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Structure based molecular design, synthesis and biological evaluation of α-pyrone analogs as anti-HSV agent

Srinivas Karampuri [a], Paromita Bag [b], Sabina Yasmin [a], Devendra Kumar Chouhan [a], Chandralata Bal [a], Debashis Mitra [c], Debprasad Chattopadhyay [b], Ashoke Sharon [a,*]

[a] Department of Applied Chemistry, Birla Institute of Technology, Mesra, Ranchi 835215, India
[b] ICMR Virus Unit, ID & BG Hospital, Beliaghata, Kolkata 700010, India
[c] National Centre for Cell Science, NCCS Complex, Pune University Campus, Ganeshkhind, Pune 411007, India

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ABSTRACT

Several options for treating Herpes Simplex Virus type 1 and type 2 are available. However, non-specific inhibition and drug resistance warrants the discovery of new anti-herpetic compounds with better therapeutic profile or different mode of action. The non-nucleoside inhibitors of HSV DNA polymerase target the site that is less important for the binding of a natural nucleoside or nucleoside inhibitors. In the present study, we have explored the possibility to find a new lead molecule based on α-pyrone analogs as non-nucleoside inhibitors using structure based modeling approach. The designed molecules were synthesized and evaluated for anti-HSV activity using MTT assay. The compound **5h** with EC_{50} 7.4 μg/ml and CC_{50} 52.5 μg/ml was moderately active against HSV when compared to acyclovir. A plaque reduction assay was also carried out and results reveal that **5h** is more effective against HSV-1 with better selective index of 12.8 than against HSV-2 (SI = 3.6). The synthesized compounds were also evaluated for anti-HIV activity, but none were active.

Herpes Simplex Virus type 1 and type 2 (HSV-1 and HSV-2) are members of Herpesviridae family, which causes several symptoms in humans including cold sores, encephalitis and are responsible for recurrent orolabial and genital infections.[1] Transmission occurs by contact with secretions from an infected person either by overt infection or asymptomatic excretion of virus. After primary infection, HSV establishes long-term latency in the ganglia of sensory nerves, from which it can reactivate episodically. There is no permanent cure for these infections till date. The goals of treatment are to accelerate lesion healing and to prevent transmission. HSV infections can be severe in immune-compromised patients, particularly those with defected cell-mediated immunity, recipients of solid organ or bone marrow transplants.[2–4] DNA replication in the virus is mediated through its DNA polymerase enzyme. Hence HSV DNA polymerase can be utilized as a target for designing anti-HSV drugs. The present treatment involves the use of mainly two types of HSV DNA polymerase inhibitors as drugs.[5–7] The first strategy includes the application of nucleoside analogs (e.g., acyclovir) or its prodrugs (e.g., valacyclovir). The nucleoside analogs require phosphorylation by viral thymidine kinase followed by host kinases to be converted into nucleoside triphosphate which is the actual inhibitor of viral DNA polymerase. The second category consists of non nucleoside analogs.[8] However, in past few years, a number of resistant virushave been isolated especially from immune-compromised patients.[9,10] In this era, where the number of immune-suppressed patients like those suffering from HIV is continuously increasing, there is an immediate need to find new drugs to treat HSV infections which have a higher efficacy or have an alternative mode of action. A number of new anti-viral drugs against HSV focusing other domains of the viral DNA polymerase are currently under research and development.[11–13] One such novel class of compounds is that of 4-oxo-dihydroquinolines (4-oxo-DHQs, Fig. 1, I) belonging to the non-nucleoside inhibitor (NNI) family.[14–16] 4-oxo-DHQs have shown high specificity index in inhibiting DNA polymerases of herpesviridae family because unrelated DNA and RNA viruses were not susceptible to their inhibitory effect.[15,16] Recently, Withaferin A (WA: **II**; Fig. 1), a naturally occurring C28-steroidal lactone from Indian ginseng[17] has been found to exert inhibitory effect via interaction with a viral DNA polymerase site that is less important for the binding of deoxynucleoside triphosphates. Therefore, it is speculated that it could be active on resistant viruses also.[18] Recently, benzylidene derivatives of bis-(4-hydroxycoumarin) (**III**) have been reported for their mild anti-HSV activity.[19] The structural inspection of these three types of molecules reveals; **I** and **III** have two close keto groups as common pharmacophoric motif (red color). Further, the compound **II** has a steroidal structure and has a α-pyrone ring.

