14}H_{20}N_6O_3$

Formula Weight: 320.35

Yield (40.5%); M.P., 204°C; MF $C_{14}H_{20}N_6O_3$ M.W. 320.35. IR (KBr, cm^{-1}) : 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.07 (s, 1H, Ar-CH), 6.74 (s, 1H, -NH), 6.74 (s, 1H, -NH), 6.74 (s, 1H, -COOH), 4.58 (s, 2H, N-CH2-), 4.41(m, 1H, -NCH-), 4.13(t, 2H, N-CH_2-), 4.03 and 3.40 (m, 2H, O-CH_2-), 3.55(m, 2H, O-CH_2-), 2.76(m, 2H, N-CH_2-), 1.53 (sextet, 2H, -CH_2-), 0.93(t, 3H, -CH_3) ; MS, (*m/z*): 343.3 (M + Na).

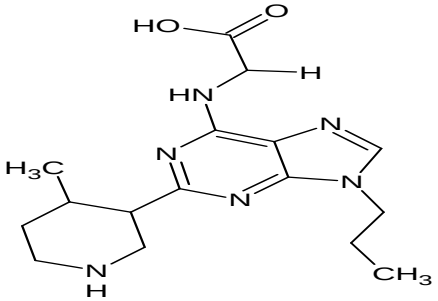
Synthesis of {[2-(piperidin-3-yl)-9-propyl-9*H*-purin-6-yl] amino} acetic acid ($R^3$3c)



Molecular Formula: $C_{15}H_{22}N_6O_2$
Formula Weight: 318.37

Yield (41.5%); M.P., 208°C; MF $C_{15}H_{22}N_6O_2$ M.W. 318.37. IR (KBr, cm^{-1}) : 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.24 (t, 1H, Ar-CH), 6.85 (s, 1H, -NH-), 6.85 (s, 1H, -NH-), 6.85 (s, 1H, -COOH), 4.58 (s, 2H,N-CH2-), 4.13(t, 2H, -NCH_2-), 3.19 and 2.85(m, 2H, NH-CH_2-), 3.06 and 2.67 (m, -NH-CH_2-), 1.89-1.65(m, 2H, -CH_2-), 1.69-1.55(m, 2H,-CH_2-), 1.53(sextet, 2H, -CH_2-), 0.93(t,3H, -CH_3-), MS, (*m/z*): 341.3 (M + Na).

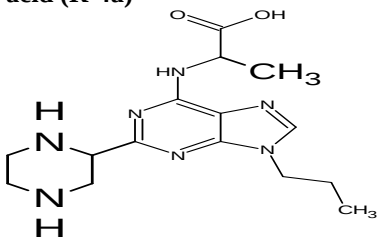
Synthesis of *N*-ethyl-2-(4-methylpiperidin-3-yl)-9-propyl-9*H*-purin-6-amino acid ($R^4$3d)



Molecular Formula: $C_{16}H_{26}N_6O_2$
Formula Weight: 334.42

Yield (43.5%); M.P., 208°C; MF $C_{16}H_{26}N_6O_2$ M.W. 334.42. IR (KBr, cm^{-1}) : 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): (-COOH) 2700 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.24 (t, 1H, Ar-CH), 6.80 (s, 1H, NH-CH-), 6.80 (s, 1H, NH-CH-), 6.80 (s, 1H, -COOH), 4.58 (s, 2H, -CH2-), 4.13 (t, 2H, -NCH_2-), 3.96 and 2.94 (m, 2H, NH-CH_2-), 3.17 and 2.77(m, 2H, NH-CH_2-), 2.58(s, 1H, -CH-), 1.75(s, 1H, -CH-), 1.52(sextet, 2H, -CH_2-),1.51 and 1.23(m, 2H, -CH_2-), 0.93(t, 2H, -CH_3), 0.93(d, 3H, -CH_3) ; MS, (*m/z*): 357.4 (M + Na).

