Sys Rev Pharm 2020;11(2):795-806
A multifaceted review journal in the field of pharmacy

The potential antiviral activity of a novel pyrimidine derivative against Herpes Simplex Virus type-1 (HSV-1)

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ABSTRACT

Context: Previous studies and the are trials to find new molecules to help in the war against viruses. We have started preparing new compounds in this field beginning with the nucleus of thiouracil for what is known from its multiple biological activity, including against viruses. objective: Evaluation antiviral activities of new synthetic pyrimidine compounds. materials and methods: Our new target compounds were prepared starting with 2-thiouracil which was hydroxy methylated to give compound 1 which in turn was chloromethylated by thionyl chloride yielding compound 2. This chloromethyl compound was then reacted with three selected aromatic amines namely, p-nitroaniline, p-fluoroaniline and 2-aminopyridine respectively, giving aminomethyl derivatives 3a-c, which were used as starting material for preparing many target compounds by reaction firstly with ß-bromopropanoic acid affording pyrimido thiazine derivatives 4a-c. They were cyclocondensed with anthranilic acid giving pyrimido quinazoline derivatives 5a-c. In another pathway, they were reacted with ß-amino ethanol affording imidazopyrimidine derivatives 6a-c. Also, they were S-methylated by methyl iodide giving methyl thiopyrimidine derivatives 7a-c alo were cyclocondensed with ethylbromoacetate and/or chloroacetyl chloride giving isomers of thiazolopyrimidine derivatives 8a-c and 9a-c. Finally, they S-alkylated by reaction with chloroacetic acid yielding acetic acid derivatives 10a-c. Results: Compound 3c and 10c exhibited a potent antiviral activity and, they showed insignificant differences as compared with other compounds. In addition, 10c exhibited higher efficacy as compared with acyclovir. Conclusion: The aryl methyl aminopyrimidine was the pharmacophore of these target compounds. Any change in the structure decreases the activity except the introduction of acetic acid moiety which potentiate the antiviral activity.

Keywords: Pyrimidine derivative, acyclovir, HSV-1, and antiviral compounds.

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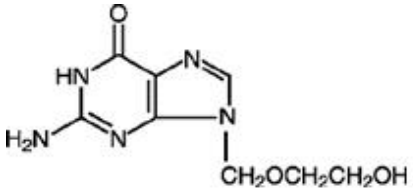
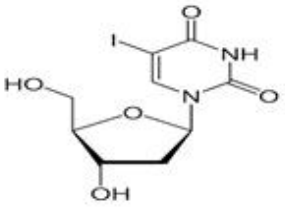
INTRODUCTION

Herpes simplex virus type 1 (HSV-1), a double-stranded DNA virus, is one of several viruses that can cause diseases worldwide [1-2]. Acyclovir is a drug of choice for treatment of HSV-1 infection, but due to poor bioavailability as well as its side effects in the treatment of viral infections that remains the major drug problems that face pharmaceutical researchers. On the other hand, our world is now subjected to the biggest threat that threatens the human being, which is the war against viruses, that infinitesimal enemy, but it has a superpower that is difficult to face. Perhaps the most important example of this is the Corona virus, the epidemic that originated from which the world was baffled and still in a fierce war against it. In this study, we offer a model in the field of antiviral agents, which Using a Vero cell line similar to acyclovir, which is known as a medical antiviral for patients with herpes simplex virus infection, chickenpox, and shingles. The new pyrimidine derivatives were manufactured against HSV-1. [3]. It is a nucleoside analogue that simulators guanosine, it acts by inhibition and inactivation of HSV-specified DNA polymerases further viral DNA synthesis [4]. In our field of study, it is possible for us, based on our previous experience with the synthesis and evaluation of pyrimidine derivatives, to start with 2-thiouracil as a pyrimidine nucleus as some thiopyrimidine arabinosides were synthesized and evaluated for their potent antiherpes virus activity. Also, 5-iodo-2'-deoxyuridine (IDU) and 9-ß-D-arabinosyl-cytosine (ara-C) were effectively used as anti-herpesvirus and also against varicella-zoster virus (VZV) infections but their clinical use had been declined due to their side effects. Moreover, some 2-thiouracil arabinosides were examined against HSV-1 and gave a promising activity as anti-HSV and VZV activities [5]. Some studies indicated that 2-thiouracil has an inhibitory effect on the growth of some culture viruses, but this inhibitory effect is very limited against animal viruses such as inhibiting the growth of the vaccine virus in tissue culture as well as its ability to disable poliovirus and 1-ß-D-arabinofuranosyl-2 Thiocytin [6-7]. Here we developed a program aimed to synthesize 5-substituted thiouracils and condensed pyrimidines in an attempt to improve the antiviral activity of 2-thiouracil using acyclovir as a standard antiviral agent. We hope that in the future we will continue to test these compounds as anti-covid-19, either through our research or in cooperation with the relevant authorities.



Acyclovir



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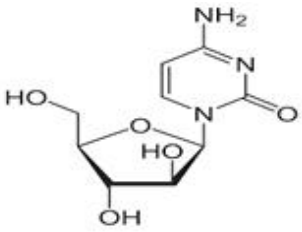


Ara-C

METHOD

All melting points are uncorrected and were measured using an Electrothermal IA 9100 apparatus (Shimadzu,Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (USA). 1HNMR spectra (300MHz) were recorded in dimethyl sulfoxide (DMSO) by employing tetramethyl silane (TMS) as an internal standard on Varian Mercury 300 MHz NMR Spectrometer (Varian, UK) and the chemical shifts (δ) were expressed as ppm against TMS as internal standard. Mass spectra were recorded on a 70 ev EI Ms-QP 1000 (Shimadzu,Japan). Microanalyses were operated using vario, Elementar apparatus (Shimadzu). The progress of all the reactions was monitored by TLC on silica gel 60 for TLC (Merck) using chloroform-methanol (3:1) as mobile phase and spots were visualized by iodine vapors or by irradiation with UV-light (254nm). **Cell line**: To choose a suitable cell line for HSV-1 incidence an African Green Ape Kidney (Vero) cell line was used. Using the lowest Dulbeco (Gibco) containing 10% of fetal bovine serum (Gibco) cells were cultured. The KOS strain of HSV-1 was tested with antiviral activity. Using the Karber method, the virus was deployed in the Vero cells and the circulating viral stock titer such as TCID 50 mL-1 was installed after the distribution of the virus stock in the titration, then stored until the date of use at a temperature of -70 ° C.

Compounds and the standard: Acyclovir was obtained from Sigma-Aldrich, and verifiedmixtures were prepared as below. Dimethyl sulfoxide was used as the solvent for tested compounds and acyclovir.

