SYNTHESIS AND STUDY OF BIOLOGICAL ACTIVITIES OF NOVEL N^2 SUBSTITUTED BIPHENYL DERIVATIVES OF VALACYCLOVIR

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ABSTRACT: New substituted biphenyl derivatives of Valacyclovir 6a-e i.e. substituted 2-((2-(([1,1'-biphenyl]-4-ylmethyl)amino)-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl 2-amino-3-methylbutanoate were synthesized by general deprotection method using Pd/C in acetic acid under hydrogrn pressure. Substituted N-protected Biphenyl derivatives were synthesized by Steglich esterification with some changes in the experimental procedure. Compound 4 was prepared by N-protection of L-Valine. Substituted 2-(([1,1'-biphenyl]-4-ylmethyl)amino)-9-((2-hydroxyethoxy)methyl)-1H-purin-6(9H)-one i.e 3a-e were synthesized by general N-alkylation of Acyclovir with 2-substituted 4-(bromomethyl)-1,1'-biphenyl using K_2CO_3 and DMF. The synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, mass and IR spectrum. The synthesized compounds were screened for their antimicrobial and anti oxidant activities by comparing with Valacyclovir and control, compounds 6a-e showed considerable activity. Among the synthesized compounds, 6b exhibited more inhibition compared with remaining synthesized compounds.

***Key Word*:** Valacyclovir, biphenyl, Steglich, Acyclovir.

INTRODUCTION

Valacyclovir is a drug that is used for viral infection. Valaciclovir is an esterified version of acyclovir that has greater oral bioavailability (about 55%) than acyclovir (10–20%). Specific antiviral drug is used for specific viral infection. Antiviral drugs do not destroy their target pathogen; instead, they inhibit their growth. Hence, the design of a safe and effective drug requires extended knowledge of the genetic and molecular functions of organisms. Researchers have been focusing on the development of effective drugs by incorporating effective pharmocophores into origin drugs or on the understanding of the structure and function of viruses to find new drugs and their bioavailability. In continuation of ongoing research work, novel biphenyl and substituted derivatives of valacyclovir have been designed, synthesized and tested against antibacterial, antimicrobial and antioxidant activity. Biphenyl and substituted biphenyls are important groups in numerous natural products and drug intermediates. Biphenyl and substituted biphenyls derivatives possess many promising biological activities, such as antihypertensive, herbicidal, antimicrobial, antioxidant, antiviral, anti-HIV and antitumor activities.

EXPERIMENTAL

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.

Synthesis

General procedure for the preparation of substituted 2-(([1,1'-biphenyl]-4-ylmethyl)amino)-9-((2-hydroxyethoxy)methyl)-1H-purin-6(9H)-one (3a-e).

Acyclovir (10g, 0.059mol) and sodium hydride (60% suspension in mineral oil, 2.50g) were taken in anhydrous N,N-dimethyl formamide (40ml) at room temperature. After stirring at room temperature for 1hr, 4-(bromomethyl)-1,1'-biphenyl (16g, 0.0649mol) 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 confirmed by TLC. After completion of reaction, charged water (100ml) and extracted to MDC (2x50ml). Finally washed MDC extract with water (2x50ml) and distilled solvents under vacuum at 50^0C and the residue was chromatographed on silica gel and the products were eluted with chloroform to give afford substituted **(3a-e)** in good yield.

***2-(([1,1'-biphenyl]-4-ylmethyl)amino)-9-((2-hydroxyethoxy)methyl)-1H-purin-6 (9H)-one* (3a):** Gummy mass. Yield: 82%. IR (KBr,v,Cm^{-1}): 3410Cm^{-1} (-OH and -NH); 1720Cm^{-1} (C=O); ^{1}H NMR(DMSO-400MHz) δ ppm: 3.38-3.45(d, 4H ,-OCH_2CH_2O-), 3.92(d, 2H, -CH_2Ph), 4.70(s, 1H, -OH), 5.35(t, 1H, -NH), 5.81(s, 2H, -OCH_2N), 7.29-7.51(m, 9H, -ArH), 7.97(s, 1H, -CH); 10.90

(s, 1H, -NH). ^{13}C NMR (DMSO-100MHz) δ ppm: 44.9, 61.0, 69.5, 70.1, 117.8, 127.4, 127.6, 127.7, 127.9, 129.2, 136.8, 138.8, 140.6, 140.8, 151, 154.1, 157.1. MS (ESI, m/z): 392.31(M^+).

