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# Novel Benzimidazo[2,1-*c*][1,4]thiazinone Derivatives with Potent Activity Against HSV-1

**Shadia A. Galal[1], Shweekar I. El- Naem[1], Ahmed O.H. El- Nezhawy[1], Mohamed. A. Ali[2], and Hoda I. El- Diwani[1]**

[1] Chemistry of Natural and Microbial Products Department, National Research Center, Cairo, Egypt
[2] Virology Lab., Water Pollution Department, National Research Center, Cairo, Egypt

The synthesis of new 2-carboxymethylsulfanylmethyl-1*H*-benzimidazole and 1,3-dihydro-4*H*-benzo[4′,5′]imidazo[2,1-*c*][1,4]thiazine-4-one-8-carboxylic acid derivatives was investigated. The antiviral activity of compounds **1**–**14** was tested against the herpes simplex virus 1. Compounds **5** and **14** showed potent activity as they inhibited virus propagation by 94.7% and 91.3% at a dose of 50 μg, respectively. Compounds **5** and **14** showed higher potency than Acyclovir at doses of 20 μg and 50 μg.

**Keywords:** Antiviral activity / Benzimidazole / Herpes simplex virus1 / Synthesis

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## Introduction

Herpes simplex virus type 1 (HSV-1) is a member of the alpha herpes virus subfamily, which is characterized by a short reproductive cycle, prompt destruction of the host cell, and the ability to establish latency in sensory ganglia [1]. HSV-1 has linear, double-stranded DNA genomes. Herpes viruses have gained increasing clinical importance because these common pathogens may be reactivated in immunocompromized transplant recipients and patients with AIDS. Generally, HSV infections have been treated successfully with Acyclovir [2]. However, drug resistant variants emerge after long-term treatment of immunocompromized patients with Acyclovir, which lead to treatment failures [3–7].

On the other hand, benzimidazole derivatives constitute a class of great pharmacological importance because of the variety of their activity, especially their antiviral activity [8–10]. 1-Aryl-1*H*,3*H*-thiazolo[3,4-*a*]benzimidazoles (TBZs) are highly active as human immunodeficiency virus type-1 (HIV-1) non-nucleoside reverse transcriptase inhibitors (NNRTIs) [11, 12]. The lead compound of this class 1-(2,6-difluorophenyl)-1*H*,3*H*-thiazolo[3,4-*a*]benzimidazole (TBZ, NSC 625487) (**I**) [13], proved to be a highly potent inhibitor of HIV-1 effect. It inhibits the replication of various strains of HIV-1, including zidovudine-resistant strain (G910-6), in a variety of human cell lines. On the other side, benzimidazole-5-carboxylic acid derivatives are specific inhibitors of the HCV polymerase [14]. Compound **II** is a representative of this class of non-nucleoside inhibitors. Benzimidazole-5-carboxamide derivatives **III** and **IV** [14, 15]. Benzimidazole derivatives as 2-bromo-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (BDCRB) and its 2-chloro analog (TCRB) 2-isopropylamino-5,6-dichloro-1-(β-L-ribofuranosyl)benzimidazole (GW1263W94, or maribavir) are also potent and selective inhibitors of β-herpes viruses as human cytomegalovirus (HCMV) replication [16] (Fig. 1).

The aim of the present work is the synthesis of new benzimidazole derivatives and studying the antiviral activity against herpes simplex virus-1.

## Results and discussion

### Chemistry

To synthesize new benzimidazole derivatives, 2-chloromethyl-1*H*-benzimidazole-5-carboxylic acid hydrochloride (**1**) was prepared as starting compound from 3,4-diaminobenzoic acid with chloroacetic acid in hydrochloric acid according to Phillips' method [17]. The treatment of compound **1** with ethanol and sulfuric acid gave the ester derivative **2**. [(Carboxymethylthio)methyl]benzimidazole derivatives **3**

**Correspondence:** Shadia A. Galal, Department of Chemistry of Natural and Microbial Products, Division of Pharmaceutical and Drug Industries Research, National Research Centre, 12622, Dokki, Cairo, Egypt.
**E-mail:** sh12galal@yahoo.com
**Fax:** 0020233370931

