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Synthesis, Characterization and Anti-microbial activity of some Novel Trisubstituted Purine Bearing Amino acid

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ABSTRACT

*A series of Trisubstited purine have been synthesized and tested for in vitro antimicrobial activity on different microorganisms. Synthesis has carried out from 9-benzyl-6-tert-butyl amine-9H-purine-2-amine [**2**].N-Boc substituted Amino acids reacted with **2** in $POCl_3$/Pyridine to give 2, 6, 9- trisubstituted purine **3a-f**. Their chemical structures were characterized using IR, ^1H NMR, MASS and elemental analysis. All compounds were tested for antimicrobial activity against S. aureus, S. pyrogenes E. coli, P. aeruginosa C. albicans, and A. clavatus. These premunary results indicate that some of compounds are exhibiting good activity.*

Keywords: Purine, amino acid, spectroscopy, Antibacterial activity.

INTRODUCTION

Purine is of great interest for several reasons, they are constituents of DNA and RNA and consequently of fundamental importance in life processes [1-3]. In the recent years of the chemistry of purine derivatives has been the subject of great interest due to the use of such ring systems as the core structure in many heterocyclic compounds covering wide range of pharmacological applications. Several reports have revealed that trisubstituted purine derivatives arrived with significant biological and pharmacological activities, such as antimicrobial, antibacterial and antifungal [1-3]. Researchers have also found that certain trisubstituted purine derivatives has shown anti-tumor and anti-viral (particularly Anti- AIDS) activity [4-8]. Apart from the pharmacological and biological activity, the purine derivatives have also part of nucleosides and nucleotides they act as hormones and neurotransmitters and are present in some co-enzymes [4-7].

Recently numbers of new purine derivatives are reported [4-7], but the area in which the condensation of amino acid with purine derivatives has not been reported so far. Hence, it was thought to explore the substituted purine-amino acid condensed heterocyclic.The reactions described in this paper have remarkable utility and valuable addition to the synthesis and manipulation of purine derivatives. The newly synthesized compounds were also screened for their antimicrobial, antibacterial and antifungal activities against a panel of gram-positive and gram-negative strains of bacteria and selected fungal strains.

MATERIALS AND METHODS

Experimental

Melting points were determined on Vigo melting point apparatus and are uncorrected. All the compounds were routinely checked for their homogeneity by TLC on silica gel plate, IR spectra were recorded in KBr pellets on Perkin-Elmer FT-IR spectrophotometer , ^{1}H NMR spectra were recorded on BRUKER spectrometer on 300 MHz in $CDCl_3$ using TMS as an internal standard and satisfactory C,H,N analysis were obtained for all the compounds. The mass spectra were recorded on FAB mass spectrometer to confirm their structure.

Antibacterial and anti-fungal activity (anti-microbial activity) was carried out by Agar cup method. The bacterial strains are identified strains and obtained from National chemical Laboratory (NCL), Pune, India.

Synthesis of 9-benzyl-6-chloro-9H-purin-2-yl amine [1]:

A mixture of 2-amino-6-chloro purine (1.0 mmol) and benzyl chloride (2.0 mmol), and potassium carbonate (2.5 mmol) were heated with stirring in 10 volume of acetonitrile at reflux temperature for 15-20 hours. Reaction was monitored on TLC ($CHCl_3$: MeOH, 9:1). Reaction mass cooled to room temperature and solid was filtered off, washed with acetonitrile. Filtrate was concentrated in vacuum to obtain crude product. It was further purified by crystallizing in methanol [8-12].