***Methyl 4'-(((9-((2-hydroxyethoxy)methyl)-6-oxo-6,9-dihydro-1H-purin-2-yl) amino)methyl)-[1,1'-biphenyl]-2-carboxylate (3b)*:** White solid. M.P:267-269^{0}C. Yield: 80%. IR (KBr,v,Cm^{-1}): 3550Cm^{-1} (-OH and –NH); 1710Cm^{-1} (-C=O); ^{1}H NMR(DMSO-400MHz) δ ppm: 3.41-3.55(d,d, 4H, -OCH_2CH_2O-), 3.87(s, 3H, -OCH_3), 3.91(d, 2H, -CH_2Ph), 4.85(s,1H, -OH), 5.82(s,2H, -OCH_2N), 7.26-8.45(m, 9H, ArH); ^{13}C NMR (DMSO-100MHz) δ ppm: 44.9, 51.5, 61.0, 70.1, 89.5, 121, 127.4, 127.5, 127.7, 133.5, 136.8, 137.4, 140.6, 154.1, 157.1, 165.0. MS (ESI, m/z): 450.40 (M^+).

***4'-(((9-((2-hydroxyethoxy)methyl)-6-oxo-6,9-dihydro-1H-purin-2-yl)amino) methyl)-[1,1'-biphenyl]-2-carbonitrile (3c)*:** white solid. M.P.: 287^{o}C. Yield: 86%. IR (KBr,v,Cm^{-1}): 3500Cm^{-1} (br,-OH and -NH); 2110Cm^{-1} (-CN) 1720Cm^{-1} (C=O); ^{1}H NMR(DMSO-400MHz) δ ppm: 3.44-3.56(dd, 4H ,-OCH_2CH_2O-), 3.98(d, 2H, -CH_2Ph), 4.81(s, 1H, -OH), 5.33(t, 1H, -NH), 5.85(s, 2H, -CH_2O), 7.29-7.97(m, 8H, -ArH), 8.01(s, 1H, -CH); 10.91 (s, 1H, -NH). ^{13}C NMR (DMSO-100MHz) δ ppm: 45.1, 61.2, 70.5, 89.5, 104.4, 117.1, 117.8, 127.4, 127.7, 128.3, 128.6, 132.7, 135.5, 136.8, 140.6, 142.8, 151.4, 154.0, 157.3. MS (ESI, m/z): 417.23(M^+).

4'-(((9-((2-hydroxyethoxy)methyl)-6-oxo-6,9-dihydro-1H-purin-2-yl)amino) methyl)-[1,1'-biphenyl]-2-carboxylic acid (3d). White solid. M.P.:301-303^{0}C. Yield: 81%. IR (KBr,v,Cm^{-1}): 3500Cm^{-1} (br,-OH and -NH); 1720Cm^{-1} (C=O); ^{1}H NMR(DMSO-400MHz) δ ppm: 3.40-3.50(dd, 4H ,-OCH_2CH_2O-), 3.92(d, 2H, -CH_2Ph), 4.81(s, 1H, -OH), 5.82(s, 2H, -OCH_2N), 7.29-7.90(m, 8H, -ArH), 8.01(s, 1H, -CH); 10.91 (s, 1H, -NH). ^{13}C NMR (DMSO-100MHz) δ ppm: 44.5, 61.0, 69.5, 70.1, 117, 121, 127.4, 130.8, 134.4, 136.8, 140.6, 151, 154.3, 157.3. MS (ESI, m/z): 436.05 (M^+).

4'-(((9-((2-hydroxyethoxy)methyl)-6-oxo-6,9-dihydro-1H-purin-2-yl)amino) methyl)-[1,1'-biphenyl]-2-carboxamide (3e) Gummy mass. Yield: 85%. IR (KBr,v,Cm^{-1}): 3500Cm^{-1} (br,-OH and -NH); 1710Cm^{-1} (C=O); ^{1}H NMR(-400MHz) δ ppm: 3.38-3.52(dd, 4H ,-OCH_2CH_2O-), 3.95(d, 2H, -CH_2Ph), 4.86(s, 1H, -OH), 5.83(s, 2H, -OCH_2N), 7.29-8.09(m, 9H, -ArH), 7.50(s, 2H, -$CONH_2$); ^{13}C NMR (DMSO-100MHz) δ ppm: 45.6, 61.2, 69.52, 70.3, 117.8, 121.2, 127.4, 132.6, 136.8, 140.8, 151, 154.1, 157.4. MS (ESI, m/z): 435.45 (M^+).