Preparation of Experimental compounds

5-Hydroxy methyl-2-thiouracil (1) and 5-Chloro methyl-2-thiouracil	Prepared as in literature (6)
5-(Arylamino methyl)-2-thioxo-2,3-dihydropyrimidin-4-(1H)-one (3a-c	A mixture of 2 (1.13 mol) and the proper aromatic amine namely, p-nitroaniline,p-fluoroaniline and 2-aminopyridine respectively and pyridine (0.016 mol) in absolute ethanol (50ml) was refluxed for 16 hours, then cooled , filtered , dried and recrystallized from DMF/water.
5-[(4-nitrophenyl)amino]methyl-2-thioxo-2,3-dihydropyrimidin-4-(1H)-one: 3a.	Yield: 73%: mp: 298 oC: IR (KBr cm-1): 3220 (NH), 3150 (CH,aromatic), 2987(CH,aliphatic),1691(C = O),1350,1551(NO2), 1270 (C = S). 1HNMR (DMSO-d6), δ:3.4(2H,s, CH2-NH), 7.2,7.5(4H,dd,Ar-H),8.2(1H,s,pyrimidine), 4.2,10.1,10.3(3H,s,NH,exchangeable with D2O),), MS: m/z (%), 278.28 (M+,18.3%), Anal. Calcd., for C11H10N4O3S: C, 47.48; H, 3.62; N, 20.13. Found: C,47.52; H,3.67; N,20.16.
5-[(4-fluorophenyl)amino]methyl-2-thioxo-2,3-dihydropyrimidin-4-(1H)-one: 3b	Yield: 76%: mp: 276 °C: IR (KBr cm-1): 3210 (NH), 3162 (CH,aromatic), 2981(CH,aliphatic),1688(C = O), 1270 (C = S). 1HNMR (DMSO-d_6), δ:3.3(2H,s, CH_2-NH), 7.1,7.3(4H,dd,Ar-H),8.1(1H,s,pyrimidine), 4.1,10.0,10.2(3H,s,NH,exchangeable with D_2O),), MS: m/z (%), 251.28 (M^+,21.7%), Anal. Calcd., for $C_{11}H_{10}FN_3OS$: C, 52.58; H, 4.01; N, 16.72. Found: C,52.52; H,4.27; N,16.83.

5-[(pyridine-2-ylamino)methyl]-2-thioxo-2,3-dihydropyrimidin-4-(1H)-one: 3c.	Yield: 80%: mp: 281 °C: IR (KBr cm-1): 3217 (NH), 3165 (CH,aromatic), 2978(CH,aliphatic),1681(C = O), 1273 (C = S), 1680(C=N). 1HNMR (DMSO-d_6), δ:3.4(2H,s, CH_2-NH), 7.0,7.3(4H,m,Ar-H),8.2(1H,s,pyrimidine), 4.2,10.1,10.3(3H,s,NH,exchangeable with D_2O),), MS: m/z (%), 234.27 (M^+,16.80%), Anal. Calcd., for $C_{10}H_{10}N_4OS$: C, 51.27; H, 4.30; N, 23.91. Found: C,51.34; H,4.26; N,23.88.
7-[arylamino]methyl]-2,3,3,9,9a-tetrahydro-4H,6H-pyrimido[2,1-b][1,3] thiazine-4,6-dione (4a-c):	A mixture of 3a-c (0.03 mol) and ß-bromopropanoic acid (0.03 mol), anhydrous sodium acetate (0.3g) and acetic anhydride (1ml) in glacial acetic acid (10ml) was refluxed for 4 h. After cooling, the reaction mixture was poured gradually with stirring on the crushed ice (about 30gm). The solid formed was filtered off, washed with water, dried and crystallized from DMF/water.
7-[(4-nitrophenyl)amino]methyl]-2,3,3,9,9a-tetrahydro-4H,6H-pyrimido[2,1-b][1,3] thiazine-4,6-dione 4a:	Yield: 69%: mp: 267 oC: IR (KBr cm-1): 3211 (NH), 3176 (CH, aromatic), 2977(CH, aliphatic),1688,1677(2C = O),1620(C=N), 1355,1555(NO_2), 1HNMR (DMSO-d6), δ2.88(t,2H,CH2),3.20(t,2H,CH2),:3.4(2H,s, CH2-NH), 7.2,7.3(4H,dd,Ar-H),8.1(1H,s,pyrimidine), 4.1 (1H,s,NH,exchangeable with D2O),), MS: m/z (%), 332.33 (M+,15.4%), Anal. Calcd., for C14H12N4O4S: C, 50.60; H, 3.64; N, 16.86. Found: C,50.62; H,3.68; N,16.80.
7-[(4-fluorophenyl)amino]methyl]-2,3,3,9,9a-tetrahydro-4H,6H-pyrimido[2,1-b][1,3] thiazine-4,6-dione 4b:	Yield: 66%: mp: 270 oC: IR (KBr cm-1): 3213 (NH), 3186 (CH, aromatic), 2971(CH,aliphatic),1687,1678(2C = O),1622(C=N), 1HNMR (DMSO-d6), δ 2.87(t,2H,CH2),3.21(t,2H,CH2),:3.4(2H,s, CH2-NH), 7.1,7.4(4H,dd,Ar-H),8.2(1H,s,pyrimidine),