* Corresponding author.
E-mail address: asharon@bitmesra.ac.in (A. Sharon).

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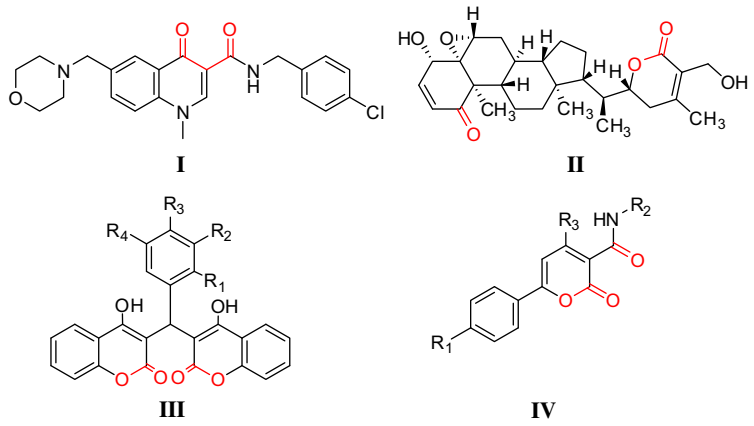


Figure 1. The chemical structure of anti-HSV compound; 4-oxo-dihydroquinoline analog (**I**), Withaferin A (WA, **II**) and benzylidene derivative of bis-(4-hydroxycoumarin) (**III**). The possible functional pharmacophore (pyran/di-keto motif) is shown in red color. The prototype molecule **IV** selected as preliminary designed molecule, consisting of pyran and di-keto motif.

In continuation to our anti-viral drug discovery program, we intended to merge these specialties together into a single skeleton to synthesize α-pyrone analogs having two close keto groups. The initially selected prototype analogs **IV** were subjected for HSV DNA polymerase binding studies. The semi-empirical binding score and binding mode were evaluated against HSV DNA polymerase in the palm region and considered as preliminary basis for the screening of the analogs for synthesis.

A model structure (Fig. 2) was prepared utilizing available PDB crystal structure of HSV DNA polymerase (PDB ID 2GV9) as starting structure.[20]

DNA polymerase is a heterodimer composed of the UL30 and UL42 gene products. The UL30 gene encodes the catalytic subunit, while the UL42 gene encodes a phosphoprotein that possesses double-stranded DNA-binding activity. The overall structure comprises of 6 structural domains; Pre $-NH_2$ domain, $-NH_2$ domain, 3′-5′ exonuclease domain, Palm, Finger and Thumb. All these domains assemble to form a disk like central hole having $-NH_2$ and –COOH termini at opposite sides of the protein. The catalytic site is situated in the palm subdomain and contains the catalytic triad of aspartic acid residues (at positions 717, 886, and 888) essential for polymerase activity.[21] Therefore, prototype analogs (**IV**) were docked using docking program Glide[22] of Schrodinger Suite[23] in the palm region to investigate the binding mode (Fig. 3, prototype analogs **5h**). The surface diagram reveals that the **5h** utilizes pyran and diketo motif to bind into the inside of the palm region cavity. Most of molecules studied had similar binding mode as that of **5h**, however interaction pattern were significantly different which lead to different G-score (relative binding score by Glide, Table 1).

A recent binding mechanism study of WA (**II**) through modeling reports the role of hydrophobic interactions mediated by Phe 718, Tyr 722, Pro 723 and Tyr 818 as well as electrostatic interaction mediated by Asn 815 and Phe 718 for stabilization of binding with target enzyme.[18] The ligand-receptor diagram (Fig. 4) of **5h** discloses similar major residues (Tyr 818, Tyr 722, Leu 721, Phe 718, Asn 815) as was reported in the case of the binding mode of anti-HSV compound WA (**II**). These interactions help the binding of **5h** into central core of HSV DNA polymerase near to palm region (Fig. 2).

