



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

VENKATA RAMANA KATLA¹, RASHEED SYED¹, MADHAVA GOLLA¹,
ADAM SHAIK² and NAGA RAJU CHAMARTHI^{1*}

¹Department of Chemistry, Sri Venkateswara University, Tirupati - 517 502, India and

²Department of Biochemistry, Sree Vidyaniketan P.G College, Tirupati - 517 502, India

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Abstract: Series of novel urea and thiourea derivatives of valaciclovir were efficiently synthesized in high yields and their antiviral activity was evaluated. (S)-2-[(2-amino-6-oxo-6,9-dihydro-3H-purin-9-yl)methoxy]ethyl 2-amino-3-methylbutanoate (valaciclovir) (**1**) was reacted with various aromatic isocyanates/thiocyanates **2a–j** in the presence of *N,N'*-dimethyl piperazine as a base in THF:pyridine (4:1) to obtain the valaciclovir urea/thiourea derivatives **3a–j**. The structures of the title compounds (**3a–j**) were confirmed by their IR, NMR (¹H and ¹³C) mass spectral data and elemental analysis. The newly synthesized compounds were screened for their antiviral activity against Tobacco mosaic virus (TMV) and antioxidant activity was evaluated by the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and superoxide dismutase (SOD) scavenging methods. The title compounds exhibited potent antiviral and good antioxidant activities.

Keywords: isocyanate; isothiocyanate; *N,N'*-dimethylpiperazine; tobacco mosaic virus; antiviral activity; antioxidant activity.

INTRODUCTION

Urea and thiourea are important functional groups in numerous natural products and drug intermediates, and are used as neutral receptor for various anions (anion complexation),¹ and building blocks for various heterocycles. Urea and thiourea derivatives possess many promising biological activities, such as herbicidal,² antimicrobial,³ antioxidant,⁴ antiviral⁵, anti-HIV⁶ and antitumor⁷ activity, while urea derivatives exhibit anti-inflammatory,⁸ antimalarial⁹ and antidiabetic activities.¹⁰ Thiourea and urea derivatives have been used as purification agents for organic and inorganic effluents, industrial, agricultural and mining wastes,¹¹ spinning mixtures, paper and paints, as well as wrinkle proofing agents for cotton and cotton polyester fabrics.^{12,13} These compounds could also be used for the

*Corresponding author. E-mail: rajuchamarthi10@gmail.com
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detoxification of super antigens from body fluids¹⁴ and for the treatment of hemoglobinopathies 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 a therapeutic agent in the treatment of tuberculosis.¹⁷ Thiourea inhibitors of plant viruses have also given rise to widespread interest in both the biological and chemical sectors.¹⁸

Valaciclovir is a prodrug 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 antivirals are used for specific viruses. Unlike most antibiotics, antiviral drugs do not destroy their target pathogen; instead, they inhibit their development. Hence, the design of a safe and effective drug requires extended knowledge of the genetic and molecular functions of organisms. Hence, researchers have been focusing on the development of effective antiviral drugs by embedding effective pharmacophores into origin drugs or on the understanding of the structure and function of viruses to find new drugs. In recent years, the impact of climate anomalies and the areas of crops affected by plant virus diseases 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 ongoing research work, novel urea and thiourea derivatives of valaciclovir have been designed, synthesized and tested against tobacco mosaic virus and antioxidant activity.

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}). ^1H - and ^{13}C -NMR spectra were recorded in $\text{DMSO}-d_6$ on a Bruker Avance III 500 MHz spectrometer operating at 500 MHz for ^1H -, 125 MHz for ^{13}C -NMR. The ^1H - 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.

Analytic and spectral data for compounds **3a–j** are given in the Supplementary material to this paper.