7- [(pyridine-2-ylamino)]methyl]-2,3,3,9,9a-tetrahydro-4H,6H-pyrimido[2,1-b][1,3] thiazine-4,6-dione 4c:	Yield: 68%: mp: 283 oC: IR (KBr cm-1): 3217 (NH), 3183 (CH, aromatic), 2976(CH,aliphatic),1686,1679(2C = O),1626(C=N), 1HNMR (DMSO-d6), δ 2.77(t,2H,CH2),3.25(t,2H,CH2),:3.4(2H,s, CH2-NH), 7.0-7.3(4H,m,Ar-H),8.2(1H,s,pyrimidine), 4.2 (1H,s,NH,exchangeable with D2O),), MS: m/z (%), 288.32 (M+,16.3%), Anal. Calcd., for C13H12N4O2S: C, 54.15; H, 4.20; N, 19.43. Found: C,54.16; H,4.38; N,19.39.
3-[(arylphenyl)amino]methyl}-4H-pyrimido[2,1-b]quinazoline-4,6-(11H)-dione 5a-c:	A mixture of 3a-b(0.03 mol) and anthranilic acid (0.03 mol) in sodium ethoxide (Na, 2mmol in 25ml absolute ethanol) was heated under reflux for 8h. After cooling, the reaction mixture was poured into acidic ice water and neutralized with HCl. The solid was filtered off, washed with water, dried and recrystallized from DMF/water.
3-[(4-nitrophenyl)amino]methyl}-4H-pyrimido[2,1-b]quinazoline-4,6-(11H)-dione 5a:	Yield: 66%: mp: 300 oC: IR (KBr cm-1): 3217 (NH), 3182 (CH, aromatic), 2981(CH, aliphatic),1681,1679(2C = O), 1619(C=N), 1350,1553(NO2), 1HNMR (DMSO-d6), δ 3.4(2H,s, CH2-NH), 7.0,7.4(8H,m,Ar-H),8.1(1H,s,pyrimidine), 4.2,8.5 (2H,s,NH,exchangeable with D2O),), MS: m/z (%), 363.32 (M+,12.7%), Anal. Calcd., for C18H13N5O4: C, 59.50; H, 3.61; N, 19.28. Found: C,59.42; H,3.68; N,19.16.
3-[(4-fluorophenyl)amino]methyl}-4H-pyrimido[2,1-b]quinazoline-4,6-(11H)-dione 5b:	Yield: 61%: mp: 308 oC: IR (KBr cm-1): 3211 (NH), 3185 (CH, aromatic), 2969(CH, aliphatic),1679,1678(2C = O), 1622(C=N), 1HNMR (DMSO-d6), δ 3.5(2H,s, CH2-NH), 7.1,7.4(8H,m,Ar-H),8.2(1H,s,pyrimidine)
3- [(pyridine-2-ylamino) methyl]-4H-pyrimido[2,1-b]quinazoline-4,6-(11H)-dione 5c:	Yield: 63%: mp: 315 oC: IR (KBr cm-1): 3222 (NH), 3187 (CH, aromatic), 2976(CH, aliphatic),1680,1679(2C = O), 1625(C=N), 1HNMR (DMSO-d6), δ 3.5(2H,s, CH2-NH), 7.0,7.3(8H,m,Ar-H),8.2(1H,s,pyrimidine), 4.3,8.5 (2H,s,NH,exchangeable with D2O),), MS: m/z (%), 319.31 (M+,18.3%), Anal. Calcd., for C17H13N5O2: C, 63.94; H, 4.10; N, 21.93. Found: C,63.85; H,4.89; N,21.99.
6-[(aryl) amino] methyl-2,3-dihydroimidazo[1,2-a] pyrimidin-5-(1H)-one 6a-c:	A mixture of 3a-c (0.03 mol),ethanol amine (3ml) in isopropyl alcohol (25ml) was refluxed for 10 h. After cooling, the reaction mixture was poured gradually with stirring on crushed ice (about 50 gm), the solid formed was filtered off, washed with water, dried and recrystallized from butanol.
6-[(4-nitrophenyl) amino] methyl}-2,3-dihydroimidazo[1,2-a] pyrimidin-5-(1H)-one 6a:	Yield: 68%: mp: 258 °C: IR (KBr cm-1): 3218 (NH), 3193 (CH, aromatic), 2982(CH, aliphatic),1681 (C = O), 1619(C=N), 1350,1552(NO_2), 1HNMR (DMSO-d_6), δ: 2.9-3.1(m,2H,CH_2),3.1-3.3(m,2H,CH_2), 3.4(2H,s, CH_2-NH), 7.0,7.2(4H,dd,Ar-H),8.1(1H,s,pyrimidine), 4.2 (1H,s,NH,exchangeable with D_2O),), MS: m/z (%), 287.27 (M^+,11.3%), Anal. Calcd., for $C_{13}H_{13}N_5O_3$: C, 54.35; H, 4.56; N, 24.38. Found: C,54.41; H,4.61; N,24.39.
6-[(4-fluorophenyl)amino]methyl}-2,3-dihydroimidazo[1,2-a]pyrimidin-5-(1H)-one 6b:	Yield: 64%: mp: 266 oC: IR (KBr cm-1): 3223 (NH), 3194 (CH, aromatic), 2975(CH, aliphatic),1683 (C = O), 1623(C=N), 1HNMR (DMSO-d6), δ: 2.8-3.1(m,2H,CH2),3.1-3.3(m,2H,CH2), 3.4(2H,s, CH2-NH), 7.1,7.3(4H,dd,Ar-H),8.1(1H,s,pyrimidine), 4.3 (1H,s,NH,exchangeable with D2O),), MS: m/z (%), 287.27 (M+,11.3%), Anal. Calcd., for Found: C,54.41; H,4.61; N,24.39.