General procedure for the preparation of 2-(((benzyloxy)carbonyl)amino)-3-methylbutanoic acid (4).
To the cooled aqueous solution of sodium hydroxide (10.24g, 0.256mol)), charged L-valine (10.0g, 0.08536mol) under stirring for 30min. Slowly added benzyl chloro formate (17.46g, 0.1024mol) over a period of 30min and maintained at 0-5^{0}C for 2hour. Reaction completion was monitored by TLC. Washed the reaction mass with toluene and separated the layers. Aqueous layer pH adjusted to 1-2 using dilute HCl and extracted the product to MDC. MDC layer washed with brine solution, evaporated to dryness resulting in oily mass which upon crystallization with cyclohexane IPA mixture results in **(4)** as white solid.

2-(((benzyloxy)carbonyl)amino)-3-methylbutanoic acid (4). White solid. Yield: 78%. IR (KBr,v,Cm^{-1}): 3600Cm^{-1} (-OH, -NH); 1715Cm^{-1} (C=O); ^{1}H NMR(DMSO-400MHz) δ ppm: 0.86-0.91(d, 6H,-2CH_3), 1.70-1.81(m, 1H, -CH), 4.25(d, 1H, -CHNH), 5.09(s, 2H, -CH_2Ph), 7.38-7.49(m, 5H, -ArH), 8.05(d, 1H, -NH), 11.0(s, 1H, -COOH); ^{13}C NMR (DMSO-100MHz) δ ppm: 18.7, 30.30, 63.5, 66.8, 127.5, 128.9, 135.8, 155.9, 174.3; MS (ESI, m/z): 252.10 (M^+).

General procedure for the preparation of substituted 2-((2-(([1,1'-biphenyl]-4-ylmethyl)amino)-6-oxo-1H-purin-9(6H)-yl) methoxy) ethyl 2-(((benzyloxy) carbonyl)amino)-3-methylbutanoate (5a-e).
To the suspension of 2-(([1,1'-biphenyl]-4-ylmethyl)amino)-9-((2-hydroxyethoxy)methyl)-1H-purin-6(9H)-one **(3a)** (1.0g, 0.0048 mol) and N-protected valine (**4**) (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 confirmed by TLC. DMF was evaporated in vacuo and the residue was washed with water. White solid slurried in ether to obtain **(5a-e)** in good yield.

2-((2-(([1,1'-biphenyl]-4-ylmethyl)amino)-6-oxo-1H-purin-9(6H)-yl)methoxy) ethyl 2-(((benzyloxy)carbonyl)amino)-3-methylbutanoate (5a). Gummy mass. Yield: 82%. IR (KBr,v,Cm^{-1}): 3400Cm^{-1} (-NH); 1715Cm^{-1} (C=O); ^{1}H NMR(DMSO-400MHz) δ ppm: 0.98-1.01(d, 6H,-2CH_3), 3.09(m, 1H, -CH), 3.65(t, 2H, -OCH_2), 3.83(s, 2H, -CH_2Ph), 3.91(s, 2H, -CH_2NH), 4.25(t, 2H, -CH_2OCO), 4.41(s, 1H, -CHNH), 5.34(s, 2H, -OCH_2N), 7.12-7.97(m, 15H, -ArH); ^{13}C NMR (DMSO-100MHz) δ ppm: 17.5, 30.4,41.8, 64,4, 65.9, 66.9, 117.6, 127.5, 129.0, 144.8, 150.8, 156.9. MS (ESI, m/z): 625.65 (M^+).

Methyl 4'-(((9-(5-isopropyl-3,6-dioxo-1-phenyl-2,7,10-trioxa-4-azaundecan-11-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)amino)methyl)-[1,1'-biphenyl]-2-carboxylate (5b). Gummy mass. Yield: 78%. IR (KBr,v,Cm^{-1}): 3450Cm^{-1} (-NH); 1750Cm^{-1} (C=O); ^{1}H NMR(DMSO-400MHz) δ ppm: 1.05-1.12(d, 6H,-2CH_3), 3.19(m, 1H, -CH), 3.68(t, 2H, -OCH_2), 3.80(s, 3H, -OCH_3), 3.90(s, 2H, -CH_2NH), 4.20(t, 2H, -CH_2OCO), 4.38(s, 1H, -CHNH), 5.40(s, 2H, -OCH_2N), 7.13-7.97(m, 14H, -ArH); ^{13}C NMR (DMSO-100MHz) δ ppm: 17.0, 30.8, 41.6, 51.3, 64.2, 65.9, 66.4, 118.6, 127.5, 129.8, 144.3, 150.8, 156.9. MS (ESI, m/z): 683.70 (M^+).

