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An Efficient Synthesis of Trisubstituted Purine via Unusual Coupling of N-Phthaloyl Derivatives of Amino Acids

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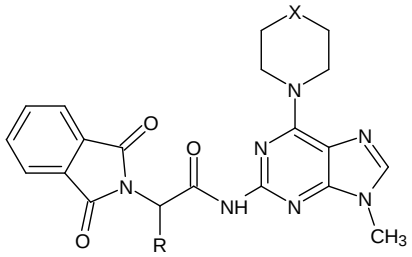
Abstract: An efficient and simple method for synthesis of 2-amide derivative of purine by coupling of N-phthaloyl derivative of amino acids with 2, 6-diamino-9-methylpurine using phosphorous oxychloride in moderate to good yield has been reported. The synthesized compounds were characterized using IR, NMR, mass spectroscopy and screened for their *in vitro* antimicrobial activity against *S. aureus, E. coli, P. aeruginosa* S. *typhimurium, F. oxysporum and A. alternata*. Synthesized compounds shows moderate antimicrobial activity against all the microorganisms
Keywords: Trisubstituted purine, N-phthaloyl amino acids, antimicrobial activity, phosphorous oxychloride.

Introduction

Purine bearing substituent at its 2, 6 and 9-position is of great interest due to potential application in medicinal chemistry. 2, 6, 9-substituted purine derivative act as CDK (cell cycle dependent kinase) inhibitor viz. Olomoucine, Roscovitine, Bohemine, Purvalanol[1-4]. Myoseverin a trisubstituted purine derivative reported as inhibitors of microtubule assembly[5]. Several examples of this class of compounds were reported to possess activity having inhibitors of Src tyrosine kinase[6], potent Hsp90 (heat shock protein 90) inhibitor[7], potent Stat3-(signal transducer and activator of transcription) binding inhibitor[8], inhibitors of p38a MAP kinase (P38 mitogen-activated protein)[9], antiviral[10], antitumor[11], sulfotransferase[12], phosphodiesterase[13], adenosine receptor antagonists[14]. They are also use for treatment of autoimmune diseases[15] and modulators of multidrug resistance[16].

Formation of an amide bond with biologically active substituted heterocycles such as purine derivatives leads to compound having broad biomedical value as therapeutics and also 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 derivatives coupled with amino acid derivatives **(Fig.1, Scheme 2, 7a-d)**, characterized using IR, ^{1}H, ^{13}C –NMR, mass analysis and screened for their *in vitro* antimicrobial activity.



X=-CH_2, or –O; R = -H, -CH_3, -CH (CH_3)$_2$, -CH_2CH (CH_3)$_2$.

Fig. I Structure of trisubstituted purine coupled with N-phthaloyl derivative amino acids 6a-h.

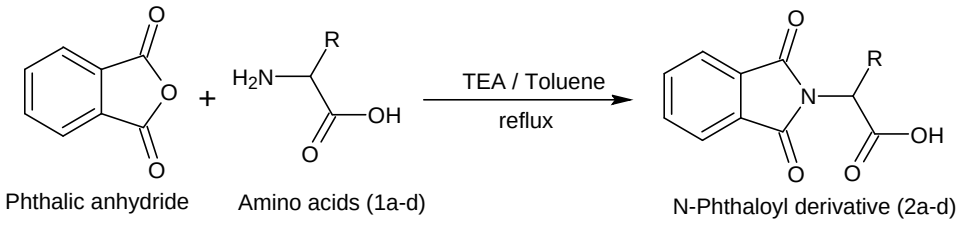
Experimental

All chemicals were purchased from commercial suppliers and used without further purification. Melting points were determined using a Veego VMP-PM melting point apparatus and are uncorrected. MS spectra were recorded on Waters Q-TOF instrument in only positive ion detection mode. ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker Avance II 500 (500MHz) NMR instrument, using either $CDCl_3$ or DMSO-d6 as a solvent and TMS as internal reference. Chemical shifts were expressed in δ values (ppm). IR spectra were recorded on Perkin Elmer spectrum 100 FT-IR spectrometer. 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 dichloromethane-methanol (9:1) solvent system and the spots were visualized under UV illumination.

