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SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL GUANINE ANALOGUES

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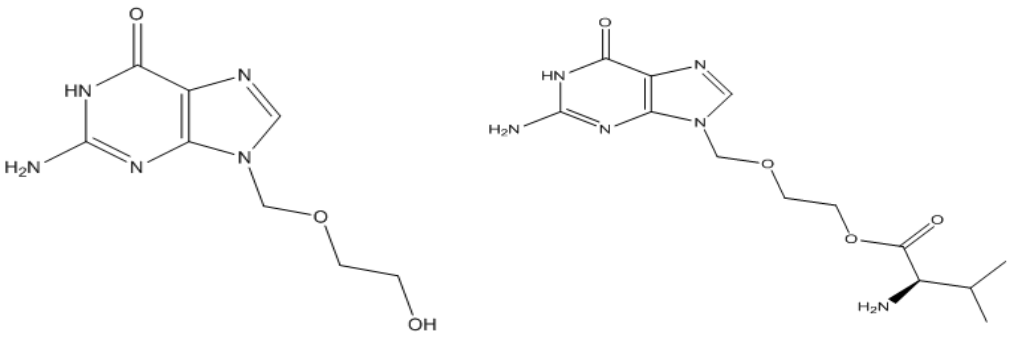
ABSTRACT

The novel substituted and potent guanines were synthesized in high yields. Guanine analogues especially acyclovir and valaciclovir for the treatment of herpes virus infections, have dominated the antiviral therapy for several decades. Valaciclovir is the L-valyl ester prodrug of acyclovir. It is used in the treatment of Herpes simplex virus and Vericella zoster virus. Herpes virus infections, especially those caused by HSV-1 and HSV-2 are the most common viral infections. In the present work, a series of novel substituted benzyl derivatives of Valacyclovir were efficiently synthesized and the structure of the compounds was confirmed by analytical spectral data. The synthesized new compounds were evaluated for their antibacterial, antifungal and antioxidant activities. Analogues synthesized in the present work exhibits strong to moderate activity. The antibacterial activity was compared with control, (gentamycin, fluconazole, and valaciclovir) ***6e*** and ***6f*** showed antibacterial, compound ***6a*** and ***6d*** showed antifungal acivity. The activity of the synthetic compounds is not significant when compared to the reference compounds gentamycin and flucanozole.

KEYWORDS: Acyclovir, Valacyclovir, HSV-1, HSV-2, Vericella zoster.

1. INTRODUCTION

Our research consortium recently became interested in guanine derivatives as a pivotal intermediate, which shares to some extent structural similarities with ACICLOVIR. The guanine ring system is an important structural motif in naturally occurring products as well as in many synthetic compounds of pharmaceutical interest. Among them 9-(2-hydroxyethoxymethyl) guanine analogues have raised considerable attentions since this class of compounds allowed an access to many demonstrated bio-active agents. Fundamental change in the field of nucleic acid chemistry and its structural evaluation during second half of twentieth century initiated not only enormous interest molecular biology and genetics but also the chemistry of nucleic acid components: nucleosides, nucleotides and oligonucleotides. 9-(2-hydroxyethoxymethyl) guanine is an antiviral drug also known as ACICLOVIR (ACV) used in the treatment of HSV-1 and HSV-2. Acyclovir is an acyclic nucleoside analogue of guanosine. It has high lipophilicity and low bioavailability. It has very limited absorption after oral administration (15-20%). This prompted the scientist to look for prodrugs of acyclovir. The possible way to increase the bioavailability is by modifying the known antiviral drug with various amino acids. Valacyclovir is one such prodrug which is derived from acyclovir by esterifying with L-valine. Valcyclovir completely converted into acyclovir upon oral administration. It is used in the treatment of herpes simplex and varicella zoster virus infections. The prodrug increases the oral bioavailability of acyclovir three to five fold. Many aciclovir analogues have been reported and widely investigated for antiviral activity.



(1) **(2)**

Fig. (1): Structure of Acyclovir (1) and Valacyclovir(2).

Considering all these facts, we have been interested in synthesizing other analogues of guanine and to study its biological activities other than antiviral activity. They were synthesized by N-alkylation of L-Valine ester followed by hydrolysis and condensation with acyclovir to study the structure and activity relationship. The analogues were synthesized by general method of N-alkylation by the condensation of substituted benzyl halides. The synthesized analogues were screened for their antibacterial, antifungal and antioxidant activities.

2. EXPERIMENTAL

2.1. MATERIALS AND METHODS

All the chemicals were purchased from Merck. They were used without further purification. Melting points were taken in open capillary tubes and are uncorrected. Reactions were monitored by thin layer chromatography (TLC) using E. Merck precoated silica gel plates (60f-254) with iodine as developing agent. IR spectra in KBr were recorded on Perkin-Elmer model 683 spectrometers. ^{1}H NMR (400MHz) and ^{13}C NMR (100MHz) spectra were recorded using tetra methyl silane (TMS) as an internal reference on Bruker spectrometer. Elemental analyses were performed on a Perkin-Elmer 2400. Mass spectra were obtained by Water-Q-TOF ultima spectrometer.

