JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Synthesis and biological screening of novel 2-((2-amino-6-oxo-1H-purin-9 (6H)-yl) methoxy) ethyl substituted benzoate .

Dr.Santhosha S. Poojary[a], Dr. Ranjini. P[b],Dr. Rekha N. D[b], Dr. N.Nanjundaswamy[c], Dr. S.Kusuma devi [c] Prof.Y.B.Basavaraju. and Dr. Devaraju*[a]

[a]*Department of Chemistry, Yuvaraja College, University of Mysore, Mysore-04, Karnataka.*

[b] Maharani Science College for woman, *P.G. Department of Biotechnology, JSS College of Arts, Commerce and Science, Mysore, Govt college Mandya, Karnataka.*

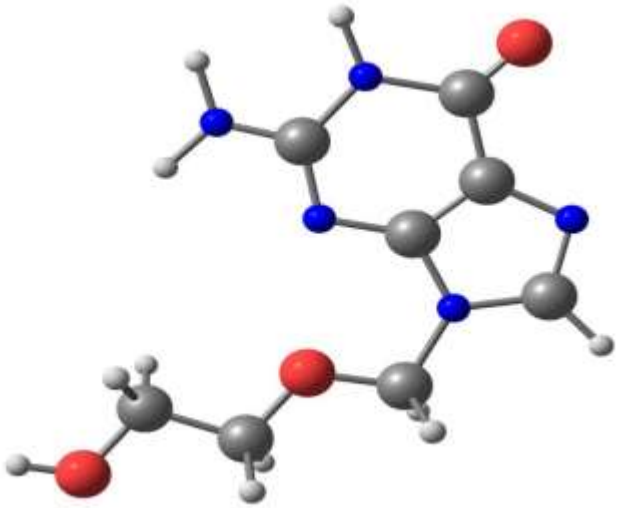
E-mail: santhu_poojary@rediffmail.com, passion49432005@gmail.com. rekha5050@rediffmail.com

Abstract: The current COVID-19 emergency warrants the urgent development of potential strategies to protect people at high risk of infection Series of novel substituted phenyl derivatives of acyclovir were efficiently synthesized in high yields and their biological activity was studied. 2-amino-9-((2-hydroxy ethoxy) methyl)-1H-purin-6(9H)-one (acyclovir) (**1**) was made to react with various substituted phenyl moiety **(2a-j)** in presence of dicyclohexyl carbodiimide and N,N-dimethylamino pyridine using N,N-dimethyl formamide to obtain the acyclovir derivatives **3a**–**j**. The structures of the synthesized compounds (**3a**–**j**) were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data and elemental analysis. The newly synthesized compounds were screened for their antimicrobial, antioxidant activity against acyclovir and antioxidant activity was evaluated by the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging methods. The title compounds exhibited potent antimicrobial and good antioxidant activities.

***Keywords*:** acyclovir, dicyclohexyl carbodiimide, N,N-dimethylamino pyridine, N,N-dimethyl formamide antimicrobial, antioxidant.

1. INTRODUCTION

The outbreak of the novel coronavirus disease, COVID-19, caused by the new coronavirus 2019-nCoV that is now officially designated as severe acute respiratory syndrome-related coronavirus SARS-CoV-2, represents a pandemic threat to global public health. [1-2]. New treatments are needed to reduce the risk of progression of disease. acylovir is an antiviral prodrug that is active against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).



