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Synthesis and biological evaluation of novel urea and thiourea derivatives of valacyclovir

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Abstract: A series of novel urea and thiourea derivatives of valacyclovir were efficiently synthesized in high yields and evaluated their antiviral activity. 2-((6-Amino-4-oxo-4,5-dihydro-1H-imidazo[4,5-c]pyridin-1-yl)methoxy)ethyl-2-amino-3-ethylbutanoate (valacyclovir) **1** is reacted with various aromatic isocyanates/thiocyanates **2** in the presence of N, N- dimethyl piperazine as a base in THF: pyridine (4:1) to obtain valacyclovir urea/thiourea derivatives **3(a-j).** The structures of the title compounds **3(a-j)** were confirmed by IR, NMR (^{1}H, ^{13}C), mass spectral and elemental analysis. The newly synthesized compounds were screened for their antiviral activity against Tobacco mosaic virus (TMV) and antioxidant activity was evaluated by DPPH, SOD and GST methods. The title compounds exhibited potent antiviral and good antioxidant activities.

Keywords: isocyanate; isothiocyanate; N, N- dimethyl piperazine; tobacco mosaic virus; antiviral; anti-oxidant activity.

INTRODUCTION

Urea and thiourea are important functional groups in numerous natural products, drug intermediates and are being used as neutral receptor for various anions (anion complexation)[1], and building blocks for various heterocycles. Urea and thiourea derivatives have been found to possess many promising biological activities such as herbicidal activity[2], antimicrobial[3], antioxidant[4], anti-viral[5], anti-HIV[6], antitumor[7], urea derivatives exhibited anti-inflammatory[8], anti-malarial[9] and antidiabetic activity[10]. Thiourea and urea have been used as purification agents for the effluent of organic and inorganic, industrial, agricultural and mining wastes[11]. These compounds are useful in agriculture, spinning mixtures, paper and paints and as wrinkle proofing agents for cotton and cotton polyester fabrics[12-13]. These compounds also could be used for

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detoxification of super antigens from body fluids[14] and for the treatment of haemoglobinopathies in the cases of sickle cell anemia and Beta (β) thalassemia[15] and thiourea derivatives were reported to be non-nucleoside inhibitors (NNIs) of the reverse transcriptase (RT) enzyme of the human immunodeficiency virus (HIV)[16]. Thiocarlide is a pharmacologically important thiourea drug used as therapeutic agent in the treatment of tuberculosis[17]. Thiourea inhibitors of plant viruses have also arisen widespread interest in both biological and chemical sectors[18]. Valacyclovir is a prodrug which is used for viral infection and an esterified version of acyclovir that has greater oral bioavailability (about 55%) than acyclovir (10–20%). Specific antivirals are used for specific viruses. Unlike most antibiotics, antiviral drugs do not destroy their target pathogen; instead they inhibit their development. So, designing of safe and effective drug is needful to that acquired extended knowledge on genetic and molecular functions of organisms. Hence, the researcher have been focusing to develop effective antiviral drugs by embedding effective pharmocophores to origin drug or to understand the structure and function of viruses to find new drug. Plant virus is a type of plant disease, known as "plants cancer". In recent years, the impact of climate anomalies and the areas of crops affected by plant virus disease are on the rise resulting in tremendous economic losses in the world. Tobacco mosaic virus (TMV) disease is an important class of common disease occurring in tobacco plants growing all over the world. In continuation of our research work, we have designed and synthesized novel urea and thiourea derivatives of valacyclovir and tested against *Tobacco mosaic virus* and evaluated their antioxidant activities.

EXPERIMENTAL

Sigma-Aldrich, Merck and Lancaster Chemicals were used as such without further purification. Solvents used for spectroscopic and other physical studies were reagent grade and were further purified by literature methods[19]. Melting points were determined by Guna Digital Melting Point apparatus using a calibrated centigrade thermometer and are uncorrected. IR spectra were obtained in KBr optics on a Perkin-Elmer Model 281-B spectrophotometer and expressed in wave numbers (cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker AVANCE III 500 MHz spectrometer operating at 500 MHz for ^{1}H, 125 MHz for ^{13}C NMR. The ^{1}H and ^{13}C chemical shifts were expressed in ppm with reference to tetramethylsilane. ESI mass spectra were recorded on a Finnigan MAT 1020 mass spectrometer. Elemental analyses were performed at University of Hyderabad, India.