2.2. Synthesis

2.2.1. General procedure for the synthesis of substituted methyl -2-(benzylamino)-3-methyl butanoate (3a-f)

L-Valine methyl ester hydrochloride (10g, 0.059mol) and potassium carbonate (12.40g, 0.089mol) were taken in N,N-dimethyl formamide (40ml). The reaction mixture was stirred at room temperature for 30min. Benzyl chloride (7.56g. 0.059mol) in N,N-dimethyl formamide (10ml) was added slowly over a period of 15min. The reaction mixture was stirred at room temperature for 10 hr. The reaction completion was monitored by TLC. After completion of reaction, charged water (100ml) and extracted to toluene (2x50ml). Finally washed with water (2x50ml) and distilled solvents under vacuum at 60^{0}C to afford substituted **(3a-f)** in good yield.

Methyl -2-(benzylamino)-3-methyl butanoate **(3a)**: Color: Gummy mass. Yield: 78%. IR (KBr,v,Cm^{-1}): 3400Cm^{-1} (-NH); 1720Cm^{-1} (C=O); 1592Cm^{-1} (aromatic C=C); ^{1}H NMR(CDCl$_3$-400MHz) δ ppm: 0.87-0.98(bs, 6H ,-CH$_3$), 2.0(m, 1H, -CH), 2.67(m, 1H, -CH), 3.44(bs, 1H, -NH), 3.68(s, 3H, -OCH$_3$), 3.82(s, 2H, -CH$_2$), 7.26-7.33(m, 5H, -ArH); ^{13}C NMR (CDCl$_3$-100MHz) δ ppm: 16.2, 32.0, 51.3, 62.0, 127.0-140.20, 175.50. MS (ESI, m/z): 257.76(M^{+}).

Methyl-2-((3-methoxylbenzyl)amino)-3-methyl butanoate **(3b)**: Color: Pasty mass. Yield: 85%. IR (KBr,v,Cm^{-1}): 3400Cm^{-1} (-NH); 1710Cm^{-1} (C=O); 1590Cm^{-1} (aromatic C=C); ^{1}H NMR(CDCl3-400MHz) δ ppm: 0.95(bs, 6H ,-CH$_3$), 2.0(m, 1H, -NH), 2.67(m, 1H, -CH), 3.48(bs, 1H, -NH), 3.68(s, 3H, -OCH$_3$), 3.82(s, 2H, -CH$_2$), 3.83(s, 3H, -OCH$_3$), 6.77-7.22(m, 4H, -ArH); ^{13}C NMR (CDCl$_3$-100MHz) δ ppm: 19.2, 31.0, 51.9, 52.2, 55.8, 64.0, 112.0, 112.6, 120.2, 129.5, 137.4, 160.4, 175.7. MS (ESI, m/z): 287.78(M^{+}).

Methyl-2-((2-bromo-4,5-dimethoxybenzyl)amino)-3-methyl butanoate **(3c)**: Color: Yellow oil. Yield: 75%. IR (KBr,v,Cm^{-1}): 3400Cm^{-1} (-NH); 1720Cm^{-1} (C=O); 1592Cm^{-1} (aromatic C=C); ^{1}H NMR(CDCl3-400MHz) δ ppm: 0.85-0.91(bs, 6H ,-CH$_3$), 2.0(m, 1H, -CH), 2.67(m, 1H, -CH), 3.44(d, 1H, -NH), 3.68(s, 3H, -OCH$_3$), 3.82(s, 2H, -CH$_2$), 3.83(s, 6H, -OCH$_3$), 6.79(s, 1H, -ArH); 7.01(S, 1H, -ArH). ^{13}C NMR (CDCl$_3$-100MHz) δ ppm: 15.2, 31.0, 48.4, 62.0, 113.2, 116.2, 119.9, 134.0, 148.5,148.6, 175.7. MS (ESI, m/z): 396.70(M^{+}).

Methyl-2-((4-fluorobenzyl)amino)-3-methyl butanoate **(3d)**: Color: Gummy mass. Yield: 83%. IR (KBr,v,Cm^{-1}): 3400Cm^{-1} (-NH); 1700Cm^{-1} (C=O); 1592Cm^{-1} (aromatic C=C); ^{1}H NMR(CDCl3-400MHz) δ ppm: 0.80-0.90(bs, 6H ,-CH$_3$), 2.0(m, 1H, -CH), 2.67(m, 1H, -CH), 3.40(d, 1H, -NH), 3.58(s, 3H, -OCH$_3$), 3.52(s, 2H, -CH$_2$), 7.12(t, 2H, -ArH); 7.39(dd, 2H, -ArH). ^{13}C NMR (CDCl$_3$-100MHz) δ ppm: 19.2, 30.0, 51.9, 64.0, 115.3, 129.5, 135.8, 161.2, 175.7. MS (ESI, m/z): 275.78(M^{+}).