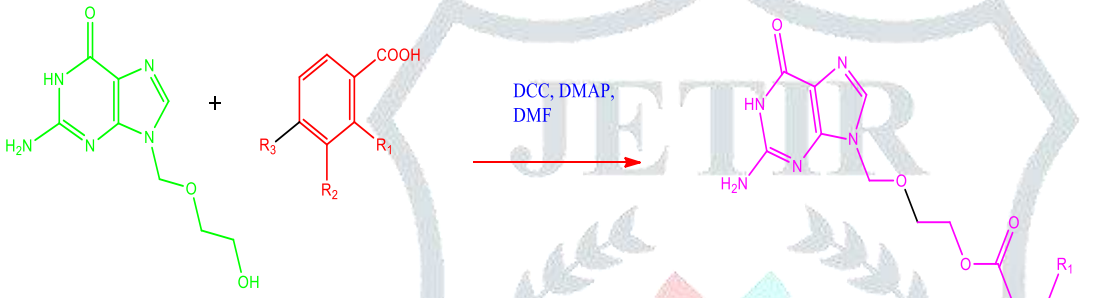
Acyclovir

Substituted phenyl moieties are important groups in numerous natural products and drug intermediates, and these derivatives possess many promising biological activities, such as herbicidal, antimicrobial, antioxidant, antiviral and antitumor, antihypertensive activities. These derivatives have also given rise to widespread interest in both the biological and chemical sectors. Acyclovir is a drug that is used for viral infection. Specific antiviral drugs are used for specific viral infection. 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 pharmocophores into origin drugs or on the understanding of the structure and function of viruses to find new drugs. Regioselective alkylation of guanine is a long lasting challenge. Considering all these facts, we have been interested in synthesizing other derivatives of acyclovir and to study its biological activities other than antiviral activity. In continuation of ongoing research work, novel substituted phenyl derivatives of acyclovir have been designed, synthesized and tested against antimicrobial, antibacterial, antioxidant activities.

2. EXPERIMENTAL

2.1. Materials and methods

The chemicals used for the research work were purchased from Merck and Sigma-Aldrich. 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 were obtained in KBr optics on a Perkin-Elmer model 683 spectrometer and expressed in wave numbers (cm^{-1}). ^{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.



Where 2a: $R_1=R_2=R_3=H$
2b: $R_1=R_2=H$, $R_3=NH_2$
2c: $R_1=R_2=H$, $R_3=Cl$
2d: $R_1=H$, $R_2=F$, $R_3=H$
2e: $R_1=H$, $R_2=OCH_3$, $R_3=H$
2f: $R_1=R_2=H$, $R_3=OH$
2g: $R_1=R_2=Cl$, $R_3=H$
2h: $R_1=R_2=H$, $R_3=CH_3$
2i: $R_1=OCH_3$, $R_2=R_3=H$
2j: $R_1=Cl$, $R_2=R_3=H$

2.2. Synthesis

2.2.1. General procedure for synthesis of title compounds 3a–j

To the cooled solution of various substituted benzoic acid **2a-j** (0.027mol) in DMF (50ml), charged dicyclohexyl carbodiimide (0.0250mol) and stirred for 30min. 2-amino-9-((2-hydroxy ethoxy) methyl)-1H-purin-6(9H)-one (acyclovir) **1** (0.022mol) and N,N-dimethylamino pyridine (0.003mol) were added at 0-5^{0}C. The suspension was stirred for 14-16hr at 0-5^{0}C and completion of the reaction was monitored by TLC using ethyl acetate: methanol (4:1) mobile phase. After completion of the reaction, filtered the dicyclohexyl urea formed during the reaction and filtrate was concentrated on a rotary-evaporator under vacuum. The solid mass

obtained was leached in water at 70-75^{0}C and the wet material was purified by recrystallization in IPA-water mixture (2:1). The structure of the title compounds **3a-j** was confirmed by spectral and elemental analysis.

***2-((2-amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl benzoate (3a)*:** Color: white solid. Yield: 69%. M.P: 198-200^{0}C. IR (KBr,v,Cm^{-1}); 3425Cm^{-1} (-NH); 1750Cm^{-1} (-C=O); 1596Cm^{-1} (aromatic –C=C); ^{1}H NMR(DMSO-400MHz) δ ppm: 3.86-3.90(t, 2H, -OCH$_2$), 4.37-4.42(t, 2H, -CH$_2$OCO), 5.85(s, 2H, -NCH$_2$O), 7.56-8.03(m, 5H, -ArH), 7.97(s, 1H, -NCHN), 8.50(s, 2H, -NH$_2$). ^{13}C NMR (DMSO-100MHz) δ ppm: 64.0, 68.8, 69.2, 117.5, 128.3, 129.9, 130.2, 133.0, 137.2, 140.4, 151.1, 157.2. MS (ESI, m/z): 329.31(M$^+$).