Yield 65%, Colour: Light yellow solid, m. p. 174-76^{o}C, Molecular Formula: $C_{12}H_{10}N_5Cl$, Molecular Weight: 259.06,

Calculated: C, 55.50; H, 3.88 ; N, 26.97; Cl,13.65
Found : C**,** 55.50; H, 3.82; N, 26.85; Cl, 13.54**,**
IR (KBr) ν max cm^{-1}: 3302.38, 3181.17 [N-H Primary amine stretching], 3077.83[=C-H Aromatic stretching] 3027.18 [C-H Alkyl stretching], 2964.60 [C-H Alkyl stretching], 1632.61 [N-H amine deformation],
^{1}H NMR: [δ $CDCl_3$]: 7.356-7.242 (multiplet, aromatic), 5.288 (CH_2 linkage), 8.232 (-CH of purine),

Synthesis of 9-benzyl-6-tert-butyl amine-9H-purine-2-amine [2]:

A mixture of 9-benzyl-6-chloro-9H-purin-2-yl amine **[1]** (1.0 mmol) and t-Butyl amine (1.5 mmol), and potassium carbonate (2.5 mmol) were heated with stirring in 10 vol of n-butanol (3 hrs, 110°C). Reaction was monitored on TLC ($CHCl_3$: MeOH, 9:1). After completion, cool to

room temperature and the solid was filtered off and washed with methanol. Filtrate was concentrated in vacuum to obtained sticky solid was triturated with hexane and filter the solid to give desire pure product [13-19].

Yield 77%, Colour: Off white solid, m.p188-90^{o}C, Molecular Formula: $C_{16}H_{20}N_6$,
Molecular Weight: 296.37
Calculated : C, 64.84 ; H, 6.80 ; N, 28.36;
Found : C**,** 64.80; H, 6.71; N, 28.31,
IR (KBr) ν max cm^{-1}: 3493.65 [N-H Primary amine stretching], 3302.67 [N-H Secondary amine stretching], 3189.90 [=C-H Aromatic stretching], 3080.70 [C-H Alkyl stretching], 1629.48 [N-H amine deformation],
^{1}H NMR: [δ CDCl$_3$]: 7.361-7.216 (multiplet, aromatic), 5.194 (CH_2 linkage), 7.806 (CH of purine), 1.10 (CH_3 of t-butyl chain), 5.83 (Aromatic NH).

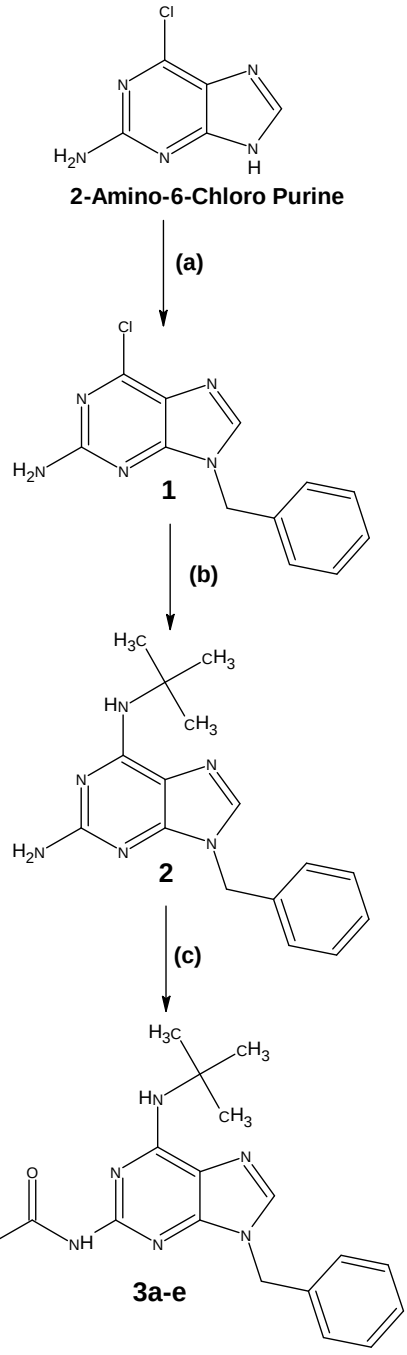
General procedure for the synthesis of 2, 6, 9-tri substituted novel derivatives of purine [3a-e]
A mixture of N-Boc Protected amino acid (0.1 mole) [20-24] and 9-benzyl-6-tert butyl amine-9H-purine-2-amine [**2**] (0.1 mole) were dissolved in anhydrous pyridine (10 volume). The solution was cooled to -10 to -15°C and phosphorous oxychloride (0.116 moles) was added [25-29]. Solution was stirred until completion. The reaction was quenched in ice water; product was extracted with ethyl acetate. Organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography to lead to the desired pure compound (3a-e). Yield and physical data (^{1}H NMR, ^{13}C NMR, and Elemental analysis) are given below