Methyl-3-methyl-2-((4-nitrobenzyl)amino)butanoate **(3e)**: Color: Yellow oil. Yield: 70%. IR (KBr,v,Cm^{-1}): 3400Cm^{-1} (-NH); 1710Cm^{-1} (C=O); 1592Cm^{-1} (aromatic

C=C); ^{1}H NMR(CDCl3-400MHz) δ ppm: 0.85-0.93(bs, 6H, -CH_3), 2.0(m, 1H, -CH), 2.67(m, 1H, -CH), 3.44(d, 1H, -NH), 3.76(s, 3H, -OCH_3), 3.62(s, 2H, -CH_2), 7.95(d, 2H, -ArH); 8.14(dd, 2H, -ArH). ^{13}C NMR ($CDCl_3$-100MHz) δ ppm: 17.2, 31.0, 51.6, 64.8, 123.7, 128.8, 146.2, 146.3, 175.7. MS (ESI, m/z): 302.75(M^+).

Methyl-2-((4-chlorobenzyl)amino)-3-methyl butanoate **(3f)**: Color: Gummy mass. Yield: 81%. IR (KBr,v,Cm^{-1}): 3400Cm^{-1} (-NH); 1720Cm^{-1} (C=O); 1592Cm^{-1} (aromatic C=C); ^{1}H NMR(CDCl3-400MHz) δ ppm: 0.98(d, 6H ,-CH_3), 2.0(m, 1H, -CH), 2.67(m, 1H, -CH), 3.44(d, 1H, -NH), 3.60(s, 3H, -OCH_3), 3.52(s, 2H, -CH_2), 7.32-7.37(m, 4H, -ArH). ^{13}C NMR ($CDCl_3$-100MHz) δ ppm: 19.2, 33.0, 50.9, 64.0, 126.6, 128.6, 130.3, 132.6, 138.3. 1735.7. MS (ESI, m/z): 292.20(M^+).

2.2.2. General procedure for the synthesis of substituted 2-(benzylamino)-3-methyl butanoic acid (4a-f)

Sodium hydroxide (1.35g, 0.0339 mol) was dissolved in methanol (20ml) and methyl -2-(benzylamino)-3-methyl butanoate **(3a-f)** (3.50g, 0.0135 mol) was added. The suspension was stirred for 8hr at 60-65^{0}C. The completion of reaction was confirmed by TLC. After completion of reaction, mixture was distilled under vacuum at 60^{0}C to obtain oily mass and dissolved in water (20ml). The compound was extracted to dichloromethane after adjusting pH 5-6 by dil. HCl, solvent was removed atmospherically at 35-40^{0}C and recrystallized in diethyl ether to get **(4a-f)** in good yield.

*2-(benzylamino)-3-methyl butanoic acid (***4a)**: Color: white solid. Yield: 84%. M.P: 188-190^{0}C. IR (KBr,v,Cm^{-1}); 3600Cm^{-1} (-OH); 1780 Cm^{-1} (-C=O); 1590Cm^{-1} (aromatic –C=C); ^{1}H NMR($CDCl_3$-400MHz) δ ppm: 0.93(d, 6H,-CH_3), 2.1(m, 1H, -CH), 2.36(m, 1H, -CH), 3.18(bs, 1H, -NH), 3.80(s, 2H, -CH_2), 7.23-7.33(m, 5H, -ArH); ^{13}C NMR ($CDCl_3$-100MHz) δ ppm: 19.2, 30.7, 51.9, 66.5, 127.0, 127.9, 128.5, 140.2, 174.7. MS (ESI, m/z): 243.10(M^+).

2-((3-methoxybenzyl)amino)-3-methylbutanoic acid **(4b)**: Color: white solid. Yield: 90%. M.P:173-176^{0}C. IR (KBr,v,Cm^{-1}): 3600Cm^{-1} (-NH); 1720Cm^{-1} (C=O); 1592Cm^{-1} (aromatic C=C); ^{1}H NMR($CDCl_3$-400MHz) δ ppm: 0.85-0.91(bs, 6H ,-CH_3), 2.0(m, 1H, -CH), 2.36(m, 1H, -CH), 3.48(bs, 1H, -NH), 3.65(s, 2H, -CH_2), 3.83(s, 6H, -OCH_3), 6.79-7.01(m, 4H, -ArH); ^{13}C NMR ($CDCl_3$-100MHz) δ ppm: 17.2, 30.1, 52.2, 55.8, 66.5, 112.0, 112.6, 120.2, 129.5, 137.4, 160.4, 173.0. MS (ESI, m/z): 273.76(M^+).

2-((2-bromo-4,5-dimethoxybenzyl)amino)-3-methylbutanoic acid **(4c)**: Color: white solid. Yield: 73%. M.P:135-137^{0}C. IR (KBr,v,Cm^{-1}): 3600Cm^{-1} (-OH); 1720Cm^{-1} (C=O); 1592Cm^{-1} (aromatic C=C); ^{1}H NMR($CDCl_3$-400MHz) δ ppm: 0.80-0.90(bs, 6H ,-CH_3), 2.10(m, 1H, -CH), 2.30(m, 1H, -CH), 3.20(bs, 1H, -NH), 3.82(d, 2H, -CH_2), 3.83(s, 6H, -OCH_3), 6.79(s, 1H, -ArH), 7.01(S, 1H, -ArH), 11.0(s, 1H, -COOH). ^{13}C NMR ($CDCl_3$-100MHz) δ ppm: 19.0, 29.0, 48.4, 56.1, 66.5, 115.2, 116.2, 119.9, 134.0, 148.5, 148.6, 174.0. MS (ESI, m/z): 382.68(M^+).