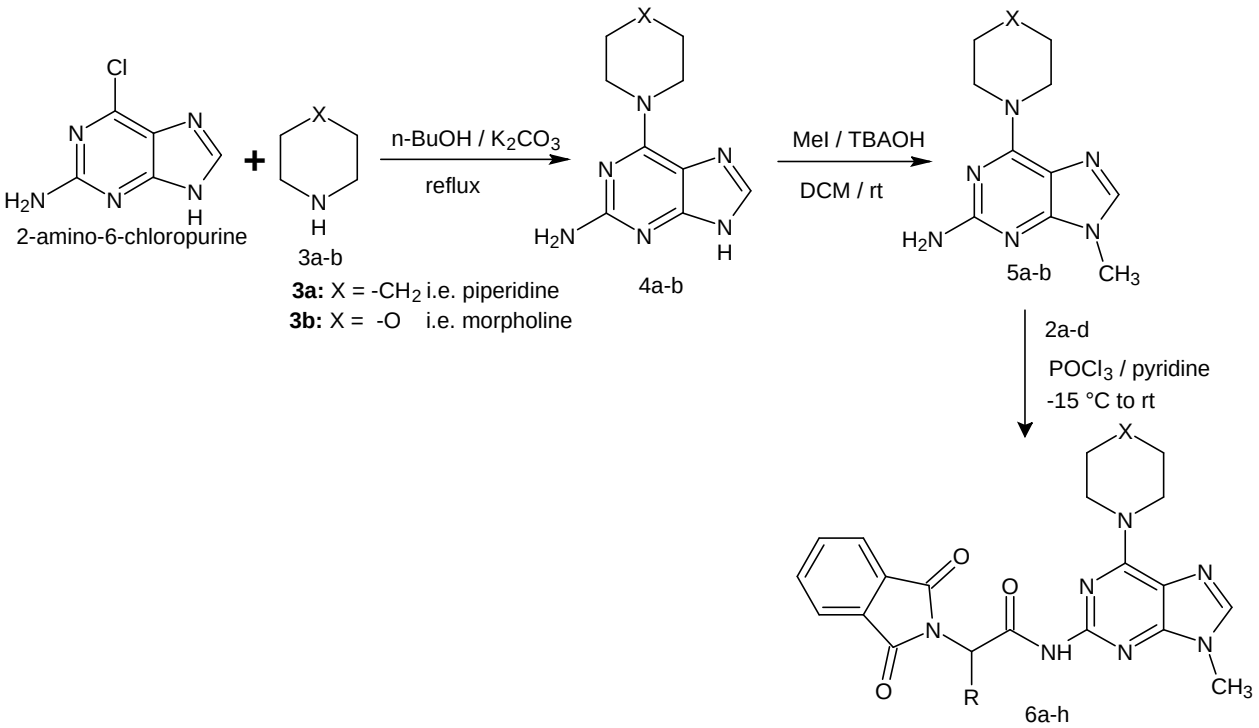
Antimicrobial Assay

Muller Hinton Agar (MHA) medium was used for growing bacterial strains. 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 bacterial 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 antibacterial 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 prepared in DMSO (0.05g in 5ml DMSO) and labeled accordingly.

After sampling, plates were incubated and after 24 hours these plates were studied for zone of inhibition.



Scheme 1: Synthesis of N-phthaloyl derivatives of amino acid 2a-d



Scheme 2: Synthesis of trisubstituted purine coupled with N-phthaloyl derivative of amino acids (6a-h).

General procedure for the synthesis of N-phthaloyl derivatives of amino acid (2a-d):-

In RBF fitted with Dean-stark apparatus and a reflux condenser, phthalic acid anhydride (1.48 g, 10 mmol) and amino acids **(1a-d)** (10 mmol) were refluxed in toluene in the presence of 0.1 ml triethylamine for 3 h. The organic solvents were removed under reduced pressure to get sticky oily mass. Water was added to oily mass, acidified with hydrochloric acid and stirred for 30 minutes to get solid. Solid was filtered off, washed with water and dried to get N-phthaloyl derivatives **2a-d**.

Physical characteristic data of the synthesized compounds are summarized in Table-1.

