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Original article

Anti-HSV-1 activity and mechanism of action of some new synthesized substituted pyrimidine, thiopyrimidine and thiazolopyrimidine derivatives

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ABSTRACT

A series of heterocyclic derivatives **2**–**16** conjugated with tetrahydronaphthalene moiety were synthesized as antiviral agents by using 1-(5,6,7,8-tetrahydronaphthalen-2-yl)ethanone as starting material. The antiviral screening showed that many of these obtained compounds have good antiviral activities comparable to Acyclovir as reference control. Two compounds **13** and **15** gave over 90% inhibition and considered to be highly promising and on confirming their activity and comparing them to antiviral activity of Acyclovir. The detailed synthesis, spectroscopic data, and antiviral screening for synthesized compounds were reported.

1. Introduction

HSV disease is distributed worldwide, it ranges from mild illness in majority of patients to a few cases of sporadic, severe and life threatening disease [1]. Acyclovir is used for its treatment but its widespread and frequent use lead to appearance of resistant cases [2], resistance also occurred to 5–10% of immunocompromised patients [3]. From here it comes clear that it is important to test new agents that can be used as antiviral compounds and also can overcome resistant strains [4]. In previous work we have found that certain substituted pyrimidine and their heterocyclic derivatives show antimicrobial and anti-inflammatory [5–8] and antitumor activities [9–11]. On the other hand, thioxopyrimidine and thiazolopyrimidine derivatives have promising biological and anticancer activities [12–14]. In addition, we have reported that certain of our newly substituted heterocyclic compounds exhibited anti-androgenic [15], anti-inflammatory [16], anticancer [17], anticonvulsant [18] and antiarrhythmic [19] activities. Recently, some new thienopyrimidinone derivatives have been synthesized and tested as analgesic, antiparkinsonian and anti-inflammatory activities [20,21]. In view of these observations and in continuation of our previous work in heterocyclic chemistry, we synthesized some new pyrimidine and thiazolopyrimidine derivatives and tested their anti-HSV-1 activities.

2. Results and discussion

2.1. Chemistry

In the present work we report the synthesis and a preliminary pharmacological activity screening of several pyrimidine derivatives based on 3-(substituted phenyl)-1-(5,6,7,8-tetrahydronaphthalen-2-yl)prop-2-en-1-one derivatives **2a**–**c**, which were prepared from 1-(5,6,7,8-tetrahydronaphthalen-2-yl)ethanone **1** as starting material according to Ref. [22]. α,β-Unsaturated ketones **2a**–**c** required for the synthesis of the corresponding oxirano derivatives (**3a**–**c**) were obtained by treatment with hydrogen peroxide (30%) in the presence of sodium hydroxide. The reaction of **3** with phenyl hydrazine in refluxing ethanol produced N-phenyl-pyrazol-4-ol derivative **4**, which was refluxed in glacial acetic acid to yield the corresponding N-phenyl pyrazolo derivative **5**. However, the latter was also prepared directly from **3** by refluxing with phenyl hydrazine in glacial acetic acid. Condensation of **3a**–**c** with thiourea in refluxing ethanolic potassium hydroxide afforded 2-thioxopyrimidinones **6a**–**c**. Compound **6b** was condensed with chloroacetic acid in a mixture of acetic acid/acetic anhydride in the presence of anhydrous sodium acetate to yield the corresponding thiazolopyrimidines **7**. Compound **7**

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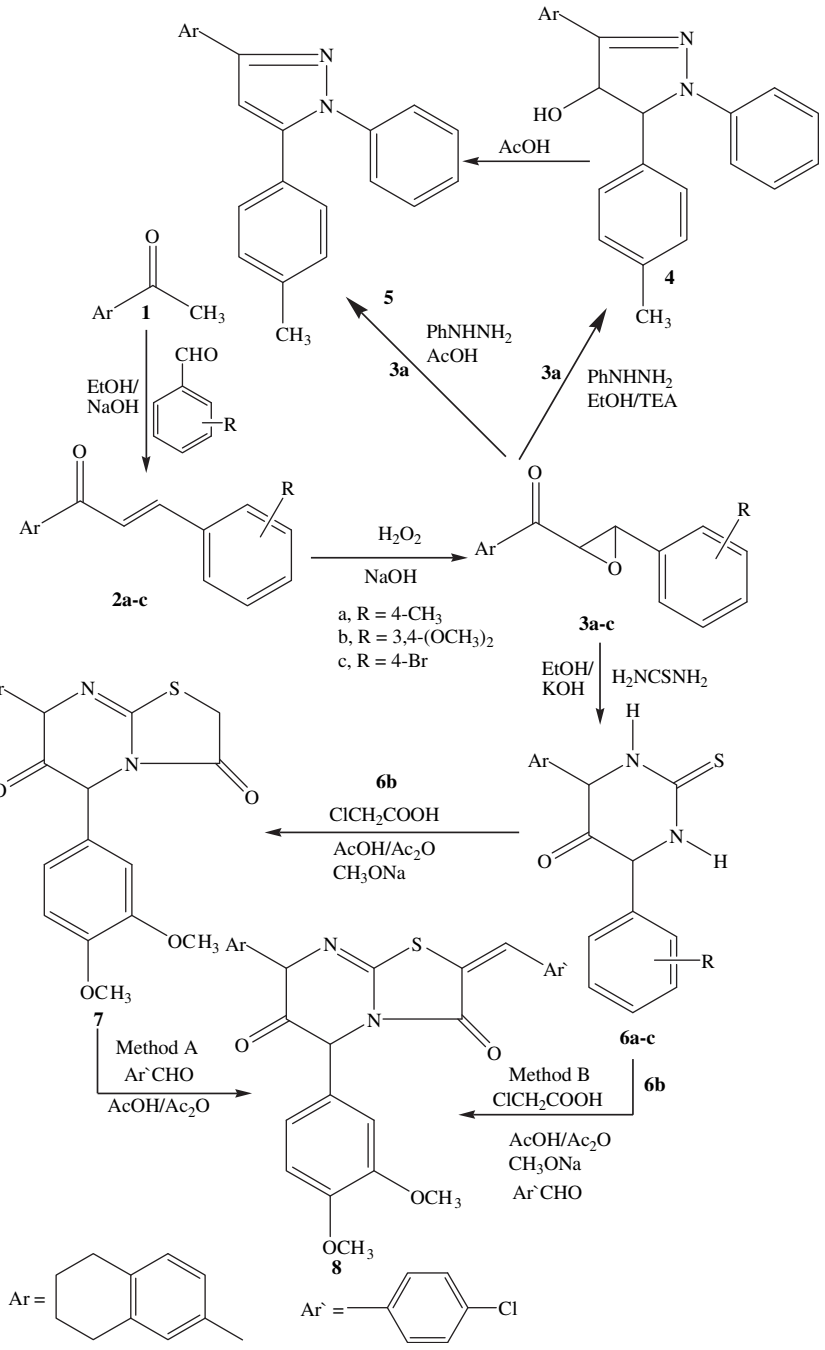
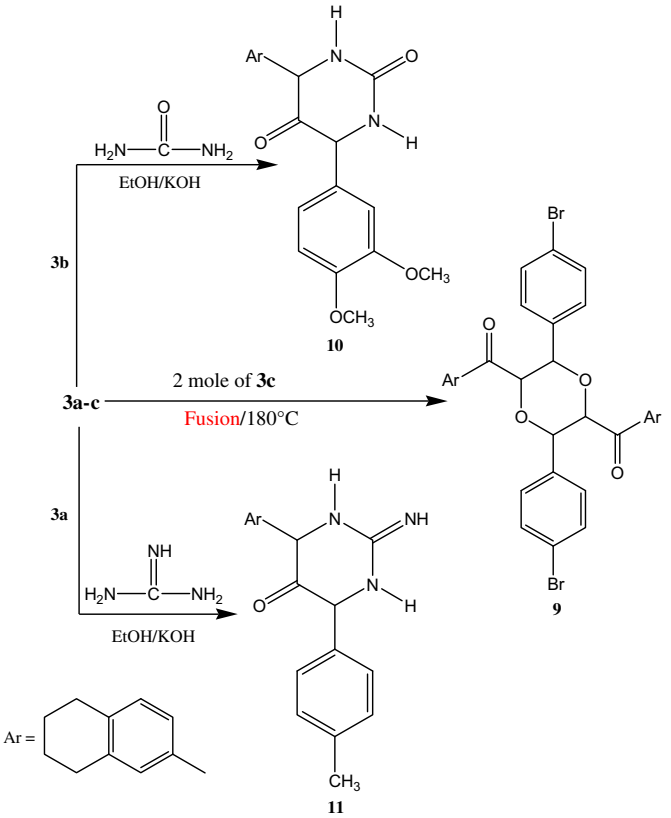
contains an active methylene group and it condensed with *p*-chlorobenzaldehyde in the presence of anhydrous sodium acetate and glacial acetic acid/acetic anhydride mixture to yield the arylmethylene **8**. However, the latter was also prepared directly from **6b** by the action of chloroacetic acid, *p*-chlorobenzaldehyde, and anhydrous sodium acetate in the presence of acetic acid/acetic anhydride mixture (Scheme 1).