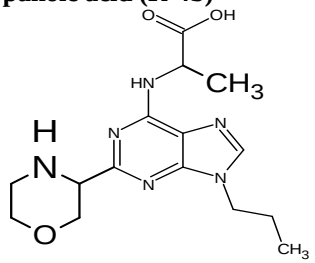
2-{[2-(piperazin-2-yl)-9-propyl-9*H*-purin-6-yl] amino} propanoic acid ($R^1$4a)



Molecular Formula $C_{15}H_{23}N_7O_2$
Formula Weight: 333.39

Yield (46.5%); M.P.,206°C; MF $C_{15}H_{23}N_7O_2$ M.W. 333.39. IR (KBr, cm^{-1}) : 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.21 (t, 1H, Ar-CH), 5.51(s, 1H, -NH-), 5.51 (s, 1H, -NH-), 5.51 (s, 1H, -NH-), 5.51 (s, 1H, -COOH), 5.14 (q, 1H, -NH-CH-), 4.13(t, 2H, -NH-CH_2-), 4.05 (m, 1H, -NH-CH-), 3.23 and 2.67(m, 2H, -NH-CH_2-), 3.14 - 3.04(m, 2H, -NH-CH_2-),2.80 – 2.65(m, 2H, -NH-CH_2-), 1.53(sextet, 2H, -CH_2-), 1.48(d, 3H, -CH_3), 0.93 (t, 3H, -CH_3), ; MS, (*m/z*): 356.3 (M + Na).

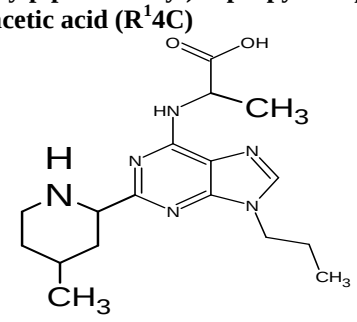
2-{[2-(morpholin-3-yl)-9-propyl-9*H*-purin-6-yl]amino}propanoic acid ($R^1$4b)



Molecular Formula: $C_{15}H_{22}N_6O_3$
Formula Weight: 334.37

Yield (46.5%); M.P.206°C; MF $C_{15}H_{23}N_7O_2$ M.W. 333.39. IR (KBr, cm^{-1}) : 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.11 (s, 1H, Ar-CH), 6.69 (s, 1H, -NH-), 6.69 (s, 1H, -NH-), 6.69 (s, 1H, -COOH), 5.14 (q, 1H, -CH_3), 4.41(m, 1H, -CH-), 4.13 (t, 1H, -NH-CH_2-), 4.05-3.40 (m, 2H, -O-CH_2-), 3.61-3.55 (m, -O-CH_2-), 2.80 - 2.70 (m, 2H, -NH-CH_2-), 1.48 (sextet, 2H, -CH_2-), 1.48 (d,3H, -CH_3), 0.93 (t, 3H, -CH_3), (*m/z*): 357.3 (M + Na).

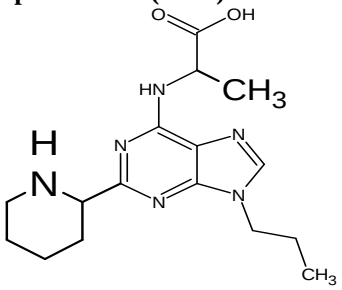
{[2-(4-methylpiperidin-2-yl)-9-propyl-9*H*-purin-6-yl]amino}acetic acid ($R^1$4C)



Molecular Formula: $C_{17}H_{26}N_6O_2$
Formula Weight: 346.43

Yield (46.5%); M.P.206°C; MF $C_{17}H_{26}N_6O_2$ M.W. 346.43. IR (KBr, cm^{-1}) : 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.13 (t, 1H, Ar-CH), 6.03 (s, 1H, -NH-), 6.03 (s, 1H, -NH-), 6.03 (s, 1H, -COOH), 4.46 (d, 1H, -CH-), 4.13 (t, 2H, -NCH_2-), 3.12(q, 2H, -NH-CH-), 2.88-2.77 (m, 2H, -NH-CH_2-), 1.72(m, 1H, -CH-), 1.60 (m, 2H, -CH_2-), 1.51 (sextet, 2H, -CH_2-), 1.48 and 1.15 (m t, 2H, -CH_2-), 1.61-1.30(m, 2H, -CH_2-), 1.17(t, 3H, -CH_3), 1.01 (d, 3H, -CH_3), 0.93(t, 3H, -CH_3), MS, (*m/z*): 369.3 (M + Na).