2-((2-(((2'-cyano-[1,1'-biphenyl]-4-yl)methyl)amino)-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl 2-(((benzyloxy)carbonyl)amino)-3-methylbutanoate (5c). White solid. Yield: 75%. IR (KBr,v,Cm^{-1}): 3450Cm^{-1} (-NH); 2100Cm^{-1} (-CN); 1750Cm^{-1} (C=O); ^{1}H NMR(DMSO-400MHz) δ ppm: 1.0-1.10(d, 6H,-2CH_3), 3.13(m, 1H, -CH), 3.65(t, 2H, -OCH_2), 3.95(s, 2H, -CH_2NH), 4.13(t, 2H,

-CH_2OCO), 4.32(s, 1H, -CHNH), 5.40(s, 2H, -OCH_2N), 7.12-7.98(m, 14H, -ArH); ^{13}C NMR (DMSO-100MHz) δ ppm: 17.3, 31.8, 41.0, 64.0, 66.4, 115.8, 118.9, 128.5, 129.9, 144.7, 150.8, 156.0. MS (ESI, m/z): 650.30 (M^+).

4'-(((9-(5-isopropyl-3,6-dioxo-1-phenyl-2,7,10-trioxa-4-azaundecan-11-yl)-6-oxo-6,9-dihydro-1H-purin-2-yl)amino)methyl)-[1,1'-biphenyl]-2-carboxylic acid (5d) White solid. Yield: 77%. IR (KBr,v,Cm^{-1}): 3550Cm^{-1} (-OH and -NH); 1720Cm^{-1} (C=O); ^1H NMR(DMSO-400MHz) δ ppm: 0.95-1.04(d, 6H,-$2CH_3$), 3.10(m, 1H, -CH), 3.53(t, 2H, -OCH_2), 3.91(s, 2H, -CH_2NH), 4.09(t, 2H, -CH_2OCO), 4.31(s, 1H, -CHNH), 5.38(s, 2H, -OCH_2N), 7.12-8.19(m, 14H, -ArH), 11.0(s, 1H, -COOH); ^{13}C NMR (DMSO-100MHz) δ ppm: 17.2, 31.5, 41.0, 63.9, 65.8, 115.3, 118.5, 128.1, 129.4, 144.9, 150.9, 156.0, 169.3. MS (ESI, m/z): 669.38 (M^+).

2-((2-(((2'-carbamoyl-[1,1'-biphenyl]-4-yl)methyl)amino)-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl 2-(((benzyloxy)carbonyl)amino)-3-methylbutanoate (5e). White solid. Yield: 76%. IR (KBr,v,Cm^{-1}): 3450Cm^{-1} (-NH); 1720Cm^{-1} (C=O); ^1H NMR(DMSO-400MHz) δ ppm: 1.0-1.09(d, 6H,-$2CH_3$), 3.11(m, 1H, -CH), 3.63(t, 2H, -OCH_2), 3.95(s, 2H, -CH_2NH), 4.13(t, 2H, -CH_2OCO), 4.32(s, 1H, -CHNH), 5.40(s, 2H, -OCH_2N), 6.05(s, 2H, -$CONH_2$), 7.12-7.80(m, 14H, -ArH); ^{13}C NMR (DMSO-100MHz) δ ppm: 17.5, 31.8, 41.0, 64.0, 66.4, 115.8, 118.9, 128.5, 129.9, 144.7, 150.8, 156.0, 168.3. MS (ESI, m/z): 668.65 (M^+).

