SYNTHESIS AND ANTIMICROBIAL EVALUATION OF 2, 6, 9-TRISUBSTITUTED PURINE COUPLED WITH L-METHIONINE DERIVATIVES AT C2 POSITION

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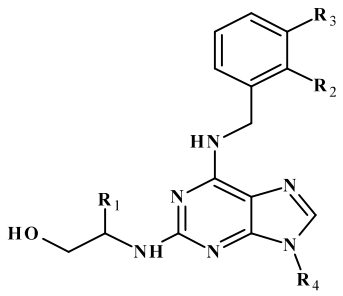
ABSTRACT

Some new 2, 6, 9-trisubstituted purine coupled with methionine derivatives at C2 position were synthesized by coupling of 2, 6-diamino-9-methyl purine with N-protected Methionine using phosphorous oxychloride in pyridine. The synthesized compounds were characterized using IR, ^{1}H, ^{13}C–NMR, mass analysis and screened for their in vitro antimicrobial activity against microorganism. Some of these compounds exhibited moderate to good activity.

Keywords: *Purine, phosphorous oxychloride, methionine, antimicrobial activity*

Introduction:

Purines are ubiquitous molecules that exist at relatively high concentrations in living organisms. Purine derivative having structural variations at its 2, 6 and 9-position is of great interest in medicinal chemistry. 2, 6, 9-trisubstituted purine (TSP) revealed a large number of highly active Cyclin-dependent kinase (CDK) inhibitors (Chang YT et al. 1999, Norman TC et al. 1996, Vesely J et al. 1994, Legraverend M et al. 1999, Sielecki TM et al. 2000). Several types of CDK inhibitors, shown in Figure 1,



1. **Olomoucine**:R_1 = R_2= R_3= -H ; R_4 = -CH_3
2. **Roscovitine**:R_1 =-CH_2CH_3; R_2= R_3= -H ; R_4 = -$CH(CH_3)_2$
3. **Olomoucine-II**:R_1 =-CH_2CH_3; R_2=-OH; R_3=-H; R_4 = -$CH(CH_3)_2$
4. **Purvalanol A**:R_1 =-CH_2CH_3; R_2=-Cl; R_3=-H; R_4 = -$CH(CH_3)_2$
5. **Purvalanol B**:R_1 =-CH_2CH_3; R_2=-Cl; R_3=-COOH; R_4 = -$CH(CH_3)_2$

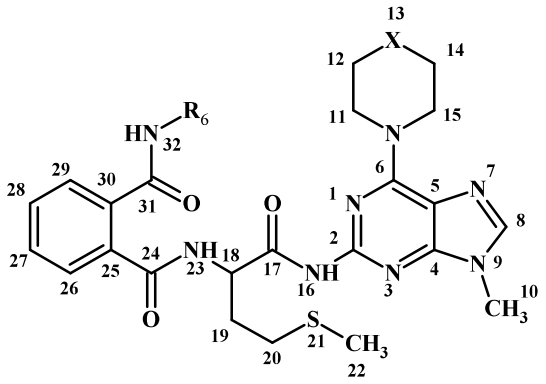
Figure 1

The first CDK inhibitor was olomoucine (Abraham and Acquarone 1995). Other derivatives with enhanced efficiency like roscovitine, purvalanol, olomoucine II was synthesized (Gray NS et al. 1998, Haesslein JL et al 2002, Elgazwy AS et al. 2010, Havlicek L et al 1997, Imbach P et al. 1999). Roscovitine is more potent and selective than olomoucine (Meijer L et al. 1997) but R-isomer of roscovitine (Whittaker SR et al. 2004, Benson C et al. 2007, Wang, S D et al. 2001, McClue SJ et al. 1997) and olomoucine II (Havlicek L et al 1997, Meijer L et al. 1997) is a more potent and selective than racemic roscovitine. In same way purvalanol A, the most potent CDK inhibitor exceeds cytotoxic activity than olomoucine II (Abraham and Acquarone 1995, Gray NS et al. 1998) while Purvalanol B is more potent in biochemical CDK assays than purvalanol A and the order of importance of the three purine ring substituents with respect to kinase inhibition is 2- > 6- > 9- (Fischer and Lane 2000).

Myosever in a TSP analogue act as microtubule assembly (Chang YT et al. 2001). TSP family currently being explored as novel

anticancer drugs (Haesslein JL et al. 2002), Inhibitors of Src tyrosine kinase for the treatment of bone diseases (Wang Y. et al. 2003), as protein A mimetics for the treatment of autoimmune diseases (Zacharie et al. 2009) as useful tools for developing potent plant mitogen-activated protein kinase inhibitors (Hyun TK et al. 2010) as inhibitors of P38 mitogen-activated protein kinase (Wan et al. 2003), as potent Hsp90 inhibitor (Taldone & Chiosis 2009), as potent stat3 binding inhibitor (Shahani et al. 2011), as antitumor (Kode et al. 2007), sulfotransferase (Chapman et al. 2002), inhibitors of phosphodiesterase 7 (PDE7) (Pitts et al. 2004) and as adenosine receptor antagonists (Hockemeyer et al. 2004) . The inhibitory activity of purine derivatives varied depending on the C2 substituent. Thus, a polar side chain at position 2 appears to be essential since it has a positive binding effect and also causes an increased solubility of the compounds (Havlicek et al. 1997).

These encouraging results led us to design other TSP as biologically relevant molecules with broad biomedical value as therapeutics. In the literature domain amino acid derivative at C2 position of purine is not available. Undoubtedly amino acid derivatives are the prominent functionalized substituent of high biological relevance. Such compounds may display biological activity and be used as building blocks in the synthesis of chemically and enzymatically stable nucleic acids–peptide/protein conjugates. In this connection, we have synthesized trisubstituted purine coupled with L-methionine derivatives and subjected to *in vitro* antimicrobial screening.