Table I: Physical parameters of N-phthaloyl derivatives of amino acids (2a-d)

Sr. No	Product code	R	MP (^{0}C)	MF	MW	Yield (%)
1	2a	-H	190	$C_{10}H_7NO_4$	205	95
2	2b	-CH_3	136	$C_{11}H_9NO_4$	219	91
3	2c	-$CH(CH_3)_2$	110	$C_{13}H_{13}NO_4$	247	91
4	2d	-$CH_2CH(CH_3)_2$	143	$C_{14}H_{12}NO_4$	261	92

***Synthesis of 2, 6-diamino-9H-purine* (4a-b):-**

2-ACP (10 mmol), piperidine/ morpholine **3a-b** (15 mmol) and K_2CO_3 (20 mmol) were heated in 30 ml n-BuOH at reflux temperature for 5-6 h. Reaction mass was filtered off and solvent was removed under reduced pressure. Sticky solid obtained was dissolved in ethyl acetate and washed with water. Solvent was removed under reduced pressure to get desired product **4a-b**. (Scheme 2)

***Synthesis of 2, 6-diamino9-methyl purine* (5a-b):-**

2, 6-diamino-9H-purine **4a-b** (10 mmol) and methyl iodide (20 mmol) were dissolved in 50 ml DCM. 40% TBAOH (10 ml) was added and reaction mass was stirred for 1 h. Organic layer was separated out, washed with water and solvent was removed under reduced pressure to get desired product **5a-b**. (Scheme 2)

***Synthesis of trisubstituted purine* (6a-h):-**

N-phthaloyl amino acid derivatives (**2a-d)** (10 mmol) and 2, *6-diamino9-methyl purine* **5a-b (**10 mmol) were dissolved in anhydrous pyridine. The solution was cooled to −15 ℃ and $POCl_3$ (11 mmol) was added drop wise under vigorous stirring. The reaction mixture then was stirred at −15 ℃ for 30 minutes. The solution was allowed to warm to room temperature and then stirred for 10-12 h at same temperature. The reaction was quenched by addition of crushed ice/water. The desired compound was extracted using ethyl acetate. The combined organic layers were dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. The crude material was further purified by column chromatography to obtain the desired trisubstituted purine **6a-h** (Scheme 2).

2-(1,3-Dioxo-13-dihydro-isoindol-2-yl)-N-(9-methyl-6-piperidin-1-yl-9H-purine-2yl)-acetamide **6a:** yield: 61 %; off white solid ; mp: 108-110 ^oC; MF: $C_{21}H_{21}N_7O_3$; MW: 419.43; IR (KBr, cm^{-1}): 3456 (N-H), 2921 (C-H), 1705, 1691 (C=O), 1615 (C=N), 1570, 1456 (C=C), 1334 (C–N); MS (*m*/*z*): $[MH]^+$ 420.77 ; ^1H NMR ($CDCl_3$, 500MHz): δ = 8.11 (s, 1H, 8CH), 7.89-7.86 (dd, 2H, Ar-CH), 7.76-7.75 (dd , 2H, Ar-CH), 7.61 (s, 1H, -CONH), 4.72 (s, 2H, $-CH_2$, αH), 4.15 (br, 4H, $-NCH_2$), 3.77 (s, 3H, $-9NCH_3$), 1.72-1.67 (m, 6H, $-CH_2$) ; ^{13}C NMR ($CDCl_3$, 125MHz): δ = 168.17 (s, >N-C=O), 153.76 (s, C_6), 151.98-151.79 (d, C_2 & C_4), 138.24 (s, C_8), 134.21 (d, Ar-CH, C_{22} & C_{23}) , 131.79 (d, Ar-C, C_{20} & C_{25}), 123.56 (d, Ar-CH, C_{21} & C_{24}), 116.92 (s, C_5), 45.73 (d, $–NCH_2$, C_{10} & C_{14}), 45.22 (s, $-CH_2$, αC_{17}), 29.85 (s, $-9NCH_3$), 25.8 (s, $-CH_2$, C_{12}), 24.51 (d, $-CH_2$, C_{11} & C_{13}).

2-(1,3-Dioxo-13-dihydro-isoindol-2-yl)-N-(9-methyl-6-piperidin-1-yl-9H-purine-2yl)-propionamide **6b:**