2-((4-fluorobenzyl)amino)-3-methylbutanoic acid **(4d)**: Color: Gummy mass. Yield: 86%. IR (KBr,v,Cm^{-1}): 3600Cm^{-1} (-NH); 1720Cm^{-1} (C=O); 1592Cm^{-1} (aromatic C=C); ^{1}H NMR(CDCl3-400MHz) δ ppm: 0.78-0.91(bs, 6H,-CH_3), 2.08(m, 1H, -CH), 2.36(m, 1H, -CH), 3.48(bs, 1H, -NH), 3.82(s, 2H, -CH_2), 7.12(t, 2H, -ArH); 7.39(dd, 2H, -ArH), 11.0(s, 1H, -COOH). ^{13}C NMR ($CDCl_3$-100MHz) δ ppm: 18.0, 30.7, 51.9, 66.5, 115.3, 129.5, 135.8, 161.2, 170.7. MS (ESI, m/z): 261.72(M^+).

3-methyl-2-((4-nitrobenzyl)amino)butanoic acid **(4e)**: Color: Yellow solid. Yield: 78%. M.P:185-187^{0}C. IR (KBr,v,Cm^{-1}): 3600Cm^{-1} (-OH); 1720Cm^{-1} (C=O); 1592Cm^{-1} (aromatic C=C); ^{1}H NMR($CDCl^3$-400MHz) δ ppm: 0.88-0.96(bs, 6H,-CH_3), 2.0(m, 1H, -CH), 2.36(m, 1H, -CH), 3.48(bs, 1H, -NH), 3.82(s, 2H, -CH_2), 7.95(d, 2H, -ArH); 8.14(dd, 2H, -ArH), 11.0(s, 1H, -COOH). ^{13}C NMR ($CDCl_3$-100MHz) δ ppm: 19.2, 30.7, 51.9, 66.5, 123.7, 128.8, 146.2, 146.3, 174.7. MS (ESI, m/z): 288.73(M^+).

2-((4-chlorobenzyl)amino)-3-methyl butanoic acid **(4f)**: Color: white solid. Yield: 88%. M.P:151-154^{0}C. IR (KBr,v,Cm^{-1}): 3600Cm^{-1} (-OH); 1720Cm^{-1} (C=O); 1592Cm^{-1} (aromatic C=C); ^{1}H NMR($CDCl^3$-400MHz) δ ppm: 0.91(d, 6H,-CH_3), 2.0(m, 1H, -CH), 2.36(m, 1H, -CH), 3.48(d, 1H, -NH), 3.82(s, 2H, -CH_2), 7.32-7.37(m, 4H, -ArH), 11.0(s, 1H, -COOH). ^{13}C NMR ($CDCl_3$-100MHz) δ ppm: 18.6, 30.6, 50.2, 64.3, 126.6, 130.3, 132.6, 138.3, 174.7. MS (ESI, m/z): 277.06(M^+).

2.2.3. General procedure for the synthesis of substituted *2-((2-amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl 2-(benzylamino)-3-methylbutanoate* (6a-f)

To the suspension of 2-(benzylamino)-3-methyl butanoic acid **(4a-f)** (1.0g, 0.0048 mol) and acyclovir (1.08g, 0.0048 mol) in DMF (25ml) charged DCC (1.46g, 0.0072 mol) and DMAP (0.17g, 0.0014 mol) and the reaction mass was stirred at 25-30^{0}C 12-14hr. The completion of the reaction was known by TLC. DMF was evaporated in vacuo and the residue was washed with water. White solid slurried in ether to obtain **(6a-f)** in good yield.

2-((2-amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl2-(benzylamino)-3-methylbutanoate **(6a)**: Color: white solid. Yield: 69%. M.P:168-170^{0}C. IR (KBr,v,Cm^{-1}); 3400Cm^{-1} (-OH); 1780Cm^{-1} (-C=O); 1590Cm^{-1} (aromatic –C=C); ^{1}H NMR(CDCl3-400MHz) δ ppm: 0.86-0.90(bs, 6H,-CH_3), 2.08-2.11(m, 1H, -CH), 2.67(m, 1H, -CH), 3.44(d, 1H, -CH), 3.82(s, 2H, -CH_2), 3.85(t, 2H, -CH_2), 4.25(t, 2H, -CH_2), 5.81(s, 2H, -CH_2), 7.23-7.33(m, 5H, -ArH), 7.97(s, 1H, -ArH), 8.56(s, 2H, -NH_2). ^{13}C NMR ($CDCl_3$-100MHz) δ ppm: 19.2, 31.0, 51.9, 64.3, 66.6,

66.8, 68.2, 117.8, 127.0, 127.9, 128.5, 140.8, 151.1, 153.9, 171.5. MS (ESI, m/z): 452.18(M^+).