X = -CH_2. Piperidine or –O: morpholine; R_6 = -cyclopropyl or cyclohexyl or methyl group

Fig. 2 Structure of trisubstituted purine

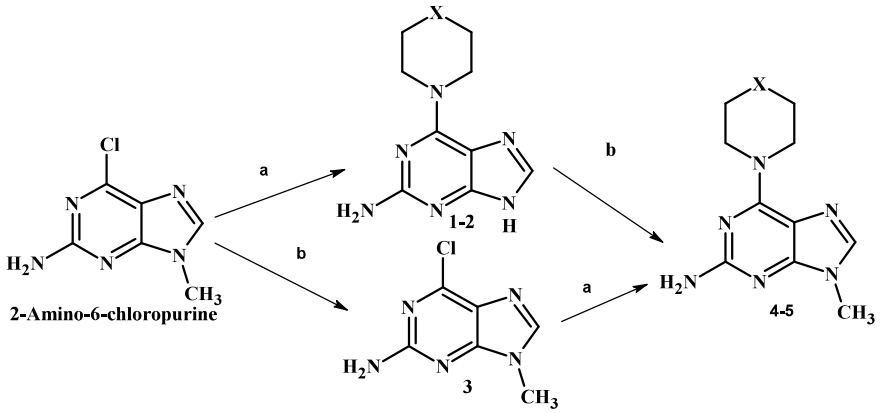
Experimental:

Reagents, instrumentation, and measurements:

Melting points were measured on a Veego VMP-PM melting point apparatus and IR spectra were recorded on Perkin Elmer Spectrum 100 FT-IR spectrometer. ^1H, and ^{13}C NMR spectra were recorded at 500.1 and 125.8 MHz respectively on a BRUKER Avance II 500 instrument with $CDCl_3$ / DMSO-d6 as solvent and TMS as internal standard. Mass spectra were recorded on a Waters Q-TOF spectrometer operating at an ionization potential of 30 eV. The course of the reactions was monitored and the purity of synthesized compounds was checked by TLC using silica gel 60 F_{254} Al-plates (Merck, Germany) in DCM: MeOH (9:1) solvent system and the spots were visualized under UV illumination.2-Amino-6-chloro purine was purchase from company name, China. L-methionine and phthalic anhydride were purchased from commercial suppliers and used without further purification. The micro-organism *Staphylococcus aureus* (NCIM 2127), *Escherichia coli* (NCIM 2065), *Pseudomonas aeruginosa* (NCIM-2036), *Salmonella typhimurium* (NCIM 2501), *Fusarium oxysporum* (NCIM 718) and *Alternaria*

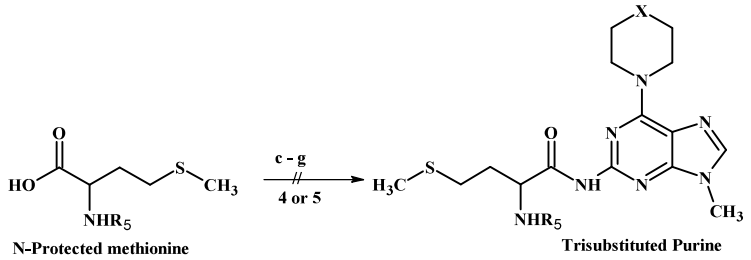
alternate (NCIM 1008) were purchased from the National Chemical Laboratory (NCL), Pune, India.

Preliminary testing of the antimicrobial activity of the newly synthesized compounds were performed by the disc diffusion method using Muller Hinton Agar (MHA) medium for growing bacterial strains and studying their antimicrobial activity. 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 such a way four test-tubes were freshly prepared for each microbial pathogen. Freshly prepared pure culture tubes slants were used for inoculation of nutrient broths. These tubes were incubated at (35±2^{0}C) for 24 hours to get bacterial suspension then used to study antimicrobial activity. The microorganisms were sprayed on the surface of MHA plate. Five wells of equal size were created using gel puncher (4mm) in each plate. These wells were then filled with the 10µl of each sample which were prepared in DMSO (10 mg / ml).



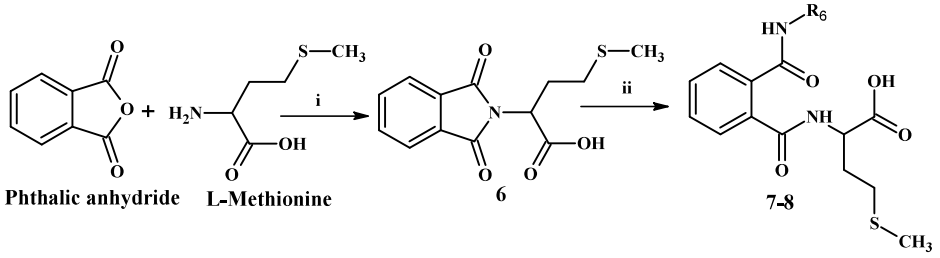
Scheme 1:- Synthesis of 2, 6-diamino-9-methyl purine

Reagents: (a) Piperidine/Morpholine , K_2CO_3, n-Butanol, reflux, 5-6 h ; (b) Methyl Iodide, 40% TBAOH, DCM, rt, 1 h.