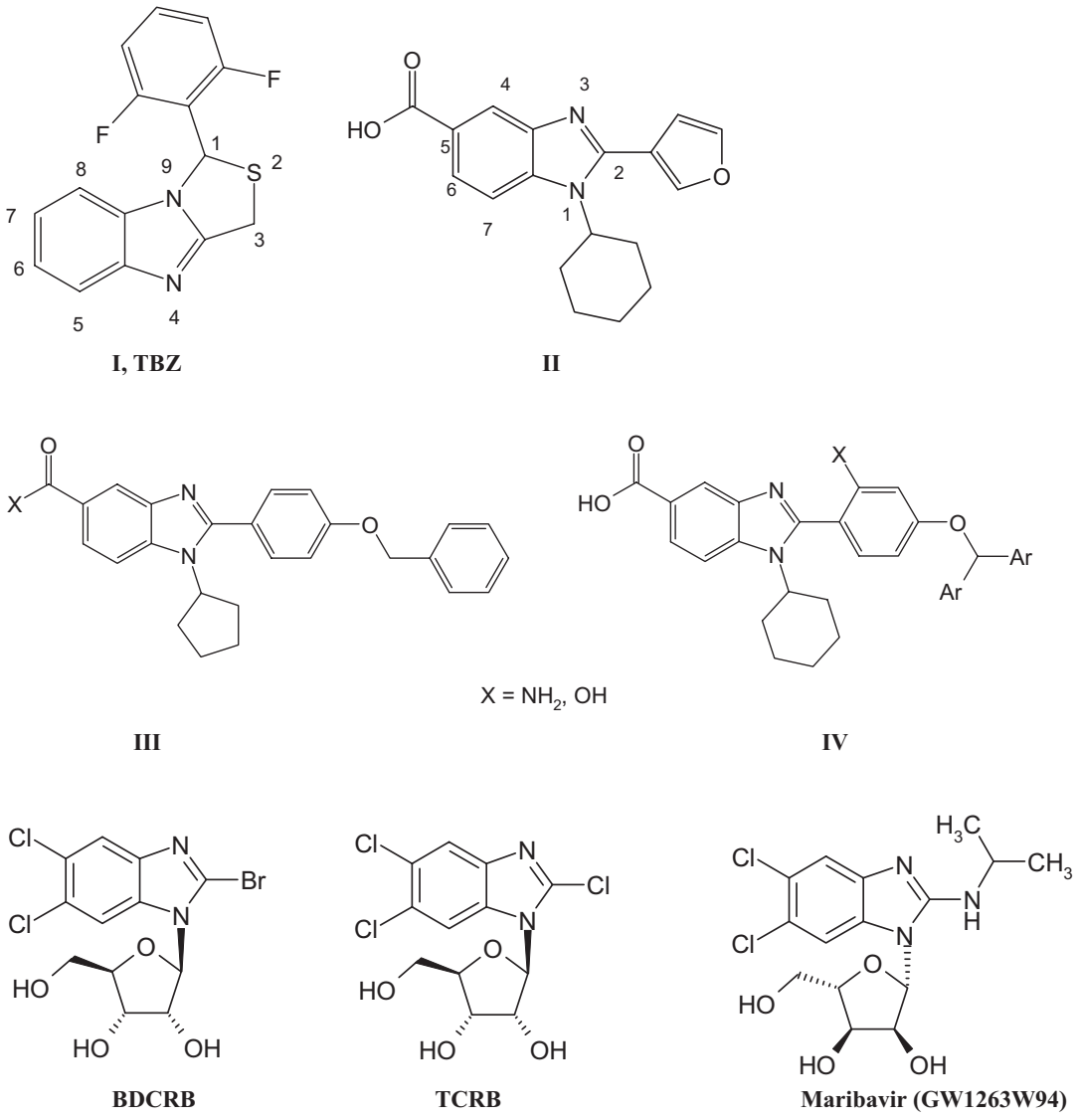



**Figure 1.** Potent antiviral benzimidazole derivatives.

and **4** were obtained by the reaction of compounds **1** and **2** with mercaptoacetic acid, respectively (Scheme 1).