2-((2-amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl-2-((3-methoxybenzyl)amino)-3-methylbutanoate **(6b)**: Color: white solid. Yield: 84%. M.P:245-248^{0}C. IR (KBr,v,Cm^{-1}); 3600Cm^{-1} (-OH); 1780Cm^{-1} (-C=O); 1590Cm^{-1} (aromatic –C=C); ^{1}H NMR(CDCl3-400MHz) δ ppm: 0.79-0.91(bs, 6H,-CH_3), 2.10(m, 1H, -NH), 2.54(m, 1H, -CH), 3.40(d, 1H, -CH), 3.65(t, 2H, -CH_2), 3.71(s, 2H, -CH_2), 3.81(s, 3H, -OCH_3), 4.15(t, 2H, -CH_2), 5.80(s, 2H, -CH_2), 6.79-7.21(m, 4H, -ArH), 7.90(s, 1H, -ArH), 8.0(s, 2H, -NH_2). ^{13}C NMR ($CDCl_3$-100MHz) δ ppm: 18.0, 30.0, 52.2, 55.8, 63.3, 66.0, 69.2, 112.0, 120.2, 129.5, 137.4, 140.8, 151.1, 153.9, 157.1, 160.4, 171.5. MS (ESI, m/z): 444.48(M^+).

2-((2-amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl-2-((2-bromo-4,5-dimethoxybenzyl)amino)-3-methylbutanoate **(6c)**: Color: white solid. Yield: 78%. M.P:180-182^{0}C. IR (KBr,v,Cm^{-1}); 3400Cm^{-1} (-NH); 1780Cm^{-1} (-C=O); 1590Cm^{-1} (aromatic –C=C); ^{1}H NMR(CDCl3-400MHz) δ ppm: 0.80-0.91(bs, 6H ,-CH_3), 2.08(m, 1H, -NH), 2.67(m, 1H, -CH), 3.44(d, 1H, -CH), 3.65(t, 2H, -CH_2), 3.82(s, 2H, -CH_2), 3.83(s, 6H, -OCH_3), 4.25(t, 2H, -CH_2), 5.81(s, 2H, -CH_2), 6.79(s, 1H, -ArH), 7.01(s, 1H, -ArH), 7.97(s, 1H, -ArH), 8.56(s, 2H, -NH_2). ^{13}C NMR ($CDCl_3$-100MHz) δ ppm: 19.2, 31.0, 48.4, 56.1, 66.6, 66.8, 69.2, 115.0, 116.2, 119.9, 134.0, 140.6, 148.5, 148.6, 151.1, 153.9, 157.1, 171.5. MS (ESI, m/z): 589.87(M^+).

2-((2-amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl-2-((4-fluorobenzyl)amino)-3-methylbutanoate **(6d)**: Color: white solid. Yield: 80%. M.P:198-200^{0}C. IR (KBr,v,Cm^{-1}); 3400Cm^{-1} (-NH); 1780Cm^{-1} (-C=O); 1590Cm^{-1} (aromatic –C=C); ^{1}H NMR(CDCl3-400MHz) δ ppm: 0.79-0.90(bs, 6H ,-CH_3), 2.0(m, 1H, -NH), 2.67(m, 1H, -CH), 3.44(d, 1H, -CH), 3.65(t, 2H, -CH_2), 3.82(s, 2H, -CH_2), 4.25(t, 2H, -CH_2), 5.81(s, 2H, -CH_2), 7.12(t, 2H, -ArH), 7.39(t, 2H, -ArH), 7.97(s, 1H, -ArH), 8.56(s, 2H, -NH_2). ^{13}C NMR ($CDCl_3$-100MHz) δ ppm: 19.2, 31.0, 64.3, 66.6, 69.2, 115.3, 117.8, 129.5, 135.8, 140.6, 151.1, 153.9, 157.1, 161.2, 171.5. MS (ESI, m/z): 468.91(M^+).

2-((2-amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl-3-methyl-2-((4-nitrobenzyl)amino)butanoate **(6e)**: Color: Yellow solid. Yield: 85%. M.P:201-203^{0}C. IR (KBr,v,Cm^{-1}); 3400Cm^{-1} (-NH); 1780Cm^{-1} (-C=O); 1590Cm^{-1} (aromatic –C=C); ^{1}H NMR(CDCl3-400MHz) δ ppm: 0.88-0.96(bs, 6H,-CH_3), 2.10(m, 1H, -NH), 2.67(m, 1H, -CH), 3.65(t, 2H, -CH_2), 3.82(s, 2H, -CH_2), 4.25(t, 2H, -CH_2), 5.81(s, 2H, -CH_2), 7.95(t, 2H, -ArH), 7.97(s, 1H, -ArH), 8.14(t, 2H, -ArH), 8.56(s, 2H, -NH_2) . ^{13}C NMR ($CDCl_3$-100MHz) δ ppm: 18.7, 30.0, 51.9, 64.3, 66.8, 69.2, 117.8, 123.7, 128.8, 140.6, 146.2, 146.3, 151.0, 153.9, 157.1, 171.0. MS (ESI, m/z): 495.92(M^+).