General procedure for synthesis of title compounds 3a–j

(*S*)-2-[(2-Amino-6-oxo-6,9-dihydro-3*H*-purin-9-yl)methoxy]ethyl 2-amino-3-methylbutanoate (valaciclovir) **1** (0.001 mol), various aromatic isocyanates/thiocyanates **2a–j** (0.001 mol) were dissolved in dry THF:pyridine (20 mL) and refluxed under stirring for 3–5 h at

about 60 °C. Identification of the product and completion of the reaction was monitored by TLC using ethyl acetate:hexane (4:1) as an eluent. After completion of the reaction, the mixture was concentrated on a rotary-evaporator and the residue was purified by column chromatography on silica gel (100–200 mesh) using petroleum ether:ethyl acetate (2:3, boiling range: 40–60 °C) as an eluent. The structures of the title compounds **3a–j** were established by spectral and elemental analysis. The obtained yields of **3a–j** were in the range 72–82 %.

Antiviral bioassay

Purification of tobacco mosaic virus (TMV). Using the Gooding method,²⁰ the upper leaves of *Nicotiana tabacum* L. inoculated with TMV were selected and ground in phosphate buffer, 6×10^{-3} mg/mL, pH 7.4, then filtered through a double layer pledget. The filtrate was centrifuged at 10000 g, treated twice with poly(ethylene glycol) 400 (PEG 400) and centrifuged. The whole procedure was performed at 4 °C. The absorbance values, A_{260} , were estimated at 260 nm using an ultraviolet spectrophotometer. The virus concentration is given:

$$\text{Virus concentration} = \frac{A_{260} \times \text{dilution ratio}}{\epsilon_{0.1\%, 260\text{ nm}} \times 1\text{ cm}} \times 100 \quad (1)$$

Curative effect of the compounds against TMV in vivo. Growing leaves of *Nicotiana tabacum* L. of the same age 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 ($500\text{ }\mu\text{g mL}^{-1}$) was smeared on the left side and the solvent was smeared on the right side for control. The local number of lesions were then counted and recorded 3–4 days after inoculation.²¹ Triplicated experiments were carried out for each compound and average value was taken as final result. The inhibition rate of the compound was then calculated according to the formula:

$$\text{Inhibition rate} = 100(x-y)/x \quad (2)$$

where x = average local lesion no. of control and y = average local lesion no. of tested sample.

Antioxidant activity

The anti-oxidant activities of the synthesized compounds were evaluated 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide radical scavenging activity methods.

DPPH radical-scavenging activity. The DPPH radical scavenging activity was measured in a reaction mixture containing 0.2 mL of DPPH (1 mM) solution, 0.8 mL of methanol (99 %) and 2 mL of tested solutions ($100\text{ }\mu\text{g/mL}$). The solution was rapidly mixed and scavenging capacity was calculated by observing the decrease in absorbance at 517 nm of the reaction mixture after half an hour at ambient temperature.²² The percentage of DPPH radical scavenging activity was calculated by the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{A_{517(\text{sample})}}{A_{517(\text{control})}}\right) \times 100 \quad (3)$$

Ascorbic acid was used as a standard for comparison of the activity. The experiments were repeated in triplicate and the average value was taken as the final result.