yield: 58 %; off white solid ; mp: 135-137 ^oC; MF: $C_{22}H_{23}N_7O_3$; MW: 433.46; IR (KBr, cm^{-1}): 3462 (N-H), 2941 (C-H), 1711, 1682 (C=O), 1627 (C=N), 1568, 1461 (C=C), 1335 (C–N); MS (*m*/*z*): $[MH]^+$ 434.20 ; ^1H NMR ($CDCl_3$, 500MHz): δ = 8.09 (s, 1H), 7.81-7.79 (dd, 2H, Ar-CH), 7.70-7.68 (dd , 2H, Ar-CH), 7.60 (s, 1H, -CONH), 4.88 (q, 1H, $-CH$, αH), 4.19 (br, 4H, $-NCH_2$), 3.75 (s, 3H, $-9NCH_3$), 1.69-1.58 (m, 9H, $-CH_2$ & $-NCH_3$). ; ^{13}C NMR ($CDCl_3$, 125MHz): δ = 168.18 (s, >N-C=O), 153.48 (s, C_6), 152.00-151.71 (d, C_4 & C_2), 138.26 (s, C_8), 134.25 (d, Ar-CH, C_{22} & C_{23}) , 131.66 (d, Ar-C, C_{20} & C_{25}), 123.7 (d, Ar-CH, C_{21} & C_{24}), 117.04 (s, C_5), 53.66 (s, -CH, αC_{17}), 45.9 (d, $–NCH_2$, C_{10} & C_{14}), 29.89 (s, $-9NCH_3$), 25.9 (s, $-CH_2$, C_{12}), 24.6 (d, $-CH_2$, C_{11} & C_{13}), 14.46 (s, $-CH_3$, C_{27})

2-(1,3-Dioxo-13-dihydro-isoindol-2-yl)-3-methyl-N-(9-methyl-6-piperidin-1-yl-9H-purine-2yl)-butyramide **6c:**

yield: 49 %; off white solid ; mp: 127-129 ^oC; MF: $C_{24}H_{27}N_7O_3$; MW: 461.51; 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*): $[MH]^+$ 462.62 ; ^1H NMR ($CDCl_3$, 500MHz): δ = 8.11 (s, 1H), 7.88-7.86 (dd, 2H, Ar-CH), 7.77-7.74 (dd , 2H, Ar-CH), 7.61 (s, 1H, -CONH), 4.84 (d, 1H, $-CH$, αH), 4.13 (br, 4H, $-NCH_2$), 3.76 (s, 3H, $-9NCH_3$), 2.78-2.74 (m, 1H, -CH), 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), 153.82 (s, C_6), 153.76-151.98 (d, C_2 & C_4), 138.24 (s, C_8), 134.81 (d, Ar-CH, C_{22} & C_{23}) , 131.72 (d, Ar-C, C_{20} & C_{25}), 123.61 (d, Ar-CH, C_{21} & C_{24}), 117.04 (s, C_5), 52.3 (s, -CH, αC_{17}), 45.5 (d, $–NCH_2$, C_{10} & C_{14}), 29.77 (s, $-9NCH_3$), 25.9 (s, $-CH_2$, C_{12}), 25.36 (s, -CH, C_{27}), 24.61 (d, $-CH_2$, C_{11} & C_{13}), 16.21 (d, $-CH_3$, C_{28} & C_{29})

2-(1,3-Dioxo-13-dihydro-isoindol-2-yl)-4-methyl-N-(9-methyl-6-piperidin-1-yl-9H-purine-2yl)-pentanoic acid amide **6d:**

yield: 59 %; off white solid ; mp: 159-161 ^oC; MF: $C_{25}H_{29}N_7O_3$; MW: 475.54; 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*): $[MH]^+$ 476.90 ; ^1H NMR ($CDCl_3$, 500MHz): δ = 8.11 (s, 1H), 7.93-7.91 (dd, 2H, Ar-CH), 7.90-7.87 (dd , 2H, Ar-CH), 7.60 (s, 1H, -CONH), 5.10 (d, 1H, $-CH$, αH), 4.19 (br, 4H, $-NCH_2$), 3.77 (s, 3H, $-9NCH_3$), 2.34 (m, 1H, -CH), 1.75-1.68 (m, 8H, $-CH_2$), 1.5 (d, 6H, $-CH_3$); ^{13}C NMR ($CDCl_3$, 125MHz): δ = 168.21 (s, >N-C=O), 153.82 (s, C_6), 152.21-151.74 (d, C_2 & C_4), 138.24 (s, C_8), 134.21 (d, Ar-CH, C_{22} & C_{23}) , 131.79 (d, Ar-C, C_{20} & C_{25}), 123.56 (d, Ar-CH, C_{21} & C_{24}), 117.04 (s, C_5), 52.18 (s, -CH, αC_{17}), 45.5 (d, $–NCH_2$, C_{10} & C_{14}), 30.1 (s, $-CH_2$, C_{27}),