2-((2-amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl 4-aminobenzoate (3b) Color: Yellow solid. Yield: 81.20%. M.P: 204-207^{0}C. IR (KBr,v,Cm^{-1}); 3410Cm^{-1} (-NH); 1750Cm^{-1} (-C=O); 1596Cm^{-1} (aromatic –C=C); ^{1}H NMR(DMSO-400MHz) δ ppm: 3.82-3.91(t, 2H, -OCH$_2$), 4.33-4.40(t, 2H, -CH$_2$OCO), 5.82(s, 2H, -NCH$_2$O), 6.26-6.35(bs, 2H, -NH$_2$Ar), 6.47-7.65(m, 4H, -ArH), 7.95(s, 1H, -NCHN), 8.52(s, 2H, -NH$_2$). ^{13}C NMR (DMSO-100MHz) δ ppm: 64.0, 68.8, 69.2, 114.5, 120.1, 128.3, 129.9, 130.7, 133.0, 137.2, 140.4, 151.1, 157.2. MS (ESI, m/z): 344.14(M$^+$).

2-((2-amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl 4-chlorobenzoate (3c)

Color: white solid. Yield: 72.50%. M.P: 196-198^{0}C. IR (KBr,v,Cm^{-1}); 3415Cm^{-1} (-NH); 1720Cm^{-1} (-C=O); 1591Cm^{-1} (aromatic –C=C); ^{1}H NMR(DMSO-400MHz) δ ppm: 3.82-3.89(t, 2H, -OCH$_2$), 4.33-4.40(t, 2H, -CH$_2$OCO), 5.82(s, 2H, -NCH$_2$O), 7.61-7.83(m, 4H, -ArH), 7.90(s, 1H, -NCHN), 8.52(s, 2H, -NH$_2$). ^{13}C NMR (DMSO-100MHz) δ ppm: 64.0, 68.8, 69.2, 117.5, 128.7, 131.2, 134.0, 138.6, 140.4, 151.6, 157.0. MS (ESI, m/z): 364.07(M$^+$).

2-((2-amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl 3-fluorobenzoate (3d)

Color: white solid. Yield: 74.50%. M.P: 206-208^{0}C. IR (KBr,v,Cm^{-1}); 3425Cm^{-1} (-NH); 1728Cm^{-1} (-C=O); 1594Cm^{-1} (aromatic –C=C); ^{1}H NMR(DMSO-400MHz) δ ppm: 3.81-3.89(t, 2H, -OCH$_2$), 4.31-4.40(t, 2H, -CH$_2$OCO), 5.81(s, 2H, -NCH$_2$O), 7.42-7.83(m, 4H, -ArH), 7.92(s, 1H, -NCHN), 8.48(s, 2H, -NH$_2$). ^{13}C NMR (DMSO-100MHz) δ ppm: 64.2, 68.3, 69.5, 114.4, 119.8, 125.5, 130.3, 137.2, 140.4, 151.1, 157.2. 162.8. MS (ESI, m/z): 347.11(M$^+$).

2-((2-amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl 3-methoxybenzoate (3e) Color: white solid. Yield: 78.0%. M.P: 199-201^{0}C. IR (KBr,v,Cm^{-1}); 3420Cm^{-1} (-NH); 1740Cm^{-1} (-C=O); 1596Cm^{-1} (aromatic –C=C); ^{1}H NMR(DMSO-400MHz) δ ppm: 3.81-3.87(m, 5H, -OCH$_2$, -OCH$_3$), 4.32-4.40(t, 2H, -CH$_2$OCO), 5.83(s, 2H, -NCH$_2$O), 7.21-7.81(m, 4H, -ArH), 7.92(s, 1H, -NCHN), 8.50(s, 2H, -NH$_2$). ^{13}C NMR (DMSO-100MHz) δ ppm: 55.8, 64.3, 68.3, 69.1, 114.3, 122.2, 129.9, 131.2, 140.4, 151.1, 160.2. MS (ESI, m/z): 359.12(M$^+$).