MolecularWeight: 453.54,
Calculated: C**,** 66.28; H, 6.86; N, 18.03
Found : C**,** 66.26; H, 6.82; N, 18.01.
IR (KBr) ν max cm^{-1}:- 3268.63 (N-H stretching >CONH group), 2974.40 (=C-H stretching aromatic), 1698.96 (>C=0 stretching of >CONH group), 1620.99 (N-H deformation of secondary amine), 1454.90 (C-H deformation of Aromatic group), 1390.73 (C-H deformation of Alkyl group)

^{1}H NMR: [δ CDCl$_3$]: 7.3-7.1 (multiplet, aromatic), 4.5 (CH_2 linkage), 8.01(CH of purine), 1.1 (- CH_3 of tert butyl), 3.85 (-CH_2 of amino acid), 1.44 (H of Boc)

^{13}C NMR (δ, CDCl$_3$): 147.9 (C_8 of Purine), 125-138 (Benzene), 58 (CH_2 of benzyl carbon), 31.0 (CH_3 of tert butyl),50.1 (-C of tert butyl), 154.(C4 of purine), 128.4 (C_5 of purine), 168 (C=O), 28.7 (CH_3 of boc), 48.2 (CH_2 of amino acid)

Scheme



Scheme 1:

a. $PhCH_2Cl$ / K_2CO_3 /Acetonitrile / Reflux,
b. Tert. Butyl amine / K_2CO_3 / n-BuOH/ Reflux,
c. N-Boc amino acid / $POCl_3$ / Pyridine / -15°C to RT

R = $-CH_3$, $-CH_2CH_3$, - CH $(CH_3)_2$, $-CH_2CH$ $(CH_3)_2$, $-CH_2Ph$

[(9-benzyl-6-tert-butylamino-9H-purin-2-ylcarbamoyl)-methyl]-carbamic acid tert-butyl ester (3a):
Yield: 64%, Color: Light brown solid, m.p. 175-76^{o}C, Molecular Formula: $C_{23}H_{31}N_7O_3$,

[1-(9-benzyl-6-tert-butylamino-9H-purin-2-ylcarbamoyl)-ethyl] -carbamic acid tert-butyl ester (3b)
Yield: 66%, Color: Light cream solid, m.p.: 177-78^{o}C, Molecular Formula: $C_{24}H_{33}N_7O_3$,
Molecular Weight: 467.26.

Calculated: C**,** 66.28; H, 6.86; N, 18.03:
Found : C, 66.26; H, 6.82; N, 18.01.
IR (KBr) Vmax cm^{-1}:- 3342.22 (N-H Stretching secondary amine), 2959.65 (C-H stretching Aromatic), 2932.36 (=C-H stretching Aromatic), 1671.43 (>C=O stretching >CONH group), 1621.63 (N-H deformation secondary amine), 1456.79 (C-H deformation of Aromatic), 1385.48 (C-H deformation of Alkyl)
^{1}H NMR: [δ $CDCl_3$]: 7.3-7.1 (multiplet, aromatic), 4.9 (CH_2 linkage), 8.01(CH of purine), 1.1 (- CH_3 of tert butyl), 4.71 (-CH of amino acid), 1.48 (-CH_3 of amino acid), 1.44 (H of Boc)
^{13}C NMR (δ, $CDCl_3$): 147.9 (C_8 of Purine), 125-138 (Benzene), 58 (CH_2 of benzyl carbon), 31.0 (CH_3 of tert butyl),50.1 (-C of tert butyl), 154.(C_4 of purine), 128.4 (C_5 of purine), 168 (C=O), 28.7 (CH_3 of boc), 53.6 (CH of amino acid), 30.9 (CH_3 of amino acid)

[1-(9-benzyl-6-tert-butylamino-9H-purin-2-ylcarbamoyl)-2-methyl propyl] -carbamic acid tert-butyl ester (3c)
Yield: 68%, Color: Cremish solid, m.p.: 177-78C; Molecular Formula: $C_{26}H_{37}N_7O_3$,
Molecular Weight: 495.30,