The oxirano derivative **3c** was fused in an oil bath at 180 °C and the obtained residue was triturated with water, then methanol to give bis(bromophenyl)dioxane derivative **9**, while compound **3b** was condensed with urea or **3a** with guanidine hydrochloride in refluxing ethanolic potassium hydroxide to afford the corresponding pyrimidinone derivatives **10** and **11**, respectively (Scheme 2).

Compound **6b** was reacted with ethyl chloroformate in refluxing alcoholic potassium hydroxide to afford ethyl carbonothioate **12**. Compound **6b** was alkylated with 3-chloroacetylacetone in the presence of potassium hydroxide to give dimethoxyphenylpyrimidin-5(4H)-one derivative **13**, which was cyclized to 2-acetylthiazolopyrimidine **14** by refluxing with acetic anhydride. Compound **14** was prepared directly from **6b** by the action of 3-chloroacetylacetone in refluxing pyridine in the presence of potassium hydroxide. Compound **6b** was condensed with 2-bromopropionic acid or 3-bromopropionic acid in a mixture of acetic acid/acetic anhydride in the presence of anhydrous sodium acetate to yield the corresponding methylthiazolo- and thiazinopyrimidines **15** and **16**, respectively (Scheme 3).



Scheme 1.



Scheme 2.

The formation of the linear cyclized products **14**–**16** are tentatively favored over the isomeric structures, due to the chemical shift (δ) of the pyrimidine proton of compounds **14**–**16**, which are deshielded by about 0.6 ppm [23], relative to the thiopyrimidinone derivative **6b**.

2.2. Anti-HSV-1 activity

2.2.1. Antiviral activity of acyclovir as our control

The Fig. 1 shows antiviral activity of Acyclovir at different concentrations from 10 to 100 μg/ml showing that our two compounds **13** and **15** have higher activity.

2.2.2. Antiviral activity

Plaque reduction assay was used to test antiviral activity of 17 chemical compounds under test, a concentration of 2 and 5 μg/mL were used in initial screening (Table 1).

Two compounds **13** and **15** gave over 90% inhibition and considered to be highly promising and on confirming their activity and comparing them to antiviral activity of Acyclovir (Fig. 1), results confirmed the activity of those two compounds and that they gave high % inhibition at low concentration.

On studying the mechanism of action of those two compounds both showed virucidal activity against HSV-1. The chemical configuration of these compounds contains heterocyclic moities which may alter virus epitopes that inhibit virus attachment. That decrease in activity in compound **13** might be due to beta-diketone moiety (Table 2).

Compound **15** also was found to inhibit viral replication this can be discussed as follows (Table 3). Many viruses have evolved their own specific enzymatic mechanisms to replicate virus nucleic acids at the expense of cellular molecules. The inhibition in replication showed by our molecule might be due to inhibition of one or more of important enzymes needed by the virus to complete its replicating cycle. There is often sufficient specificity in virus polymerases to provide a target for a specific antiviral agent, and this method has produced the majority of the specific antiviral drugs currently in use.

Nucleoside analogues are in fact pro-drugs, since they need to be phosphorylated before becoming effective.

3. Conclusion

A series of heterocyclic derivatives conjugated with tetrahydronaphthalene moiety were synthesized as antiviral agents by using 1-(5,6,7,8-tetrahydronaphthalen-2-yl)ethanone as starting material. The antiviral screening showed that many of these obtained compounds have good antiviral activities comparable to Acyclovir as reference control. Two compounds **13** and **15** gave over 90% inhibition and considered to be highly promising and on confirming their activity and comparing them to antiviral activity of Acyclovir.