29.75 (s, -9NCH_3), 25.7 (s, -CH_2, C_{12}), 25.28 (s, -CH, C_{28}), 24.61 (d, -CH_2, C_{11} & C_{13}), 19.33 (d, -CH_3, C_{29} & C_{30})

2-(1, 3-Dioxo-13-dihydro-isoindol-2-yl)-N-(9-methyl-6- morpholin-4-yl-9H-purine-2yl)-acetamide **6e:**

yield: 50 %; off white solid ; mp: 120-122 ^oC; MF: $C_{20}H_{19}N_7O_4$; MW: 421.40; 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*): $[MH]^+$ 422.63 ; ^1H NMR ($CDCl_3$, 500MHz): δ = 8.10 (s, 1H), 7.88-7.85 (dd, 2H, Ar-CH), 7.75-7.72 (dd , 2H, Ar-CH), 7.60 (s, 1H, -CONH), 4.78 (s, 2H, -CH_2, αH), 4.28 (br, 4H, -OCH_2), 3.83-3.77 (m, 4H, -NCH_2), 3.72 (s, 3H, -9NCH_3) ; ^{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), 134.99 (d, Ar-CH, C_{22} & C_{23}) , 131.72 (d, Ar-C, C_{20} & C_{25}), 123.58 (d, Ar-CH, C_{21} & C_{24}), 117.04 (s, C_5), 66.64 (d, -CH_2, C_{11} & C_{13}), 45.71 (d, –NCH_2, C_{10} & C_{14}), 45.11 (s, -CH_2, αC_{17}), 29.85 (s, -9NCH_3).

2-(1,3-Dioxo-13-dihydro-isoindol-2-yl)-N-(9-methyl-6- morpholin-4-yl-9H-purine-2yl)-propionamide **6f:**

yield: 63 %; off white solid ; mp: 143-145 ^oC; MF: $C_{21}H_{21}N_7O_4$; MW: 435.43; IR (KBr, cm^{-1}): 3455 (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 ; ^1H NMR ($CDCl_3$, 500MHz): δ = 8.03 (s, 1H), 7.79-7.77 (dd, 2H, Ar-CH), 7.69-7.67 (dd , 2H, Ar-CH), 7.61 (s, 1H, -CONH), 4.82 (q, 1H, -CH, αH), 4.16 (br, 4H, -OCH_2), 3.68-3.60 (m, 4H, -NCH_2), 3.58 (s, 3H, -9NCH_3), 1.65 (d, 3H,-CH_3); ^{13}C NMR ($CDCl_3$, 125MHz): δ = 168.11 (s, >N-C=O), 153.82 (s, C_6), 152.21-151.74 (d, C_4 & C_5), 138.81 (s, C_8), 134.30 (d, Ar-CH, C_{22} & C_{23}) , 131.72 (d, Ar-C, C_{20} & C_{25}), 123.61 (d, Ar-CH, C_{21} & C_{24}), 117.04 (s, C_5), 66.99 (d, -CH_2, C_{11} & C_{13}), 54.42 (s, -CH, αC_{17}), 45.65 (d, –NCH_2, C_{10} & C_{14}), 29.85 (s, -9NCH_3), 15.46 (s, -CH_3, C_{27})

2-(1,3-Dioxo-13-dihydro-isoindol-2-yl)-3-methyl-N-(9-methyl-6-morpholin-4-yl-9H-purine-2yl)-butyramide **6g:**

yield: 55 %; off white solid ; mp: 105-107 ^oC; MF: $C_{23}H_{25}N_7O_4$; MW: 463.48; IR (KBr, cm^{-1}): 3462 (N-H), 2941 (C-H), 1711, 1682 (C=O), 1627 (C=N), 1568, 1461 (C=C), 1335 (C–N); MS (*m/z*): $[MH]^+$ 464.11 ; ^1H NMR ($CDCl_3$, 500MHz): δ = 8.11 (s, 1H), 7.89-7.87 (dd, 2H, Ar-CH), 7.77-7.75 (dd , 2H, Ar-CH), 7.62 (s, 1H, -CONH), 4.85 (d, 1H, -CH, αH), 4.27 (br, 4H, -OCH_2), 3.83-3.77 (m, 4H, -NCH_2), 3.77 (s, 3H, -9NCH_3), 2.72-2.65 (m, 1H, -CH), 1.16-1.10 (d, 3H,CH_3), 0.93-0.92 (d, 3H, -CH_3); ^{13}C NMR ($CDCl_3$, 125MHz): δ = 168.38 (s, >N-C=O), 153.83 (s, C_1), 152.21-151.73 (d, C_4 & C_5), 138.81 (s, C_8), 133.79 (d, Ar-CH, C_{22} & C_{23}) , 132.21 (d, Ar-C, C_{20} & C_{25}), 123.11 (d, Ar-CH, C_{21} & C_{24}), 116.91 (s, C_5), 68.33 (d, -CH_2, C_{11} & C_{13}), 53.15 (s, -CH, αC_{17}), 48.93 (d, –NCH_2, C_{10} & C_{14}), 29.76 (s, -9NCH_3), 25.51 (s, -CH, C_{27}), 16.51 (d, -CH_3, C_{28} & C_{29})