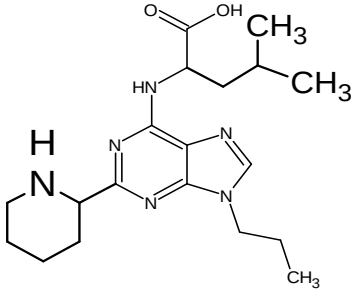
2-{[2-(piperidin-2-yl)-9-propyl-9*H*-purin-6-yl]amino}propanoic acid ($R^1$4d)



Molecular Formula: $C_{16}H_{24}N_6O_2$
Formula Weight: 332.40

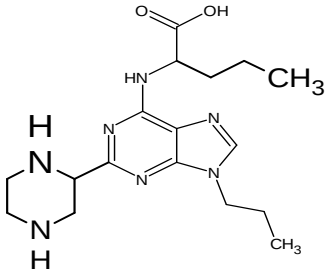
Yield (46.9%); M.P.203°C; MF **$C_{16}H_{24}N_6O_2$** M.W. 332.40. IR (KBr, cm^{-1}) : 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.21 (t, 1H, Ar-CH), 7.12 (s, 1H, -NH-), 7.12 (s, 1H, -NH-), 7.12 (s, 1H, -COOH), 5.14 (q, 1H, -CH-), 4.39-4.38 (mm, 1H, -CH-), 4.13(t, 2H, N-CH_2-), 2.87 – 2.77 (m, 2H, -N-CH_2-), 1.87-1.68(m, 6H, -CH_2-), 1.53-1.48 (sextet, 2H, -CH_2-), 1.46 (d, 3H, -CH_3), 0.93 (t, 3H, -CH_3), MS, (*m/z*): 355.4 (M + Na).

4-methyl 2-{[2-(4-methyl 1- piperidine 2-yl)-9-propyl-9*H*-purin-6-yl]amino}pentanoic acid (R^1 4e)



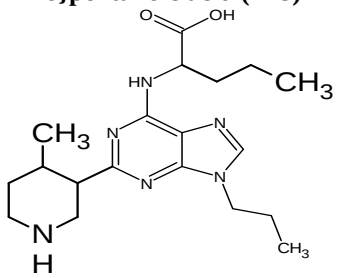
Molecular Formula: $C_{19}H_{30}N_6O_2$
Formula Weight: 374.48
Yield (37.6%); M.P.189°C; MF $C_{19}H_{30}N_6O_2$ M.W. 374.48 IR (KBr, cm^{-1}) : 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): -COOH 2850 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 9.7 (s, 1H, -NH), 9.71 (s, 1H, -COOH), 8.50 (t, 1H, N-CH-N), 4.11 (t, 2H, -CH_2-), 3.65 (m, 1H, N-CH-), 1.82 – 1.65 (m, 2H, -CH_2-), 1.50 (sextet, -CH_2), 0.91 (t, 2H, -CH_3), 0.82-0.85 (tt, 6H, -CH_3), 7.12 (s, 1H, -NH-), MS, (*m/z*): 397.4(M + Na).

2-{[2-(4-methyl 1- piperazin 2-yl)-9-propyl-9*H*-purin-6-yl]amino}pentanoic acid
(R´a)



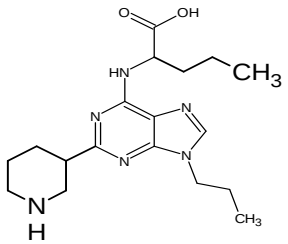
Molecular Formula: $C_{17}H_{27}N_7O_2$
Formula Weight: 361.44
Yield (37.0%); M.P.183-184°C; MF **$C_{17}H_{27}N_7O_2$** M.W. 361.44 IR (KBr, cm^{-1}) :: 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.08 (s, 1H, Ar-CH), 7.7 (dd, 2H, Ar-CH), 7.6 (s, 1H, -CONH), 3.3-3.5 (t, 6H, -CH2 q,2H –CH2) 2.8 (q, 1H) 4.0 (q,1H), 2.2 – 2.3 (m, 2H, J13.5 Hz, J24.0), 1.5 (m, 2H, J11.5 Hz, J25.5 Hz,-CH_2),2.5 (t,2H) 1.8 (t,3H CH3) MS, (*m/z*): 384.4 (M + Na).