General Procedure for synthesis of title compounds ***3(a-j)***

2-((6-Amino-4-oxo-4, 5-dihydro-1H-imidazo [4,5-c] pyridin-1-yl) methoxy) ethyl-2-amino-3-ethylbutanoate (valacyclovir) **1** (0.001 mol), various aromatic isocyanates/ thiocyanates **(2)** (0.001 mol) were dissolved in dry THF:py (20 mL) and refluxed with stirring for 3-5 h at about 60 ^{o}C. Identification of the product and completion of the reaction was monitored by TLC using ethyl acetate: hexane (4:1). After completion of the reaction, the mixture was concentrated in a rota-evaporator and the residue was purified by column

chromatography on silica gel (100–200 mesh) using petroleum ether-ethyl acetate (2:3) as eluent. The structures of the title compounds **3(a-j)** were established by spectral and elemental analysis. The obtained yields of **3(a-j)** are in the range of 72-82%.

2-((2-Amino-6-oxo-1H-purine-9(6H)-yl)methoxy)ethyl-3-methyl-2-(3-phenylthioureido) butanoate ***(3a):*** Yield: 82%; m.p. 144-146 °C; Anal. Calcd. for $C_{20}H_{25}N_7O_4S$: C, 52.27; H, 5.48; N, 21.34. Found C, 52.29, H, 5.42; N, 21.25; IR (KBr, cm^{-1}): 3412 (N-H), 3138 (NH_2), 1183 (C=S), 724 (C-S); ^{1}H-NMR (300 MHz, DMSO-d_6, δ/ppm): 10.8 (1H, s, N-H), 8.21 (1H, s, Ar-H), 7.86 (2H, s, NH_2), 7.68-6.94 (m, 5H, Ar-H), 5.24 (2H, *t*, CH_2), 5.18 (2H, *t*, CH_2), 4.58 (2H, s, CH_2), 4.20 (1H, s, NH-Ar), 3.41 (1H, s, NH-C=S), 2.84 (1H, d, H-*ipr*, *J* = 5.2 Hz), 1.64-1.48 (1H, m, (CH-$(CH_3)_2$), 1.24 (6H, d, (CH-$(CH_3)_2$, *J* = 5.6 Hz); ^{13}C-NMR (300 MHz, DMSO-d_6, δ/ppm): 166.8 (C_{20} of C=S), 159.8 (C_{15} of C=O), 158.2 (C_4 of HN-C=O), 152.4 (C_6 of NH_2), 146.2, 142.4, 136.2, 128.2, 126.8, 126.4, 124.6, 126.2, 118.6, 67.4, 65.2 (C_{16} of NH), 64.2, 61.2, 32.4, 19.4, 18.5; MS (m/z): 459 M^+.

2-((2-Amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl2-(3-(4-fluorophenyl)thioureido)-3-methylbutanoate ***(3b):*** Yield: 80%; m.p. 150-152 °C; Anal. Calcd. for $C_{20}H_{24}N_7O_4S$: C, 50.31; H, 5.07; N, 20.53. Found C, 50.25, H, 5.01; N, 20.48; IR (KBr, cm^{-1}): 3416 (N-H), 3118 (NH_2), 1188 (C=S), 728 (C-S); ^{1}H-NMR (300 MHz, DMSO-d_6, δ/ppm): 11.2 (1H, s, N-H), 8.12 (1H, s, Ar-H), 8.02 (2H, s, NH_2), 7.62-6.92 (m, 4H, Ar-H), 5.12 (2H, *t*, CH_2), 4.96 (2H, *t*, CH_2), 4.32 (2H, *s*, CH_2), 4.16 (1H, s, NH-Ar), 3.21 (1H, s, NH-C=S), 2.04 (1H, d, H-ip^r, *J* = 4.6 Hz), 1.92-1.82 (1H, m, (CH-$(CH_3)_2$), 1.12 (6H, d, (CH-$(CH_3)_2$, *J* = 5.2 Hz), ^{13}C-NMR (300 MHz, DMSO-d_6, δ/ppm): 168.2 (C_{20} of C=S), 159.2 (C_{15} of C=O), 156.2 (C_4 of HN-C=O), 153.8 (C_6 of NH_2), 144.2, 139.6, 135.4, 129.2, 128.6, 127.5, 127.2, 125.4, 116.4, 66.8, 65.7, 65.4(C_{16} of NH), 60.6, 33.6, 19.6, 19.2; MS (m/z): 477 M^+.