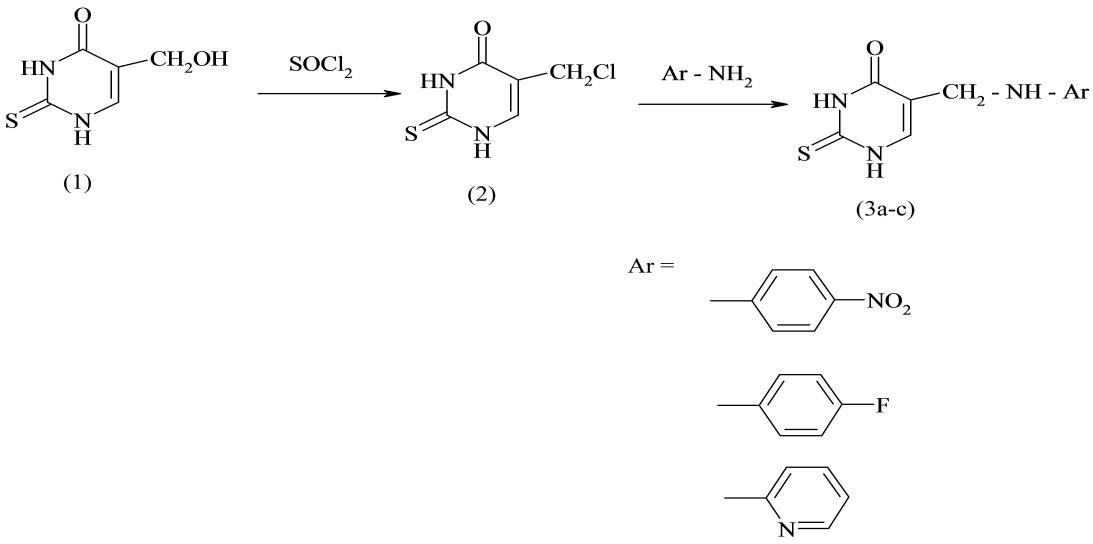
6- [(pyridine-2-ylamino)]methyl]-2,3-dihydroimidazo[1,2-a]pyrimidin-5-(1H)-one 6c:	Yield: 65%: mp: 282 oC: IR (KBr cm-1): 3230 (NH), 3197 (CH, aromatic), 2981(CH, aliphatic),1685 (C = O), 1620(C=N), 1HNMR (DMSO-d6), δ: 2.7-3.2(m,2H,CH2),3.0-3.1(m,2H,CH2), 3.4(2H,s, CH2-NH), 7.0,7.2(4H,m,Ar-H),8.1(1H,s,pyrimidine), 4.4 (1H,s,NH,exchangeable with D2O),), MS: m/z (%), 245.28 (M+,19.4%), Anal. Calcd., for C12H15N5O: C, 58.76; H, 6.16; N, 28.55. Found: C,58.81; H,6.21; N,28.38.
2-(methyl thio)-5-[(aryl)amino] methyl pyrimidin-4-(3H)-one 7a-c:	To a solution of 3a-c (0.03 mol) in sodium ethoxide / ethanol mixture (25 ml, 0.03 mol), methyl iodide (0.03 mol) was added and refluxed for 3h. The reaction mixture was cooled and poured on crushed ice (about 30 gm), neutralized with dil HCl, the solid formed was filtered off, washed with water, dried and recrystallized from DMF/ water.
2-(methylthio)-5-[(4-nitrophenyl) amino] methyl pyrimidin-4-(3H)-one 7a:	Yield: 72%: mp: 298 oC: IR (KBr cm-1): 3217 (NH), 3186 (CH, aromatic), 2991(CH, aliphatic),1685 (C = O), 1623(C=N), 1350,1552(NO2), 1HNMR (DMSO-d6), δ: 1.8(s,CH3), 3.4(2H,s, CH2-NH), 7.1,7.3(4H,dd,Ar-H),8.1(1H,s,pyrimidine), 4.2,10.5 (2H,s,NH,exchangeable with D2O),), MS: m/z (%), 292.31 (M+,24.2%), Anal. Calcd., for C12H12N4O3S: C, 49.31; H, 4.14; N, 19.17. Found: C,49.41; H,4.32; N,19.29.
2-(methyl thio)-5-[(4-fluorophenyl)amino]methyl pyrimidin-4-(3H)-one 7b	Yield: 75%: mp: 267 oC: IR (KBr cm-1): 3218 (NH), 3171 (CH, aromatic), 2983(CH, aliphatic),1686 (C = O), 1622(C=N), 1HNMR (DMSO-d6), δ: 1.9(s,CH3), 3.3(2H,s, CH2-NH), 7.2,7.4(4H,dd,Ar-H),8.2(1H,s,pyrimidine), 4.3,10.4 (2H,s,NH,exchangeable with D2O),), MS: m/z (%), 292.31 (M+,24.2%), Anal. Calcd., | Found: C,54.40; H,4.52; N,15.89.

2-(methylthio)-5-[(pyridine-2-ylamino]methyl]pyrimidin-4-(3H)-one 7c:	Yield: 72%: mp: 270 oC: IR (KBr cm-1): 3219 (NH), 3182 (CH, aromatic), 2977(CH, aliphatic),1685 (C = O), 1623(C=N), 1HNMR (DMSO-d6), δ: 1.8(s,CH3), 3.4(2H,s, CH2-NH), 7.0,7.3(4H,m,Ar-H),8.1(1H,s,pyrimidine), 4.2,10.5 (2H,s,NH,exchangeable with D2O),), MS: m/z (%), 248.30 (M+,23.6%), Anal. Calcd., for C11H12N4OS: C, 53.21; H, 4.87; N, 22.56. Found: C,53.30; H,4.72; N,22.49.
6-[(aryl)amino]methyl-5H-[1,3]thiazolo[3,2-a]pyrimidine-3,5-(2H)-dione.8a-c:	To an ice cold solution of 3a-c (0.03 mole) in 40 ml dry DMF, tri-ethylamine (0.03 mole) and ethylbromoacetate (0.03 mole) were added successfully. The reaction mixture was heated in a water bath at 70 OC for 6 hours, cooled poured into ice / cold water and extracted with methylene chloride 3x30 ml, the organic layer was dried on anhydrous sodium sulphate, filtered off and the filtrate was evaporated to dryness. The residue was washed with 10% sodium carbonate solution and the undissolved solid was filtered off, dried and crystallized from DMF/water.
6-[(4-nitrophenyl)amino]methyl-5H-[1,3]thiazolo[3,2-a]pyrimidine-3,5-(2H)-dione.8a:	Yield: 75%: mp: 307 oC: IR (KBr cm-1): 3225 (NH), 3167 (CH, aromatic), 2969(CH, aliphatic),1684,1689 (2C = O), 1624(C=N), 1350,1552(NO2), 1HNMR (DMSO-d6), δ: 2.7 (s,CH2), 3.4(2H,s, CH2-NH), 7.2,7.4(4H,dd,Ar-H),8.1(1H,s,pyrimidine), 4.2 (1H,s,NH,exchangeable with D2O),), MS: m/z (%), 308.30 (M+,24.2%), Anal. Calcd., for C13H10N4O4S: C, 49.05; H, 3.17; N, 17.60. Found: C,49.11; H,3.22; N,17.82.