2-((2-amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl 4-hydroxybenzoate (3f) Color: white solid. Yield: 69%. M.P: 198-200^{0}C. IR (KBr,v,Cm^{-1}); 3425Cm^{-1} (-NH); 1750Cm^{-1} (-C=O); 1596Cm^{-1} (aromatic –C=C); ^{1}H NMR(DMSO-400MHz) δ ppm: 3.86-3.90(t, 2H, -OCH_2), 4.37-4.42(t, 2H, -CH_2OCO), 5.33(bs, 1H, -OH), 5.85(s, 2H, -NCH_2O), 6.86-7.89(m, 4H, -ArH), 7.91(s, 1H, -NCHN), 8.48(s, 2H, -NH_2). ^{13}C NMR (DMSO-100MHz) δ ppm: 64.1, 68.2, 69.5, 115.7, 117.2, 122.5, 128.3, 129.9, 130.2, 131.3. 137.2, 140.4, 151.1, 162.80. MS (ESI, m/z): 345.15(M^+).

2-((2-amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl 2,3-dichlorobenzoate (3g) Color: white solid. Yield: 66%. M.P: 194-196^{0}C. IR (KBr,v,Cm^{-1}); 3410Cm^{-1} (-NH); 1710Cm^{-1} (-C=O); 1590Cm^{-1} (aromatic –C=C); ^{1}H NMR(DMSO-400MHz) δ ppm: 3.86-3.90(t, 2H, -OCH_2), 4.37-4.42(t, 2H, -CH_2OCO), 5.85(s, 2H, -NCH_2O), 7.36-8.00(m, 3H, -ArH), 7.90(s, 1H, -NCHN), 8.50(s, 2H, -NH_2). ^{13}C NMR (DMSO-100MHz) δ ppm: 64.0, 68.8, 69.2, 117.5, 128.3, 129.9, 130.2, 133.1. 137.2, 142.4, 151.1, 157.2. MS (ESI, m/z): 398.08(M^+).

2-((2-amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl 4-methylbenzoate (3h) Color: white solid. Yield: 69%. M.P: 198-200^{0}C. IR (KBr,v,Cm^{-1}); 3425Cm^{-1} (-NH); 1750Cm^{-1} (-C=O); 1596Cm^{-1} (aromatic –C=C); ^{1}H NMR(DMSO-400MHz) δ ppm: 2.35(s, 2H, -CH_3Ph), 3.81-3.92(t, 2H, -OCH_2), 4.32-4.40(t, 2H, -CH_2OCO), 5.81(s, 2H, -NCH_2O), 7.34-7.93(m, 4H, -ArH), 7.92(s, 1H, -NCHN), 8.48(s, 2H, -NH_2). ^{13}C NMR (DMSO-100MHz) δ ppm: 21.3, 64.2, 68.5, 69.1, 117.5, 128.9, 129.8, 133.0. 137.2, 140.4, 151.1, 157.0. MS (ESI, m/z): 343.13(M^+).

2-((2-amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl 2-methoxybenzoate (3i) Color: white solid. Yield: 78.0%. M.P: 199-201^{0}C. IR (KBr,v,Cm^{-1}); 3410Cm^{-1} (-NH); 1748Cm^{-1} (-C=O); 1600Cm^{-1} (aromatic –C=C); ^{1}H NMR(DMSO-400MHz) δ ppm: 3.80-3.88(m, 5H, -OCH_2, -OCH_3), 4.31-4.38(t, 2H, -CH_2OCO), 5.81(s, 2H, -NCH_2O), 7.11-7.54(m, 4H, -ArH), 7.90(s, 1H, -NCHN), 8.50(s, 2H, -NH_2). ^{13}C NMR (DMSO-100MHz) δ ppm: 55.5, 64.3, 68.3, 69.1, 114.3, 122.2, 129.9, 131.2, 140.4, 151.1, 160.2. MS (ESI, m/z): 359.12(M^+).