2-((2-Amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl2-(3-(4-chlorophenyl)thioureido)-3-methylbutanoate ***(3c):*** Yield: 79%; m.p. 148-150 °C; Anal. Calcd. for $C_{20}H_{24}ClN_7O_4S$: C, 48.63; H, 4.90; N, 19.85, Found C, 48.71, H, 4.83; N, 19.68; IR (KBr, cm^{-1}): 3422 (N-H), 3130 (NH_2), 1194 (C=S), 732 (C-S); ^{1}H-NMR (300 MHz DMSO-d_6, δ/ppm): 11.6 (1H, s, N-H), 8.32 (1H, s, Ar-H), 7.64-6.82 (m, 4H, Ar-H), 7.54 (2H, s, NH_2), 5.24 (2H, *t*, CH_2), 4.82 (2H, *t*, CH_2), 4.32 (1H, s, NH-Ar), 4.28 (2H, *s*, CH_2), 4.2 (1H, s, NH-C=S), 2.42 (1H, d, H-ip^r , *J* =4.6 Hz), 1.42-1.34 (1H, (CH-$(CH_3)_2$), 1.22 (6H, d, (CH-$(CH_3)_2$, *J* = 5.4 Hz); ^{13}C NMR (300 MHz DMSO-d_6, δ/ppm): 174.6 (C_{20} of C=S), 171.6 (C_{15} of C=O), 154.2 (C_6 of NH_2), 152.5 (C_4 of HN-C=O), 145.6, 141.5, 135.4, 129.2, 128.6, 127.5, 127.2, 125.4, 118.2, 69.2, 67.2 (C_{16} of NH), 66.4, 61.5, 31.4, 19.7, 19.4; MS (m/z): 493 M^+.

2-((2-Amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl3-methyl-2-(3-(4-nitrophenyl)thioureido) butanoate ***(3d):*** Yield: 78%; m.p. 128-130 °C; Anal. Calcd. for $C_{20}H_{24}N_8O_6S$: C, 47.61; H, 4.79; N, 22.21, Found C, 47.56, H, 4.75; N, 22.17; IR (KBr, cm^{-1}): 3428 (N-H), 3116 (NH_2), 1206 (C=S), 738 (C-S); ^{1}H-NMR (300 MHz, DMSO-d_6, δ/ppm): 11.4 (1H, s, N-H), 8.42 (1H, s, Ar-H), 7.72 (2H, *s*, NH_2), 7.52-6.88 (m, 4H, Ar-H), 5.18 (2H, *t*, CH_2), 4.64 (2H, *t*, CH_2), 4.62 (1H, s, NH-C=S), 4.56 (1H, s, NH-Ar), 4.32 (2H, *s*, CH_2), 2.26 (1H, d, H-ip^r, *J* = 4.6 Hz), 1.42-1.26 (1H, m, (CH-$(CH_3)_2$), 1.08 (6H, d, (CH-$(CH_3)_2$ *J* = 5.2 Hz), ^{13}C-NMR (300 MHz DMSO-d_6, δ/ppm): 182.4 (C_{20} of C=S), 172.4 (C_{15} of C=O), 155.4 (C_6 of NH_2), 151.6 (C_4 of HN-C=O), 146.2, 140.4, 136.2, 128.7, 128.4, 128.2, 128.0, 127.4, 117.4, 68.6, 67.2, 66.2 (C_{16} of NH), 63.7, 30.6, 19.5, 19.2; MS (m/z): 504 M^+.

2-((2-Amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl2-(3-(3-bromophenyl)thioureido)-3-methyl butanoate ***(3e):*** Yield: 76%; m.p. 110-112 °C; Anal. Calcd. for $C_{20}H_{24}N_8O_6S$: C, 44.61; H, 4.49; N, 18.21, Found C, 44.51, H, 4.36; N, 18.32; IR (KBr, cm^{-1}): 3426 (N-H), 3112 (NH_2), 1198 (C=S), 720 (C-S); ^{1}H-NMR (300 MHz DMSO-d_6, δ/ppm): 10.9 (1H, s, N-

H), 8.28 (1H, s, Ar-H), 7.84 (2H, *s*, NH_2), 7.32-6.94 (m, 4H, Ar-H), 5.24 (2H, *t*, CH_2), 4.82 (1H, s, NH-C=S), 4.46 (2H, *t*, CH_2), 4.32 (1H, s, NH-Ar), 4.12 (2H, *s*, CH_2), 2.12 (1H, d, H-ip^r, *J* =5.8 Hz), 1.42-1.24 (1H, m, (CH-(CH_3)$_2$), 1.14 (6H, d, (CH-(CH_3)$_2$, *J* = 6.4 Hz); ^{13}C-NMR (300 MHz DMSO-d_6, δ/ppm): 172.8 (C_{20} of C=S), 171.6 (C_{15} of C=O), 157.2 (C_6 of NH_2), 152.4 (C_4 of HN-C=O), 147.6, 138.6, 137.4, 127.6, 127.2, 126.8, 126.4, 124.6, 116.8, 69.2, 68.4, 65.4 (C_{16} of NH), 65.2, 31.2, 18.9, 18.6; MS (m/z): 537 M^+.