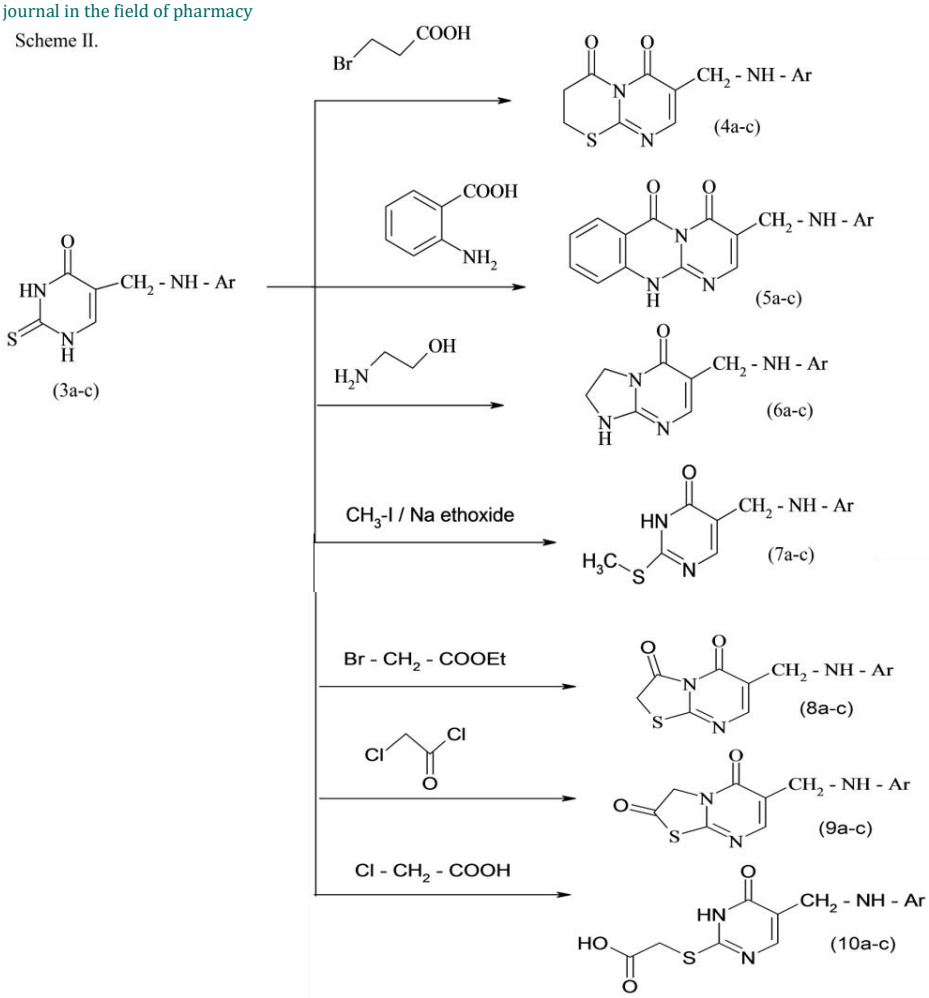
6-[(4-fluorophenyl)amino]methyl-5H-[1,3]thiazolo[3,2-a]pyrimidine-3,5-(2H)-dione.8b:	Yield: 72%: mp: 312 oC: IR (KBr cm-1): 3235 (NH), 3189 (CH, aromatic), 2989(CH, aliphatic),1685,1686 (2C = O), 1622(C=N), 1HNMR (DMSO-d6), δ: 2.8 (s,CH2), 3.3(2H,s, CH2-NH), 7.1,7.3(4H,dd,Ar-H),8.2(1H,s,pyrimidine), 4.3 (1H,s,NH,exchangeable with D2O),), MS: m/z (%), 291.30 (M+,21.7%), Anal. Calcd., for C13H10FN3O2S: C, 53.60; H, 3.46; N, 14.42. Found: C,53.71; H,3.42; N,14.52.
6-[(pyridine-2-ylamino)methyl] -5H-[1,3]thiazolo[3,2-a]pyrimidine-3,5-(2H)-dione.8c:	Yield: 78%: mp: 317 oC: IR (KBr cm-1): 3243 (NH), 3198 (CH, aromatic), 2978(CH, aliphatic),1684,1684 (2C = O), 1625(C=N), 1HNMR (DMSO-d6), δ: 2.7 (s,CH2), 3.2(2H,s, CH2-NH), 7.0,7.2(4H,m,Ar-H),8.2(1H,s,pyrimidine), 4.3 (1H,s,NH,exchangeable with D2O),), MS: m/z (%), 274.29 (M+,16.3%), Anal. Calcd., for C12H10N4O2S: C, 52.54; H, 3.67; N, 20.43. Found: C,52.69; H,3.72; N,20.52.
6-[(aryl)amino]methyl}-5H-[1,3]thiazolo[3,2-a]pyrimidine-2,5-(3H)-dione.9a-c:	To an ice cold solution of 3a-c (0.03 mole) in 40 ml dry DMF, tri-ethylamine (0.03 mole) and chloroacetylchloride (0.03 mole) were added successfully. The reaction mixture was heated in a water bath at 70 OC for 6 hours, cooled poured into ice / cold water and extracted with methylene chloride 3x30 ml, the organic layer was dried on anhydrous sodium sulphate, filtered off and the filtrate was evaporated to dryness. The residue was washed with 10% sodium carbonate solution and the undissolved solid was filtered off, dried and crystallized from DMF.
6-[(4-nitrophenyl)amino]methyl-5H-[1,3]thiazolo[3,2-a]pyrimidine-2,5-(3H)-dione.9a:	Yield: 77%: mp: 301 oC: IR (KBr cm-1): 3226 (NH), 3169 (CH, aromatic), 2975(CH, aliphatic),1683,1688 (2C = O), 1627(C=N), 1351,1558(NO2), 1HNMR (DMSO-d6), δ: 2.8 (s,CH2), 3.3(2H,s, CH2-NH), 7.2,7.3(4H,dd,Ar-H),8.2(1H,s,pyrimidine), 4.3 (1H,s,NH,exchangeable with D2O),), MS: m/z (%), 308.30 (M+,21.7%), Anal. Calcd., for C13H10N4O4S: C, 49.05; H, 3.17; N, 17.60. Found: C,49.02; H,3.25; N,17.73.
6-[(4-fluorophenyl)amino]methyl-5H-[1,3]thiazolo[3,2-a]pyrimidine-2,5-(3H)-dione.9b:	Yield: 72%: mp: 309 oC: IR (KBr cm-1): 3242 (NH), 3178 (CH, aromatic), 2982(CH, aliphatic),1684,1687 (2C = O), 1627(C=N), 1HNMR (DMSO-d6), δ: 2.7 (s,CH2), 3.4(2H,s, CH2-NH), 7.2,7.4(4H,dd,Ar-H),8.1(1H,s,pyrimidine), 4.4 (1H,s,NH,exchangeable with D2O),), MS: m/z (%), 291.30 (M+,24.3%), Anal. Calcd., for C13H10FN3O2S: C, 53.60; H, 3.46; N, 14.42. Found: C,53.66; H,3.35; N,14.37.
6-[(pyridine-2-ylamino)methyl] -5H-[1,3]thiazolo[3,2-a]pyrimidine-2,5-(3H)-dione.9c:	Yield: 73%: mp: 320 oC: IR (KBr cm-1): 3245 (NH), 3173 (CH, aromatic), 2972(CH, aliphatic),1686,1687 (2C = O), 1626(C=N), 1HNMR (DMSO-d6), δ: 2.6 (s,CH2), 3.3(2H,s, CH2-NH), 7.0,7.2(4H,m,Ar-H),8.1(1H,s,pyrimidine), 4.4 (1H,s,NH,exchangeable with D2O),), MS: m/z (%), 274.29 (M+,13.9%), Anal. Calcd., for C12H10N4O2S: C, 52.54; H, 3.67; N, 20.43. Found: C,52.56; H,3.59; N,20.37.
[(5-[(aryl)amino]methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio] acetic acid.10a-c:	To a solution of monochloroacetic acid(0.03 mole) and sodium hydroxide (0.03 mole) in 5ml water, a solution of 3a-c (0.03 mole) and sodium hydroxide (0.03 mole)in 10 ml water was added. The reaction mixture was stirred at ambient temperature for 3 hours and rendered acidic with hydrochloric acid (6N). The