4. Experimental

4.1. Chemistry

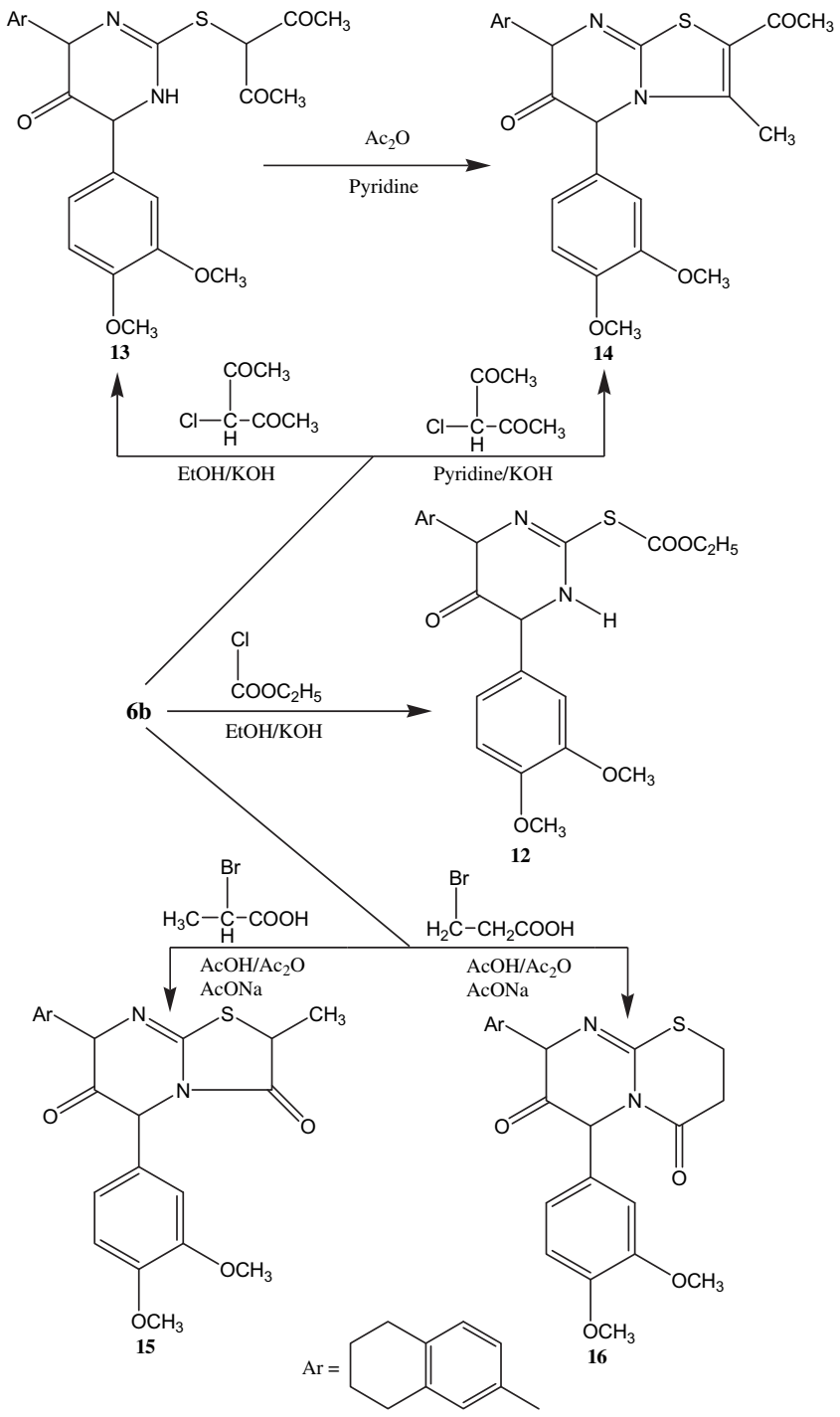
Melting points were determined on open glass capillaries using a Electrothermal IA 9000 digital melting point apparatus. Elemental analyses were performed on Elementar, Vario EL, Microanalytical Unit, National Research Center, Cairo Egypt and were found within ±0.4% of the theoretical values. Infrared spectra were recorded on Carl Zeiss Spectrophotometer model UR 10 using the KBr disc technique. ^{1}H NMR spectra were recorded on Varian Gemini 270 MHz spectrometer (DMSO-d_6) and the chemical shifts are given in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard. The mass spectra were measured using a Finnigan SSQ 7000 mass spectrometer. Follow up of the reactions and checking the purity of the compounds was made by TLC on silica gel-precoated aluminum sheets (Type 60 F_{254}, Merck, Darmstadt, Germany).

4.1.1. Synthesis of 3-(substituted phenyl)-1-(5,6,7,8-tetrahydronaphthalen-2-yl)prop-2-en-1-ones **2a**–**c**

A mixture of **1** (1.74 g, 10 mmol) and aromatic aldehydes, namely, 4-methylbenzaldehyde, 3,4-dimethoxybenzaldehyde and 4-bromobenzaldehyde (10 mmol) in alcoholic sodium hydroxide (50 mL, 10%) was stirred for 12 h at room temperature. The reaction mixture was poured onto ice-water, the solid formed was collected by filtration, dried and crystallized to give **2a** [22], **2b** and **2c**, respectively.

*4.1.1.1. 3-(3,4-Dimethoxyphenyl)-1-(5,6,7,8-tetrahydronaphthalen-2-yl)prop-2-en-1-one (**2b**).* Yield 95%, mp. 85 °C (EtOH); IR (KBr, cm^{-1}): 3145 (Ar-CH), 2932 (aliph-CH), 1670 (C=O); ^{1}H NMR (DMSO-d_6): δ 1.68–1.70 (m, 4H, 2 CH_2-tetraline), 2.80–2.82 (m, 4H, 2 CH_2-tetraline), 3.75, 3.80 (2s, 6H, 2OCH_3), 6.90–7.90 (m, 8H, Ar-H + CH=CH); Mass: *m*/*z* (%): 322 (M^+, base peak, 100). Analysis calculated for $C_{21}H_{22}O_3$ (322.40): C, 78.23; H, 6.88; O, 14.89. Found: C, 78.18; H, 6.82.

*4.1.1.2. 3-(4-Bromophenyl)-1-(5,6,7,8-tetrahydronaphthalen-2-yl)prop-2-en-1-one (**2c**).* Yield 98%, mp. 115 °C (EtOH); IR (KBr, cm^{-1}): 3138 (Ar-CH), 2918 (aliph-CH), 1674 (C=O); ^{1}H NMR (DMSO-d_6): δ 1.66–1.68 (m, 4H, 2 CH_2-tetraline), 2.78–2.80 (m, 4H, 2 CH_2-tetraline), 6.96–7.95 (m, 9H, Ar-H + CH=CH); MS (EI, 70 eV):



Scheme 3.

m/z = 341 (M^+, 32), 210 (base peak, 100). Analysis calculated for $C_{19}H_{17}BrO$ (341.24): C, 66.87; H, 5.02. Found: C, 66.81; H, 5.00.