The in silico analysis using QikProp were also conducted to assess the drug like properties of selected molecules (Table 1). None of the molecules violated Lipinski's rule[24] of five as well as Jorgensen's rule[25,26] of three (Table 1). Thus, the in silico studies supported the selection of analogs for synthesis to access the anti-HSV potential.

Ketene dithioacetals are versatile reagents which have been extensively utilized in literature for the synthesis of diverse class of molecules.[27–32] Appropriate acetophenones were used as an economic starting material for the reaction with CS_2 to form the

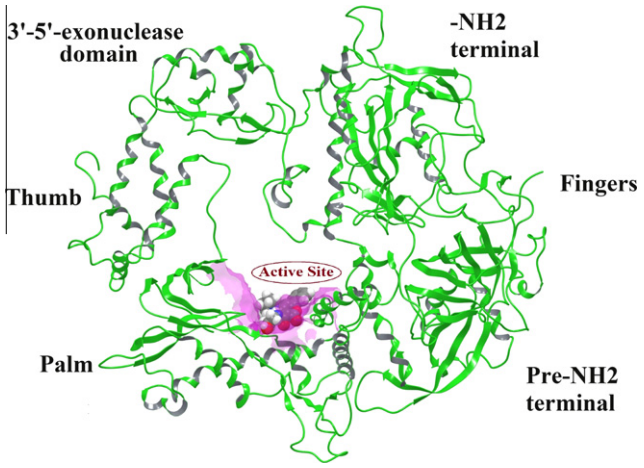


Figure 2. Model structure of HSV DNA polymerase showing 3′-5′-exonuclease domain, palm, finger and thumb domain. The docked pose of prototype molecule **IV** (**5h**) was used to show the active site at HSV-polymerase palm region. Overall the active site is located in the central core of enzyme near to palm surface.

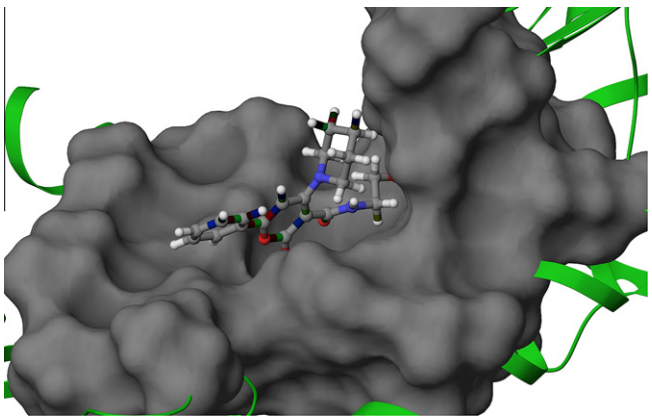


Figure 3. Surface diagram showing active site grooves for the binding of **5h** into the inside of the palm region cavity.

Table 1
In silico binding analysis and drug like properties (DLP) evaluation of prototype analogs (**IV**)

IV	R^1	R^2	R^3	G-Score[a]	DLP violation[b]	
					#5[c]	#3[d]
5a	H	C_2H_4OH	C_4H_8N	−5.3	0	0
5b	F	C_2H_4OH	$C_5H_{10}N$	−4.0	0	0
5c	H	C_6H_5	C_4H_8N	−2.8	0	0
5d	H	4-Cl-C_6H_4	C_4H_8N	−2.6	0	1
5e	H	4-Br-C_6H_4	C_4H_8N	−2.7	0	1
5f	H	4-F-C_6H_4	C_4H_8N	−2.6	0	1
5g	H	4-Me-C_6H_4	C_4H_8N	−2.7	0	1
5h	H	C_2H_4OH	$C_5H_{10}N$	−3.7	0	0
5i	F	C_2H_4OH	C_4H_8N	−4.9	0	0

[a] G-Score (Glide Score): The minimized poses are rescored using Schrödinger's proprietary GlideScore scoring function and the binding affinity can be estimated by G-Score.
[b] DLP: Drug Like Properties.
[c] #**5**: Number of violations of Lipinski's rule of five. The rules are: molecular weight MW <500, QPlogPo/w <5, donorHB ⩽ 5, accptHB ⩽ 10. Compounds that satisfy these rules are considered drug-like.
[d] #**3**: Number of violations of Jorgensen's rule of three. The three rules are: QPlogS >−5.7, QPPCaco > 22 nm/s, Primary Metabolites <7. Compounds with fewer violations of these rules are more likely to be orally available.