General procedure for the preparation of substituted 2-((2-(([1,1'-biphenyl]-4-ylmethyl)amino)-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl2-amino-3-methyl butanoate (6a-e).
To the solution of *2-((2-(([1,1'-biphenyl]-4-ylmethyl)amino)-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl 2-(((benzyloxy)carbonyl)amino)-3-methylbutanoate* ***(5a)*** (5g, 0.008mole) in IPA and DM water was taken in an autoclave, charged acetic acid (0.72g, 0.012mol) and 10% Pd/C (0.125g) at 25-30^{0}C. Pressurized the reaction with 1-2Kgs hydrogen gas and gradually warmed to 30-35^{0}C for 1-2 hr. Reaction completion was monitored by TLC. Catalyst was filtered and washed with IPA. Concentrated the filtrate under reduced pressure to obtain solid residue which was purified using column chromatography on silica gel (100-200mesh) using ethyl acetate: hexane as an eluent.

2-((2-(([1,1'-biphenyl]-4-ylmethyl)amino)-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl 2-amino-3-methylbutanoate (6a). White solid. Yield: 75%. IR (KBr,v,Cm^{-1}): 3455Cm^{-1} (-NH); 1750Cm^{-1} (C=O); ^1H NMR(DMSO-400MHz) δ ppm: 0.98-1.07(d, 6H,-$2CH_3$), 2.71(m, 1H, -CH), 3.45(d, 1H, -$CHNH_2$), 3.63(t, 2H, -OCH_2), 4.25(t, 2H, -CH_2OCO), 3.90(s, 2H, -CH_2NH), 4.24(t, 2H, -CH_2OCO), 5.81(s, 2H, -OCH_2N), 7.12-7.97(m, 10H, -ArH); ^{13}C NMR (DMSO-100MHz) δ ppm: 17.3, 33.2, 41.8, 59.5, 64.0, 65.7, 66.9, 118.9, 127.5, 129.9, 136.5, 140.7, 156.0, 170.7; MS (ESI, m/z): 491.65 (M^+).

Methyl 4'-(((9-((2-((2-amino-3-methylbutanoyl)oxy)ethoxy)methyl)-6-oxo-6,9-dihydro-1H-purin-2-yl)amino)methyl)-[1,1'-biphenyl]-2-carboxylate (6b) White solid. Yield: 78%. IR (KBr,v,Cm^{-1}): 3400Cm^{-1} (-NH); 1760Cm^{-1} (C=O); ^1H NMR(DMSO-400MHz) δ ppm: 0.98-1.08(d, 6H,-$2CH_3$), 2.55(s, 3H, -$COCH_3$), 2.70(m, 1H, -CH), 3.40(d, 1H, -$CHNH_2$), 3.65(t, 2H, -OCH_2), 4.21(t, 2H, -CH_2OCO), 3.91(s, 2H, -CH_2NH), 4.25(t, 2H, -CH_2OCO), 5.75(s, 2H, -OCH_2N), 7.10-7.98(m, 9H, -ArH); ^{13}C NMR (DMSO-100MHz) δ ppm: 17.5, 29.6, 33.0, 41.8, 59.5, 64.0, 65.6, 66.9, 118.9, 123.5, 129.9, 136.5, 144.7, 156.0, 171.0, 199.0; MS (ESI, m/z): 533.50 (M^+).

2-((2-(((2'-cyano-[1,1'-biphenyl]-4-yl)methyl)amino)-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl 2-amino-3-methylbutanoate (6c) White solid. Yield: 70%. IR (KBr,v,Cm^{-1}): 3450Cm^{-1} (-NH); 2120Cm^{-1} (-CN); 1752Cm^{-1} (C=O); ^1H NMR(DMSO-400MHz) δ ppm: 0.99-1.09(d, 6H,-$2CH_3$), 2.72(m, 1H, -CH), 3.43(d, 1H, -$CHNH_2$), 3.63(t, 2H, -OCH_2), 4.23(t, 2H, -CH_2OCO), 3.90(s, 2H, -CH_2NH), 4.24(t, 2H, -CH_2OCO), 5.88(s, 2H, -OCH_2N), 7.13-7.98(m, 9H, -ArH); ^{13}C NMR (DMSO-100MHz) δ ppm: 17.2, 33.3, 41.8, 59.5, 64.6, 65.7, 66.9, 104.5, 115.8, 118.9, 127.5, 129.9, 136.5, 140.7, 156.9, 171.5; MS (ESI, m/z): 516.6(M^+).