2-(1,3-Dioxo-13-dihydro-isoindol-2-yl)-4-methyl-N-(9-methyl-6-morpholin-4-yl-9H-purine-2yl)-pentanoic acid amide **6h:**

yield: 39 %; off white solid ; mp: 128-130 ^oC; MF: $C_{24}H_{27}N_7O_4$; MW: 477.51; IR (KBr, cm^{-1}): 3462 (N-H), 2941 (C-H), 1711, 1682 (C=O), 1627 (C=N), 1568, 1461 (C=C), 1335 (C–N); MS (*m/z*): $[MH]^+$ 478.75 ; ^1H NMR ($CDCl_3$, 500MHz): δ = 8.09 (s, 1H), 7.89-7.86 (dd, 2H, Ar-CH), 7.77-7.75 (dd , 2H, Ar-CH), 7.62 (s, 1H, -CONH), 5.05 (d, 1H, -CH, αH), 4.28 (br, 4H, -OCH_2), 3.85-3.79 (m, 4H, -NCH_2), 3.74 (s, 3H, -9NCH_3), 2.32 (m, 1H, -CH), 1.75 (m, 2H, -CH_2), 1.4 (d, 6H, -CH_3), ^{13}C NMR ($CDCl_3$, 125MHz): δ = 168.17 (s, >N-C=O), 153.74 (s, C_1), 152.00-151.71 (d, C_4 & C_5), 138.26 (s, C_8), 134.3 (d, Ar-CH, C_{22} & C_{23}) , 131.72 (d, Ar-C, C_{20} & C_{25}), 123.62 (d, Ar-CH, C_{21} & C_{24}), 116.45 (s, C_5), 67.00 (d, -CH_2, C_{11} & C_{13}), 54.32 (s, -CH, αC_{17}), 46.12 (d, –NCH_2, C_{10} & C_{14}), 29.45 (s, -CH_2, C_{27}), 29.20 (s, -9NCH_3), 25.51 (s, -CH, C_{28}), 19.56 (d, -CH_3, C_{29} & C_{30})

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 II.

Table II. In *vitro* antimicrobial activities of all synthesized compounds **6a-h**

Compound code	Zone of inhibition in mm					
	Bacteria*				Fungi#	
	Gram +ve	Gram -ve				
	S. *aurous*	E. *coli*	P. *aeruginosa*	S. *typhimurium*	*F. oxysporum*	*A. alternata*
6a	8	9	11	8	19	17
6b	7	8	8	8	18	17
6c	9	10	9	9	17	16
6d	9	8	8	8	15	14
6e	10	11	10	8	22	16
6f	10	11	11	9	18	16
6g	12	8	13	11	22	21
6h	9	10	10	10	18	21
Amplicilline	20	11	-	-		
Chloramphenicol	17	20	12	12		
Nystatin	-	-	-	-	70	50

*Less active: 3–7 mm; moderately active: 8–12 mm; highly active: 13–17 mm; –: No inhibition or inhibition less than 5 mm; –: NT: not tested.
Less active: 15–20 mm; moderately active: 20-25 mm; highly active: 25-307 mm; –: NT: not tested

Fig II: In *vitro* antibacterial activities of all synthesized compounds **6a-h**

