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SYNTHESIS AND ANTIMICROBIAL EVALUATION OF PURINE DERIVATIVE COUPLED WITH PROTECTED AMINO ACIDS

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ABSTRACT:
Some new tri-substituted purine derivatives were synthesized by coupling of purine with Boc protected Amino acid derivatives 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, amino acid derivatives, antimicrobial activity*

INTRODUCTION:

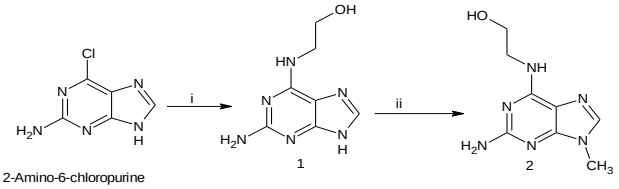
Purine derivative having is of great interest in medicinal chemistry. Tri-substituted purine revealed a large number of highly active Cyclin-dependent kinase (CDK) inhibitors[1-2]. Tri-substituted purine family currently being explored as novel anticancer drugs[3-4], Inhibitors of Src tyrosine kinase for the treatment of bone diseases[5], as protein A mimetics for the treatment of autoimmune diseases as useful tools for developing potent plant mitogen-activated protein kinase inhibitors as inhibitors of P38 mitogen-activated protein kinase[6], as potent Hsp90 inhibitor[6], as potent stat3 binding inhibitor[7].

These encouraging results led us to design other Trisubstitured purine as biologically relevant molecules with broad biomedical value as therapeutics. We have synthesized trisubstituted purine coupled with protected amino acid derivatives and subjected to *in vitro* antimicrobial screening.

METHOD AND MATERIAL:

Reagents, instrumentation, and measurements:

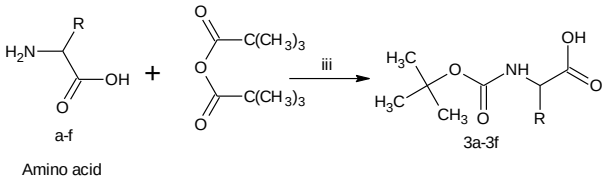
Melting points were measured on a Veego VMP-PM melting point apparatus and IR spectra were recorded on Perkin Elmer Spectrum 100 FT-IR spectrometer. ^{1}H, and ^{13}C NMR spectra were recorded at 500.1 and 125.8 MHz respectively on a BRUKER Avance II 500 instrument with $CDCl_3$ / DMSO-d6 as solvent 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. Amino acid derivatives and Boc 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.



2-Amino-6-chloropurine

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

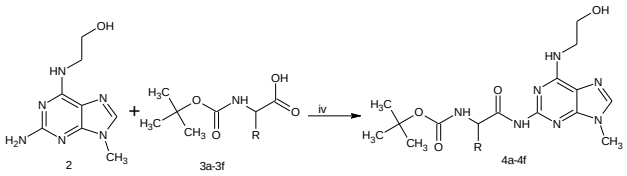
Reagents: (i) Amino Ethanol , K_2CO_3, n-Butanol, reflux, 5-6 h ; (ii) Methyl Iodide, 40% TBAOH, DCM, rt, 1 h.



Reagents: (iii) NaOH, Water/Dioxane, 12 h

R; a = -H, b = $-CH_3$, c = $-CH(CH_3)_2$, d = $-CHCH(CH_3)_2$, e = $-CH_2Ph$, f = $-CH_2SCH_3$,

Scheme 2: Synthesis of Boc derivatives of amino acids



Reagents: (iv) $POCl_3$, pyridine, -15°C, 10-12 h

Scheme 3: Synthesis of trisubstituted purine derivatives

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

2-amino-6-chloropurine (10 mmol), was suspended in n-BuOH, Amino ethanol (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 **2**. Further purification is done by crystallization in ethanol. (Scheme 1)

Synthesis of Boc amino acid derivatives (3a-3f)