2-((2-Amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl2-(3-(4-bromophenyl)ureido)-3-methyl butanoate ***(3f):*** Yield: 77%; m.p. 118-120 °C; Anal. Calcd. for $C_{20}H_{24}BrN_7O_5$: C, 45.99; H, 4.63; N, 15.30, Found C, 45.86, H, 4.52; N, 15.26; IR (KBr, cm^{-1}): 3432 (N-H), 3109 (NH_2), 1656 (C=O), 1070 (C-O); ^{1}H-NMR (300 MHz, DMSO-d_6, δ/ppm): 11.2 (1H, s, N-H), 8.36 (1H, s, Ar-H), 7.62 (2H, *s*, NH_2), 7.46-6.87 (m, 4H, Ar-H), 5.12 (2H, *t*, CH_2), 4.64 (1H, NH-C=S), 4.32 (2H, *t*, CH_2), 4.26 (1H, s, NH-Ar), 4.06 (2H, *s*, CH_2), 2.24 (1H, d, H-ip^r, *J* = 6.4 Hz), 1.36- 1.24 (1H, m, (CH-(CH_3)$_2$), 1.22 (6H, d, (CH-(CH_3)$_2$, *J* = 4.6 Hz); ^{13}C-NMR (300 MHz, DMSO-d_6, δ/ppm): 169.2 (C_{15} of C=O), 158.4 (C_6 of NH_2), 151.6 (C_4 of HN-C=O), 148.4 (C_{20} of C=O), 145.8, 137.5, 136.2, 128.2, 127.9, 127.6, 126.8, 123.8, 118.2, 69.5, 68.4, 66.5 (C_{16} of NH), 64.8, 30.6, 19.4, 18.8; MS (m/z): 522 M^+.

2-((2-Amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl2-(3-(4-fluorophenyl)ureido)-3-methyl butanoate ***(3g):*** Yield: 81%; m.p. 106-108 °C; Anal. Calcd. for $C_{20}H_{24}BrN_7O_5$: C, 52.06; H, 5.24; N, 21.25, Found C, 52.01, H, 5.17; N, 21.18; IR (KBr, cm^{-1}): 3440 (N-H), 3152 (NH_2), 1648 (C=O), 1035 (C-O); ^{1}H-NMR (300 MHz, DMSO-d_6, δ/ppm): 12.4 (1H, s, N-H), 8.02 (1H, s, Ar-H), 7.92-6.74 (m, 4H, Ar-H), 7.76 (2H, *s*, NH_2), 5.64 (2H, *t*, CH_2), 4.86 (1H, NH-C=S), 4.36 (1H, NH-Ar),) 4.28 (2H, *t*, CH_2), 4.12 (2H, *s*, CH_2), 2.12 (1H, d, H-ip^r, *J* = 5.6 Hz), 1.82-1.64 (1H, m, (CH-(CH_3)$_2$), 1.38 (6H, d, (CH-(CH_3)$_2$, *J* = 6.8); ^{13}C-NMR (300 MHz, DMSO-d_6, δ/ppm): 170.4 (C_{15} of C=O), 157.2 (C_6 of NH_2), 153.4 (C4 of HN-C=O), 152.6 (C_{20} of C=O), 146.4, 139.8, 139.4, 127.2, 126.6, 125.8, 125.2, 124.6, 117.5, 68.2, 67.6, 67.2, 65.2, (C_{16} of NH), 31.6, 19.8, 19.4; MS (m/z) : 461 M^+.

2-((2-Amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl2-(3-(3,4-dichlorophenyl)ureido)-3-methylbutanoate ***(3h):*** Yield: 75%; m.p. 102-104 °C; Anal. Calcd. For $C_{20}H_{23}Cl_2N_7O_5$: C, 46.89; H, 4.52; N, 19.14, Found C, 46.80, H, 4.46; N, 19.10; IR (KBr, cm^{-1}): 3438 (N-H), 3112 (NH_2), 1652 (C=O), 1074 (C-O); ^{1}H-NMR (300 MHz, DMSO-d_6, δ/ppm): 12.2 (1H, s, N-H), 8.12 (1H, s, Ar-H), 7.94-6.84 (m, 3H, Ar-H), 7.62 (2H, *s*, NH_2), 5.52 (2H, *t*, CH_2), 4.74 (1H, s, NH-C=S), 4.32 (2H, *t*, CH_2), 4.28 (1H, s, NH-Ar), 4.14 (2H, *s*, CH_2), 2.22 (1H, d, H-ip^r, *J* = 5.6 Hz), 1.86-1.64 (1H, m, (CH-(CH_3)$_2$), 1.26 (6H, d, (CH-(CH_3)$_2$, *J* = 6.2 Hz); ^{13}C-NMR (300 MHz, DMSO-d_6, δ/ppm): 171.5 (C_{15} of C=O), 158.4 (C_6 of NH_2), 155.6 (C_4 of C=O), 150.8 (C_{20} of C=O), 148.2, 138.2, 136.8, 127.4, 126.8, 126.6, 126.2, 125.8, 118.2, 69.2, 67.4, 66.4 (C_{16} of NH), 62.6, 19.6, 32.4, 19.2; MS (m/z): 512 M^+.