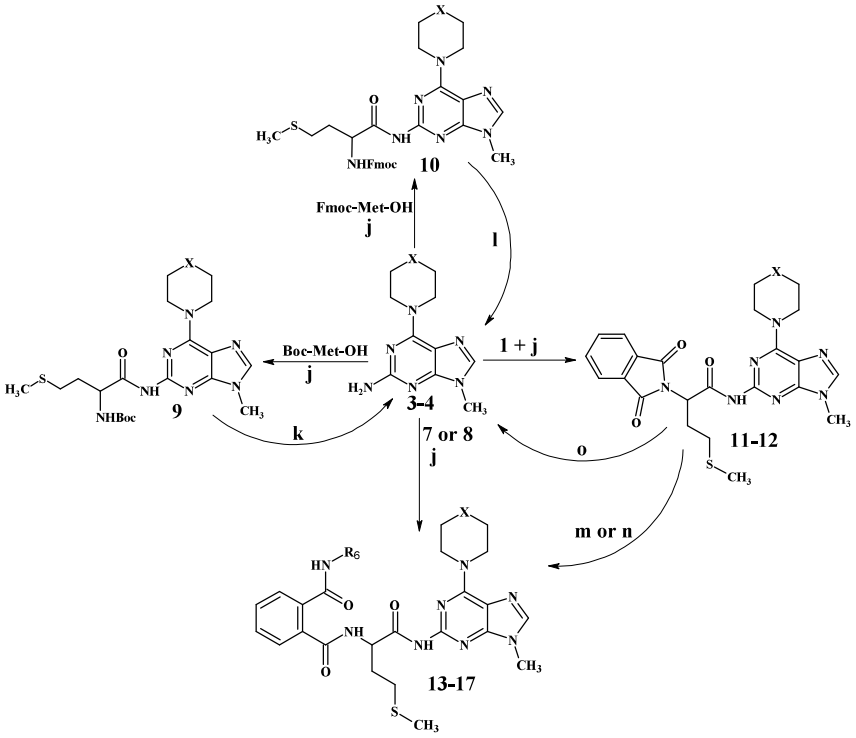
Scheme 2:- Conventional coupling of N-protected L-Methionine with 2, 6-diamino-9-methyl purine

Reagents: R_5= -Boc, -Fmoc or -Pht; R_5= piperidine or morpholine (c) (i) Thionyl chloride (ii) base/solvent (d) Ethyl chloroformate, DCC; (e) CDI/MDC; (f) HOBt, DCC (g) (i) benzotriazole (ii) DCC



Reagents: 7: R_6 =cyclohexyl; 8: R_6= cyclopropyl (i) Triethylamine, toluene, reflux, 3 h (ii) amine, DCM: MeOH, 10-12 h;

Scheme 3: Synthesis of N-Phthaloyl and carboxamide derivatives of L-methionine



9-11: X =-O: morpholine. 12: X =-CH_2: piperidine

13: X = -O: morpholine; R_6 = cyclohexyl; 14: X = -O: morpholine; R_6 = cyclopropyl;

15: X =-CH_2: piperidine; R_6 = cyclohexyl; 16: X =-CH_2: piperidine; R_6 =-cyclopropyl;

17: X = -O: morpholine; R_6 = methyl;

Reagents: (j) $POCl_3$, pyridine, -15°C, 10-12 h; (k) TFA/MDC or HCl/dioxane (l) piperidine / DMF, rt, 10-12 h, (m) cyclohexyl or cyclopropyl /DMF, rt, 10-12 h, (n) $MeNH_2$/EtOH, 0°C, 2h, (o) NH_2-NH_2/EtOH, reflux or $MeNH_2$/EtOH, rt

Scheme 4: Synthesis of trisubstituted purine derivatives

Synthesis of 9methyl-purine-2, 6-diamine (4-5)

2-amino-6-chloropurine (10 mmol), was suspended in n-BuOH, piperidine / morpholine (15 mmol) and anhydrous K_2CO_3 (20 mmol) were added and heated at reflux temperature for 5-6 h. Inorganic solid was filtered off, solvent was removed under reduced pressure to obtain sticky solid which was further dissolved in ethyl acetate and washed with water. Solvent was removed under reduced pressure to get crude product further dissolved in DCM. The mixture of 40% aqueous TBAOH (10 ml), and methyl iodide (20 mmol) was added and stirred vigorously for 1 h. Organic and aq layer was separated out, washed with water and solvent was removed under reduced pressure to get crude product **4-5**. Further purification is done by crystallization in ethanol. (Scheme 1)

Synthesis of N-Phthaloyl methionine (6)

In RBF fitted with Dean-stark apparatus and a reflux condenser, Phthalic acid anhydride (0.1 mol) and L-methionine (0.1 mol) were refluxed in 150 ml toluene in presence of 1.3 ml triethylamine for 2 hours. The organic solvents were removed in vacuo. The solid residue so obtained was then stirred with 200 ml of cold water and 2 ml of hydrochloric acid for 30 minutes. Product was obtained by filtration.

Yield 85%, white solid; mp 98 °C; MF: $C_{13}H_{13}NO_4S$; MW: 279.31; MS (EI) m/z 280.48 (M+1), 302.58 (M + Na); IR (KBr)/cm^{-1}: 3275 (O-H), 2933, 2929 (C=C), 1770, 1713 (-COO), 1693 (N-C=O) 1468 (CH=), 720 (C-S-C)
^{1}H NMR $CDCl_3$: 7.89-7.87 (dd, 2H, Ar-H), 7.76-7.75 (dd, Ar-H), 5.2-5.18 (t, 1H, -CH), 2.61-2.52 (m, 4H, -CH_2) , 2.08 (s, 3H, -S-CH_3); ^{13}C NMR $CDCl_3$: 175.04 (-COO), 167.57 (>N-C=O), 134.36 (Ar-C), 123.70 (Ar-CH), 131.66 (Ar-CH), 50.58 (-CH), 30.76 (-CH_2), 27.72 (-SCH_2), 15.26 (-SCH_3)

Synthesis of 2-Benzamido-4-(methylsulfanyl) butanoic acid-2-cycloalkyl carboxamide (7-8)

N-Phthaloyl methionine (0.1 mol) was dissolved in 30 ml of methanol: dichloromethane (1:2). Cyclopropyl /cyclohexylamine (0.2 mol) was added and stirred at room temperature for 10-12h. Organic solvent was removed under reduced pressure, an oily residue was obtained which was triturated with hexane and then was stirred in ethyl acetate: hexane mixture to get carboxamide 2-3 (Scheme 1).