4'-(((9-((2-((2-amino-3-methylbutanoyl)oxy)ethoxy)methyl)-6-oxo-6,9-dihydro-1H-purin-2-yl)amino)methyl)-[1,1'-biphenyl]-2-carboxylic acid (6d) White solid. Yield: 75%. IR (KBr,v,Cm^{-1}): 3600Cm^{-1} (-OH,-NH); 1750Cm^{-1} (C=O); ^1H NMR(DMSO-400MHz) δ ppm: 1.02-1.09(d, 6H,-$2CH_3$), 2.70(m, 1H, -CH), 3.45(d, 1H, -$CHNH_2$), 3.65(t, 2H, -OCH_2), 4.22(t, 2H, -CH_2OCO), 3.90(s, 2H, -CH_2NH), 4.24(t, 2H, -CH_2OCO), 5.86(s, 2H, -OCH_2N), 7.12-7.95(m, 9H, -ArH), 11.0(s, 1H, -COOH); ^{13}C NMR (DMSO-100MHz) δ ppm: 17.2, 33.0, 41.6, 59.8, 64.2, 65.6, 66.5, 118.1, 127.4, 130.2, 136.5, 140.4, 156.0, 169.4, 171.6; MS (ESI, m/z): 535.49 (M^+).

2-((2-(((2'-carbamoyl-[1,1'-biphenyl]-4-yl)methyl)amino)-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl 2-amino-3-methylbutanoate (6e) White solid. Yield: 79%. IR (KBr,v,Cm^{-1}): 3450Cm^{-1} (-NH); 1750Cm^{-1} (C=O); ^1H NMR(DMSO-400MHz) δ ppm: 0.99-1.08(d, 6H,-$2CH_3$), 2.70(m, 1H, -CH), 3.42(d, 1H, -$CHNH_2$), 3.61(t, 2H, -OCH_2), 4.20(t, 2H, -CH_2OCO), 3.91(s, 2H, -CH_2NH), 4.24(t, 2H, -CH_2OCO), 5.80(s, 2H, -OCH_2N), 6.0(s, 2H, -$CONH_2$) 7.10-7.95(m, 10H, -ArH); ^{13}C NMR (DMSO-100MHz) δ ppm: 17.3, 33.2, 41.8, 59.5, 64.0, 65.7, 66.9, 118.9, 127.5, 129.9, 136.5, 140.7, 156.0, 168.1, 171.5; MS (ESI, m/z): 533.58 (M^+).

Biological Evaluation
Antimicrobial Evaluation
The newly synthesized N^2 biphenyl substituted valacyclovir ***6a*** to ***6e*** 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 and their activity were compared with valacyclovir. 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 24h 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***
Valacyclovir	49	-	-	-	-
6a	80	51	-	35	31
6b	50	23	-	-	-
6c	-	-	10	35	-
6d	92	-	-	-	18
6e	53	-	-	-	-
Gentamycin	0.80	0.55	0.72	1.3	
Flucanozole	-	-	-	-	0.75

Antimicrobial 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 (valacyclovir) is active only against *P.fluorescence* which is a Gram –ve bacteria; compound ***6e*** again showing much specificity in killing Gram-ve bacteria like the valacyclovir. 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.

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).

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. The percentage of scavenging nitric oxide radical ranges from 26.1ug/mL to 39.51 ug/mL which are not so significant when compared to the standard (BHT), which showed the IC_{50} value of 6.4 ug/mL.

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 517nm 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. Compound valacyclovir and compound 6a-e showed potent DPPH scavenging activity with IC_{50} value of 44.9µg/mL to 73.75µg/mL compared to the reference compound BHT with an IC_{50} value of 42.5 µg/mL.

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, 80 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 20ºC 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