2-((2-Amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl2-(3-(3-chloro-4-fluorophenyl)ureido)-3-methylbutanoate ***(3i):*** Yield: 72%; m.p. 100-102 °C; Anal. Calcd. For $C_{20}H_{23}ClFN_7O_5$: C, 48.44; H, 4.67; N, 19.77, Found C, 48.39, H, 4.58; N, 19.72; IR (KBr, cm^{-1}): 3442 (N-H), 3132 (NH_2), 1668 (C=O), 1035 (C-O); ^{1}H-NMR (300 MHz, DMSO-d_6, δ/ppm): 11.9 (1H, s, N-H), 8.24 (1H, s, Ar-H), 7.86-6.92 (m, 3H, Ar-H), 7.74 (2H, *s*, NH_2), 5.42 (2H, *t*, CH_2), 4.62 (1H, s, NH-C=S), 4.46 (2H, *t*, CH_2), 4.16 (1H, s, NH-Ar), 4.24 (2H, *s*, CH_2), 2.34 (1H, d, H-ip^r, *J* = 5.7 Hz), 1.91-1.74 (1H, (CH-(CH_3)$_2$), 1.34 (6H, d, (CH-(CH_3)$_2$, *J* = 5.2 Hz); ^{13}C-NMR (DMSO-d_6, δ/ppm): 172.4 (C_{15} of C=O), 159.6 (C_6 of NH_2), 156.2 (C_4 of HN-C=O), 152.6 (C_{20} of C=O), 149.5, 139.7, 139.2, 128.5, 127.7, 126.8, 126.2, 125.2, 119.4, 69.6, 68.2, 67.6 (C_{16} of NH), 63.7, 31.8, 20.2, 19.8; MS (m/z): 496 M^+.

2-((2-Amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl2-(3-(4-chloro-3-(trifluoromethyl) phenyl) ureido)-3-methylbutanoate ***(3j):*** Yield: 74%; m.p. 122-124 ^{o}C; Anal. Calcd. For $C_{21}H_{23}ClF_3N_7O_5$: C, 46.20; H, 4.25; N, 17.96, Found C, 46.14, H, 4.18; N, 17.89; IR (KBr, cm^{-1}): 3448 (N-H), 3142 (NH_2), 1672 (C=O), 1053 (C-O); ^{1}H-NMR (300 MHz, DMSO-d_6, δ/ppm): 12.2 (1H, s, N-H), 8.22 (1H, s, Ar-H), 7.92-6.74 (m, 3H, Ar-H), 7.58 (2H, *s*, NH_2), 5.32 (2H, *t*, CH_2), 4.56 (1H, s, NH-C=S), 4.54 (2H, *t*, CH_2), 4.36 (2H, *s*, CH_2), 4.28 (1H, s, NH-Ar), 2.28 (1H, d, H-*ip*^{r}, *J* = 4.4Hz), 1.84-1.62 (1H, m, (CH-$(CH_3)_2$), 1.26 (6H, d, (CH-$(CH_3)_2$, *J* = 6.6 Hz), ^{13}C-NMR (300 MHz, DMSO-d_6, δ/ppm): 171.6 (C_{15} of C=O), 157.6 (C_4 of HNC=O), 156.4 (C_6 of NH_2), 151.2 (C_{20} of C=O), 148.8, 140.8, 139.4, 128.2, 127.9, 126.5, 125.8, 124.8, 118.2, 68.6, 67.4, 66.4 (C_{16} of NH), 62.6, 30.4, 19.8, 19.2; MS (m/z): 545 M^{+}.

Antiviral bioassay

Purification of Tobacco mosaic virus (TMV). Using Gooding's method[20], upper leaves of Nicotiana tobacum L inoculated with TMV were selected and ground in phosphate buffer, then filtered through a double layer pledget. The filtrate was centrifuged at 10,000 g, treated twice with PEG 400 and centrifuged again. The whole experiment was carried out at 4 °C. Absorbance values were estimated at 260 nm using an ultraviolet spectrophotometer.

$$Virus\ Concentration = \frac{A260\ X\ dilution\ ratio}{E1cm\ (0.1\%, 260\,nm)} \times 100$$

Curative effect of compounds against TMV in vivo

Growing leaves of Nicotiana tobacum L of the same ages were selected. TMV (concentration of 6×10^{-3} mg/mL) was dipped and inoculated on the whole leaves, then the leaves were washed with water and dried. The compound solution was smeared on the left side and the solvent was smeared on the right side for control. The local lesion numbers were then counted and recorded 3-4 days after inoculation[21]. For each compound, three repetitions were measured. The inhibition rate of the compound was then calculated according to the appropriate formula ('av' means average).