*4.1.2. Synthesis of (3-(substituted phenyl)oxiren-2-yl)(5,6,7,8-tetrahydronaphthalen-2-yl)methanones **3a–c***

To a solution of **2a–c** (10 mmol) in a mixture of acetone/methanol (50 mL, 3:2 ratio) and sodium hydroxide (2 g) in few drops of water, hydrogen peroxide (6 mL, 30%) was added drop wise with stirring for 15 min at 0 °C. The reaction mixture was stirred for 3 h at room temperature, then left overnight at −5 °C, then poured onto ice-water. The solid formed was collected by filtration, dried and crystallized to give **3a**, **3b** and **3c**, respectively.

*4.1.2.1. (3-(4-Methylphenyl)oxiren-2-yl)(5,6,7,8-tetrahydronaphthalen-2-yl)methanone (**3a**).* Yield 93%, mp. 67 °C (EtOH); IR (KBr, cm^{-1}): 1688 (C═O); ^{1}H NMR (DMSO-d_6): δ 1.65–1.68 (m, 4H, 2 CH_2-tetraline), 2.18 (s, 3H, CH_3), 2.79–2.80 (m, 4H, 2 CH_2-tetraline), 4.05, 4.82 (2s, 2H, 2CH-epoxy), 6.85–7.85 (m, 7H, Ar-H); MS (EI, 70 eV): m/z = 292 (M^+, 46), 159 (base peak, 100). Analysis calculated for $C_{20}H_{20}O_2$ (292.37): C, 82.16; H, 6.89. Found: C, 82.12; H, 6.84.

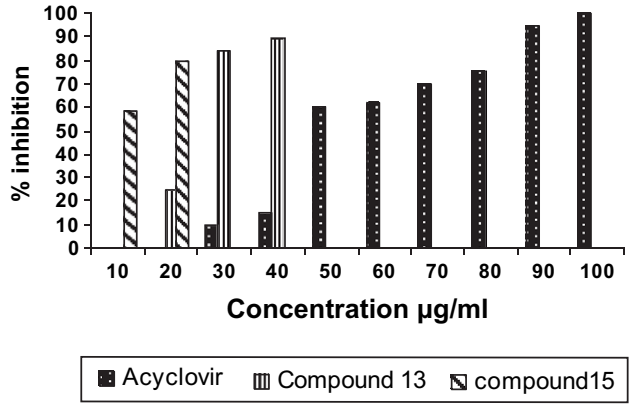


Fig. 1. Comparison between Acyclovir and active compounds.

*4.1.2.2. (3-(3,4-Dimethoxyphenyl)oxiren-2-yl)(5,6,7,8-tetrahydronaphthalen-2-yl)methanone (**3b**).* Yield 92%, mp. 122 °C (EtOH), IR (KBr, cm^{-1}): 1690 (C═O); ^{1}H NMR (DMSO-d_6): δ 1.66–1.70 (m, 4H, 2 CH_2-tetraline), 2.78–2.82 (m, 4H, 2 CH_2-tetraline), 3.81, 3.85 (2s, 6H, 2OCH_3), 4.10, 4.80 (2s, 2H, 2CH-epoxy), 6.90–7.80 (m, 6H, Ar-H); MS (EI, 70 eV): m/z = 338 (M^+, 77), 159 (base peak, 100).

Table 1
Anti-HSV-1 activity of selected newly synthesized compounds.

Code	Concentration µg/ml	Viral count (control)	Viral count (Extract)	% inhibition
3a	2	0.84×10^7	0.84×10^7	0
	5		0.84×10^7	0
3b	2	0.66×10^7	0.50×10^7	24
	5		0.28×10^7	57
3c	2	0.66×10^7	0.46×10^7	30
	5		0.54×10^7	18
4	2	0.84×10^7	0.20×10^7	76
	5		0.20×10^7	76
5	2	0.66×10^7	0.64×10^7	3
	5		0.54×10^7	26
6a	2	0.84×10^7	0.84×10^7	0
	5		0.28×10^7	66
6b	2	0.66×10^7	0.48×10^7	27
	5		0.30×10^7	54
7	2	0.66×10^7	0.66×10^7	0
	5		0.66×10^7	0
8	2	0.84×10^7	0.84×10^7	0
	5		0.84×10^7	0
9	2	0.66×10^7	0.66×10^7	0
	5		0.66×10^7	0
10	2	0.66×10^7	0.44×10^7	33
	5		0.52×10^7	21
11	2	0.84×10^7	0.80×10^7	4
	5		0.84×10^7	0
12	2	0.84×10^7	0.32×10^7	61
	5		0.68×10^7	19
13	2	0.84×10^7	0.08×10^7	90
	5		0.04×10^7	95
14	2	0.84×10^7	0.84×10^7	0
	5		0.72×10^7	14
15	2	0.84×10^7	0.08×10^7	90
	5		0	100
16	2	0.66×10^7	0.64×10^7	3
	5		0.46×10^7	30

Table 2
Mechanism of action of compound **13**.

Activity	Concentration µg/ml	Viral count (control)	Viral count (Extract)	% inhibition
Virucidal	30	0.84×10^7	0.6×10^7	28
	40		CPE	
Adsorbtion	30	0.4×10^7	0.4×10^7	0
	40		0.4×10^7	0
Replication	30	0.1×10^7	0.1×10^7	0
	40		0.1×10^7	0

Analysis calculated for $C_{21}H_{22}O_4$ (338.4): C, 74.54; H, 6.55. Found: C, 74.50; H, 6.51.

*4.1.2.3. (3-(4-Bromophenyl)oxiran-2-yl)(5,6,7,8-tetrahydronaphthalen-2-yl)methanone (**3c**).* Yield 96%, mp. 65 °C (EtOH); IR (KBr, cm^{-1}): 1685 (C═O); ^{1}H NMR (DMSO-d_6): δ 1.69–1.72 (m, 4H, 2 CH_2-tetraline), 2.79–2.80 (m, 4H, 2 CH_2-tetraline), 4.08, 4.80 (2s, 2H, 2CH-epoxy), 6.80–7.80 (m, 7H, Ar-H); MS (EI, 70 eV): m/z = 357 (M^+, 36), 159 (base peak, 100). Analysis calculated for $C_{19}H_{17}BrO_2$ (357.24): C, 63.88; H, 4.80. Found: C, 63.82; H, 4.876.