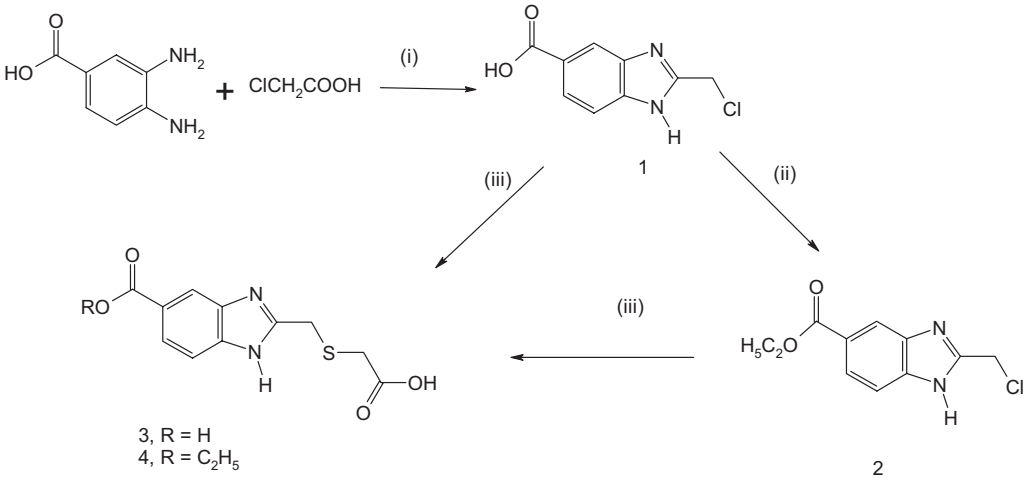
On the other hand, cyclization of compounds **3** and **4** yielded compounds **5** and **6**. The presence of carboxylic acid and carboxylic ethyl ester function groups at position 8, respectively, was confirmed by the downfield shift of H6 in compounds **5** and **6** by the influence of the vicinal carbonyl group [18].

Oxidation of compounds **5** and **6** using hydrogen peroxide and glacial acetic acid yielded compounds **7** and **8**, respectively (Scheme 2).

Condensation of compound **5** with pyridine-3-carbaldehyde, benzo[1,3]dioxole-5-carbaldehyde, 1*H*-indole-3-carbaldehyde or furan-2-carbaldehyde, was performed in acetic anhydride and pyridine to afford compounds **9–12,** respectively, where selective condensation took place at position 1. This was proved by $^{1}$H-NMR spectra of compounds **9–11** by the disappearance of the singlet at 4.3 ppm with respect to compound **5**.

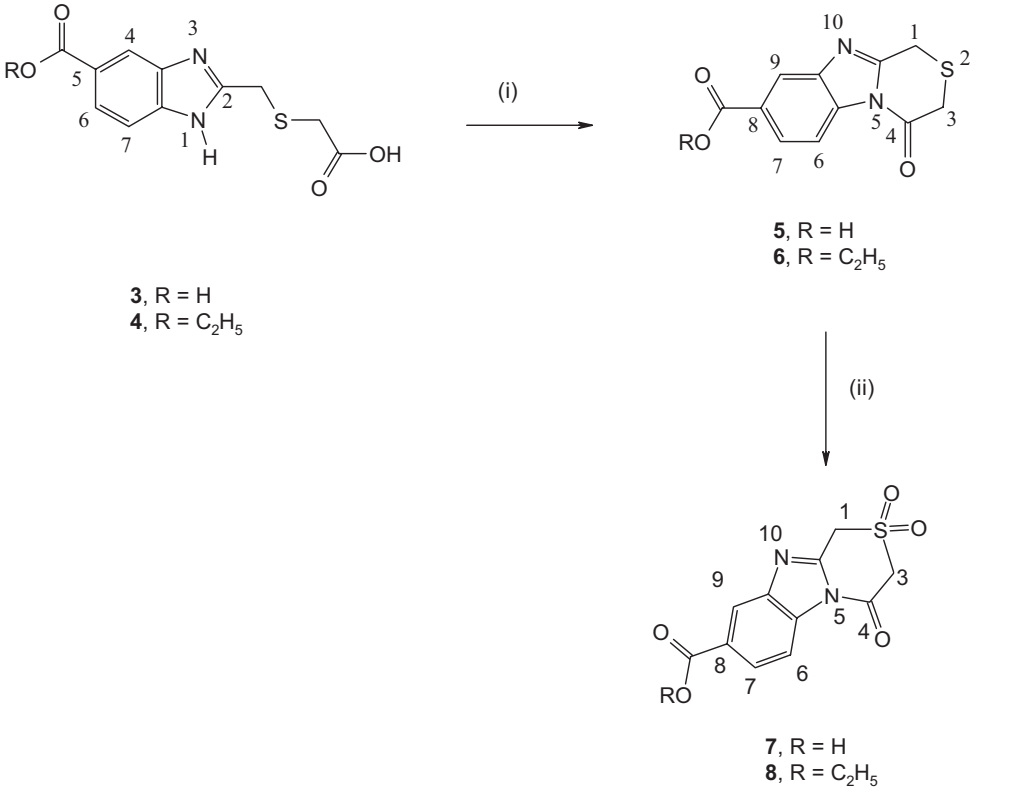
On the other hand, condensation of compound **5** with furan-2-carbaldehyde occurred on the both positions, 1 and 3, to yield compound **12** which may be attributed