Antioxidant activity

Antioxidant activity was evaluated by three methods, DPPH, Super Oxide radical scavenging activities and GST method. Scavenging capacity was measured spectrophotometrically by monitoring the decrease in absorbance at 517 nm.

DPPH radical-scavenging activity

The DPPH radical scavenging activity was measured in a reaction mixture containing 1 mM DPPH radical solution 0.1 mL, 99% ethanol 0.8 mL and 0.1 mL of each one of the title compounds prepared by dissolving the compound in methanol. The solution was rapidly mixed and scavenging capacity was measured spectrophotometrically by monitoring the decrease in absorbance at 517 nm[22]. The antioxidant activity of test compounds was expressed as IC_{50}, which was defined as the concentrations of test compounds required for inhibition of the formation of DPPH radicals by 50 %.

$$DPPH\ \ radical\ scavenging\ activity\ (\%) = 1 - \frac{Absorbance\ of\ \ sample\ at\ 517\ nm}{Absorbance\ of\ \ controlat\ 517nm} \times 100$$

Superoxide radical scavenging activity

Superoxide radicals were identified by spectrophotometric method to study the effect of various concentrations of test compounds on the reduction of nitroblue tetrazolium (NBT),

according to a previously described procedure[23]. Superoxide radicals were generated in a non-enzymatic phenazine methosulfate–nicotinamide adenine dinucleotide (PMS/NADH) system. The non-enzymatic generation of superoxide radicals was measured in reaction mixtures containing various concentrations of test compounds, PMS (15 µM), NADH (73 µM), and NBT (50 µM) in phosphate buffer (20 mM, pH 7.4). After incubation for 5 min at ambient temperature, the color was read at 560 nm against blank samples. The superoxide radical-scavenging activity was expressed as the IC_{50} value.

Reactive oxygen species (ROS), such as superoxide anion radical (O_2^-), hydroxyl radicals ($HO^{\cdot}$) and peroxy radicals ($ROO^{\cdot}$) are produced as a part of normal metabolic process. The compounds **3(a-j)** showed high antioxidant activity by scavenging the free radicals and superoxide radicals and the data are given in Table II.

GST method

Glutathione-S-transferases (GSTs,' EC 2.5.1.18) are a group of multifunctional proteins that exist in complicated but distinct isozyme forms in various plant tissues. Different isozymes from a given tissue can be distinguished by their catalytic and immunological properties and by their primary structure. The GSTs are well known for their role in the first step of mercapturic acid formation. They catalyze the nucleophilic attack of GSH on electrophilic centers in a wide variety of organic compounds. However, in recent years, the glutathione (GSH) peroxidase activity associated with certain forms of GSTs has attracted greater attention.

GST activity in fresh leaves (control) extract was assessed spectrophotometrically according to the method of Habig and others[24] using GSH (2.4 mmol/L) and CDNB (1mmol/L) as substrate. Assay was initiated with 50 µL enzyme in 0.5 mL phosphate buffer (0.1 mol/L), pH 6.5, at 25°C. One unit of enzyme activity is defined as the amount of enzyme catalyzing the oxidation of 1µmol of substrate (CDNB)/ mL /min at 25°C. The above method is followed to estimate GST activity in viral affected tobacco leaves and to estimate the GSH level, homogenate was prepared from tobacco leaves and centrifuged at 9,615 g for 30 minutes. An equal volume of 5% perchloric acid was mixed with supernatant extract and centrifuged at 805 g for 10 minutes at 4°C. The reaction mixture (2 mL), 100 µL supernatant, 1.88 mL of 0.1 mol/L potassium phosphate buffer, pH 8.0 and 0.02 mL 4% DTNB were mixed. Incubation of the reaction mixture was carried out at room temperature for 3 minutes, and absorbance of the developed color was recorded at 517 nm[25]. Distilled water was used as a blank. A standard graph was prepared using GSH. All experiments were set up in duplicate and the results were expressed as the mean of two independent trials. Each treatment group consisted of 5 leaves, and the mean value of each data was compared with the mean value of a control group of equal size. The significant values were calculated using the student t- test.