2-Benzamido-4-(methylsulfanyl) butanoic acid-2-cyclohexyl carboxamide (7)

Yield 65%; white solid crystal; mp 126 ^{o}C; MF: $C_{19}H_{26}N_2O_4S$; MW: 378.48; MS (EI) m/z 379.21 (M+1), IR (KBr)/cm^{-1}: 3458 (-NH), 3207 (O-H), 2939, 2858 (C=C) , 1776, 1716(-COO), 1629 (N-C=O), 1468 (CH=), 720 (C-S-C); ^{1}H NMR $CDCl_3$: 7.81-7.79 (dd, 2H, Hz, Ar-H), 7.7-7.68 (dd, 2H, Ar-H), 4.75 (t, 1H, -CH), 3.71 (m, 1H, -NCH) 2.51-2.49 (m, 4H, -CH_2), 2.05 (s, 3H, -SCH_3), 1.24-1.11(m, 10H, -CH_2); ^{13}C NMR $CDCl_3$: 174.53 (-COO), 168.38 (>N-C=O), 133.79 (Ar-C), 123.11(Ar-CH), 132.21(Ar-CH), 54.00 (-CH), 50.10(-CH, cyclohexyl), 31.84 (-CH2), 30.74 (-SCH_2), 29.20 , 24.61-24.45(-CH_2, cyclohexyl), 15.45(-SCH_3).

2-Benzamido-4-(methylsulfanyl) butanoic acid-2-cyclopropyl carboxamide (8)

Yield 75%; off white solid; mp 101 ^{o}C; MF: $C_{16}H_{20}N_2O_4S$; MW: 336.40; MS (EI) m/z 337.21(M+1) ; IR (KBr)/cm^{-1}: : 3475 (-NH, O-H), 2919, 2879 (C=C) , 1776, 1716(-COO), 1629 (N-C=O) 1487 (CH=), 721 (C-S-C); ^{1}H NMR $CDCl_3$: 7.89-7.86 (dd, 2H, Hz, Ar-H), 7.77-7.75 (dd, 2H, Ar-H), 4.71 (t, 1H, -CH), 2.87 (m, 1H, -NCH) 2.61-2.59 (m, 4H, -CH_2), 2.06 (s, 3H, -SCH_3), 0.74-0.58(m, 4H, -CH_2); ^{13}C NMR $CDCl_3$: 174.45 (-COO), 168.51 (>N-C=O), 133.68 (Ar-C), 132.16 (Ar-CH), 123.28 (Ar-CH), 54.23 (-CH), 31.26 (-CH, cyclopropyl), 30.27 (-CH_2), 27.16 (-SCH_2), 15.26 (-SCH_3), 6.61-6.51 (-CH_2, cyclopropyl).

Synthesis of 9-12

N-Phthaloyl methionine 1 or Boc-Met-OH or Fmoc-Met-OH (1 mmol) and 4 or 5 was dissolved in 30 ml anhydrous pyridine. The solution was cooled to −15 °C and phosphorus oxychloride (1.1 mmol) was added drop wise under vigorous stirring. The reaction mixture was stirred at −15 °C for 30 minutes and then it was allowed to stir at room temperature for 10-12 h. The reaction was quenched by addition of crushed ice. Product was extracted using ethyl acetate. The combined organic layers were dried over anhydrous sodium sulphate and concentrated under reduced pressure to get crude product. Further purified by column chromatography to obtain trisubstituted purine 6-9.

[1(9-Methyl-6-morpholin-yl-9H-purin-2-ylcarbamoyl)-3-methylsulfanyl-propyl]-carbamic acid tert butyl ester (9)

Yield: 58 %; off white solid; mp: 75-77 ^{o}C ; MF: $C_{30}H_{31}N_7O_4S$; MW: 465.56; MS (EI) m/z 466.11 (M+1), 488.10 (M +Na); IR (KBr, cm^{-1}): 3462 (-NH, O-H), 2941 (C=C) , 1777, 1711 (N-C=O), 1627 (-COO) 1461 (CH=), 718 (C-S-C); ^{1}H NMR (DMSO-d6, 500MHz): δ = 10.008 (s, 1H, -NH, exchangeable), 8.027 (s, 1H, 8-C), 7.102-7.087 (d, 1H, Ar), 4.408 (br, 1H, CH), 4.198 (br, 4H, -OCH_2), 3.7-3.672 (m, 4H, -NCH_2), 3.37 (s, 3H, 9-NCH_3), 2.502 (m, 4H,-S-CH_2), 2.03 (s, 3H,-S-CH_3), 1.375 (s, 9H, -CH_3); ^{13}C NMR (DMSO-d6, 125MHz): δ = 156.03 (>N-C=O, Boc), 153.52 (C_6), 152.50-152.42 (C_2 & C_4), 140.65 (C_8), 116.45 (C_5), 78.60 (>C<, Boc), 66.66 (C_{12} & C_{14}), 54.78 (C_{18}), 31.96 (C_{19}), 30.42 (C_{20}), 29.94 (C_{10}), 28.65 (CH_3, Boc), 15.07 (C_{22}).