In RBF charge amino acid (0.1 mol) and dissolve Water/Dioxane solution. Sodium Hydroxide (0.15 mol) added and stir to clear solution. Reaction mass cooled to below 10°C. Boc anhydride added dropwise below 10°C. After addition reaction mass stir at room temperature till completion. Reaction is monitored over TLC. After reaction completion, solvent distilled out below 40°C under vacuum. RM cooled to 10-15°C. Water added and pH adjusted to 2-3 using pre-cooled dilute HCl. Product extract using Ethyl acetate. Ethyl acetate layer wash with water followed by brine solution. Solvent evaporated to give sticky mass. Hexane added and stir solid appeared. Filtered the solid and wash with Hexane to get pure Boc amino acid which is directly use as it is without further purification .

Synthesis of Final Product (4a-4f)

Boc Amino acid (3a-3f) (1 mmol) and **2** 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 **4a-4f**.

Tert-Butyl [2-({6-[(2-Hydroxyethyl)Amino]-9-Methyl-9H-Purin-2-Yl}Amino)-2-Oxoethyl] Carbamate 4a:

yield: 60 %; off white solid ; mp: 105-108 °C; MF: $C_{15}H_{23}N_7O_4$; MW: 365.38; IR (KBr, cm^{-1}): 3455 (N-H), 2920 (C-H), 1700, 1691 (C=O), 1611 (C=N), 1572, 1455 (C=C), 1333 (C–N); MS (*m/z*): $[MH]^+$ 366 .77 ; ^1H NMR ($CDCl_3$, 500MHz): δ = 8.1 (s, 1H, CH),

7.61 (s, 1H, -CONH), 4.72 (s, 2H, $-CH_2$, αH), 4.15 (br, 2H, $-OCH_2$), 3.7 (br, 2H, $-NCH_2$), 3.77 (s, 3H, $-NCH_3$), 1.37 (s, 9H, $-CH_3$) ; ^{13}C NMR ($CDCl_3$, 125MHz): δ = 168.17 (s, >N-C=O, Boc), 153.76 (s, C_6), 151.98-151.79 (d, C_2 & C_4), 138.24 (s, C_8), 116.92 (s, C_5), 78.60 (s, >C<, Boc), 66.6 (s, $-OCH_2$), 45.73 (s, $–NCH_2$,), 45.22 (s, $-CH_2$, αC), 29.85 (s, $-9NCH_3$), 28.65 (CH_3, Boc),

Tert-Butyl [1-({6-[(Hydroxyethyl) Amino]-9-Methyl-9H-Purin-2-Yl} Amino)-1-Oxopropan-2-Yl]Carbamate 4b:

yield: 52 %; off white solid ; mp: 130-132 °C; MF: $C_{16}H_{25}N_7O_4$; MW: 3795.38; IR (KBr, cm^{-1}): 3460 (N-H), 2942 (C-H), 1710, 1685 (C=O), 1629 (C=N), 1560, 1463 (C=C), 1331 (C–N); MS (*m/z*): $[MH]^+$ 380.20 ; ^1H NMR ($CDCl_3$, 500MHz): δ = 8.1 (s, 1H, CH), 7.61 (s, 1H, -CONH), 4.88 (m, -H, -CH, αH), 4.15 (br, 2H, $-OCH_2$), 3.7 (br, 2H, $-NCH_2$), 3.77 (s, 3H, $-NCH_3$), 1.69 (d, 3H, $-CH_3$). 1.37 (s, 9H, $-CH_3$) ; ^{13}C NMR ($CDCl_3$, 125MHz): δ = 168.17 (s, >N-C=O, Boc), 153.76 (s, C_6), 151.98-151.79 (d, C_2 & C_4), 138.24 (s, C_8), 116.92 (s, C_5), 78.60 (s, >C<, Boc), 66.6 (s, $-OCH_2$), 53.22 (s, -CH, αC), 45.73 (s, $–NCH_2$,), 29.85 (s, $-9NCH_3$), 28.65 (CH_3, Boc), 14.46 (s, $-CH_3$)