RESULTS AND DISCUSSION

Chemistry

The synthesis of title compounds was accomplished by reacting 2-((6-amino-4-oxo-4, 5-dihydro-1H-imidazo[4,5-c]pyridin-1-yl)methoxy)ethyl-2-amino-3-ethylbutanoate (valacyclovir) **(1)** with various isocynates/isothiocynates **2(a-j)** and N, N- dimethyl piperazine as a base in THF:py solvent (20 mL) at 60 ^{o}C. The progress of the reaction was monitored by TLC. The resulting **3(a-j)** title compounds were obtained in high yields in 3-5 hrs (Scheme 1). The chemical

structures of the title compounds **3(a-j)** were deduced by IR, NMR (^{1}H, ^{13}C), mass spectral and elemental analysis. IR absorptions were observed in the regions 1183-1206, 1648-1672 and 3412-3448 cm^{-1}, C=**S**, C=**O**, and N-**H** respectively for **3(a-j).** The ^{1}H NMR spectra exhibited broad signals for NH protons at 10.8-12.4 ppm. ^{13}C NMR chemical shifts were observed in the regions, δ 166.8-182.4 for C=**S** and δ 148.4-152.6 for C=**O**.

Antiviral activity

The newly synthesized derivatives **3(a-j)** were screened for their antiviral activity against *Tobacco mosaic virus* (TMV) by the Goodings method[20]. The bioassay results obtained at 50 µg/mL using valacyclovir as the control are presented in Table I. It is clear that the title compounds **3(a-j)** showed high antiviral activities against *Tobacco mosaic virus*. Among the compounds, **3a** and **3e** derivatives bearing thiourea group exhibited high TMV inhibition.

Antioxidant activity

The title compounds exhibited good anti-oxidant activity as it was performed by using three methods DPPH, SOD and GST. The antioxidant activity of title compounds **(3a-j)** exhibited high results in DPPH method and reliable results were observed in SOD and GST methods. Among the title compounds **3(a-j),** the **3e, 3a** exhibited high antioxidant values in the three methods, 81.68±1.12 (DPPH), 76.19±1.37 (SOD), 76.02±1.95 (GST) and 79.47±1.34 (DPPH),75.07±1.73 (SOD) and 74.08±1.13 (GST) respectively, when compared to Ascorbic acid. The compounds **3f, 3i** exhibited next higher antioxidant values, 78.02±1.45 (DPPH), 73.15±1.15 (SOD), 74.64±1.09 (GST) and 76.84±1.08 (DPPH), 72.07±1.24 (SOD), 70.82±1.73 (GST) when compared to standard. The title compounds **(3a, 3e) and (3f, 3i)** derivatives bearing thiourea and urea groups respectively exhibited high antioxidant activity and remaining title compounds showed good to moderate results. The antioxidant activity of the title compounds was compared to the standard reference ascorbate.

CONCLUSION

Synthesis of valacyclovir 2-((6-amino-4-oxo-4,5-dihydro-1H-imidazo[4,5-c]pyridin-1-yl)methoxy)ethyl-2-amino-3-ethylbutanoate **1** derivatives of thiourea and urea was accomplished by reacting various aromatic isocyanates/thiocyanates **2** in the presence of N,N-dimethyl piperazine as a base in high yields (72-82%) and in short reaction times. The title compounds **3(a-j)** exhibited good antiviral and promising antioxidant activities.

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И З В О Д

СИНТЕЗА И БИОЛОШКА АКТИВНОСТ УРЕА И ТИОУРЕА ДЕРИВАТА ВАЛАЦИКЛОВИРА

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Синтетисана је серија уреа и тиоуреа деривата валацикловира и испитивана је њихова активност. 2-((6-амино-4-оксо-4,5-дихидро-1H-имидазо[4,5-c]пиридин-1-ил)метокси)етил-2-амино-3-етилбутаноат (валацикловир) је реаговао са различитим ароматичним изоцијанатима/тиоцијанатима у присуству H,H-диметил пиперазина дајући уреа/тиоуреа деривате валациколовира. Структуре добијених једињења су утврђене методама IR, NMR (^{1}H, ^{13}C), MS и елементалном анализом. За новосинтетисана једињења испитана је активност спрам вируса Tobacco mosaic (TMV), као и антиоксидативна активност методама DPPH, SOD и GST. Сва једињења су испољила значајну антивирусну и антиоксидативну активност.

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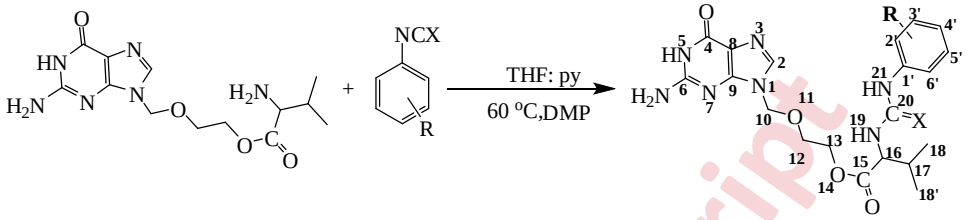
Table I: Viral inhibitory activity of title compounds **3(a-j)** against TMV.