Calculated: C**,** 63.01; H, 7.52, N, 19.78
Found : C, 63.03; H, 7.48; N, 19.71
IR (KBr) Vmax cm^{-1}: 3419.79 (N-H Stretching secondary amine), 2933.32 (=C-H stretching Aromati), 2854.74 (C-H Stretching Alkyl group), 1694.24 (>C=O stretching CONH group), 1593.88 (N-H deformation secondary amine), 1456.25 (C-H deformation of Aromatic), 1367.67 (C-H deformation of Alkyl)
^{1}H NMR: [δ $CDCl_3$]: 7.3-7.1 (multiplet, aromatic), 5.01 (CH_2 linkage), 8.05(CH of purine), 1.00-1.40 (H of CH_3), 4.5 (H of CH), 2.68 (H of CH_2)

[1-(9-benzyl-6-tert-butylamino-9H-purin-2-ylcarbamoyl)-3-methyl butyl] -carbamic acid tert-butyl ester] (3d)
Yield: 65%, Color: Light yellow, m.p.: 177-79^{o}C, Molecular Formula: $C_{27}H_{39}N_7O_3$,
Mol. Weight: 509.31,

Calculated: C**,** 66.28; H, 6.86; N, 18.03
Found : C, 66.26; H, 6.82; N, 18.01.
IR (KBr) Vmax cm^{-} 3306.99 (N-H stretching >CONH group), 2957.92 (C-H stretching secondary aromatic), 2871.49 (C-H stretching Alkyl), 1695.12 (>C=O stretching Boc protected

amine), 1621.91 (>C=O stretching >CONH group), 1384.69 (C-N stretching secondary aromatic amine), 1469.81 (C-H deformation of Alkyl group)
^{1}H NMR: [δ $CDCl_3$]: 7.3-7.1 (multiplet, aromatic), 4.9 (CH_2 linkage), 7.968 (CH of purine), 1.00-1.40 (H of CH_3), 4.53 (H of CH), 1.8 (H of CH_2)

[1-(9-benzyl-6-tert-butylamino-9H-purin-2-ylcarbamoyl)-2-phenyl ethyl] -carbamic acid tert-butyl ester (3e)
Yield: 74%, Color: Light cream solid, m.p. 178-80^{o}C, Molecular Formula: $C_{30}H_{37}N_7O_3$, Molecular Weight: 543.30,

Calculated: C, 66.28; H, 6.86; N, 18.03
Found : C, 66.26; H, 6.82; N, 18.01.
IR (KBr) Vmax cm^{-1} 3493.65 (N-H Stretching Secondary amine), 3306.99 (N-H stretching >CONH group), 3189.90 (=C-H stretching aromatic), 3080.70 (C-H stretching Alkyl), 1629.48 (>C=O stretching >CONH group), 1568.46 (N-H deformation of secondary amine), 1496.02 (C-H deformation of aromatic), 1464.48 (C-H deformation of Alkyl group)
^{1}H NMR: [δ $CDCl_3$]: 7.354-7.155 (multiplet, aromatic), 4.49 (CH_2 linkage), 3.737 (CH_2 linkage Amino acid), 8.014 (CH of purine), 1.474 (CH_3 of Boc), 1.2 (CH_3 of tert Butyl), 5.334 (H of CH),
^{13}C NMR (δ, $CDCl_3$): 128-152 (C of Purine), 125-130 (>CH of Benzene), 58 (CH_2 of purine benzyl carbon), 38 (CH_2 of benzyl carbon of amino acid), 31 (CH_3 of tert butyl), 28 (CH_3 of BOC), 158 (C=O of BOC), 172 (C=O of amino acid), 70 (C of BOC), 50 (C of tert butyl)

Biological Evaluation
Anti-bacterial activity of 3a-e:
The study has been conducted according to the method adopted by Cruickshank et al [5]**.** Nutrient agar broth was melted in a water bath and cooked to 45 °C with gentle shaking to bring about uniform cooling. It was inoculated with 0.5-0.6 ml of 24 hour old culture especially and mixed well by gentle shaking before pouring on the sterilized Petri dish (25 ml each). The poured material was allowed to set (1.5 hour) and there after the “cups” was made by punching into the agar surface with a sterile cork borer and soaping out the punched part of agar. Into this “cups” 0.1 ml of test solution (prepared by dissolving 100 ml of sample in 10 ml DMF) was added by sterile micropipette. The plates were noted. The antibacterial activities of all compounds are compared against Ampicilin as a standard drug.