Tert-Butyl [1-({6-[(2-Hydroxyethyl)Amino]-9-Methyl-9H-Purin-2-Yl}Amino)-3-Methyl-1-Oxobutan-2-Yl]Carbamate 4c:

yield: 45 %; off white solid ; mp: 120-122 °C; MF: $C_{18}H_{29}N_7O_4$; MW: 407.46; IR (KBr, cm^{-1}): 3462 (N-H), 2941 (C-H), 1723, 1676 (C=O), 1627 (C=N), 1568, 1461 (C=C), 1335 (C–N); MS (*m/z*): $[MH]^+$ 408.62 ; ^1H NMR ($CDCl_3$, 500MHz): ^1H NMR ($CDCl_3$, 500MHz): δ = 8.1 (s, 1H, CH), 4.84 (m, -CH, αH), 4.15 (br, 2H, $-OCH_2$), 3.7 (br, 2H, $-NCH_2$), 3.77 (s, 3H, $-NCH_3$), 2.78-2.74 (m, 1H, -CH), 1.69 (d, 3H, $-CH_3$), 1.37 (s, 9H, $-CH_3$), 1.18-1.16 (d, 3H,CH_3), 0.93-0.92 (d, 3H, $-CH_3$) ; ^{13}C NMR ($CDCl_3$, 125MHz): δ = 168.17 (s, >N-C=O, Boc), 153.82 (s, C_6), 153.76-151.98 (d, C_2 & C_4), 138.24 (s, C_8), 117.04 (s, C_5), 78.60 (s, >C<, Boc), 66.6 (s, $-OCH_2$), 52.3 (s, -CH, αC), 45.73 (s, $–NCH_2$,), 29.85 (s, $-9NCH_3$), 28.65 (CH_3, Boc), 25.36 (s, -CH), 16.21 (d, $-CH_3$), 14.46 (s, $-CH_3$)

Tert-Butyl [1-({6-[(Hydroxyethyl)Amino]-9-Methyl-9h-Purin-2-Yl}Amino)-4-Methyl-1-Oxopentan-2-Yl]Carbamate 6d:

yield: 59 %; off white solid ; mp: 155-160 °C; MF: $C_{19}H_{31}N_7O_4$; MW: 421.49; IR (KBr, cm^{-1}): 3462 (N-H), 2951 (C-H), 1713, 1675 (C=O), 1625 (C=N), 1571, 1455 (C=C), 1333 (C–N); MS (*m/z*): $[MH]^+$ 422.90 ; ^1H NMR ($CDCl_3$, 500MHz): δ = 8.1 (s, 1H, CH), 5.1 (d, -CH, αH), 4.19 (br, 2H, $-OCH_2$), 3.9 (br, 2H, $-NCH_2$), 3.77 (s, 3H, $-NCH_3$), 2.34 (m, 1H, -CH), 1.5 (d, 6H, $-CH_3$), 1.37 (s, 9H, $-CH_3$) ; ^{13}C NMR ($CDCl_3$, 125MHz): δ = 168.21 (s, >N-C=O, Boc), 153.82 (s, C_6), 152.21-151.748 (d, C_2 & C_4), 138.24 (s, C_8), 117.04 (s, C_5), 78.60 (s, >C<, Boc), 66.6 (s, $-OCH_2$), 52.18 (s, -CH, αC), 45.5 (s, $–NCH_2$,), 30.1 (s, $-9NCH_3$), 28.65 (CH_3, Boc), 25.28 (s, -CH), 24.61 (d, $-CH_2$), 19.33 (d, $-CH_3$)