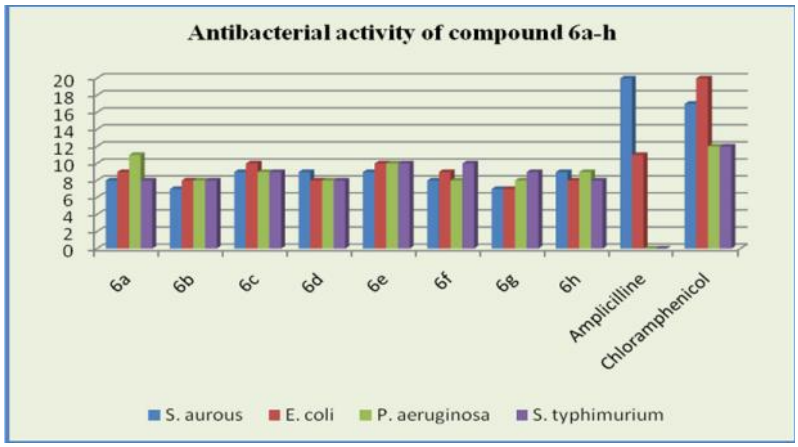


Fig.III In *vitro* antifungal activities of all synthesized compounds **6a-h**

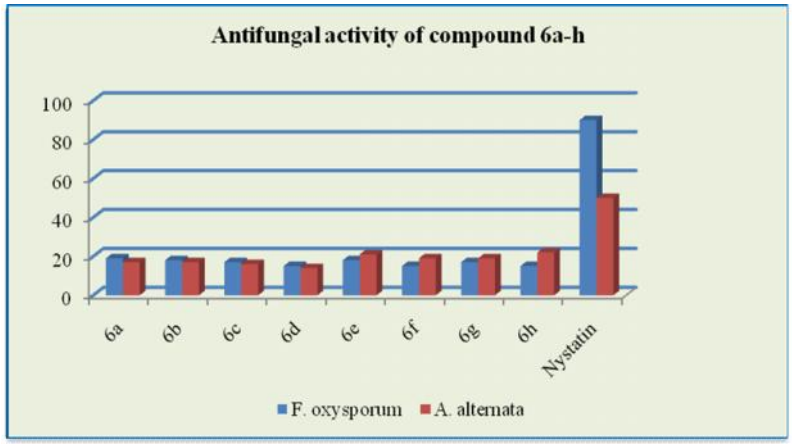
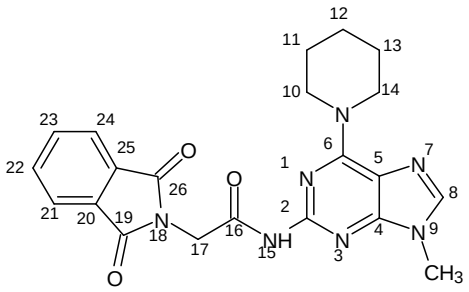
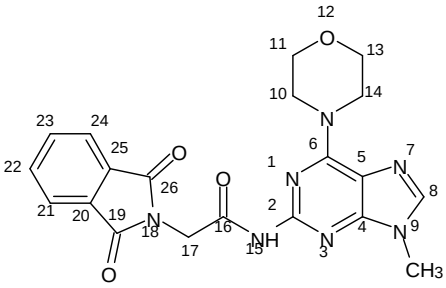


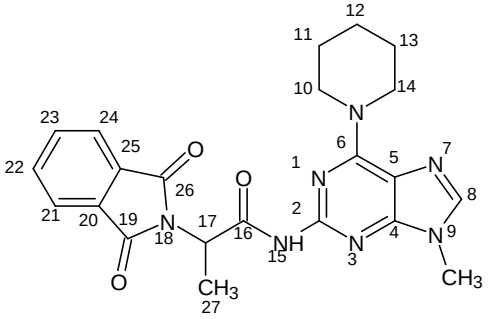
Fig IV: Structure of all synthesized trisubstituted purine 6a-h



6a



6e



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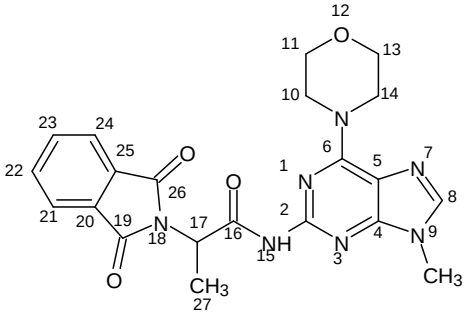