Antifungal activity of 3a-e:
The fungicidal activity of all the compounds was studied at 1000 ppm concentration in vitro. Plant pathogenic organisms used were **C. albicans** and **A. clavatus**. The antifungal activity of all the compounds was measured on each of these plant pathogenic strains on a potato dextrose agar (PDA) medium such a PDA medium contained potato 200 gm., dextrose 20 gm., agar 20 gm., and water 1 liter. Five days old cultures were employed. The compounds to be tested were suspended (1000 ppm) in a PDA medium and autoclaved at 120 ^{0}C for 15 min and at 15 atm. pressure. These media were poured into sterile Petri plates and the organisms were inoculated after cooling the Petri plates. The percentage inhibition for fungi was calculated after five days using the formula given below.

$$\text{Percentage of inhibition} = \frac{100\ (X - Y)}{X}$$

Where, X = Area of colony in control plate.
Y = Area of colony in test plate.

Table-.1: Antibacterial activity of compounds 3a-e.

Compound No.	Zone of inhibition (in mm)			
	Gram +ve		Gram -ve	
	S. aureus	*S. pyrogenes*	*E. coli*	*P. aeruginosa*
3a	08	11	10	09
3b	11	11	11	11
3c	12	13	10	12
3d	11	11	11	11
3e	13	14	12	14
Amplicilline	10	13	11	14
Chloramphenicol	12	14	10	13
Ciprofloxacin	17	19	16	19
Norfloxacin	19	22	18	19

Table -2: Antifungal activity of compounds 3a-e

Compound No.	*C. albicans*	*A. clavatus*
3a	17	17
3b	19	14
3c	17	17
3d	21	20
3e	23	22
Greseofulvin	18	21
Nystatin	18	21

RESULTS AND DISCUSSION

New trisubstituted purines have been synthesized by the reaction of -benzyl-6-tert-butyl amine-9H-purine-2-amine [2]. With various Boc-protected amino acid in 65 to 75% yield. All the trisubstituted Purine have low melting point i.e. below 200°C. The structures of compounds are confirmed by IR, NMR and Mass Spectral data and future supported by correct elemental analysis. Newly synthesized compounds 3a-e was shown significant microbial activities.

Table 1 of antibacterial activity show that the compound 3a,3c and 3e have more active in S. pyrogenes compare to S. aureus in Gram +ve while 3a more active in E-coli compare to P. aeruginosa and 3c and 3e are more active in P. aeruginosa compare to E-coli in Gram –ve. 3b and 3d having same activity in Gram +ve as well as in Gram –ve. Table 2 of Antifungal activity show that the compound 3a,3c and 3e have more active in C.albicans compare to A.clavatus while 3b and 3d having same activity in both.

CONCLUSION

Newly synthesized trisubstituted purines 3a-e have been tested for their anti bacterial activity against gram positive bacteria S. aureus and S. pyrogenes while gram negative bacteria E. coli and P. aeruginosa. By punching into the agar surface with a sterile cork borer and soaping out the punched part of agar. Into this "cups" 0.1 ml of test solution, prepared by dissolving 100 ml of sample in 10 ml DMF. Amplicilline, Chloramphenicol, Ciprofloxacin Norfloxacin Greseofulvin and Nystatin were used as a reference compound. **3a** shown moderate activity against gram positive and gram negative bacteria compared to **3b, 3c, 3d** and **3e** were shown good activity against gram positive and gram negative bacteria. Same compounds were tested for their anti fungal activity against A. clavatus and C. albicans using cup-plate method. The compound 3a, 3b and 3c show moderate activity while 3d and 3e show good anti fungal activity

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