2-((2-amino-6-oxo-1H-purin-9(6H)-yl)methoxy)ethyl 2-chlorobenzoate (3j)

Color: white solid. Yield: 75.00%. M.P: 195-198^{0}C. IR (KBr,v,Cm^{-1}); 3415Cm^{-1} (-NH); 1720Cm^{-1} (-C=O); 1598Cm^{-1} (aromatic –C=C); ^{1}H NMR(DMSO-400MHz) δ ppm: 3.80-3.86(t, 2H, -OCH_2), 4.31-4.42(t, 2H, -CH_2OCO), 5.80(s, 2H, -NCH_2O), 7.44-7.56(m, 4H, -ArH), 7.80(s, 1H, -NCHN), 8.42(s, 2H, -NH_2). ^{13}C NMR (DMSO-100MHz) δ ppm: 64.0, 68.8, 69.2, 117.5, 128.7, 131.2, 134.0, 138.6, 140.4, 151.6, 157.0. MS (ESI, m/z): 364.07(M^+).

2.3. Biological evaluation

2.3.1. Antimicrobial evaluation: An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria and antifungals are used against fungi. They can also be classified according to their function. Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are called biostatic. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis. Based on the principle applied, antimicrobial testing methods are divided into 2 parts. They are dilution (both dilution and agar dilution) and disc diffusion (Stokes method and Kirby- Bauer method) Disc diffusion method is the most commonly used to study the antimicrobial activity.

Paper discs impregnated with the test substances were placed on the surface of the Muller Hinton agar medium inoculated with the target organisms. The plates were incubated and the zones of inhibition around each disc were measured. The synthesized compounds **3(a-j)** were screened for antibacterial and antifungal activity by disc diffusion method against gram- positive bacteria *(Bacilus, Subtilius, Streptococcus),* gram negative bacteria *(Escherichia coli, Proteus)* and against fungus *C.albican.*

MIC (Minimum inhibitory concentration) is the lowest concentration of an antimicrobial compound that inhibits the visible growth of microorganisms after overnight incubation. MIC values can be obtained by a number of standard test procedures. Determination of minimum inhibitory concentration is important in diagnostic laboratories to study the resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents.

Petriplates, DMF, nutrient agar medium and sterile discs were the materials required for the determination of microbial study. The synthesized compounds were screened for their antibacterial activity against Gram-positive bacteria (Bacilus subtilius, Streptococcus) and Gram-negative bacteria (Escherichia coli, Proteus) in DMF by disc diffusion method and agar used as a nutrient medium. Antifungal activity of the synthesized compounds was determined against diploid fungus candida albican. The sterile nutrient agar medium (15ml) in each petridishes was uniformly smeared with Gram-positive and Gram-negative bacteria cultures and fungi. Sterile discs of 10mm diameter (Hi-media) was placed in the petriplates to which different concentrations of drug (20, 40, 80, 100µg/ml) of the synthesized compounds were added. Antibacterial activity of the synthesized compound was compared with Gentamycin used as positive control as well as with Acyclovir. Flucanozole is used as positive control for the comparison of antifungal activity. For each treatment, three replicates were maintained. The plates were incubated at 37^0C for 24hours and the zone of inhibition was determined.

Compounds	Minimum inhibitory concentration (µg/mL)				
	S. aureus	*B.subtilius*	*E.coli*	*Proteus*	*C. albicans*
3a	1.45	1.53	1.39	1.84	2.51
3b	3.80	1.59	1.36	1.36	6.90
3c	1.42	1.62	1.43	1.55	1.39
3d	1.53	2.69	1.30	1.30	1.31
3e	4.20	-	2.95	4.43	2.50
3f	2.84	1.70	1.54	2.52	1.83
3g	1.48	1.54	1.30	1.34	1.35
3h	1.32	1.65	1.43	1.40	1.49
3i	4.54	-	2.41	4.67	2.67
3j	1.41	1.73	1.56	1.43	1.40
Acyclovir	3.80	1.78	2.26	2.82	1.91
Gentamycin	0.80	0.55	0.72	1.3	-
Fluconazole	-	-	-	-	0.75

Table-1: Anti-microbial activities of synthesized compounds 3(a-j).