*4.1.3. Synthesis of 1-phenyl-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-5-p-tolyl-4,5-dihydro-1H-pyrazol-4-ol (**4**)*

A mixture of **3a** (2.9 g, 10 mmol) and phenyl hydrazine (10 mL) in absolute ethanol (30 mL) in the presence of triethylamine (0.5 mL) was refluxed for 3 h. The solvent was concentrated under reduced pressure, poured onto cold water, acidified with dilute hydrochloric acid, the formed precipitate was filtered off, dried and crystallized to give **4**. Yield 74%, mp. 76 °C (EtOH/pet. ether); IR (KBr, cm^{-1}): 3440 (OH), 1600 (C═N); ^{1}H NMR (DMSO-d_6): δ 1.70–1.72 (m, 4H, 2 CH_2-tetraline), 2.30 (s, 3H, CH_3), 2.77–2.80 (m, 4H, 2 CH_2-tetraline), 6.30 (d, 1H, CH-pyrazole), 6.70 (t, 1H, CH-pyrazole), 7.00–7.70 (m, 8H, Ar-H + OH); MS (EI, 70 eV): m/z = 382 (M^+, base peak, 100). Analysis calculated for $C_{26}H_{26}N_2O$ (382.50): C, 81.64; H, 6.85; N, 7.32. Found: C, 81.60; H, 6.80; N, 7.28.

*4.1.4. Synthesis of 1-phenyl-3-(5,6,7,8-tetrahydronaphthalen-2-yl)-5-p-tolyl-1H-pyrazole (**5**)*

4.1.4.1. Method A. A mixture of **3a** (2.9 g, 10 mmol) and phenyl hydrazine (10 mL) in glacial acetic acid (30 mL) was refluxed for 3 h. The reaction mixture was poured onto ice-water, the formed precipitate was filtered off, dried and crystallized to give **5**. Yield 92%, mp. 102 °C (MeOH); IR (KBr, cm^{-1}): 1600 (C═N); ^{1}H NMR (DMSO-d_6): δ 1.65–1.69 (m, 4H, 2 CH_2-tetraline), 2.28 (s, 3H, CH_3), 2.76–2.82 (m, 4H, 2 CH_2-tetraline), 7.00 (s, 1H, CH-pyrazole), 7.10–7.70 (m, 7H, Ar-H); MS (EI, 70 eV): m/z = 364 (M^+, base peak, 100). Analysis calculated for $C_{26}H_{24}N_2$ (364.48): C, 85.68; H, 6.64; N, 7.69. Found: C, 85.62; H, 6.60; N, 7.63.

4.1.4.2. Method B. A solution of **4** (3.8 g, 10 mmol) in glacial acetic acid (20 mL) was refluxed for 2 h. The reaction mixture was concentrated under reduced pressure, then poured onto ice-water,

Table 3
Mechanism of action of compound **15**.

Activity	Concentration µg/ml	Viral count (control)	Viral count (Extract)	% inhibition
Virucidal	20	0.84×10^7	0.06×10^7	92
	30		0.04×10^7	95
Adsorbtion	20	0.4×10^7	0.4×10^7	0
	30		0.4 x10^7	0
Replication	20	0.1×10^7	0.04×10^7	60
	30		0.02×10^7	80

the formed precipitate was filtered off, dried and crystallized to give **5** in 88% yield. The crystallized product was identified by m. p., mixed m. p. and TLC in comparison with authentic sample from Method A.

*4.1.5. Synthesis of 4-(4-substituted phenyl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-thioxo-tetrahydropyrimidin-5(6H)-ones **6a–c***

A mixture of **3a–c** (10 mmol) and thiourea (0.8 g, 10 mmol) in ethanolic potassium hydroxide (30 mL, 4%) was refluxed for 2 h. The reaction mixture was concentrated under reduced pressure, the residue was triturated with diluted hydrochloric acid. The solid formed was collected by filtration, and crystallized to give **6a**, **6b** and **6c**, respectively.

*4.1.5.1. 4-(4-Methylphenyl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-thioxo-tetrahydropyrimidin-5(6H)-one (**6a**).* Yield 95%, mp. 220 °C (dioxane); IR (KBr, cm^{-1}): 3300, 3200 (2NH), 1710 (C═O), 1252 (C═S); ^{1}H NMR (DMSO-d_6): δ 1.70–1.72 (m, 4H, 2 CH_2-tetraline), 2.30 (s, 3H, CH_3), 2.78–2.80 (m, 4H, 2 CH_2-tetraline), 3.00, 3.40 (2d, 2H, 2 CH-pyrimidinone) [23], 6.89–7.40 (m, 7H, Ar-H), 10.65, 11.40 (2s, 2H, 2 NH exchangeable with D_2O); MS (EI, 70 eV): m/z = 350 (M^+, 70), 245 (base peak, 100). Analysis calculated for $C_{21}H_{22}N_2OS$ (350.48): C, 71.97; H, 6.33; N, 7.99; S, 9.15. Found: C, 71.92; H, 6.28; N, 7.95; S, 9.10.

*4.1.5.2. 4-(3,4-Dimethoxyphenyl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-thioxo-tetrahydro-pyrimidin-5(6H)-one (**6b**).* Yield 97%, mp. 231 °C (dioxane); IR (KBr, cm^{-1}): 3350, 3220 (2NH), 1715 (C═O), 1245 (C═S); ^{1}H NMR (DMSO-d_6): δ 1.68–1.70 (m, 4H, 2 CH_2-tetraline), 2.78–2.80 (m, 4H, 2 CH_2-tetraline), 3.10, 3.40 (2d, 2H, 2 CH-pyrimidinone), 3.70, 3.80 (2s, 6H, 2OCH_3), 6.95–7.50 (m, 6H, Ar-H), 10.70, 11.60 (2s, 2H, 2 NH exchangeable with D_2O); MS (EI, 70 eV): m/z = 396 (M^+, 62), 292 (base peak, 100). Analysis calculated for $C_{22}H_{24}N_2O_3S$ (396.50): C, 66.64; H, 6.10; N, 7.07; S, 8.09. Found: C, 66.58; H, 6.02; N, 7.00; S, 8.00.

*4.1.5.3. 4-(4-Bromophenyl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-2-thioxo-tetrahydropyrimidin-5(6H)-one (**6c**).* Yield 92%, mp. 242 °C (MeOH); IR (KBr, cm^{-1}): 3360, 3225 (2NH), 1718 (C═O), 1250 (C═S); ^{1}H NMR (DMSO-d_6): δ 1.70–1.72 (m, 4H, 2 CH_2-tetraline), 2.80–2.84 (m, 4H, 2 CH_2-tetraline), 3.08, 3.42 (2d, 2H, 2 CH-pyrimidinone), 6.95–7.48 (m, 7H, Ar-H), 10.60, 11.38 (2s, 2H, 2 NH exchangeable with D_2O); MS (EI, 70 eV): m/z = 415 (M^+, 35), 231 (base peak, 100). Analysis calculated for $C_{20}H_{19}BrN_2OS$ (415.35): C, 57.83; H, 4.61; N, 6.74; S, 7.72. Found: C, 57.76; H, 4.54; N, 6.70; S, 7.68.