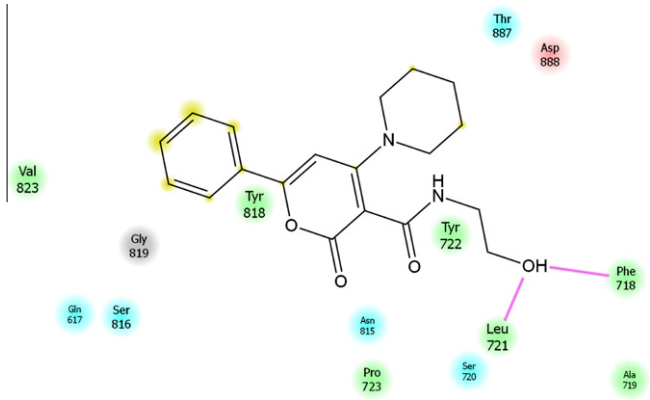


Figure 4. Ligand-receptor diagram showing the major hot spot residues (hydrophobic: Val 823, Tyr 818, Tyr 722, Leu 721, Phe 718, Pro 723, Ala 719; acidic: Asp 888; hydrophilic: Asn 815, Ser 720, Ser 816, Gln 617, and Thr 887) causing the binding of **5h** into central core of HSV DNA polymerase near to palm region (see Fig. 2).

ketene dimercapto intermediate in situ followed by addition of methyl iodide to yield substituted ketene dithioacetals (**2a**–**b**, Scheme 1).[33]

One-pot synthetic approach was applied to yield the α-pyrone derivates (**3a**–**b**) having carboxylic acid group at C-3 position from **2a**–**b** using diethyl malonate as a carbanion source in presence of NaH.[34] In brief, the generated diethyl malonate carbanion formed C–C bond with ketene dithioacetal followed by abstraction of another active methylene proton from the intermediate to facilitate ring closure to form α-pyrone C-3 ester derivate, which undergone hydrolysis in situ. 2-(7-Aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HATU) as a coupling reagent[35] was used to couple amines with the carboxyl group of **3a**–**b** to generate respective amides (**4a**–**g**) in more than 70% yield.[36] The methylthio group at C-4 of **4a**–**g** was replaced with secondary amines[37] by heating in dioxane to yield the designed molecules **5a**–**i**.[38] All the intermediates were characterized by spectroscopic analysis. The physical and spectroscopic analysis data for **5a**–**i** are summarized in Table 2.

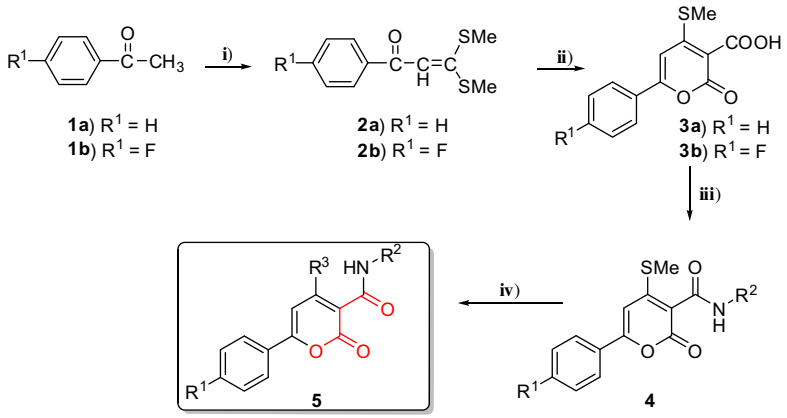
The antiviral activity of **5a**–**i** against HSV-1 was evaluated by MTT assay on African green monkey kidney cell (Vero cells, ATCC, Manassas, VA, USA) and was compared with Acyclovir. Compounds **5b**, **5d**–**f** and **5i** were either not properly soluble in DMSO or formed crystal with the media. Therefore, their cytopathic effect could not be determined. The % of inhibition of virus yield was initially determined for **5a**, **5c**, **5g** and **5h** at the concentration of 20 μg/ml. Our results show that these compounds can reduce the virus yield in 62–89% range (Table 3). These compounds were also tested for their ability to inhibit cytopathic effect caused by HSV-1 as well as their toxicity to Vero cells. The EC_{50} and CC_{50}, respectively, were calculated on the basis of these results (Table 3).