[1(9-Methyl-6-morpholin-yl-9H-purin-2-ylcarbamoyl)-3-methylsulfanyl-propyl]-carbamic acid 9H-fluoren-9-ylmethyl ester (10)

Yield: 72%; off white solid; mp: 157-159^{o}C; MF: $C_{30}H_{33}N_7O_4S$; MW: 587.69; MS (EI) m/z 588.46 (M+1), 610.47 (M +Na); IR (KBr, cm^{-1}): 3442 (-NH, O-H), 2921, 2865 (C=C) , 1771, 1714(-COO), 1666 (N-C=O), 1468 (CH=), 721 (C-S-C); ^{1}H NMR($CDCl_3$):-8.18 (br, 1H, CH), 7.776-7.762 (d, 2H, Ar-H), 7.677 (s, 1H, -NH), 7.635-7.60 (t, 2H, Ar-H), 7.418-7.391 (t, 2H, Ar-H), 7.33-7.316 (t, 2H, Ar-H), 5.813 (s, 1H), 4.426-4.413 (d, 2H, -CH_2), 4.250 -4.222(t, 1H, -CH), 3.82 (s, 4H, -CH_2), 3.764 (s, 3H, -NCH_3), 2.648-2.605 (q, 2H, -S-CH_2), 2.264-2.252 (d, 1H, -CH_2), 2.095 (s, 3H, -SCH_3), 2.037-2.022 (d, 1H, CH_2); ^{13}CNMR:-159.97 (C_2), 153.54-153.39 (C_4 & C_6), 156.47 (>N-C=O), 143.74-143.54 (Ar-C, Fmoc), 141.29 (Ar-C, Fmoc), 127.75 (Ar-CH, Fmoc), 127.08 (Ar-CH, Fmoc), 125.02 (Ar-CH, Fmoc), 120.00 (Ar-CH, Fmoc), 67.12 (-OCH_2, Fmoc), 66.65 (C_{11} & C_{14}), 52.99 (C_{18}), 47.07 (-CH, Fmoc), 31.44 (C_{19}), 29.93 (C_{10}), 29.89 (C_{20}), 14.34 (C_{22})

2-(1, 3-dioxo-1, 3-dihydro-2H-isoindol-2-yl)-N-(9-methyl-6-morpholin-4-yl-9H-purin-2-yl)-4-methylsulfanyl butyramide (11)

Yield: 52 %; white solid crystal; mp: 104-106 ^{o}C ; MF: $C_{23}H_{25}N_7O_4S$; MW: 495.44; MS (EI) m/z 496.22 (M+1), 518.22 (M+ Na); IR (KBr, cm^{-1}): 3440 (-NH, O-H), 2919, 2856 (C=C) , 1774, 1716 (N-C=O), 1649 (-COO) 1465 (CH=), 720 (C-S-C); ^{1}H NMR (DMSO d6, 500MHz): δ = 10.44 (s, 1H, -NH, exchangeable), 8.031 (s, 1H, -CH), 7.87(m, 4H, Ar-CH), 5.25 (br, 1H, -CH), 4.16 (br, 4H, -OCH_2), 3.68-3.65 (m, 4H, -NCH_2), 3.6 (s, 3H, 9-CH_3), 2.55-2.47 (m, 4H,-CH_2), 2.01 (s, 3H,-S-CH_3); ^{13}C NMR ($CDCl_3$, 125MHz): δ = 168.16 (>N-C=O), 153.82 (C_6), 152.21-151.74 (C_2 & C_4), 138.81 (C_8), 134.3(C_{25} and C_{30}), 131.72 (C_{27} and C_{28}), 123.61 (C_{26} & C_{29}), 117.04 (C_5), 66.99 (C_{12} & C_{14}), 54.33 (C_{18}), 31.16 (C_{19}), 29.85 (C_{10}), 27.82 (C_{20}), 15.46 (C_{22}).

2-(1, 3-dioxo-1, 3-dihydro-2H-isoindol-2-yl)-N-(9-methyl-6-piperidin-1-yl-9H-purin-2-yl)-4-methylsulfanyl butyramide (12)

Yield: 68%; off white solid ; m.p. : 139-141 ^{o}C ; MF: $C_{24}H_{27}N_7O_3S$; MW: 493.58; MS (EI) m/z 494.23 (M+1), 516.23 (M+ Na); IR (KBr, cm^{-1}): 3444 (-NH, O-H), 2936 (C=C) , 1772, 1716 (N-C=O), 1667 (-COO) 1465 (CH=), 718 (C-S-C); ^{1}H NMR ($CDCl_3$, 500MHz): δ = 8.14

(s, 1H, C_8-CH), 7.89-785 (dd, 2H, Ar-CH), 7.75-7.72 (dd, 2H, Ar), 7.61-7.6 (d, 1H, -CH). 5.8 (br, 1H, CH), 4.18 (br, 4H, -CH_2), 3.75 (s, 3H, -CH_3), 2.82-2.51 (m, 4H,-CH_2), 2.01(s, 3H,-SCH_3), 1.72-1.13 (m. 6H, -CH_2); ^{13}C NMR ($CDCl_3$, 500MHz): δ = 168.21 (>N-C=O), 153.76 (C_6), 151.98-151.79 (C_2 & C_4), 138.24 (C_8), 134.36 (C_{25} and C_{30}), 131.66 (C_{27} and C_{28}), 123.7 (C_{26} & C_{29}), 116.92 (C_5), 54.36 (C_{18}), 31.21(C_{19}), 29.83 (C_{10}), 27.85 (C_{20}), 26.09(C_{22}), 24.73, (C_{22}), 22.86 (C_2), 15.44 (C_{22}).