*4.1.6. Synthesis of 5-(3,4-dimethoxyphenyl)-7-(5,6,7,8-tetrahydronaphthalen-2-yl)-2H-thiazolo[3,2-a]pyrimidine-3,6(5H,7H)-dione (**7**)*

A mixture of **6b** (4.0 g, 10 mmol), chlororacetic acid (1.0 g, 10 mmol) and anhydrous sodium acetate (1.6 g, 20 mmol) in glacial acetic acid (30 mL) and acetic anhydride (10 mL) was refluxed for 3 h. The reaction mixture was poured into water, the formed solid was filtered off and crystallized to give **7**. Yield 66%, mp. 153 °C (*Et*OH); IR (KBr, cm^{-1}): 1710 (C═O), 1700 (C═O); ^{1}H NMR (DMSO-d_6): δ 1.65–1.68 (m, 4H, 2 CH_2-tetraline), 2.75–2.80 (m, 4H, 2 CH_2-tetraline), 3.60 (s, 2H, CH_2), 3.70, 3.85 (2d, 2H, 2 CH-pyrimidinone), 3.80, 3.88 (2s, 6H, 2OCH_3), 6.90–7.40 (m, 6H, Ar-H); MS (EI, 70 eV): m/z = 436 (M^+, 10), 288 (base peak, 100). Analysis calculated for $C_{24}H_{24}N_2O_4S$ (436.52): C, 66.03; H, 5.54; N, 6.42; S, 7.35. Found: C, 65.95; H, 5.50; N, 6.36; S, 7.30.

*4.1.7. 2-(4-Chlorobenzylidene)-5-(3,4-dimethoxyphenyl)-7-(5,6,7,8-tetrahydronaphthalen-2-yl)-2H-thiazolo[3,2-a]pyrimidine-3,6(5H,7H)-dione (**8**)*

4.1.7.1. Method A. A mixture of **7** (4.4 g, 10 mmol) and 4-chlorobenzaldehyde (1.4 g, 10 mmol) in glacial acetic acid/acetic anhydride (40 mL, 3:1) was refluxed for 3 h, allowed to cool, then poured onto water. The solid formed was collected by filtration and crystallized to give **8**. Yield 82%, mp. 195 °C (EtOH); IR (KBr, cm^{-1}): 1730 (C═O), 1710 (C═O); ^{1}H NMR (DMSO-d_6): δ 1.70–1.72 (m, 4H, 2 CH_2-tetraline), 2.74–2.78 (m, 4H, 2 CH_2-tetraline), 3.70, 3.75 (2s, 6H, OCH_3), 3.72, 3.90 (2d, 2H, 2 CH-pyrimidinone), 6.85–7.40 (m, 11H, Ar-H + CH-arylidine); MS (EI, 70 eV): m/z = 559 (M^+, 0.3), 151 (base peak, 100). Analysis calculated for $C_{31}H_{27}ClN_2O_4S$ (559.08): C, 66.60; H, 4.87; Cl, 6.34; N, 5.01; S, 5.74. Found: C, 66.55; H, 4.82; Cl, 6.28; N, 4.95; S, 5.68.

4.1.7.2. Method B. To a mixture of **6b** (4.4 g, 10 mmol), chloroacetic acid (1.0 g, 10 mmol) and anhydrous sodium acetate (1.60 g, 20 mmol) in glacial acetic acid/acetic anhydride (40 mL, 3:1, ratio), 4-chlorobenzaldehyde (10 mmol) was added. The reaction mixture was heated under reflux for 2 h, then cooled and poured into water. The solid formed was collected by filtration and crystallized to yield **8** in 84% yield. The crystallized product was identified by m. p., mixed m. p. and TLC in comparison with authentic sample from Method A.

*4.1.8. (3,6-Bis(4-bromophenyl)-1,4-dioxane-2,5-diyl)bis((5,6,7,8-tetrahydronaphthalen-2-yl)methanone) (**9**)*

Compound **3c** (3.6 g, 1 mmol) was fused in an oil bath at 180 °C for 4 h. The obtained residue was triturated with water, the formed precipitate was filtered off, washed with water, dried and crystallized to give **9**. Yield 82%, mp. 135 °C (MeOH); IR (KBr, cm^{-1}): 1685 (2C═O); ^{1}H NMR (DMSO-d_6): δ 1.65–1.69 (m, 4H, 2 CH_2-tetraline), 2.78–2.80 (m, 4H, 2 CH_2-tetraline), 5.35–5.55 (m, 4H, 4CH-dioxane), 6.90–7.80 (m, 14H, Ar-H); MS (EI, 70 eV): m/z = 714 (M^+, 10), 159 (base peak, 100). Analysis calculated for $C_{38}H_{34}Br_2O_4$ (714.48): C, 63.88; H, 4.80. Found: C, 63.80; H, 4.75.

*4.1.9. Synthesis of Tetrahydropyrimidinone **10** and **11***

A mixture of **3b** (10 mmol) and urea (10 mmol) or **3a** (10 mmol) and guanidine (10 mmol) in ethanolic potassium hydroxide (50 mL, 4%) was refluxed for 2 h. The solvent was concentrated under reduced pressure, the reaction mixture was poured onto acidified water. The solid formed was collected by filtration, dried and crystallized to give **10** and **11**, respectively.

*4.1.9.1. 4-(3,4-Dimethoxyphenyl)-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-tetrahydropyrimidine-2,5-dione (**10**).* Yield 78%, mp. 230 °C (EtOH); IR (KBr, cm^{-1}): 3250, 3400 (2NH), 1720, 1700 (2C═O); ^{1}H NMR (DMSO-d_6): δ 1.66–1.70 (m, 4H, 2 CH_2-tetraline), 2.76–2.79 (m, 4H, 2 CH_2-tetraline), 3.78, 3.85 (2d, 6H, 2OCH_3), 5.50, 5.70 (2s, 2H, 2 CH-pyrimidinone), 6.70–7.30 (m, 6H, Ar-H), 10.60, 11.40 (2s, 2H, 2 NH exchangeable with D_2O); MS (EI, 70 eV): m/z = 380 (M^+, 56), 158 (base peak, 100). Analysis calculated for $C_{22}H_{24}N_2O_4$ (380.44): C, 69.46; H, 6.36; N, 7.36. Found: C, 69.40; H, 6.30; N, 7.30.