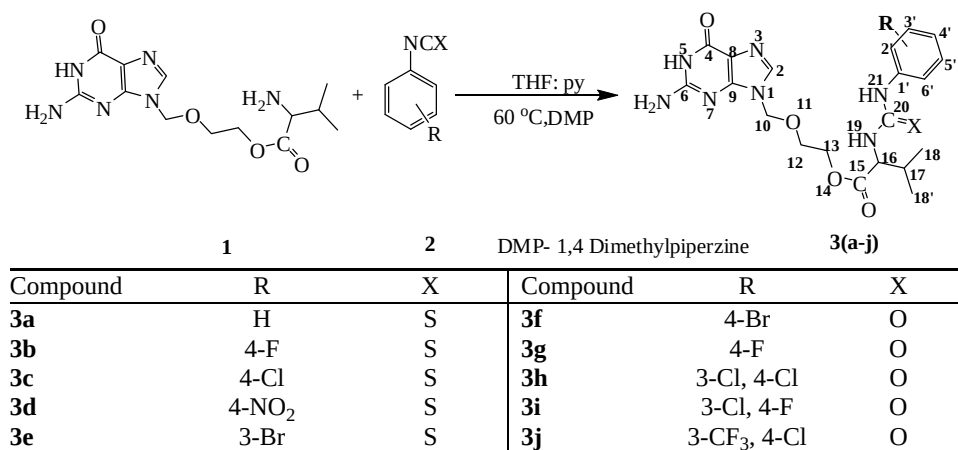
Superoxide radical scavenging activity (SRSA). Superoxide radicals were identified by a spectrophotometric method to study the effect of various concentrations of the test compounds on the reduction of nitroblue tetrazolium (NBT), according to a previously described procedure.²³ The superoxide radicals 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 3 mL of 100 $\mu\text{g mL}^{-1}$ solution of test compounds, 400 μL of PMS (15 μM), 1 mL of NADH (460 μM) and 1 mL of NBT (150 μM) in phosphate buffer (20 mM, pH 7.4), and then incubated for 5 min at ambient temperature. The change in absorbance was read at 560 nm against blank samples. The percentage of superoxide radical scavenging activity was measured using the Eq. (3). Ascorbic acid was used as a standard.

RESULTS AND DISCUSSION

Chemistry

The synthesis of title compounds was accomplished by reacting (S)-2-[(2-amino-6-oxo-6,9-dihydro-3H-purin-9-yl)methoxy]ethyl 2-amino-3-methylbutanoate (valaciclovir, **1**) with various isocyanates/isothiocyanates **2a–j** in the presence of *N,N'*-dimethylpiperazine as a base in THF:pyridine solvent (20 mL) at 60 °C. The progress of the reaction was monitored by TLC. The resulting title compounds **3a–j** were obtained in high yields in 3–5 h (Scheme 1). The chemical structures of the title compounds **3a–j** were deduced by IR, NMR (^1H and ^{13}C), mass spectral and elemental analysis, the results of which are given in the Supplementary material to this paper. IR absorptions bands for **3a–j** were observed in the regions 1183–1206, 1648–1672 and 3412–3448 cm^{-1} , assigned to C=S, C=O and N–H, respectively. The ^1H -NMR spectra exhibited broad signals for NH protons at 10.8–12.4 ppm. ^{13}C -NMR chemical shifts were observed in the δ regions 166.8–182.4 ppm for C=S and 148.4–152.6 for C=O.



Scheme 1. Synthesis of urea and thiourea derivatives of valaciclovir (**3a–j**).

Antiviral activity

The newly synthesized derivatives **3a–j** were screened for their antiviral activity against tobacco mosaic virus (TMV) by the Gooding method.²⁰ The bioassay results obtained at 500 $\mu\text{g mL}^{-1}$ of the synthesized compounds and vala-

ciclovir as the positive control are shown in Table I. It is clear that the title compounds **3a–j** showed high antiviral activities against the tobacco mosaic virus. Among the compounds **3a–j**, thiourea derivative **3a** bind to phenyl ring and **3e** bearing 3-bromophenyl ring exhibited high TMV inhibition.

TABLE I. TMV inhibitory activity of title compounds **3a–j** in concentration of 500 $\mu\text{g mL}^{-1}$

Compound	Inhibition rate, %
3a	87.2 $\pm$ 0.06
3b	84.4 $\pm$ 0.12
3c	82.3 $\pm$ 0.14
3d	79.1 $\pm$ 0.17
3e	89.2 $\pm$ 0.05
3f	83.2 $\pm$ 0.08
3g	80.4 $\pm$ 0.13
3h	78.1 $\pm$ 0.09
3i	85.4 $\pm$ 0.07
3j	77.2 $\pm$ 0.02
Valaciclovir (positive control)	91.3 $\pm$ 0.05