The compound **5h** was moderately active with EC_{50} 7.4 ± 0.4 μg/ml compared to 2.1 ± 0.1 μg/ml for acyclovir. The CC_{50} of **5h** was 52.5 μg/ml and selectivity index (SI) 7.1. Compounds **5c** and **5g** were not found significant. The compound **5a** was found to have comparable activity with **5h**, but with less SI of 3.1 over 7.1. The specificity of **5h** for HSV-1 or HSV-2 was determined by plaque reduction assay. The test was used to access the antiviral activity against HSV-1 and HSV-2, using acyclovir and DMSO (0.1%) as positive and negative control, respectively. The results revealed that **5h** at a concentration of 4.1 and 14.5 μg/ml inhibited 50% plaque formation by HSV-1 and HSV-2, (Figs. 5a and 5b). The results also showed that **5h** is more effective against HSV-1 (Table 4), with better selective index of 12.8 than against HSV-2 (SI = 3.6).

To build a model structure, PDB crystal structure of HSV DNA polymerase (PDB: 2GV9) was obtained from protein databank. The structure was optimized and prepared using Protein Preparation Wizard (PPW) module in Maestro interface of Schrodinger suit 2011.[23] As the crystal structure consists two identical polypeptide chains, one chain was deleted and the other was modeled for further studies. The obtained structure was submitted for a short minimization through Impref module of PPW followed by a MacroModel[39] minimization employing OPLS2005 force field with 5000 iterations. A dynamic simulation of 1.2 ns was performed through Desmond[40] in explicit solvent system to observe the conformational behavior of structure in presence of water as a solvent. The average structure obtained was minimized through MacroModel.[39] The final structure (Fig. 3) thus obtained was considered to investigate the binding profile with docking studies. Receptor grid was generated using Glide receptor grid generation utility of Schrodinger's suite 2011. The grid was generated around the catalytic traid of aspartic acid residues (717, 886 and 888). All ligands (**IV**, Fig. 1) were built in Maestro interface of Schrodinger suite 2011. Finally all compounds were treated in LigPrep module of Schrodinger suite 2011 using OPLS2005 force field followed by conformational search analysis through MacroModel using MMFFs force field to obtain the most favored conformations. The lowest energy conformer was selected for each ligand to perform docking studies. The lowest energy conformer of compound **5a**–**i** was submitted for drug like properties analysis using QikProp module of Schrodinger. The reference (WA) was also prepared for docking with the same protocol mentioned above. Glide module of Schrodinger suite 2011 was used to perform docking analysis. All ligands and reference were docked at the predefined receptor grid using XP mode. Overall, the estimated essential in silico properties of compounds were summarized in Table 1.

African green monkey kidney cells (Vero cells, ATCC, Manassas, VA, USA) were grown and maintained in Eagle's minimum essential medium (EMEM), supplemented with 5–10% fetal bovine serum (FBS).[41] The standard control strain HSV-2G (ATCC-734) and HSV-1F (ATCC-733) were purchased from the ATCC. After plaque purification, the virus was grown and the virus stocks were stored at −80 °C for future use[42] and whenever required the virus stocks were grown on Vero cells to determine the titers and used for further study.

Cell toxicity was monitored by determining the effect of the test compounds on cell viability.[43] For this Vero cells were cultured



5a) R^1 = H, R^2 = C_2H_4OH, R^3 = C_4H_8N
5b) R^1 = F, R^2 = C_2H_4OH, R^3 = C_4H_8N
5c) R^1 = H, R^2 = C_6H_5, R^3 = C_4H_8N
5d) R^1 = H, R^2 = 4-Cl-C_6H_4, R^3 = C_4H_8N
5e) R^1 = H, R^2 = 4-Br-C_6H_4, R^3 = C_4H_8N
5f) R^1= H, R^2 = 4-F-C_6H_4, R^3 = C_4H_8N
5g) R^1 = H, R^2 = 4-Me-C_6H_4, R^3 = C_4H_8N
5h) R^1 = H, R^2 = C_2H_4OH, R^3 = $C_5H_{10}N$
5i) R^1 = F, R^2 = C_2H_4OH, R^3 = $C_5H_{10}N$