2-((2-amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl-2-((4-chlorobenzyl)amino)-3-methyl butanoate hydrochloride **(6f)**: Color: white solid. Yield: 71%. M.P:160-162^{0}C. IR (KBr,v,Cm^{-1}); 3400Cm^{-1} (-NH); 1780Cm^{-1} (-C=O); 1580Cm^{-1} (aromatic –C=C); ^{1}H NMR(CDCl3-400MHz) δ ppm: 0.81-0.91(bs, 6H ,-CH_3), 2.07(m, 1H, -NH), 2.58(m, 1H, -CH), 3.55(t, 2H, -CH_2), 3.64(s, 2H, -CH_2), 4.45(t, 2H, -CH_2), 5.1(s, 2H, -CH_2), 7.32-7.37(M, 4H, -ArH), 7.95(s, 1H, -ArH), 8.56(s, 2H, -NH_2). ^{13}C NMR ($CDCl_3$-100MHz) δ ppm: 18.2, 31.0, 56.9, 64.3, 66.6, 69.2, 117.8, 128.6, 130.3, 132.6, 138.3, 140.6, 151.0, 153.9, 157.1, 171.5. MS (ESI, m/z): 485.36(M^+).

2.3. Biological evaluation

2.3.1. Antimicrobial Evaluation

The newly synthesized guanine analogues, compounds ***6a*** to ***6f*** were evaluated *in vitro* for antibacterial activity against *B.subtilis* and Micrococcus as examples of Gram-positive bacteria and *Pseudomonas fluorescence* and Proteus as examples of Gram-negative bacteria. They were also evaluated *in vitro* for their antifungal activity against *Candida albicans*. Inhibition zone diameter (IZD) in cm was used as criterion for the antimicrobial activity using disc diffusion method. Gentamycin and Flucanazole were used as reference drugs for antibacterial and antifungal activity respectively. Microbes were grown in Nutrient Broth (NB, Merck) medium at 37°C for 24h. The bacterial number in the final inoculums was adjusted to 106 CFU/ml. A bacterial lawn was prepared by pouring 0.1 ml of bacterial suspension onto each plate of Nutrient Agar medium (NA, Merck), spread by a sterile cotton swab, and allowed to remain in contact for 1 min. Compounds of different concentrations (20µg,40µg,80µg and 100µg) were prepared in order to impregnate the paper discs. The sterile filter paper discs containing novel compounds (6-mm diameter) were then placed on the bacterial lawn. The Petri dishes were subsequently incubated at 37°C for 24 h and the inhibition zone around each disc was measured in cm. As positive controls, Gentamycin and Flucanozole, containing discs were used.

Table 1: Antibacterial and antifungal activities of synthesized compounds.

Compounds	Minimum inhibitory concentration (µ gms)				
	P. fluroscence	***Micrococcus***	***B.subtilis***	***Proteus***	***C.albicans***
Valciclovir	49	-	-	-	-
6a	87	54	-	25	36
6b	94	33	-	-	-
6c	-	-	6	31	-
6d	92	-	-	-	13
6e	57	-	-	-	-
6f	84	-	-	-	-
Gentamycin	0.80	0.55	0.72	1.3	
Flucanozole	-	-	-	-	0.75

RESULTS

Anti microbial activity is the capacity of the compounds to kill the microorganisms. Table 1 reveals that, compound ***6a*** is acting both on Gram +ve and Gram -ve bacterias and there by showing non specificity. Standard (valciclovir) is active only against *P.fluorescence* which is a Gram –ve bacteria; compound ***6e*** and compound ***6f*** are again showing much specificity in killing Gram-ve bacteria like the valciclovir. Compound ***6b*** and compound ***6c*** are acting both on Gram+ve and Gram-ve bacteria, showing non specificity. Among the compounds studied compound ***6a*** and compound ***6d*** are showing anti fungal activity. The activity of the synthetic compounds is not significant when compared to the reference compounds Gentamycin and Flucanozole.

2.3.2. Antioxidant evaluation

Antioxidants are compounds of exogenous or endogenous in nature which either prevent the generation of toxic oxidants or intercept any that are generated and inactivate them and thereby block the propagation of chain reaction produced by these oxidants (Rangan U and Bulkley GB, 1993).

2.3.3. DPPH radical scavenging assay

DPPH radical scavenging activity was carried out according to Scherer R et al. Method (Scherer R and Godoy HT, 2009). Briefly, 1mL of DPPH solution (0.1mM in 95% ethanol) was mixed with different aliquots of 10-100ng of novel compounds. After vigorous shaking, the mixture was allowed to stand for 20 min. at room temperature. Absorbance of the resulting solution was measured at 517 nm with a UV-VIS spectrophotometer (HITACHI, U-2900). Butylated hydroxyl toluene (BHT) was used as positive control. Radical scavenging potential was expressed as IC_{50} value, which represents the sample concentration at which 50% of the DPPH radicals were scavenged.

2.3.4. Nitric oxide radical scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction. Sodium nitroprusside in phosphate buffer at physiological pH spontaneously generates nitric oxide, which in turn reacts with oxygen to produce nitrite ions that can be estimated by the Griess reagent (Marcocci L et al., 1994). Nitric oxide scavengers compete with oxygen, leading to reduced production of nitric oxide. Sodium nitroprusside (5mM) in phosphate buffered saline was mixed with different aliquots of 20-100µg of novel compounds and incubated at 25ºC for 1 hr. The absorbance of the colour formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm and referred to the absorbance of BHT treated in the same way with the Griess reagent. The radical scavenging potential was calculated and expressed as IC_{50} value.