[(5-[(4-nitrophenyl)amino]methyl}-6-oxo-1,6-dihydropyrimidin-2-yl)thio] acetic acid.10a:	Yield: 77%: mp: 316 oC: IR (KBr cm-1): 3290 (NH,OH,v.b due to H.b), 3178 (CH, aromatic), 2985(CH, aliphatic),1680,1735 (2C = O), 1627(C=N), 1351,1558(NO2), 1HNMR (DMSO-d6), δ: 2.9 (s,CH2), 3.3(2H,s, CH2-NH), 7.2,7.4(4H,dd,Ar-H),8.1(1H,s,pyrimidine), 4.3,10.5 (2H,s,2NH,exchangeable with D2O),), MS: m/z (%), 336.32 (M+,31.3%), Anal. Calcd., for C13H12N4O5S: C, 46.43; H, 3.60; N, 16.66. Found: C,46.32; H,3.59; N,16.70.
[(5-[(4-fluorophenyl)amino]methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio] acetic acid.10b	Yield: 73%: mp: 319 oC: IR (KBr cm-1): 3295 (NH,OH,v.b due to H.b), 3183 (CH, aromatic), 2969(CH, aliphatic),1686,1737 (2C = O), 1628(C=N), 1HNMR (DMSO-d6), δ: 2.9 (s,CH2), 3.4(2H,s,CH2-NH), 7.1,7.3(4H,dd,Ar-H),8.2(1H,s,pyrimidine), 4.3,10.6 (2H,s,2NH,exchangeable with D2O),), MS: m/z (%), 336.32 (M+,31.3%), Anal. Calcd., for C13H12FN3O3S: C, 50.48; H, 3.91; N, 13.58. Found: C,50.42; H,3.89; N,13.42.
(6-oxo-5-[(pyridine-2-yl)methyl]-1,6-dihydropyrimidin-2-yl-thio) acetic acid. 10c	Yield: 76%: mp: 322 oC: IR (KBr cm-1): 3289 (NH,OH,v.b due to H.b), 3185 (CH, aromatic), 2973(CH, aliphatic),1682,1734 (2C = O), 1629(C=N), 1HNMR (DMSO-d6), δ: 2.9 (s,CH2), 3.4(2H,s, CH2-NH), 7.0,7.2(4H,m,Ar-H),8.1(1H,s,pyrimidine), 4.4,10.5 (2H,s,2NH,exchangeable with D2O),), MS: m/z (%), 392.31 (M+,33.4%), Anal. Calcd., for C12H12N4O3S: C, 49.31; H, 4.14; N, 19.17. Found: C,49.42; H,4.19; N,19.29.

Scheme I.



Scheme II.



EVALUATION OF ANTVIRAL ACTIVITY

To determine the antiviral effect, use a quasi-quantitative cytopathic inhibition test for each compound tested. During the examination and briefly, the Vero cells grew in a microplate of a 96-well cell (2 x 103 cells / wells).Use 5% carbon dioxide and at 37 ° C incubate the cultured plates until the cells show 80% confluence. Then, from each well, the center of the farm was removed and 100 TC1D50 were added from the virus suspension, various concentrations from the lowest concentration to the highest concentration of the tested compounds and the addition of acyclovir from non-cellu1ar concentrations to each well from the small cell p1ate. For each concentration of tested compounds and acyc1ovir 4 we11s were selected. For the virus contro1. 100 TC1D50 with the highest amount of Dimethyl sulfoxide which did not display cytotoxicity were added to 4 wells. A1so, in each microp1ate, 4 wells were treated with Dimethyl sulfoxide without virus as a negative contro1 for virus. Furthermore, 4 wel1s of each row were treated with the highest 1evel of tested compounds which did not beforedisplay cytotoxicity. The p1ates were 1ncubated at 37oC 1n a moistened CO2 atmosphere and be thereexamineddaily for cytopathic presentation up to 5 days post 1nfection. The degree of 1nhibition was expressed as percent yie1d of virus contro1 (% virus contro1 = CPE experimenta1 group/CPE virus contro1 x 100) (8)

Statistical analysis

We used mean ± SEM to express the data and One-way ANOVA followed by Tukey's post hoc. We also used $P < 0.05$ to find the difference in antihypertensive activities between different compounds. Analysis was done by using SPSS software update version and the graphs were draw by GraphPad Prism software v8.0.2.

RESULTS

Primary screening Different in antiviral activity of new synthetic compounds and acyclovir were tested on African green monkey kidney cell line (Vero) to find the effective compound. All the newly synthesized compounds were screened for antiviral activity. Acyclovir was used as standard reference drug for screening of antiviral activity. The results of antiviral activity of newly synthesized compounds as compared with acyclovir showed that acyclovir, 3c, and 10c (99.87 ± 0.09, 99.83 ± 0.12, and 99.73 ± 0.15 respectively) have insignificant difference ($p>0.05$) and they have maximum potency as compared with others compounds see tables 1, 2 and figures 1,2. We found that 10c (99.73 ± 0.15) has highest potency and efficacy as compared other newly synthesized compounds. There is significant difference ($p<0.001$) between others compounds and acyclovir table 4. The EC50 of 10c is insignificant difference ($p>0.05$) as compared with acyclovir, and also there is significant difference ($p>0.05$) between 10c and other compounds except 3c where it slightly elevated than 10c table 3 and figure 3.

Table 1: Antiviral activity of 3a-c compounds vs acyclovir presented by Mean± SEM, EC50 and CPE % values

Dose (µg/mL)	3a	3b	3c	Acyclovir
	Mean ± Std. Error	Mean ± Std. Error	Mean ± Std. Error	Mean ± Std. Error
10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
16	0.00 ± 0.00	0.00 ± 0.00	15.17 ± 0.22	29.30 ± 0.15
22	10.33 ± 0.60	0.00 ± 0.00	27.83 ± 0.12	49.97 ± 0.24
28	25.40 ± 2.95	8.20 ± 0.31	40.27 ± 0.48	70.00 ± 0.20
34	30.30 ± 2.80	14.13 ± 0.35	65.20 ± 0.31	76.80 ± 0.26
40	34.90 ± 2.69	17.87 ± 0.24	81.97 ± 0.32	92.00 ± 0.23
46	50.07 ± 2.71	25.93 ± 0.55	99.83 ± 0.12	99.87 ± 0.09
52	65.07 ± 2.72	60.03 ± 0.20		
58	98.67 ± 0.88	72.07 ± 0.35		
64		80.13 ± 0.35		
70		99.12.00 ± 0.35		
76				
EC50	51.63	62.53	27.68	17.42
logEC50	1.713	1.796	1.442	1.241