4a) R^1 = H, R^2 = C_2H_4OH
4b) R^1 = F, R^2 = C_2H_4OH
4c) R_1 = H, R^2 = C_6H_5
4d) R_1 = H, R^2 = 4-Cl-C_6H_4
4e) R_1 = H, R^2 = 4-Br-C_6H_4
4f) R_1 = H, R^2 = 4-F-C_6H_4
4g) R_1 = H, R^2 = 4-Me-C_6H_4

Scheme 1. Reagents and conditions: (i) NaH, THF, CS_2, MeI, 0–5 °C, 4 h; (ii) NaH, diethylmalonate, dioxane, 0–70 °C, 6 h; (iii) R^2NH_2, HATU, DMF, rt, 6 h; (iv) R^3H, dioxane, 70 °C, 4 h.

Table 2
The analytical data of synthesized compounds

Sl. No.	Yield (%)	MP (°C)	Spectral data
5a	55	186–187	MS-ESI (m/z): [M^+] 329.1; IR: (KBr) (ν, cm^{-1}) 3270.2 (NH), 1659.8 and 1525.8 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.88–1.90 (m, 4H), 3.26 (t, J = 6 Hz, 2H), 3.47 (t, J = 6 Hz, 2H), 3.54–3.57 (m, 4H), 4.60–4.63 (m, 1H, OH), 6.80 (s, 1H), 7.51–7.53 (m, 3H), 7.90–7.91 (m, 2H), 8.07 (s, 1H, NH)
5b	46	165–166	MS-ESI (m/z): [M^+] 347.1; IR: (KBr) (ν, cm^{-1}), ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.88–1.90 (m, 4H), 3.23 (t, J = 6 Hz, 2H), 3.45 (t, J = 6 Hz, 2H), 3.47–3.49 (m,4H), 4.60–4.63 (m,1H, OH), 7.34–7.38 (m, 2H), 7.79 (s, 1H), 7.95–7.99 (m, 2H), 8.08 (s, 1H, NH)
5c	44	195–196	MS-ESI (m/z): [M^+] 361.1; IR: (KBr) (ν, cm^{-1}) 3275.1 (NH), 1656.8 and 1521.8 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.88–1.90 (m, 4H), 3.48–3.51 (m,4H), 6.85 (s, 1H), 7.03–7.07 (m,1H), 7.53–7.55 (m, 2H) , 7.66–7.7 (m, 3H), 7.92–7.93 (m, 2H), 7.94–8.0 (m, 2H), 10.25 (s, 1H-NH)
5d	53	198–199	MS-ESI (m/z): [M^+] 394.8, IR: (KBr) (ν, cm^{-1}) 3236.55 (NH), 1658.8 and 1525.7 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.88–1.91 (m, 4H), 3.50–3.53 (m, 4H), 6.86 (s,1H), 7.36–7.40 (m, 2H), 7.53–7.55 (m, 3H), 7.70–7.73 (m, 2H), 7.92–7.95 (m, 2H), 10.42 (s, 1H-NH)
5e	55	229–230	MS-ESI (m/z): [M^+] 437.6; IR: (KBr) (ν, cm^{-1}) 3234.6 (NH), 1658.8, 1531.5 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.98–2.01 (m, 4H), 3.60–3.63 (m, 4H), 7.33 (s, 1H), 7.62–7.66 (m, 7H), 8.05–8.12 (m, 2H), 10.79 (s, 1H-NH)
5f	43	208–209	MS-ESI (m/z): [M^+] 379.0; IR: (KBr) (ν, cm^{-1}) 3255.8 (NH), 1660.7 and 1510.2 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) 1.92–1.95 (m, 4H), 3.70–3.74 (m, 4H) 7.17–7.22 (m, 3H), 7.58–7.62 (m, 3H), 7.67–7.70 (m, 2H), 8.03–8.05 (m, 2H), 10.82 (s, 1H-NH).
5g	56	230–231	MS-ESI (m/z): [M^++1] 375.0; IR: (KBr) (ν, cm^{-1}) 3333.0 (NH), 1670.3 and 1519.9 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.80–1.83 (s, 4H), 3.61–3.64 (m,4H), 6.90 (s, 1H), 7.2–7.5 (m, 2H), 7.41–7.60 (m, 5H), 8.01–8.12 (m, 2H), 10.2 (s, 1H-NH).
5h	55	167–168	MS-ESI (m/z): [M^++1] 343.2; IR: (KBr) (ν, cm^{-1}) 3276.3 (NH), 1654.1 and 1531.3 (C=O); ^{1}H NMR: (400 MHz, DMSO-d_6) δ 1.60–1.68 (m, 6H), 3.25 (t, J = 6 Hz, 2H), 3.47 (t, J = 6 Hz, 2H), 3.48–3.51 (m,4H), 4.60–4.63 (m, 1H, OH), 7.00 (s, 1H), 7.51–7.53 (m, 3H), 7.93–7.94 (m, 2H), 8.19 (s, 1H-NH).
5i	56	193–194	MS-ESI (m/z): [M^+] 361.1; IR: (KBr) (ν, cm^{-1}) 3270.2 (NH), 1662.1 and 1560.3 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.49–1.51 (m, 6H), 3.51 (m, 4H), 3.37 (t, J = 6 Hz, 2H), 3.47 (t, J = 5.2 Hz, 2H), 4.70–4.71 (m, 1H, OH), 5.92 (s, 1H), 7.30–7.32 (m, 2H), 7.48–7.50 (m, 2H), 8.17 (s, 1H, NH).