Synthesis of 13-16

Reaction of Carboxamide derivative 7-8 (1 mmol) with 4-5 in $pocl_3$/pyridine will give direct product 13-16

Similarly 11-12 (10 mmol) was dissolve in DMF. Cyclopropyl or cyclohexyl (20 mmol) was added and stirred for 10-12 hrs. Distilled of solvent. Water was added, stir for 1h and filtered off to get crude product. Further purified by column chromatography to obtain the desired trisubstituted purine 13-16 (Scheme 3)

N-Cyclohexyl-N-[(1-(9-methyl-6-morpholin-4-yl-9H-purin-2-ylcarbamoyl)-3-methylsulfanyl-propyl]–phthalamide (13):

Yield: 55%. Appearance: Off white solid; m.p: 50-52 ^oC; MF: $C_{29}H_{38}N_8O_4S$; MW: 594.72;MS (EI) m/z 379.21 (M+1) ; IR (KBr, cm^{-1}): 3455 (-NH, O-H), 2966 (C=C) , 1765, 1718 (N-C=O), 1666 (-COO) 1445 (CH=), 720 (C-S-C);); ^{1}H NMR ($CDCl_3$, 500MHz): δ = 8.09 (s, 1H, 8-H), 7.89-7.86 (dd 2H, Ar-H), 7.77-7.75 (dd, 2H, Ar-H), 7.62 (s, 1H, -NH), 5.80 (s, 1H, -CH), 4.27 (br, 4H, -CH_2), 3.83-3.81 (t, 4H, -CH_2), 3.77(s, 3H, 9N-CH_3), 2.81-2.79(m, 1H, -CH), 2.62-2.54 (m, 4H,-S-CH_2), 2.07 (s, 3H,-S-CH_3), 1.59-1.13 (m, 10H, -CH_2); ^{13}C NMR ($CDCl_3$, 500MHz): δ = 169.15 (>N-C=O), 167.83 (>N-C=O), 153.74 (C_6), 152.00-151.71(C_2 & C_4), 138.26 (C_8), 135.21-134.25 (C_{25} & C_{30}), 130.41-130.12 (C_{27} & C_{28}), 128.52-128.27 (C_{26} & C_{29}), 116.91 (C_5), 68.33 (C_{12} & C_{14}), 53.16 (C_{18}), 48.93 (-CH, Cyclohexyl), 32.81 (-CH_2, Cyclohexyl), 30.81 (C_{19}), 29.76 (C_{10}), 26.14 (C_{20}), 25.51, 24.74, 22.74 (-CH_2, Cyclohexyl), 15.70 (C_{22}).

N-Cyclopropyl-N-[(1-(9-methyl-6-morpholin-4-yl-9H-purin-2-ylcarbamoyl)-3-methylsulfanyl-propyl]–Phthalamide (14):

Yield: 52%. Appearance: Off white solid; mp: 55-58 oC; MF: C26H32N8O4S; MW: 552.64;MS (EI) m/z 575.20 (M +Na) ; IR (KBr, cm-1): 3462 (-NH, O-H), 2941 (C-H), 1771, 1711(C=O), 1682 (N-C=O), 1627 (C=N), 1568, 1461 (C=C), 1335 (C–N); 1H NMR (CDCl3, 500MHz): δ = 2.66 (t, 2H, CH2), 2.20 (S, 3H , CH3), 2.19 (td, 2H, CH2), 4.37 (t ,1H, CH), 7.57(ddd, 1H, Ar-H), 7.56 (ddd, 1H, Ar-H), 7.60 (ddd, 1H, Ar-H), 7.60(ddd, 1H, Ar-H), 2.98 (tt, 1H,CH), 3.77 (ddd, 1H,CH), 3.79 (ddd, 1H,CH), 3.79 (ddd, 1H,CH), 3.77 (ddd, 1H,CH), 7.58 (s,1H,CH), 3.68 (s,3H, CH3), 0.47(dddd ,1H,CH), 0.42 (tdd, 1H,CH), 0.42 (tdd, 1H,CH), 0.47 (dddd, 1H,CH), 3.69 (ddd, 1H,CH), 3.72 (ddd, 1H,CH), 3.72 (ddd, 1H,CH), 3.69 (ddd, 1H,CH), ^{13}C NMR ($CDCl_3$, 500MHz): δ = 168.17 (>N-C=O), 168.17 (>N-C=O), 153.83 (C_6), 151.73 (d, C_2 & C_4), 138.81 (C_8), 134.30 (C_{25} & C_{30}), 131.72 (C_{27} & C_{28}), 130.36 (C_{26} & C_{29}), 123.62 (C_5), 67.00 (C_{12} & C_{14}), 54.32 (C_{18}), 31.17 (C_{19}), 30.24 (C_{20}), 29.85 (C_{10}), 27.82 (-CH, Cyclopropyl), 15.46 (C_{22}), 6.64 (-CH_2, Cyclopropyl)

N-Cyclohexyl-N-[(1-(9-methyl-6-piperidin-1-yl-9H-purin-2-ylcarbamoyl)-3-methylsulfanyl-propyl]–Phthalamide (15):

Yield: 65%. Appearance: Off white solid; mp: 128-130 ^oC; MF: $C_{30}H_{40}N_8O_3S$; MW: 592.75; MS (EI) m/z 593.32 (M+1), 615.31 (M+ Na); IR (KBr, cm^{-1}): 3444 (-NH, O-H), 2972 (C=C) , 1774, 1716 (N-C=O), 1653 (-COO) 1465 (CH=), 721 (C-S-C); ^{1}H NMR (DMSO-d_6, 500MHz): δ = 10.09 (s, 1H,-NH. exchangeable), 8.489-8.473 (d, 1H, -NH, exchangeable), 8.09-8.08 (1H, -NH, exchangeable), 8.00 (s, 1H, 8H), 7.53-7.46 (m, 4H, Ar-H), 4.9 (s, 1H, -CH), 4.27 (br, 4H, -CH_2), 3.66 (s, 1H, -CH_3), 3.64-3.62 (m, 1H, -CH), 2.64-2.61 (m, 4H,-CH_2), 2.1 (s, 3H,-