2-{[2-(4-methyl 1- piperidine 2-yl)-9-propyl-9*H*-purin-6-yl]amino}pentanoic acid (R´b)



Molecular Formula: $C_{19}H_{30}N_6O_2$
Formula Weight: 374.48
Yield (31.0%); M.P.169-171°C; MF $C_{19}H_{30}N_6O_2$ M.W. 374.48 IR (KBr, cm^{-1}) :: 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.08 (s, 1H, Ar-CH), 7.6 (d, 2H, Ar-NH), 7.89 (dd, 2H, Ar-CH), 7.6 (s, 1H, -CONH), 1.2 (d,2H) 3.8 (q,2H) 3.3-3.5 (t, 4H, -CH2 q,2H –CH2) 2.8 (q, 1H) 4.0 (q,1H), 2.2 – 2.3 (m, 2H, J13.5 Hz, J24.0), 1.5-1.8 (m, 2H, J11.5 Hz, J25.5 Hz,-CH_2,CH2),2.5 (t,2H) ,1.8 (t,3H CH3) 1.1 (q,2H, t, 2H)
MS, (*m/z*): 397.5(M + Na).

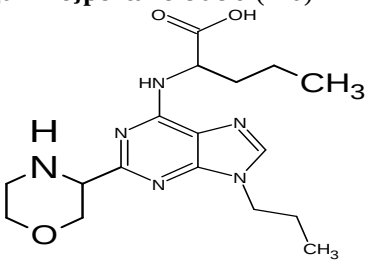
2-{[2-(Piperidine 2-yl)-9-propyl-9*H*-purin-6-yl]amino}pentanoic acid
(R´c)



Molecular Formula: $C_{18}H_{28}N_6O_2$
Formula Weight: 360.45

Yield (33.0%); M.P.171-174°C; MF $C_{17}H_{27}N_7O_2$ M.W. 361.44 IR (KBr, cm^{-1}) :: 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.08 (s, 1H, Ar-CH), 7.6 (d, 1H, Ar-NH), 7.6 (s, 1H, -CONH), 1.2 (d,2H) 3.8 (q,2H) 3.3-3.5 (t, 4H, -CH2), 2.4-2.8 (q, 2H,m,1H) 3.8 -4.0 (q,1H,q 2H), 2.2 – 2.3 (m, 2H, J13.5 Hz, J24.0), 1.5-1.8 (m, 2H, J11.5 Hz, J25.5 Hz,-CH_2,CH2),2.5 (t,2H) ,1.8 (t,3H CH3) 1.1-1.0 (t,3H, t, 2H)
MS, (*m/z)*: 383.4 (M + Na).

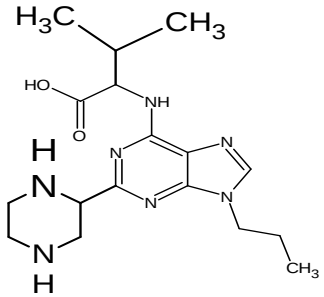
2-{[2-(4-methyl 1- morpholine 2-yl)-9-propyl-9*H*-purin-6-yl]amino}pentanoic acid (R´d)



Molecular Formula: $C_{17}H_{26}N_6O_3$
Formula Weight: 362.42

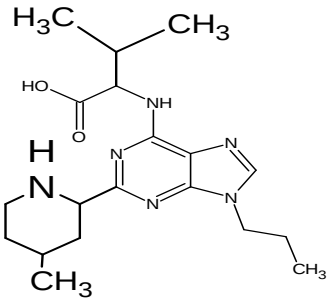
Yield (30.0%); M.P.160-162°C; MF $C_{17}H_{26}N_6O_3$ M.W. 362.42 IR (KBr, cm^{-1}) :: 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.08 (s, 1H, Ar-CH), 7.6 (d, 1H, Ar-H), 1.5 (m,2H),2.5 (t,2H), 1.1 (t,3H),3.5(q,2H),3.6(t,2H) 3.8(d,2H),2.8(q,1H), 4.0 (q,1H), 3.8 (q,2H), 1.8 (t,3H), MS, (*m/z)*: 385.4 (M + Na).