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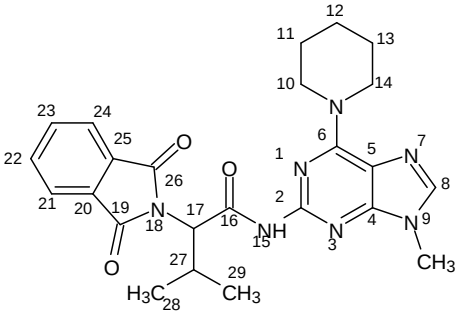

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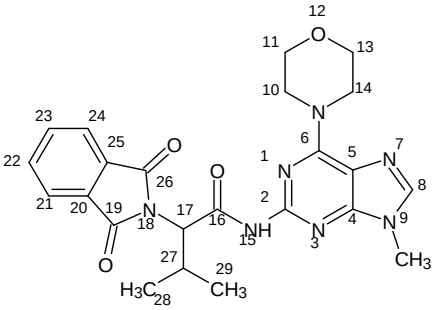

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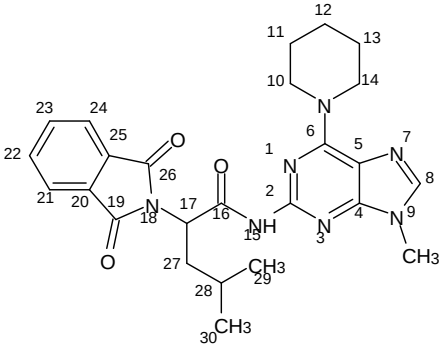

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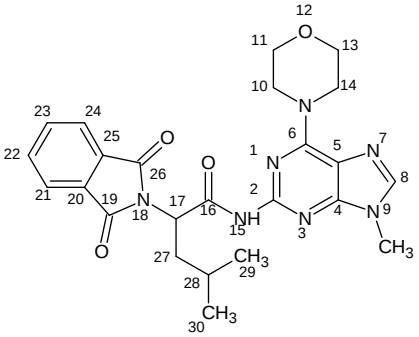

6h

Results and Discussion:

The general strategy for the synthesis of trisubstituted purine using 2-amino-6-chloropurine (2-ACP) is carried out by reaction of any amine at position C6 and then alkylation at 9N-position and finally coupling of amino acid derivatives at most difficult and unreactive site i.e. C2 position.

The amination of 2-amino-6-chloropurine can be achieved by various synthetic technique reported in the literature using solvent like ethanol, n-butanol (n-BuOH)[15], acetonirtile[17], 1,4-Dioxane, dimethylformamide[18] or dimethyl sulphoxide[19] and base like triethylamine, *N*,*N*-dimethyl cyclohexylamine or diisopropylethylamine [20] at higher temperature. 2-6-diamino-9H-purine (4a-b) were synthesized by reaction of 2-ACP with 2° amine viz morpholine/piperidine using potassium carbonate (K_2CO_3) as base and n-BuOH as solvent at reflux temperature[21] followed by N9 methylation using 40% aq. solution of tetrabutylammonium hydroxide (TBAOH) [2] as base in dichloromethane (DCM) to yield 5a-b. For the synthesis of targeted molecule the non-classical coupling system phosphorous oxychloride ($POCl_3$) in pyridine was used[22-23].

We have tried coupling of N-phthaloyl derivatives 2a-d of amino acid with 2, 6-diamino-9-methyl-purine 5a-b in $POCl_3$/pyridine shown in Scheme 2. Synthesis of N-phthaloyl 2a-d was carried out using reported method in literature[24] i.e. reaction of phthalic anhydride and amino acids 1a-d in toluene at reflux temperature in presence of triethylamine shown in scheme 1.

Moreover, the structures of the products were elucidated by MS, ^{1}H-NMR, ^{13}C-NMR and IR spectral analysis. ^{1}H-NMR spectra of all the compounds was quite simple and proton at C8 position of purine of the entire synthesized compound found in the region of 8.05 - 8.11 ppm depending on the substituent. The aromatic protons of phenyl ring appear as a multiplet in the region of 7.89 -7.75 ppm. The C_2 carbon of purine ring appears in the region 153.76-153.83, C_4 & C_6 at 151.61-153.7 C_8 at 136.26-140.0 and C_5 at 116.90-117.08. In IR spectrum C=O stretch appears in the region of 1723-1676 cm^{-1}.

The results obtained in antimicrobial assay are shown in table II. The entire synthesized compound showed moderate antimicrobial activity against all the microorganisms.

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