2.3.2. Antioxidant activity:

The compounds which are capable of either delay or inhibit the oxidation process which occur under the influence of atmospheric oxygen or reactive oxygen species. The synthesized compounds were screened for their anti-oxidant assay using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and nitric oxide scavenging activity methods.

DPPH radical-scavenging activity: An anti-oxidant compound reacts with DPPH radical that can donate proton and get reduced. DPPH when acted upon by an anti-oxidant is converted in to diphenyl picryl hydrazine. This chemical change of DPPH can be identified by its original purple color to light yellow color. This can be quantified spectrophotometrically at 540nm to indicate the extent of DPPH scavenging activity by the synthesized compounds. DPPH solution, ethanol and diluted solutions of synthesized analogues are the materials used for the above purpose. 1ml of DPPH solution (0.1mM, in rectified spirit) was mixed with different concentrations of compounds (20, 40, 80, 100µg/ml), shaken and incubated for 20min at room temperature. The absorbance was recorded at 517nm against a blank solution. The radical scavenging activity was measured as the decrease in the absorbance of DPPH and calculated using the following equation.

DPPH radical scavenging activity (%) =

$$\frac{\text{Absorbance of control} - \text{Absorbance of sample} \quad x \quad 100}{\text{Absorbance of control}}$$

Where,

Absorbance of control = Absorbance of ethanol and DPPH radical.

Absorbance of sample = Absorbance of DPPH radical + sample extract standard.

Compounds	Avg. IC_{50} value (µg/mL)
3a	28.4
3b	27.0
3c	29.6
3d	27.8
3e	39.3
3f	26.8
3g	26.3
3h	25.6
3i	25.1
3j	28.4
Acyclovir	35.3
BHT	42.5

Table-2: Percentage IC_{50} values of 3(a-j) in DPPH radical scavenging activity.

Nitric oxide radical scavenging assay: Sodium nitroprusside used to generate nitric oxide and is measured by Griess reagent. Nitric oxide generated from aqueous solution of sodium nitroprusside interacts with oxygen to produce nitrite ions that can be estimated by Griess reagent. Nitric oxide scavengers compete with oxygen leading to reduced production of nitric oxide. The absorbance of the chromophore in presence of scavengers were studied at 540nm and expressed as percentage reduction of nitric oxide. Sodium nitroprusside (5mM) in phosphate buffer saline was mixed with different concentrations of the compounds (20, 40, 80, 100µg/mL) and incubated at 25^0C for 2hours and were reacted with Griess reagent.

Compounds	Avg. IC_{50} value (µg/mL)
3a	43.3
3b	24.8
3c	27.5
3d	20.5
3e	28.5
3f	33.9
3g	18.6
3h	16.4
3i	43.9
3j	22.5
Acyclovir	36.3
BHT	6.4

Table-3: Percentage IC_{50} values of 3(a-j) in nitric oxide radical scavenging activity.

3.0. RESULTS AND DISCUSSION

3.1. Chemistry

The title compounds was synthesized by reacting 2-amino-9-((2-hydroxy ethoxy) methyl)-1H-purin-6(9H)-one (acyclovir) **1** with various substituted benzoic acids **2a**–**j** in the presence of dicyclohexyl carbodiimide as coupling agent with catalytic amount of N,N-dimethylamino pyridine using N,N-dimethyl formamide as reaction medium at 0-5^{0}C. The progress of the reaction was monitored by TLC. The resulting title compounds **3a**–**j** was obtained in high yields with reaction time of 14-16hr. (Scheme 1). The chemical structures of the title compounds **3a**–**j** were confirmed by IR, ^{1}H NMR, ^{13}C NMR, mass spectral and elemental analysis. IR absorptions bands for **3a**–**j** were observed in the regions 1650–1690 and 3400–3440 cm^{-1}, assigned to C=O and N–H, respectively. The ^{1}H-NMR spectra shows signals for aromatic protons at δ values of 6.80–7.93ppm. ^{13}C-NMR chemical shifts were observed in the *δ* regions 141.4–150.6 for C=O.