CH_3), 2.06-1.51 (m, 16H, -CH_2); ^{13}C NMR (DMSO-d_6, 500MHz): δ = 168.8 (>N-C=O), 167.59 (>N-C=O). 153.48 (C_6), 152.41 (C_2 & C_4), 140.15 (C_8), 136.54-136.05 (C_{25} & C_{30}), 129.95-129.78 (C_{27} & C_{28}), 128.36-128.26 (C_{26} & C_{29}), 116.45 (C_5), 53.66 (C_{18}) 48.65(-CH, Cyclohexyl), 32.59-32.51 (-CH_2, Cyclohexyl), 32.23 (C_{19}), 30.39 (C_{20}), 29.89 (C_{10}), 26.22 (C_{13}), 25.57 (C_{12} & C_{14}), 25.15, 24.74 (-CH_2, Cyclohexyl), 15.06 (C_{22}).

N-Cyclopropyl-N-[(1-(9-methyl-6- piperidin-1-yl-9H-purin-2-ylcarbamoyl)-3-methylsulfanyl-propyl] –Phthalamide (16):

Yield: 50 %. Appearance: White solid crystal; mp: 78-80 ^{o}C; MF: $C_{27}H_{34}N_8O_3S$; MW: 550.67; MS (EI) m/z 551.22 (M+1), 573.18 (M+ Na); IR (KBr, cm^{-1}): 3446 (-NH, O-H), 2919 (C-H) , 1771, 1715(C=O) , 1649 (N-C=O)1465 (CH=), 720 (C-S-C);; 1H NMR ($CDCl_3$, 500MHz): δ = 2.66 (t,2H, CH_2), 2.20 (s, 3H, CH_3), 2.19 (m, 2H, CH_2), 4.37 (t, 1H, CH), 7.57 (m, 1H, CH), 7.56 (m, 1H, CH), 7.60 (m, 1H,CH), 7.60 (m, 1H, CH), 2.99 (m, 1H,CH), 3.29 (m, 1H,CH), 3.70 (m, 1H,CH), 3.70 (m, 1H, CH), 3.29 (m, 1H,CH), 7.58 (S, 1H, CH), 3.68 (s, 3H,CH_3), 0.47(m, 1H, CH), 0.43 (m, 1H,CH), 0.43 (m, 1H, CH), 0.48 (m, 1H,CH), 1.55 (m, 1H,CH), 2.10 (1H, m, CH), 2.09 (m, 1H,CH), 1.55 (m, 1H,CH), 1.53 (m, 1H,CH), 1.482 (M, 1H, CH)

Synthesis of N-Methyl-N-[(1-(9-methyl-6-morpholin-4-yl-9H-purin-2-ylcarbamoyl)-3-methylsulfanyl-propyl]–phthalamide (17):

12 (10 mmol) was dissolve in ethanol and cooled 0°C. Methyl amine in ethanol (20 mmol) was added and stir for 3-4 hr. Distilled off solvent. Water was added, stir for 1h and filtered off to get crude product. Further purified by column chromatography to obtain the desired trisubstituted purine 17 (Scheme 3)

Yield: 35%. Off white solid; m.p: 95-98 ^{o}C; MF: $C_{24}H_{30}N_8O_4S$; MW: 526.61; MS (EI) m/z 527.34 (M+1), 549.33 (M+ Na); IR (KBr, cm^{-1}): 3441 (-NH, O-H), 2946 (C=C) , 1777, 1714 (N-C=O), 1657 (-COO) 1466 (CH=), 721 (C-S-C); ^{1}H NMR (DMSO-d6, 500MHz): δ = 10.14 (s, 1H, -NH exchangeable), 8.518-8.504 (s, 1H, -NH exchangeable), 8.241 (s, 1H, -NH exchangeable), 8.043 (s, 1H, 8-H), 7.504 (m, 4H, Ar-H), 4.819 (br, 1H, -CH), 4.208 (br, 4H, -CH_2), 3.701-3.679 (d, 4H, -CH_2), 3.394 (s, 3H, 9N-CH_3), 2.717 (s, 3H, -CH_3), 2.633 (s, 1H, -CH_2), 2.503 (m, 2H, -S-CH_2), 2.066 (s, 3H,-S-CH_3), 1.924 (s, 1H, -CH_2); ^{13}C NMR (DMSO-d6, 500MHz): δ = 168.93-168.79 (>NC=O), 153.52 (C_6), 152.52-152.36 (C_2 & C_4), 140.74 (C_8), 136.43-136.03 (C_{25} & C_{30}), 130.01-129.95 (C_{27} & C_{28}), 128.40-127.92 (C_{26} & C_{29}), 116.56 (C_5), 66.67 (C_{12} & C_{14}), 53.62 (C_{18}), 32.06 (C_9), 30.35 (C_{20}) , 29.95 (C_{10}), 26.64 (-CONH-**C**H_3), 15.06 (C_{22}).