*4.1.9.2. 4-(3,4-Dimethoxyphenyl)-2-imino-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-tetrahydro-pyrimidin-5(6H)-one (**11**).* Yield 72%, mp. > 300 °C (dioxane); IR (KBr, cm^{-1}): 3180, 3320, 3348 (3NH), 1710 (C═O), (C═N); ^{1}H NMR (DMSO-d_6): δ 1.70–1.72 (m, 4H, 2 CH_2-tetraline), 2.30 (s, 3H, CH_3),2.80–2.82 (m, 4H, 2 CH_2-tetraline), 3.11, 3.30 (2s, 2H, 2 CH-pyrimidinone), 7.00–7.30 (m, 7H, Ar-H), 7.50, 7.70, 8.80 (3s, 3H, 3 NH exchangeable with D_2O); MS (EI, 70 eV): m/z = 379 (M^+, base peak, 100). Analysis calculated for $C_{22}H_{25}N_3O_3$ (379.45): C, 69.64; H, 6.64; N, 11.07. Found: C, 69.58; H, 6.60; N, 11.00.

*4.1.10. Synthesis of S-6-(3,4-dimethoxyphenyl)-5-oxo-4-(5,6,7,8-tetrahydronaphthalen-2-yl)-1,4,5,6-tetrahydro-pyrimidin-2-yl O-ethyl carbonothioate (**12**)*

A mixture of **6b** (4.0 g, 10 mmol) and ethyl chloroformate (1.3 g, 10 mmol) in alcoholic potassium hydroxide (30 mL, 2%) was

refluxed for 2 h. The reaction mixture was poured onto acidified ice-water, the solid formed was filtered off, dried and crystallized to give **12**. Yield 88%, mp. 202 °C (EtOH), IR (KBr, cm^{-1}): 3300 (NH), 1740 (C═O, ester), 1720 (C═O, thiopyrimidone); ^{1}H NMR (DMSO-d_6): δ 1.35 (t, 3H, CH_3), 1.66–1.70 (m, 4H, 2 CH_2-tetraline), 2.78–2.82 (m, 4H, 2 CH_2-tetraline), 3.00, 3.40 (2d, 2H, 2 CH-pyrimidinone), 3.60, 3.65 (2s, 6H, 2OCH_3), 4.15 (q, 2H, CH_2), 6.85–7.40 (m, 6H, Ar-H), 8.95 (s, 1H, NH exchangeable with D_2O); MS (EI, 70 eV): m/z = 468 (M^+, 2), 396 (base peak, 100). Analysis calculated for $C_{25}H_{28}N_2O_5S$ (468.57): C, 64.08; H, 6.02; N, 5.98; S, 6.84. Found: C, 65.96; H, 5.95; N, 5.92; S, 6.80.

*4.1.11. Synthesis of 2-(2,4-dioxopentan-3-ylthio)-1,6-dihydro-4-(1,2,3,4-tetrahydronaphthalen-6-yl)-6-(3,4-dimethoxyphenyl)pyrimidin-5(4H)-one (**13**)*

To a solution of **6b** (4.0 g, 10 mmol) in alcoholic potassium hydroxide (50 mL, 2%), 3-chloroacetylacetone (1.40 g, 10 mmol) was added drop wise with stirring. The reaction mixture was stirred at room temperature for 1 h, then poured onto water, the formed solid was filtered off, dried and crystallized to give **13**. Yield 72%, mp. 226 °C (dioxane); IR (KBr, cm^{-1}): 3220 (NH), 1715 (C═O), 1685 (2C═O); ^{1}H NMR (DMSO-d_6): δ 1.67–1.70 (m, 4H, 2 CH_2-tetraline), 2.77–2.80 (m, 4H, 2 CH_2-tetraline), 2.55, 2.65 (2s, 6H, 2 $COCH_3$), 3.70, 3.72 (2s, 6H, 2OCH_3), 4.45, 4.65 (2d, 2H, 2 CH-pyrimidinone), 4.55 (s, 1H, CH), 6.90–7.40 (m, 6H, Ar-H), 8.20 (s, 1H, NH exchangeable with D_2O); MS (EI, 70 eV): m/z = 494 (M^+, 15), 269 (base peak, 100). Analysis calculated for $C_{27}H_{30}N_2O_5S$ (494.60): C, 65.57; H, 6.11; N, 5.66; S, 6.48. Found: C, 65.50; H, 5.98; N, 5.60; S, 6.44.

*4.1.12. Synthesis of 2-acetyl-5-(3,4-dimethoxyphenyl)-3-methyl-7-(5,6,7,8-tetrahydronaphthalen-2-yl)-5H-thiazolo[3,2-a]pyrimidin-6(7H)-one (**14**)*

4.1.12.1. Method A. A mixture of **6b** (4.0 g, 10 mmol) and 3-chloroacetylacetone (1.4 g, 10 mmol) in pyridine (20 mL) in the presence of potassium hydroxide (10 mmol) was heated under reflux for 4 h. The reaction mixture was poured onto acidified cold water, the formed solid was filtered off, dried and crystallized to give **14**. Yield 70%, mp. 165 °C (DMF/H_2O); IR (KBr, cm^{-1}): 1710 (C═O), 1690 (C═O); ^{1}H NMR (DMSO-d_6): δ 1.60 (s, 3H, CH_3), 1.65–1.68 (m, 4H, 2 CH_2-tetraline), 2.30 (s, 3H, CH_3), 2.78–2.80 (m, 4H, 2 CH_2-tetraline), 3.50, 3.65 (2d, 2H, 2 CH-pyrimidinone), 3.65, 3.68 (2s, 6H, 2OCH_3), 7.10–8.00 (m, 7H, Ar-H); MS (EI, 70 eV): m/z = 476 (M^+, base peak, 100). Analysis calculated for $C_{27}H_{28}N_2O_4S$ (476.59): C, 68.04; H, 5.92; N, 5.88; S, 6.73. Found: C, 67.96; H, 5.88; N, 5.82; S, 6.66.

4.1.12.2. Method B. A mixture of **13** (4.0 g, 10 mmol) and acetic anhydride (20 mL) in dry pyridine (30 mL) was heated on a water bath for 6 h. The reaction mixture was poured onto cold water, the formed solid was filtered off, dried and crystallized to give **14** in 65% yield. The crystallized product was identified by m. p., mixed m. p. and TLC in comparison with authentic sample from Method A.

*4.1.13. Synthesis of thiazolo- and thiazinopyrimidines **15** and **16***

A mixture of **6b** (4.0 g, 10 mmol), 2-bromopropanoic acid or 3-bromopropanoic acid (10 mmol) and anhydrous sodium acetate (2 g) in glacial acetic acid (30 mL) and acetic anhydride (10 mL) was refluxed for 4 h. The reaction mixture was poured into cold water, the formed solid was filtered off, dried and crystallized to give **15** and **16**, respectively.