4.0. CONCLUSION

The substituted phenyl derivatives of 2-amino-9-((2-hydroxy ethoxy) methyl)-1H-purin-6(9H)-one (acyclovir) was accomplished by reacting various substituted benzoic acids in the presence dicyclohexyl carbodiimide as

coupling agent with catalytic amount of N,N-dimethylamino pyridine using N,N-dimethyl formamide reaction medium in high yields. The title compounds exhibits good antimicrobial and promising antioxidant activities. Synthesized compounds show moderate antimicrobial activity compared to acyclovir and not significant when compared with Gentamycin and Fluconazole. All the compounds shows significant scavenging activity of the DPPH radicals compared to the reference compound BHT. They also show scavenging activity for nitric oxide radicals where scavenging activity was not significant compared to reference compound BHT.

Acknowledgement:

The authors are thankful to Institute of Excellence (IOE), University of Mysore, Mysore for providing spectral data.

REFERENCES.

1. Gorbalenya, A. E. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. **Nature Microbiology 2020**, DOI: 10.1038/s41564-020-0695-z
2. Kupferschmidt, K.; Cohen, J. Will novel virus go pandemic or be contained?. Science **2020**, 367 (6478), 610– 611, DOI: 10.1126/**science**.367.6478.610
3. David W. Kimberlin. Acyclovir Derivatives and Other New Antiviral Agents, **Seminars in Pediatric Infectious Diseases, Vol. 12(13), 224-234.**
4. Estep Kimberly G. et al., Synthesis and Structure-Activity Relationships of 6-Heterocyclic-Substituted Purines; **J. Med. Chem**., 38:2582-2595 (1995).
5. Noelle et al. Potential Purine Antagonists; **J. Am. Chem. Society, 81, 5997-6007 (1959).**
6. Alhede J., A simple and Efficient Synthesis of 9-Substituted Guanines; **Org. Chem. 1991, 2139.**
7. Ashwell et al, An Improved route to Guanines Substituted at N-9; **J. Chem. Soc., (14), 955-956 (1990).**
8. Jose Luis Garcia Ruano, Alejandro Parro, Jose Aleman, Francisco Yuste and Virginia M. Mastranzo; Monoalkylation of Primary Amines and N-Sulfinylamides; **Chem. Commun., 2009, 404-406.**
9. Balzarini J., Schols D., Baba I., Field H. J., and D Clerq E. Antiviral Drugs - a short story of their discovery and development; **Microbial Today 31, 58-61 (2004).**
10. Beauchamp L. M. and Krenitsky T. A., Acyclovir prodrugs: the road to Valciclovir. **Drugs future 18, 619-628 (1993).**
11. Ralph N. Salvatore, Cheol Hwan Yoon and Kyong Woon Jung; Synthesis of secondary amines; **Tetrahedron, 57, 7785-7811 (2001).**

12. Bisacchi et al., Synthesis and antiviral activity of enantiomeric forms of Nucleoside Analogues. **J. Med. Chem., vol. 34, No. 4, pp. 1415-1421 (1991).**
13. Hans Rene Bjorsvik, Hanno Priebe, Jan Cervinka, Arne W. Aabye; A Selective Process for N-Alkylation in Competition with O-alkylation; **Organic Process Research and Development 2001, 5, 472-478.**
14. Greene, T. W. Protective group in Organic Synthesis; **Wiley: New York, 1981.**
15. Chu-Pei Xu, Zhen-Hua Xiao, Bi-Quin Zuho Pei-Qiang Huang; Efficient and chemoselective alkylation of amines/amino acids using alcohols as alkylating reagents under mild condition; **The Royal Society of Chemistry 2010.**
16. Bernfeld P, Amylases: alpha and beta methods, **Enzymol, 1955, 1: 149-158.**
17. Mueller J.H. and Hinton J, A protein free medium for primary isolation of the gonococcus and meningococcus, **proceedings of the society for experimental biology and medicine, 1941, 48, 330.**
18. Shivi Bhatia and Monika Gupta, 1,3,4-Oxadiazole as antimicrobial agents. **An overview, journal of Chemical and Pharmaceutical research, 2011, 3(3), 137-147.**