Results and discussion

The synthesis of target molecule (TM) is carried out using readily available starting material 2-amino-6-chloropurine (2-ACP) using general strategy i.e. first synthesis of 2, 6-diamino-9-methyl purine (4-5) and then coupling at C2 position. 2, 6-diamino-9-methyl purine (**4-5**) was synthesis by alkylation at 9N position using methyl iodide and 40% TBAOH in DCM (Havlicek et al. 1997), followed by amination of C6 position using K_2CO_3 in n-Butanol at reflux temperature (Zacharie et al. 2009)) or vice versa(Scheme 1). The introduction of substituent at the 2-position was very difficult as it is most unreactive site and required harsh reaction condition. It is observed that coupling of acid group of amino acid derivatives with C2-amino group is not working using standard coupling reagents. The use of well-known conventional coupling methods and reagents such as mixed anhydrides, carbonyl di-imidazole (CDI), acid chloride method, and dicyclohexyl carbodimide (DCC) (Christian & Virginie 2005), benzotriazole coupling method (Katritzky et al. 2008) were investigated but all coupling methods were almost completely ineffective (Scheme 2). Coupling of Boc-protected amino acid with heterocyclic amine using $POCl_3$ in pyridine was reported (Quelever G et al 2004), so we tried the same for coupling of Boc-methionine with 9-methyl-6-(morpholin-4-yl)-9*H*-purin-2-amine (4)

followed by deprotection in acidic medium (Shendage DM et al. 2004). But it is observed that during deprotection the amide bond between amino acid and purine also cleave (Urban J et al. 1996) and get starting material back (scheme 4). Same result was obtained by using Fmoc-protected methionine and deprotection with piperidine in DMF (Atherton E. et al. 1981). Finally we tried coupling with phthalamide protected L-methionine and then ring opening reaction with cycloalkyl amine in DMF (Okunrobo and Usifoh, 2006) to get target molecule. The target molecule was also synthesized by direct coupling of carboxamide derivative of L-methionine.

For further study we tried the deprotection of phthalamide group with hydrazine hydrate (Curley OMS et al. 2003) and also with methyl amine (Roehrig S et al. 2005). It is observed that using hydrazine hydrate, cleavage of amide bond takes place and same result was obtained using methylamine in ethanol at room temperature, but at lower temperature (0°C) we get methyl carboxamide product (17).

The aim of this work was to synthesize novel derivative of trisubstituted purine. An efficient methodology has been established for the synthesis of novel trisubstituted purine by using $POCl_3$ in pyridine for the coupling of methionine derivative with 2-amino purine compounds at normal reaction temperature and conditions. The reactions were completed in 10-12 h and products were obtained in good yield after simple work up and purification using column chromatograph.

Moreover, the structures of the products were elucidated by MS, ^{1}H-NMR, ^{13}C-NMR and IR. ^{1}H-NMR spectra of all the compounds was quite simple and proton of C8 position of purine of the entire synthesized compound found in the region of 8.0-8.1 ppm and carbon at 138.0 ppm in $CDCl_3$ while 140.0 ppm in DMSO-d6. In DMSO-d6 distinct peak of –NH proton are observed at 10.0, 8.4, 8.0 ppm and are exchangeable in D_2O, while in $CDCl_3$ only one peak at 7.6 is observed. α-carbon shows distinct displacement in N-protected methionine (6 & 9-12) (50.0 ppm) and in its alkyl carboxamide derivatives (7-8 & 13-17) (54.0 ppm). CMR of N-**C**H_2 of morpholine derivative (9-11 and 13, 14, 17) was not observed (expected at 45.0 ppm) but it shows clear triplet at 3.8-3.7 in PMR. The aromatic protons of phthalamide ring appear as a double doublet in the region of 7.7-7.89 ppm depending on the aromatic substituent. In IR spectrum the peak appears in the region of 1715-1766 cm^{-1}

Biological assays

Compounds: Test compounds were dissolved in DMSO at an initial concentration of 1 mg/ml and then were serially diluted in culture medium.

Bacterial strains: *Staphylococcus aurous, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimurium.*

Fungal stains: *Fusarium oxysporum* and *Alternaria alternate*

Antimicrobial 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 and results were compared with the standard 0.3% Amplicilline and Chloramphenicol as antibacterial agent. While *in vitro* antifungal activities were evaluated against the fungal strains *F. oxysporum* and *A. alternate* and results were compared with antifungal agent Nystatin. Results were summarized in Table 1.

Table 1. *In vitro* antimicrobial activities of trisubstituted purine 9-17.

Sr. No.	Compounds	Zone of inhibition in mm					
		Bacteria				**Fungi**	
		Gram +	**Gram –**				
		S. aureus	*E. coli*	*P. aeruginosa*	*S. typhimurium*	*F. oxysporum*	*A. alternata*
1	9	12	12	11	10	37	28
2	10	12	12	10	11	35	27
3	11	11	11	12	11	36	29
4	12	10	11	10	9	33	25
5	13	**20**	12	12	11	**55**	**42**
6	14	19	12	11	11	52	37
7	15	**20**	11	12	11	**58**	**41**
8	16	19	11	12	11	50	36
9	17	19	15	10	10	51	40
10	Amplicilline	20	11	NT	NT	NT	NT
11	Chloramphenicol	17	20	12	12	NT	NT
12	Nystatin	NT	NT	NT	NT	70	50

*Less active: 6–12 mm; moderately active: 13–19 mm; highly active: 20–30 mm; –: No inhibition or inhibition less than 5 mm; NT: not tested.

The antimicrobial results of the compounds shown in Table 1 revealed that all trisubstituted derivatives of purine 9-17 show good to moderate activity. Among the tested compounds, the compound N-Cyclohexyl-N-[(1-(9-methyl-6-morpholin-4-yl-9H-purin-2-ylcarbamoyl)-3-methylsulfanyl-propyl]–phthalamide (13)and N-Cyclohexyl-N-[(1-(9-methyl-6-piperidin-1-yl-9H-purin-2-ylcarbamoyl)-3-methylsulfanyl-propyl]–Phthalamide (15)having cyclohexyl ring showed excellent activity against bacteria *S. aureus* as well as both fungi *F. oxysporum* and *A. alternata* .

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Supporting Information Available:

Experimental procedures and analytical data for the synthesis and characterization of the compounds depicted in Schemes 1, 2 and 3. (49 pages).

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