2.3.5. Ferrous ion chelating assay

Ferrous ion chelating ability was measured according to Gordon M.H.1990 et al, method. For the mechanism of the anti-oxidant action, three sets of test tube were taken. One tube was taken as control to this $FeCl_3$ (200 mM) and $K_3Fe(CN)_6$ (400 mM) were added and the volume was made up to 1 ml by adding distilled water. For the second tube, EDTA (40 mM), $FeCl_3$ (200 mM) and $K_3Fe(CN)_6$ (400 mM) were added and the volume was made up to 1 ml by adding distilled water. For the third one, test compounds (STD and compound 1 to 6) with concentrations 20, 40, 60,8 and 100 µg, $FeCl_3$ (200 mM) and $K_3Fe(CN)_6$ (400 mM) were added and the volume was made up to 1 ml by adding distilled water. The tube was incubated for 10 min at 20^{o}C and read the absorbance at 700 nm and ion chelating ability was calculated. The anti-oxidant activity of all the compounds was compared with that of BHT. Radical scavenging activity was expressed as percentage activity using the formula: [(Control absorbance-sample absorbance)/control absorbance)] × 100.

Table 2: Antioxidant activities of the synthesized compounds.

IC50 values (µg/mL or ng/mL)			
Compounds	**NO radical scavenging assay ug/mL**	**DPPH radical scavenging assay**	**Ferrous ion scavenging assay ug/mL**
BHT	6.4	42.5 ug/mL	51.6
Valciclovir	43.3	44.1 ng/mL	66.25
6a	24.5	63.75 ng/mL	65
6b	26.5	68.75 ng/mL	67.5
6c	22.5	60 ng/mL	68.75
6d	27.5l	76.92 ng/mL	63.5
6e	35	48.3 ng/mL	44.16
6f	19.5	54.5 ng/mL	65

RESULTS

DPPH radical scavenging assay

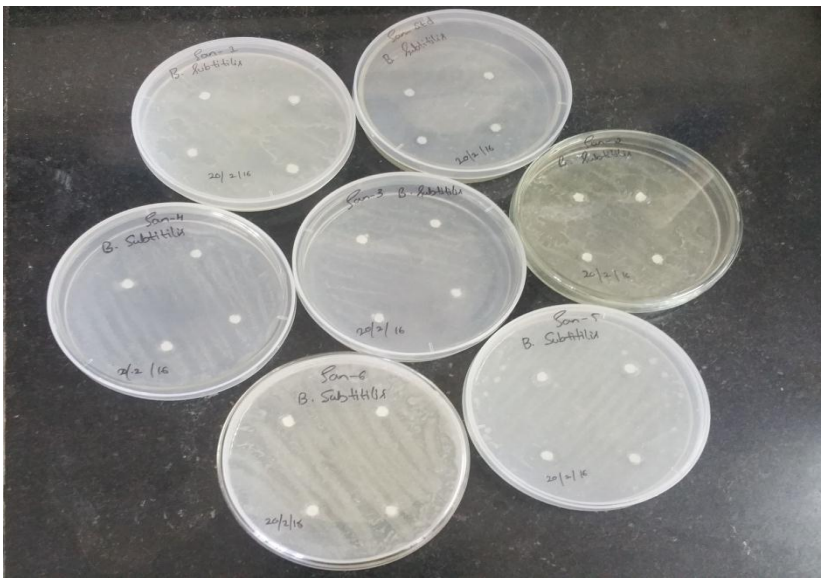
Compound valciclovir (STD) and compound 1 to 6 showed potent DPPH scavenging activity with an IC_{50} value of 44.1 ng/mL to 76.92 ng/mL/mL, compared to the reference compound BHT with an IC_{50} value of 42.5 ug/mL/mL.

Nitric oxide radical scavenging assay

The percentage of scavenging nitric oxide radical ranges from 19.5 ug/mL to 43.3 ug/mL which are not so significant when compared to the standard (BHT), which showed the IC_{50} value of 6.4 ug/mL.