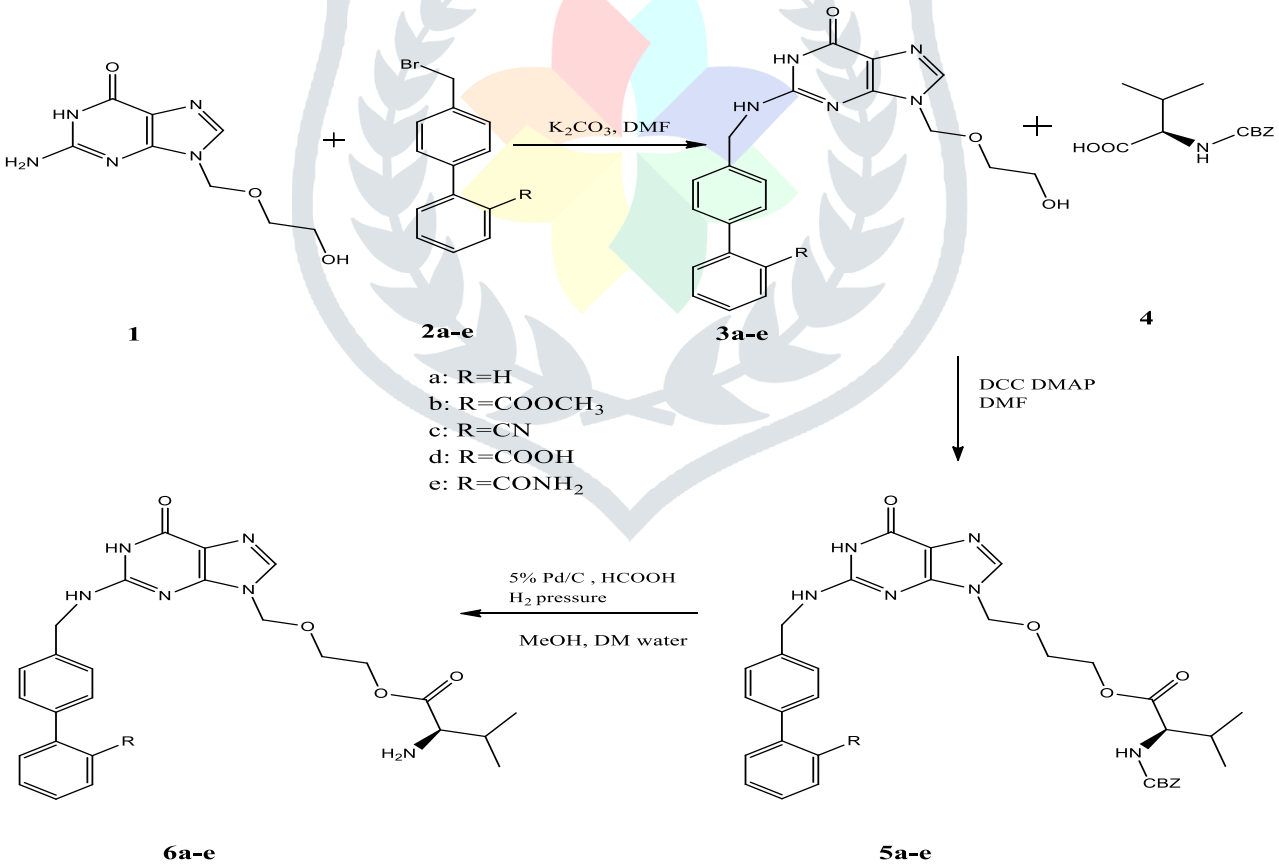
absorbance-sample absorbance)/control absorbance)] × 100. Ferrous ion radical scavenging activity is a corrective approach to prevent oxidative stress-induced disorder and tested for compounds ***6a*** to ***6e***, only compound ***6e*** showed significant ferrous ion scavenging activity compared to the reference compound BHT, other showed moderate activity of scavenging the ferrous ion.

Table 2: Antioxidant activities of the synthesized compounds.

Compounds	IC_{50} values (µg/mL)		
	NO radical scavenging assay	DPPH radical scavenging assay	Ferrous ion scavenging assay
BHT	6.4	42.5	55.6
Valacyclovir	41.3	44.9	69.25
6a	28.5	66.75	66.0
6b	26.1	73.75	69.5
6c	33.5	60.7	66.75
6d	39.5l	73.92	64.55
6e	35.84	68.3	47.31

RESULTS AND DISCUSSION

Newly synthesized N^2 substituted biphenyl derivatives of valacyclovir (***6a-e***) is a 3 step process obtained in good yields using cost effective and readily available chemicals. Acyclovir **(1)** made to react with substituted bibenzyl halide **(*2a-e*)** in DMF and sodium hydride (60%) as base. The compounds **(*4a-e*)** were obtained by condensation of **(*3a-e*)** with N-protected L-valine **(4)** by Steglich esterification using DCC and DMAP in DMF medium. Compounds **(*6a-e*)** were obtained by deprotection of **(5*a-e*)** using Pd/C, acetic acid in ethanol medium under hydrogen pressure. The products were characterized by IR, ^{1}H NMR, ^{13}C NMR mass spectral analysis. 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. Also they 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.



Scheme-1: Synthetic pathway for compounds 6(a-e).

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