*4.1.13.1. 5-(3,4-Dimethoxyphenyl)-2-methyl-7-(5,6,7,8-tetrahydronaphthalen-2-yl)-2H-thiazolo-[3,2-a]pyrimidine-3,6(5H,7H)-dione (**15**).* Yield 56%, mp. 162 °C (MeOH); IR (KBr, cm^{-1}): 1710 (C═O), 1690 (C═O); ^{1}H NMR (DMSO-d_6): δ 1.45 (d, 3H, CH_3), 1.68–1.70 (m, 4H, 2 CH_2-tetraline), 2.78–2.82 (m, 4H, 2 CH_2-tetraline), 3.60, 3.85 (2d, 2H, 2 CH-pyrimidinone), 3.70, 3.75 (2s, 6H, 2OCH_3), 3.90 (q, 1H, CH), 6.60–7.30 (m, 6H, Ar-H); MS (EI, 70 eV): m/z = 450 (M^+, 12), 269 (base peak, 100). Analysis calculated for $C_{25}H_{26}N_2O_4S$ (450.55): C, 66.64; H, 5.82; N, 6.22; S, 7.12. Found: C, 66.59; H, 5.75; N, 6.12; S, 7.05.

*4.1.13.2. 6-(3,4-Dimethoxyphenyl)-8-(5,6,7,8-tetrahydronaphthalen-2-yl)-2,3-dihydropyrimido[2,1-b][1,3]thiazine-4,7(6H,8H)-dione (**16**).* Yield 62%, mp. 182 °C (MeOH); IR (KBr, cm^{-1}): 1725 (C═O), 1710 (C═O); ^{1}H NMR (DMSO-d_6): δ 1.68–1.70 (m, 4H, 2 CH_2-tetraline), 2.60–2.75 (m, 4H, 2CH_2-thiazine), 2.78–2.79 (m, 4H, 2 CH_2-tetraline), 3.60, 3.90 (2d, 2H, 2 CH-pyrimidinone), 3.68, 3.70 (2s, 6H, 2OCH_3), 6.70–7.80 (m, 6H, Ar-H); MS (EI, 70 eV): m/z = 450 (M^+, 6), 151 (base peak, 100). Analysis calculated for $C_{25}H_{26}N_2O_4S$ (450.55): C, 66.64; H, 5.82; N, 6.22; S, 7.12. Found: C, 66.60; H, 5.76; N, 6.14; S, 7.05.

4.2. Antiviral assay

4.2.1. Material and methods

Compounds (1 mg) were dissolved in 1 mL (10% DMSO and 90% deionized water) and sterilized by 1% antibiotic–antimycotic mixture.

4.2.1.1. Cells. Vero cell line was used. The cells were propagated in DMEM medium (Applichem,Germany) supplemented with 10% fetal bovine serum (Sigma), 1% antibiotic–antimycotic mixture (PAA Laboratories GmbH, Austria). The pH was adjusted to 7.2–7.4 using 7.5% sodium bicarbonate solution. The mixture was sterilized by filtration through 0.2 μm pore size nitrocellulose membrane.

4.2.1.2. Plaque reduction assay. Assay was carried out according to the method of Tebas et al., [24] in a six well plate where vero cells (10^5 cell/ml) were cultivated for 2 days at 37 °C. HSV-1 was diluted to give 10^4 PFU/well and mixed with the safe concentration of the compound or different concentrations of acyclovir (used as positive control) and incubated for 1 h at 37 °C before being added to the cells. Growth medium was removed from the cell culture plates and virus-extract or virus- acyclovir mixtures were inoculated (100 μL/well), After 1 h contact time for virus adsorption, 3 mL of Dulbecco's Modified Eagles Media (DMEM) supplemented with 2% agarose was added onto the cell monolayer, plates were left to solidify and incubated at 37 °C till formation of viral plaques. Formalin (10%) was added for 2 h then plates were stained with crystal violet. Control wells were included where untreated virus was incubated with Vero cells and finally plaques were counted and percentage reduction in plaques formation in comparison to control wells was recorded as following:

$$\%\ \text{inhibition} = \text{viral count(untreated)} - \text{viral count(treated)}/\text{viral count(untreated)} \times 100$$

4.2.1.3. Mechanism of virus inhibition. Possible mechanism of HSV-1 inhibition by the compound was studied at three different levels:

4.2.1.3.1. Viral replication [25]. Assay was carried out in a 6 well plate where Vero cells were cultivated (10^5 cell/ml) for 2 days at 37 °C. Virus was diluted to give 10^4 PFU/well and applied directly to the cells and incubated for 1 h at 37 °C, unadsorbed viral particles were removed by washing cells three successive times by supplements free-medium. Compound was applied at different concentration, after 1 h contact time, 3 mL of DMEM medium

supplemented with 2% agarose was added to the cell monolayer. Plates were left to solidify and incubated at 37 °C till appearance of viral plaques. Cell monolayers were fixed in 10% formalin solution for 2 h, and stained with crystal violet. Control wells were included where Vero cells were incubated with the virus and finally plaques were counted and percentage reduction in plaques formation in comparison to control wells was recorded as above mentioned.

4.2.1.3.2. Viral adsorption [26]. Vero cells were cultivated in a 6 well plate (10^5 cell/ml) for 2 days at 37 °C. Compound was applied at different concentrations in 200 μL medium without supplements and coincubated with the cells for 2 h at 4 °C. Unadsorbed extract was removed by washing cells three successive times with supplements free-medium then HSV-1 virus diluted to give 10^4 PFU/well was coincubated with the pretreated cells for 1 h followed by adding 3 mL DMEM supplemented with 2% agarose. Plates were left to solidify then incubated at 37 °C to allow formation of viral plaques, fixed and stained as above mentioned to calculate percentage reduction in plaques formation in comparison to control wells where untreated Vero cells were directly infected with HSV-1.

4.2.1.3.3. Virucidal [27]. Assay was carried out in a 6 well plate where Vero cells were cultivated (10^5 cell/ml) for 2 days at 37 °C. A volume of 200 μl serum free DMEMcontaining 10^7 PFU forming HSV-1 was added to the concentration of compound resulting in viral inhibition, after 1 h incubation, the mixture was diluted using serum free-medium 3 times each 10 fold that still allows existence of viral particles to grow on Vero cells but leaves nearly no extract and 100 μL of each dilution were added to the Vero cell monolayer. After 1 h contact time, DMEM overlayer was added to cell monolayer. Plates were left to solidify then incubated at 37 °C to allow formation of viral plaques, fixed and stained as above mentioned to calculate percentage reduction in plaques formation in comparison to control wells where cell were infected with virus that was not pretreated with the compound.

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