onto 96-well plate at 1.0×10^5 cells/ml. Different concentrations of test compounds and standard drug acyclovir were added to culture wells at a final volume 100 µl in each well. Each particular concentration was made in triplicate. DMSO (0.1%) was used as a negative control. After incubation at 37 °C with 5% CO_2 for 2 days, 10 µl MTT reagent was added to each well. After 4 h of incubation at 37 °C, the formazan formed was dissolved by adding 0.04 N HCl in Isopropanol, and the absorbance was determined at 570 nm with a reference wavelength of 690 nm by an ELISA reader. Data were calculated as the percentage of cell viability using the formula: [(sample absorbance-blank)/mean media control absorbance)]/100. The 50% cytotoxic concentration (CC_{50}) for Vero cells was determined with respect to cell control.[42,44]

Table 3
Antiviral activity of **5a**, **5c**, **5g** and **5h**

Compound	% of inhibition of viral yield	CC_{50}[a]	EC_{50}[b]	SI[c]
5a	70 ± 1	30.0	9.7 ± 0.5	3.1
5c	79 ± 2	37.5	12.5 ± 0.6	3.0
5g	62 ± 2	56.5	20.9 ± 1.1	2.7
5h	89 ± 1	52.5	7.4 ± 0.4	7.1
ACV	94 ± 1	130.0	2.1 ± 0.1	61.9

[a] The 50% cytotoxic concentration for Vero cells in µg/ml.
[b] Concentration of compound in µg/ml producing 50% inhibition of virus induced cytopathic effect. This experiment was repeated for three times.
[c] Selectivity index (SI) = CC_{50}/EC_{50}.

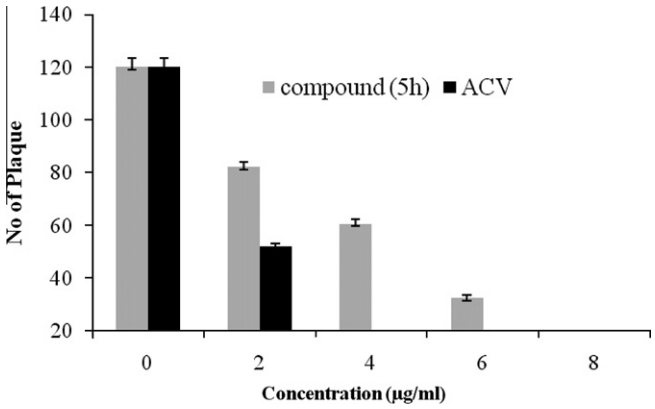


Figure 5a. Plaque reduction assay of HSV-1 at different concentrations of **5h** and ACV.