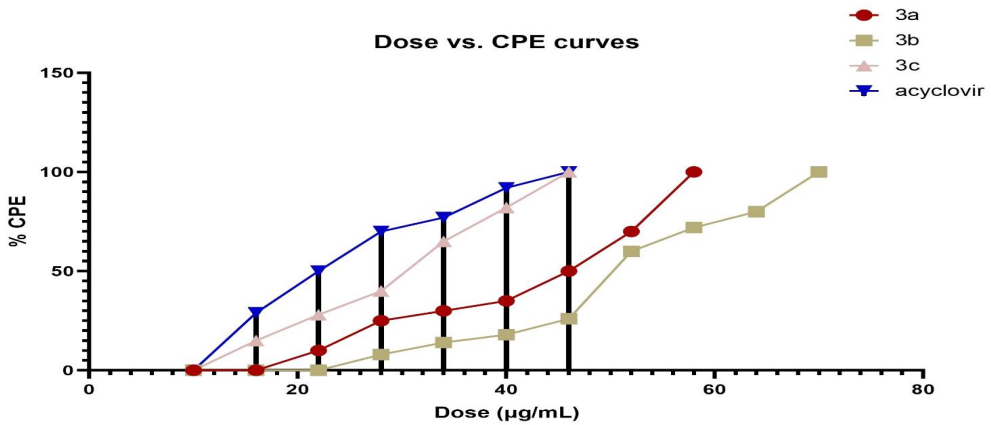


Figure 1: Antiviral activity of 3a-c compounds vs acyclovir

Table 2: Antiviral activity of 10a-c compounds vs acyclovir presented by Mean± SEM, EC50 and CPE % values

Dose (µg/mL)	10a	10b	10c	Acyclovir
	Mean ± Std. Error	Mean ± Std. Error	Mean ± Std. Error	Mean ± Std. Error
10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
16	20.10 ± 0.15	20.00 ± 0.23	23.00 ± 0.23	29.30 ± 0.15
22	28.93 ± 0.29	25.97 ± 0.26	59.83 ± 0.27	49.97 ± 0.24
28	54.97 ± 0.09	51.07 ± 0.29	71.97 ± 0.38	70.00 ± 0.20
34	67.10 ± 0.21	69.07 ± 0.12	78.10 ± 0.15	76.80 ± 0.26
40	89.97 ± 0.20	88.00 ± 0.29	99.73 ± 0.15	92.00 ± 0.23
46	99.87 ± 0.09	99.67 ± 0.24		99.87 ± 0.09
EC50	41.23	23.67	24.43	18.37
logEC50	1.615	1.374	1.388	1.241

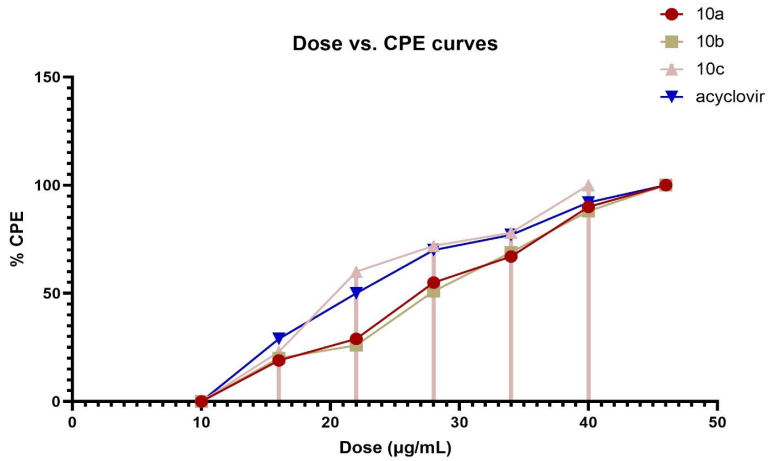


Figure 2: Antiviral activity of 10a-c compounds vs acyclovir

Table 3: Antiviral activity of 3c and 10c compounds vs acyclovir presented by Mean± SEM, EC50 and CPE % values

Dose (µg/mL)	3c	10c	Acyclovir
	Mean ± Std. Error	Mean ± Std. Error	Mean ± Std. Error
10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
16	15.17 ± 0.22	23.00 ± 0.23	29.30 ± 0.15
22	27.83 ± 0.12	59.83 ± 0.27	49.97 ± 0.24
28	40.27 ± 0.48	71.97 ± 0.38	70.00 ± 0.20
34	65.20 ± 0.31	78.10 ± 0.15	76.80 ± 0.26
40	81.97 ± 0.32	99.73 ± 0.15	92.00 ± 0.23
46	99.83 ± 0.12		99.87 ± 0.09
EC50	27.68	24.43	17.42
logEC50	1.442	1.388	1.241

Table 4: Antiviral activity of other compounds vs acyclovir presented by EC50 and CPE % values

Dose	Compounds (% CPE)																		
(µg/mL)	4a	4b	4c	5a	5b	5c	6a	6b	6c	7a	7b	7c	8a	8b	8c	9a	9b	9c	acyclovir
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29
22	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50
28	0	28	30	0	20	10	10	22	18	0	0	12	18	12	12	20	14	14	70
34	12	40	45	20	28	20	20	34	22	16	10	22	32	28	21	34	6	22	77
40	25	65	65	28	35	32	32	45	26	26	23	36	43	32	33	46	34	36	92
46	30	82	80	35	38	50	50	52	46	36	46	45	51	41	67	55	44	67	100
52	42	100	100	38	45	87	87	66	48	44	55	58	69	55	90	67	56	95	
58	62			45	65	100	100	72	74	58	66	67	88	72	100	75	88	100	
64	70			65	87			77	78	70	88	88	100	100		100	100		
70	82			87	100			82	85	82	92	100							
76	100			100				100	100	100	100								
EC50	17.42	64.56	37.63	39.28	65.95	49.1	49.38	49.38	37.78	46.03	53.13	43.83	46.98	40.32	53.37	43.59	41.01	52.67	18.37
logEC50	1.241	1.81	1.576	1.594	1.819	1.691	1.694	1.694	1.577	1.663	1.725	1.642	1.672	1.606	1.727	1.639	1.613	1.722	1.264