Ferrous ion chelating assay

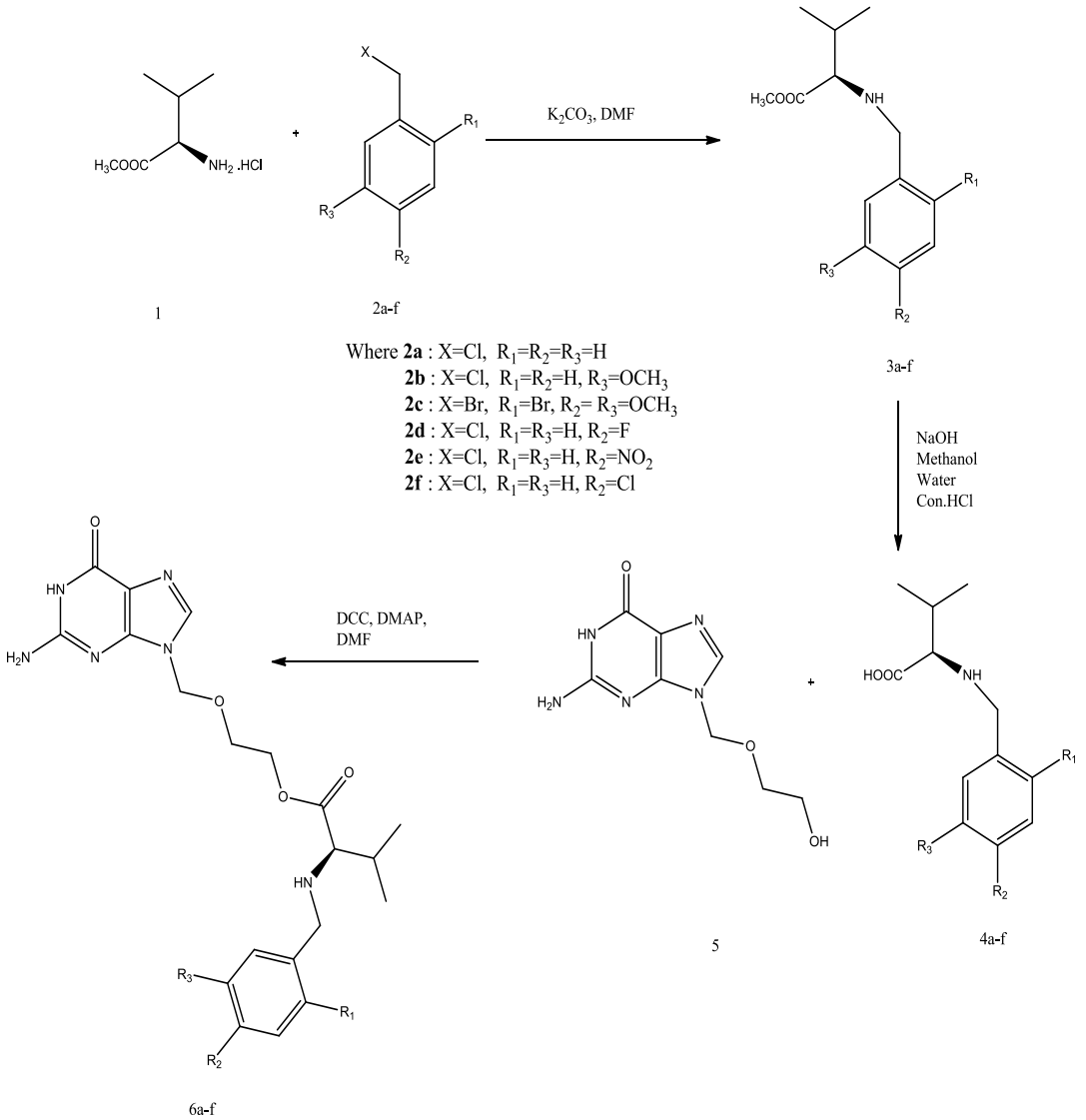
Ferrous ion radical scavenging activity is a corrective approach to prevent oxidative stress-induced disorder and tested for compounds ***6a*** to ***6f***, only compound ***6e*** showed significant ferrous ion scavenging activity compared to the reference compound BHT, other showed moderate activity of scavenging the ferrous ion.



3.0. RESULTS AND DISCUSSION

3.1. Chemistry

The compounds **(*6a-f*)** were synthesized by the following method. **(scheme 1)**. Compounds (***3a-f***) were synthesized by N-alkylation of commercially available materials such as L-valine methyl ester **(1)** substituted benzyl halide **(*2a-f*)** in DMF potassium carbonate base. The products were obtained in high yields. The compounds **(*4a-f*)** were obtained by saponification of **(*3a-f*)** using sodium hydroxide in water and methanol. Compounds **(*6a-f*)** were prepared by the condensation of **(*4a-f*)** with acyclovir **(5)** in DMF medium using DCC and DMAP as dehydrating agents at 25-30^{0}C. The products were characterized by IR, ^{1}H NMR, ^{13}C NMR mass spectral analysis.



Scheme-1: Synthesis of new substituted guanine analogues.

4. CONCLUSION

Synthesized different analogues of guanine (***6a-f***) in 3 step process in good yields using cost effective and readily available chemicals. N-alkylation of amino ester can be easily achieved using potassium carbonate as base. Saponification of N-alkylated amino ester to corresponding acid is achieved using sodium hydroxide as base. Condensation of N-alkylated amino acid with acyclovir carried out using DCC and DMAP. The structures of the synthesized compounds were confirmed and characterized by analytical and spectral data. Among the synthesized analogues, compound ***6a*** and ***6d*** showing potent antifungal activity, the activity of the synthetic compounds is improved significant when compared to the reference compounds Gentamycin and Flucanozole. Compound valciclovir (STD) and compound 1 to 6 showed potential DPPH scavenging activity compared to the reference compound BHT. The percentages of scavenging nitric oxide radical which are not much progressive when compared to the standard (BHT). Only compound ***6e*** showed significant ferrous ion scavenging activity compared to the reference compound BHT, other showed moderate activity of scavenging the ferrous ion.

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