4-methyl-2-[2-(4-methyl 1- piperazin 2-yl (9-propyl-9*H*-purin-6-yl)amino]butanoic acid
(R´´a)



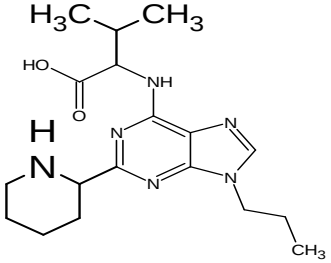
Molecular Formula: **$C_{17}H_{27}N_7O_2$**
Formula Weight: **361.43**
Yield (34.0%); M.P.159-162°C; MF $C_{17}H_{27}N_7O_2$ M.W. 361.43 IR (KBr, cm^{-1}) :: 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.08 (s, 1H, Ar-CH), 7.6 (d, 1H, Ar-H), 1.5 (m,2H),2.5 (t,2H), 1.1 (t,3H),3.5 (t,2H),3.3 (t,2H) 2.8 (q,2H), 4.0 (q,1H), 3.8 (q,2H), 1.4 (m,1H), 1.2 (d 6H) MS, (*m/z)*: 381.4 (M + Na).

4-methyl-2-[2-(4-methyl 1- piperidine 2-yl (9-propyl-9*H*-purin-6-yl) amino]butanoic acid
(R´´b)



Molecular Formula: $C_{19}H_{30}N_6O_2$
Formula Weight: 374.48
Yield (34.0%); M.P.159-161°C; MF $C_{19}H_{30}N_6O_2$ M.W. 374.48
IR (KBr, cm^{-1}) :: 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.08 (s, 1H, Ar-CH), 7.6 (d, 1H, Ar-H), 1.5 (m,2H),2.5 (t,2H), 1.1 (t ,2H) 3.5 (q,2H), 1.1 (m,2H, 2H) 1.2 (d,2H) 1.1 (t,2H) 2.8 (q,1H), 4.0 (t,1H),1.4 (m,1H),1.2(d,6 H)
MS, (*m/z)*: 397.5 (M + Na).

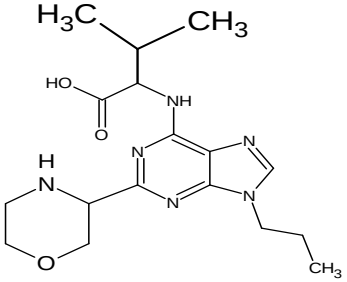
4-methyl-2-[2-(piperidine 2-yl (9-propyl-9*H*-purin-6-yl)amino]butanoic acid (R´´C)



Molecular Formula: $C_{18}H_{28}N_6O_2$

Formula Weight: 360.44
Yield (30.0%); M.P.153-155°C; MF $C_{18}H_{28}N_6O_2$ M.W. 360.44
IR (KBr, cm^{-1}) :: 3455 (N-H), 2939 (C-H), 1713, 1686 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.08 (s, 1H, Ar-CH), 7.6 (d, 1H, Ar-H), 1.5 (m,2H),2.5 (t,2H), 1.1 (t ,2H) 3.5 (q,2H), 1.1 (m,2H, 2H) 1.1 (q,2H) 3.6 (t,2H) 3.8(d,2H),2.8(q,1H), 4.0 (t,1H),1.4 (m,1H),1.2(d,6 H) MS, (*m/z)*: 383.5 (M + Na).