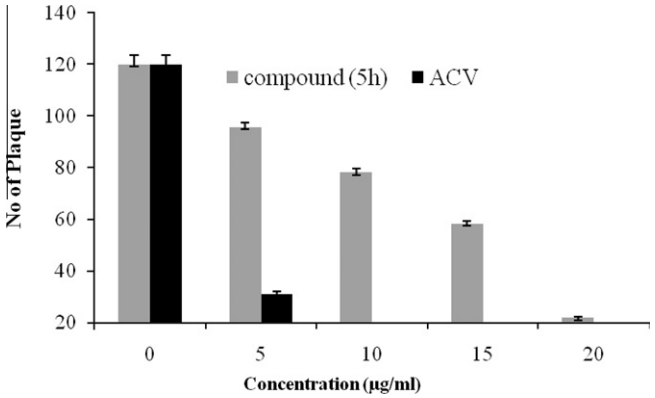


Figure 5b. Plaque reduction assay of HSV-2 at different concentrations of **5h** and ACV.

Table 4
Assessment of anti-HSV activity of **5h** by plaque reduction assay on vero cell

Compound	EC_{50}		SI	
	HSV-1	HSV-2	HSV-1	HSV-2
5h	4.1 ± 0.5	14.5 ± 0.5	12.8	3.6
ACV	1.6 ± 0.2	1.8 ± 0.3	81.3	72.2

The antiviral activity against HSV-1 was evaluated by MTT assay.[45] Vero cells were seeded onto 96-well plates with a concentration of 1.0×10^5cells/ml. After incubation at 37 °C in 5% CO_2 for 6 h, the virus at (0.5 MOI) was added and incubated for 1 h. Different concentrations of test compounds and standard drug acyclovir were added to culture wells at a final volume 100 µl in each well. Each particular concentration was made in triplicate. DMSO (0.1%) was used as a negative control and acyclovir as a positive control. After 3 days incubation at 37 °C in 5% CO2, the MTT test was carried out as described above. The % of viral inhibition was calculated as: $[(A_{tv} - A_{cv})/(A_{cd} - A_{cv})]/100$. A_{tv} indicates the absorbance of test compounds with virus infected cells. A_{cv} and A_{cd} indicate the absorbance of the virus control and the absorbance of the cell control. The concentration which achieved 50% inhibition of virus-induced cytopathic effects (EC_{50}) was determined. The amount of virus used in each experiment was based on infected target cells of 0.5 MOI of both viruses to produce 50% MTT formazan products as in uninfected control cells.[46]

For viral plaque reduction assay, serial dilutions of compounds in EMEM was added to the infected cells (MOI: 0.5 of HSV-1 or HSV-2) and incubated at room temperature. After 1–2 h of incubation, the cells were washed with fresh EMEM and overlaid with methylcellulose, so that the virus can spread via cell-to-cell route to form plaques. The plaques that developed after 2–3 days of incubation were stained with crystal violet. The effective concentration of test compounds that inhibited the number of viral plaques by 50% (EC_{50}) was interpolated from the dose-response curves.[46]

We have rationally designed novel α-pyrone analogs using structure based in silico approach along with drug like properties estimation for the synthesis and biological evaluation. Compound **5h** was found to be moderately active with respect to Acyclovir against HSV-1 as well as has limited cytotoxicity. Therefore, we selected **5h** as our lead for future optimization and detailed structure activity relationship. The synthesized compounds were also evaluated for anti-HIV activity on MT-4 cell using MTT assay and none of the compounds were found significant.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.07.098.

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    To a solution of appropriate 2*H*-pyran-4-methylthio-3-carboxylic acid (**3a**–**b**, 3.8 mmol) in 15 ml DMF, HATU (4.19 mmol) and diisopropylethylamine (15.2 mmol) was added and stirred for 15 min. The appropriate amine (4.5 mmol) was added and stirred for 5 h at room temperature. The reaction was monitored by TLC and then poured into ice water with stirring. The organic layer was separated and the water layer was extracted with DCM. The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was recrystallized with methanol to get yellow crystalline compound.
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