Compound No.	Concentration (µg/mL)	Inhibition rate
3a	0.5	87.25± 0.06
3b	0.5	84.42±0.12
3c	0.5	82.31±0.14
3d	0.5	79.11±0.17
3e	0.5	89.24±0.05
3f	0.5	83.25±0.08
3g	0.5	80.36±0.13
3h	0.5	78.12±0.09
3i	0.5	85.42±0.07
3j	0.5	77.21±0.015
Valacyclovir (positive control)	0.5	91.32±0.05

Table II: Antioxidant activities of the title compounds **3(a-j).**

Entry	DPPH	GST	SOD
3a	79.47±1.34	74.08±1.13	75.07±1.73
3b	72.34±1.36	70.03±1.08	71.12±1.55
3c	73.13±1.21	71.05±1.14	70.54±1.57
3d	75.48±1.84	72.06±1.03	71.02±1.86
3e	81.68±1.12	76.02±1.95	76.19±1.37
3f	78.02±1.45	73.15±1.15	74.64±1.09
3g	74.87±1.15	69.14±1.16	72.45±1.64
3h	71.94±1.73	68.07±1.07	69.08±1.88
3i	76.84±1.08	72.07±1.24	70.82±1.73
3j	70.69±1.58	67.08±1.09	68.56±1.01
Ascorbic acid	83.42 ± 1.65	77.05±1.05	78.51± 1.43

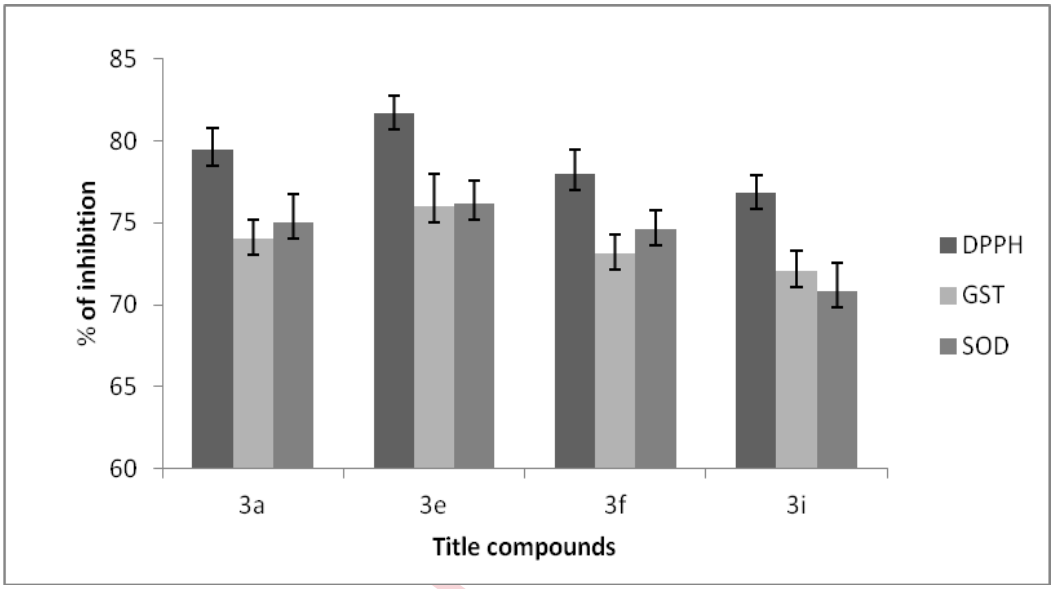
Scheme 1: Synthesis of urea and thiourea derivatives of valacyclovir **3(a-j).**



1 **2** DMP- 1,4 Dimethylpiperzine **3(a-j)**

Compound	R	X
3a	H	S
3b	4-F	S
3c	4-Cl	S
3d	4-NO_2	S
3e	3-Br	S
3f	4-Br	O
3g	4-F	O
3h	3-Cl, 4-Cl	O
3i	3-Cl, 4-F	O
3j	3-CF_3, 4-Cl	O

Graph 1: The potent antioxidant activity of the compounds **3a**, **3e, 3f** and **3i** with standard deviation.



Caption for Tables

Table I: Viral inhibitory activity of title compounds **3(a-j)** against TMV.

Table II: Antioxidant activities of the title compounds **3(a-j).**

Caption for Scheme

Scheme 1: Synthesis of urea and thiourea derivatives of valacyclovir **3(a-j).**

Caption for graph

Graph 1: The potent antioxidant activity of the compounds **3a**, **3e, 3f** and **3i** with standard deviation.