Antioxidant activity

Antioxidant activity of the synthesized compounds **3a–j** was investigated using DPPH and SRSA scavenging activity methods and the percentage of the scavenging activity are shown in Table II. The bio-screening data revealed that all the title compounds showed potent to moderate antioxidant activity in tested methods. In tested methods, valaciclovir thiourea derivative **3a** bearing phenyl ring, **3e** bonded to 3-bromophenyl ring, urea derivative **3f** containing 4-bromophenyl ring and **3i** attached to 3-chloro-4-fluorophenyl ring exhibited promising antioxidant activity quite close to the standard, ascorbic acid. In overall observation of the antioxidant activity of the synthesized compounds, thiourea derivatives showed better activity than urea derivatives.

TABLE II. Antioxidant activities (%) of the title compounds **3(a–j)**

Entry	DPPH	SRSA
3a	79.5 $\pm$ 1.3	75.1 $\pm$ 1.7
3b	72.3 $\pm$ 1.4	71.1 $\pm$ 1.6
3c	73.1 $\pm$ 1.2	70.5 $\pm$ 1.6
3d	75.5 $\pm$ 1.8	71.0 $\pm$ 1.9
3e	81.7 $\pm$ 1.1	76.2 $\pm$ 1.4
3f	78.0 $\pm$ 1.4	74.6 $\pm$ 1.1
3g	74.9 $\pm$ 1.2	72.4 $\pm$ 1.6
3h	71.9 $\pm$ 1.7	69.1 $\pm$ 1.9
3i	76.8 $\pm$ 1.1	70.8 $\pm$ 1.7
3j	70.7 $\pm$ 1.6	68.6 $\pm$ 1.0
Ascorbic acid	83.4 $\pm$ 1.6	78.5 $\pm$ 1.4

CONCLUSIONS

Synthesis of (S)-2-[(2-amino-6-oxo-6,9-dihydro-3H-purin-9-yl)methoxy]ethyl 2-amino-3-methylbutanoate (valaciclovir) derivatives of thiourea and urea was accomplished by reacting various aromatic isocyanates/thiocyanates in the presence of *N,N'*-dimethylpiperazine as a base in high yields (72–82 %) and in short reaction times. The obtained compounds exhibited good antiviral and promising antioxidant activities.

SUPPLEMENTARY MATERIAL

Analytic and spectral data for compounds **3a–j** are available electronically from <http://www.shd.org.rs/JSCS/>, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА И БИОЛОШКА АКТИВНОСТ УРЕА И ТИОУРЕА ДЕРИВАТА
БАЛАЦИКЛОВИРА

VENKATA RAMANA KATLA¹, RASHEED SYED¹, MADHAVA GOLLA¹, ADAM SHAIK² и NAGA RAJU CHAMARTHI¹

¹Department of Chemistry, Sri Venkateswara University, Tirupati - 517 502, India и ²Department of Biochemistry, Sree Vidyaniketan P.G College, Tirupati - 517 502, India

Синтетисана је серија уреа и тиоуреа деривата валацикловира и испитивана је њихова активност. (S)-2-[(2-Амино-6-оксо-6,9-дихидро-3H-пурин-9-ил)метокси]етил-2-амино-3-метилбутаноат (валацикловир) је реаговао са различитим ароматичним изоцијанатима/тиоцијанатима у присуству *N,N'*-диметилпиперазина дајући уреа/тиоуреа деривате валацикловира. Структуре добијених једињења су утврђене методама IR, NMR (¹H и ¹³C), MS и елементалном анализом. За новосинтетисана једињења испитана је активност спрам вируса мозаика дувана (TMV), као и антиоксидативна активност методама ца примрном 1,1-дифенил-2-пикрилхидразил радикала (DPPH) и супероксид-дисмутаза (SOD). Сва једињења су испољила значајну антивирусну и антиоксидативну активност.

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