Tert-Butyl [1-({6-[(Hydroxyethyl)Amino]-9-Methyl-9H-Purin-2-Yl}Amino)-1-Oxo-3-Phenylpropan-2-Yl]Carbamate 4e:

yield: 56 %; off white solid ; mp: 128-135 °C; MF: $C_{22}H_{29}N_7O_4$; MW: 455.51; IR (KBr, cm^{-1}): 3466 (N-H), 2935 (C-H), 1705, 1688 (C=O), 1631 (C=N), 1559, 1463 (C=C), 1333 (C–N); MS (*m/z*): $[MH]^+$ 456.63 ; ^1H NMR ($CDCl_3$, 500MHz): δ = 8.10 (s, 1H), 7.60 (s, 1H, -CONH), 7.3-7.09 (m, 5H, Ar-CH), 5.2 (s, 1H, -CH, αH), 4.25 (br, 4H, $-OCH_2$), 3.83-3.77 (m, 4H, $-NCH_2$), 3.72 (s, 3H, $-9NCH_3$), 3.4-3.36 (dd, 1H, -CH2), 3.27-3.22(dd, 1H, -CH2), 1.76 (s. 2H, -CH2) ; ^{13}C NMR ($CDCl_3$, 125MHz): δ = 168.16 (s, >N-C=O), 153.83 (s, C_6), 152.20-151.79 (d, C_2 & C_4), 140.79 (s, C_8), 138.8 (s, 1H, Ar-C), 128.88 (d, 1H, Ar-CH), 128.56 (d, 1H, Ar-CH), 127.81 (s, 1H, Ar-CH),117.04 (s, C_5), 56.0 (s, -CH), 37.88 (s, $-CH_2$), 29.85 (s, $-9NCH_3$), 28.66 (CH_3, Boc),

Tert-butyl [1-({6-[(2-hydroxyethyl)amino]-9-methyl-9H-purin-2-yl}amino)-3-(methylsulfanyl)-1-oxopropan-2-yl]carbamate 4f:

yield: 60 %; off white solid ; mp: 141-143 ^{o}C; MF: $C_{17}H_{27}N_7O_4S$; MW: 425.50; IR (KBr, cm^{-1}): 3451 (N-H), 2931 (C-H), 1705, 1690 (C=O), 1635 (C=N), 1562, 1466 (C=C), 1338 (C–N); MS (*m/z*): $[MH]^+$ 436.61 ; δ = 10.008 (s, 1H, -NH), 8.027 (s, 1H, 8-C), 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 (-OCH2), 54.78 (-αCH), 31.96 (-CH_2), 30.42 (-CH_2), 29.94 (-NCH_3), 28.65 (CH_3, Boc), 15.07 (-SCH_3).

RESULTS AND DISCUSSION

The synthesis of final product 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 (**2**) was synthesis by amination of C6 position using K_2CO_3 in n-Butanol at reflux temperature followed by alkylation at 9N position using methyl iodide and 40% TBAOH in DCM (Scheme 1). Coupling of Boc-protected amino acid with heterocyclic amine using $POCl_3$ in pyridine was reported, so we tried the same for coupling of Boc-amino acid derivatives with 9-methyl-6-aminoethanol-9*H*-purin-2-amine (2)

The aim of this work was to synthesize novel derivative of tri substituted purine. An efficient methodology has been established for the synthesis of novel tri substituted purine by using $POCl_3$ in pyridine for the coupling of amino acid derivatives 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.

Microbial Analysis:

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.

The antimicrobial results of the compounds shown in Table 1 revealed that all trisubstituted derivatives of purine 3a-3f show good to moderate activity. Among the tested compounds, the compound tert-butyl [1-({6-[(hydroxyethyl)amino]-9-methyl-9h-purin-2-yl}amino)-1-oxo-3-phenylpropan-2-yl]carbamate (4e) having phenyl group showed excellent activity against bacteria *S. aureus*aswell asboth fungi *F. oxysporum* and *A. alternata*.

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Table 1. *In vitro* antimicrobial activities of trisubstituted purine 4a-4f.

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	4a	11	11	10	09	33	23
2	4b	11	11	10	10	32	21
3	4c	10	11	10	10	33	27
4	4d	10	10	11	10	32	23
5	4e	**16**	11	11	10	**51**	**38**
6	4f	15	10	10	11	45	32
7	Amplicilline	20	11	NT	NT	NT	NT
8	Chloramphenicol	17	20	12	12	NT	NT
9	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.