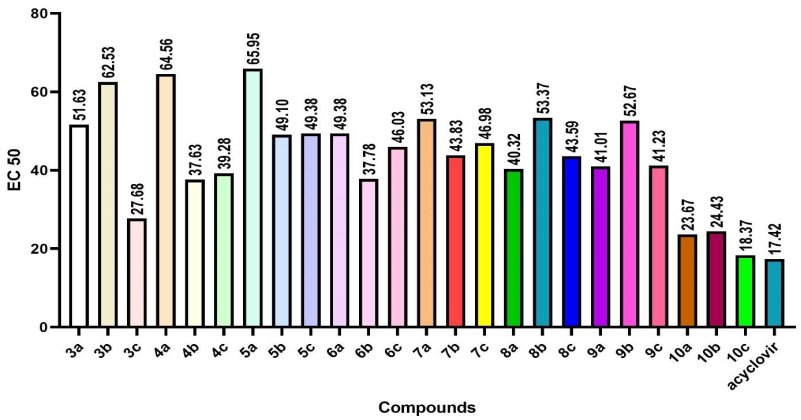
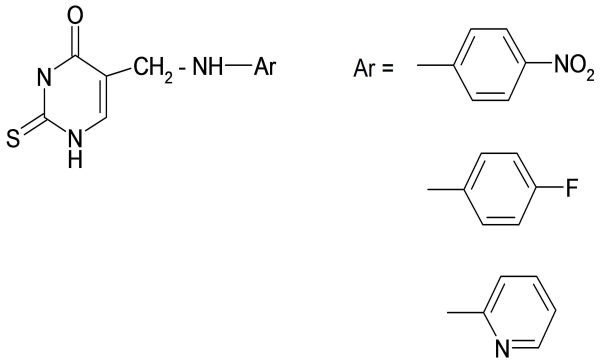
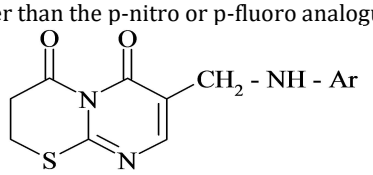


Figure 3:EC50 of synthetic compounds vs acyclovir

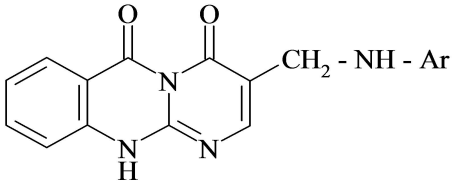
DISCUSSION



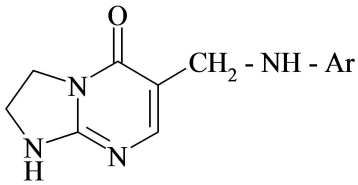
The herpes simplex virus, also known as HSV, is an infection that causes herpes. Herpes can appear in various parts of the body, most commonly on genitals or mucus. HSV-1which primarily causes oral herpes and generally responsible for cold sores and fever blisters around the mouth and on the face [9]. There is currently no cure for this virus. Treatment focuses on getting rid of sores and limiting outbreaks some medications are allowed such as acyclovir, famicyclovir and valacyclovir and others [10]. In our study we synthesized and developed some new pyrimidine derivatives and they were examined against vero cells. 2-thiouracil was the block unit in these syntheses. it was hydroxyl methylated by paraformaldehyde in basic medium (KOH) to give 5-hydroxymethyl-2-thiouracil which in turn chlorinated with thionyl chloride to give chloromethyl derivative which was the target starting material for the subsequent work. This chloromethyl derivative was firstly reacted with three aromatic amines in presence of anhydrous pyridine as an acid binder to give amino methyl derivatives (3a-c) that showed promising antiviral effects against vero cells especially when 2-aminopyridine was a selected aromatic amine. Potent activity was maximized when the aryl group is a pyridine ring 3c as compared to the p-nitrophenyl analogue 3a and p- fluorophenyl one 3b and give same potency as compare with acyclovir.In another reaction, 4a-c compounds were cyclocondensed with β-bromopropanoic acid giving pyrimidothiazine derivatives(4a-c) of a moderate antiviral activity. The introduction of thiazine ring decreased the antiviral activity comparable to acyclovir, but the pyridinyl group is still better than the p-nitro or p-fluoro analogues.



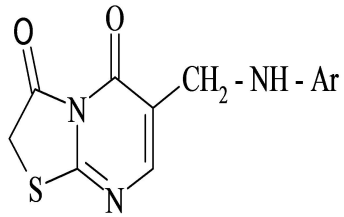
On the other hand, compounds 3a-c were reacted with anthranilic acid affording the tricyclic compounds pyrimidoquinazoline derivatives (5a-c) of also moderate antiviral activity. The tricyclic system attenuated the activity comparable to acyclovir but the pyridinyl group is still the first choice.



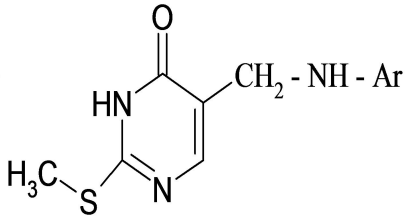
Furthermore, 3a-c were again reacted with β-aminoethanol yielding imidazopyrimidine derivatives (6a-c) of moderate antiviral activity. The imidazole ring decreased the antiviral activity to very reliable extent comparable to acyclovir, but a pyridinyl group is a first choice



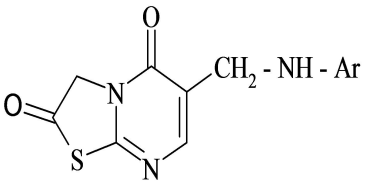
Also, compounds 3a-c were S-methylated by methyl iodide in sod. ethoxide/ethanol mixture yielding methyl thiopyrimidine derivatives (7a-c) of the least antiviral activity. S-methylationdramatically decreased the antiviral activity to give the least reactive targeted compounds. In addition, isomers of thiazolo pyrimidine derivatives (8a-c) and (9a-c) were obtained by reaction of 3a-c compounds with ethylbromoacetate and/or chloroacetyl chloride respectively of moderate antiviral activity but lesser than acyclovir.



7a-c:

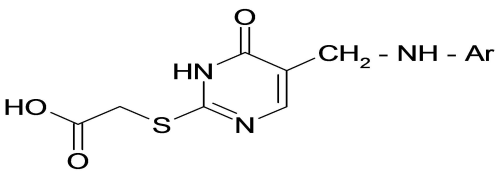


8a-c



9a-c

Finally, acetic acid derivatives (10a-c) were obtained by reaction of 3a-c compounds with chloroacetic acid to give the most potent derivatives of comparable activities to acyclovir especially when 2-aminopyridine was used as a selected aromatic amine in the synthesis of active targeted compounds.Introduction of acetic acid potentiate the antiviral activity as compared to acyclovir especially when the aryl group is a pyridine.



CONCLUSION

Substitution of 2-thiouracil at the 5th position by aryl aminomethyl group gives active antiviral agents which could be considered as a pharmacophore for these series.

Any manipulation in the structure decreases the antiviral activity except the introduction of acetic acid that gives more potent and high efficacy compounds comparable to acyclovir.

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