4-methyl-2-[2-morpholine 2-yl) (9-propyl-9*H*-purin-6-yl)amino]butanoic acid (R´´d)



Molecular Formula: $C_{17}H_{26}N_6O_3$
Formula Weight: 362.42

Yield (20.0%); M.P.150-151°C; MF $C_{19}H_{30}N_6O_2$ M.W. 362.42
IR (KBr, cm^{-1}) :: 3457 (N-H), 2936 (C-H), 1715, 1687 (C=O): 1H NMR spectrum in $CDCl_3$ (δ ppm) ; 8.08 (s, 1H, Ar-CH), 7.6 (d, 1H, Ar-H), 1.5 (m,2H),2.5 (t,2H), 1.1 (t ,2H) 3.6 (t,2H) 3.5 (q,2H),3.6(t,2H) 3.8(d,2H),2.8(q,1H), 4.0 (t,1H),1.4 (m,1H),1.2(d,6 H) 3.8 (q,2H), 1.8 (t,3H), MS, (*m/z)*: 382.5 (M + Na).

EXPERIMENTAL BIOLOGICAL PART

Preliminary testing of the antibacterial activity of the newly synthesized compounds was performed by the disc diffusion method using Muller Hinton Agar (MHA) medium. In hard glass screw cap test tube, sterile slants of MHA were prepared. Stored pure cultures were transferred to the freshly prepared MHA slants separately for each organism using sterilized inoculating loop. In this way, four test tubes were freshly prepared for each bacterial pathogen.

Freshly prepared pure culture tubes slants were used for inoculation of nutrient broths. These tubes were incubated at (35±5°C) for 24 hours to get bacterial suspensions used to study antibacterial activity. This is also suitable for antiviral activity. The microorganisms were spared on the surface of MHA plate. Five wells of equal size were created using gel puncher (4 mm) in each plate.

These wells were then filled with 10 µL of each sample) and labeled accordingly. DMSO was used as a solvent. The micro-organisms of *Staphylococcus aureus* NCIM 2127 (*S. aureus*), *Escherichia coli* NCIM 2065 (*E. coli*), *Pseudomonas aeruginosa* NCIM-2036 (*P. aeruginosa)* and *Salmonella typhimurium* NCIM 2501 (*S. typhimurium*) were purchased from the National Chemical Laboratory (NCL), Pune, India.

RESULTS AND DISCUSSION

All the synthesized compounds were characterized using various spectroscopic techniques. IR spectra showed characteristic bands & Stretching frequencies of acid 2800 to 2900 cm^{-1} and NH- 3400 to 3500 cm^{-1}. The 1H spectrum was carried out at 500 MHz and showed characteristics pattern of peaks. Aromatic protons of Purine derivatives appeared at 7.85 –7.66 ppm. Electron ionization mass spectrometric fragmentation pattern of all compounds was the same.

Biological Assays

All the synthesized compounds were evaluated *in vitro* for their antibacterial activities against *S. aureus as* examples of Gram positive bacteria and *E. coli, P. aeruginosa and S. typhimurium as* examples of Gram negative bacteria. They were also evaluated *in vitro* for their antifungal activities against the *F. oxysporum* and *A. alternate* fungal strains. The results were compared with the standard 0.3% Amplicilline and Chloramphenicol as antibacterial agent while Nystatin was used as reference drugs as antifungal agent. Results were summarized in Table 1.

Table I. In *vitro* antimicrobial activities of all synthesized compounds

Compound code	Zone of inhibition in mm					
	Bacteria				Fungi	
	Gram +ve	Gram -ve				
	S. *aurous*	E. *coli*	P. *aeruginosa*	S. *typhi*	*F. oxysporum*	*A. alternata*
R´´a	18	10	11	10	49	38
R´´b	17	10	10	11	38	35
R´´c	13	7	8	9	22	26
R´´d	12	6	7	8	23	24
R´ a	18	11	12	11	53	33
R´b,c,d	19	10	11	11	38	32
Amplicilline	20	11	-	-	-	-
Chloramphenicol	17	20	12	12	-	-
Nystatin	-	-	-	-	70	50

CONCLUSION

In summary, we have disclosed the rational design of a series of potent novel Purine derivatives **(** The biological data indicate that Purine derivatives having are more active than other functional group. Also 9 th position of Purine derivatives is more reactive than the 6 th position

ACKNOWLEDGEMENTS

The authors are thankful to the Head of the Department of Chemistry, for providing the necessary facilities to carry out the research work. Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan, India. and kind and consistent guidance and encouragement from Dr. Kartik Vyas.

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