(i) 6 N HCl, reflux, 3 h. (ii) Absolute ethanol, catalytic amount of conc. $H_2SO_4$, reflux, 4 h. (iii) Mercaptoacetic acid, DMF or acetone, reflux, 4 h.

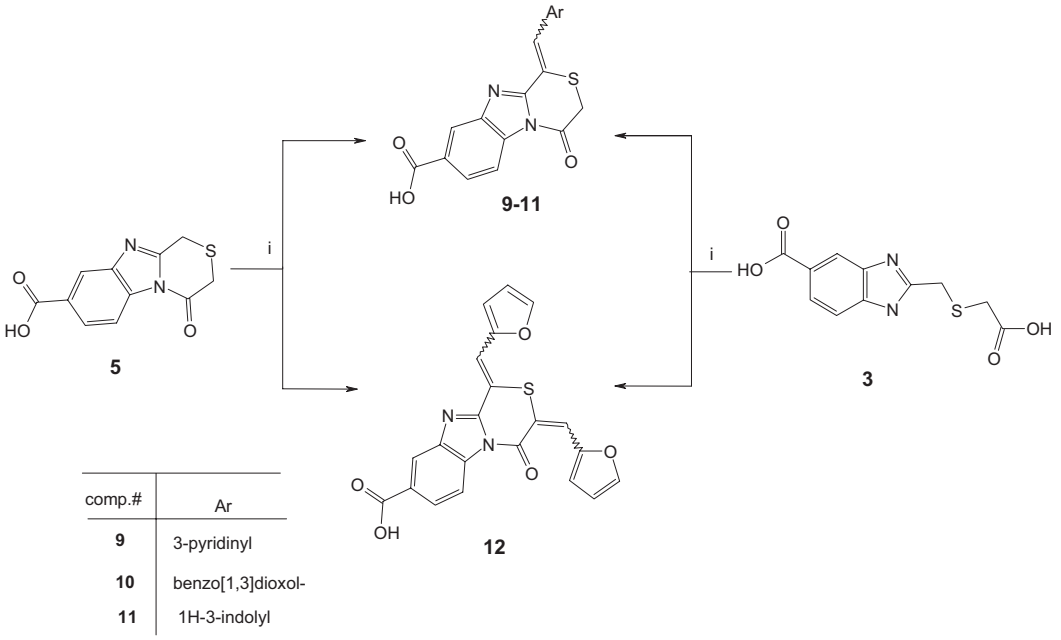
**Scheme 1.** Synthesis of compounds **1–4**.




(i) Pyridine, acetic anhydride, stirring at 90°C, 0.5 h. (ii) Glacial acetic acid, hydrogen peroxides 30%, stirring at room temperature, 24 h.

**Scheme 2.** Synthesis of compounds **5–8**.




i) Pyridine, acetic anhydride, stirring at 90°C for 0.5 h.

**Scheme 3.** Synthesis of compounds **9–12**.

to the size or activity of furan-2-carbaldehyde by comparison with pyridine-3-carbaldehyde, benzo[1,3]dioxole-5-carbaldehyde, 1*H*-indole-3-carbaldehyde.

Also, the latter condensation compounds **9–12** were obtained in one-pot reaction of compound **3** and the appropriate aldehyde by using the same conditions (Scheme 3).

Compound **4** reacted with 2-aminothiazole in ethanol in the presence of sodium ethoxide to afford compound **13** which was recyclized by acetic anhydride and sodium acetate to give the 8-substituted amide **14** (Scheme 4).

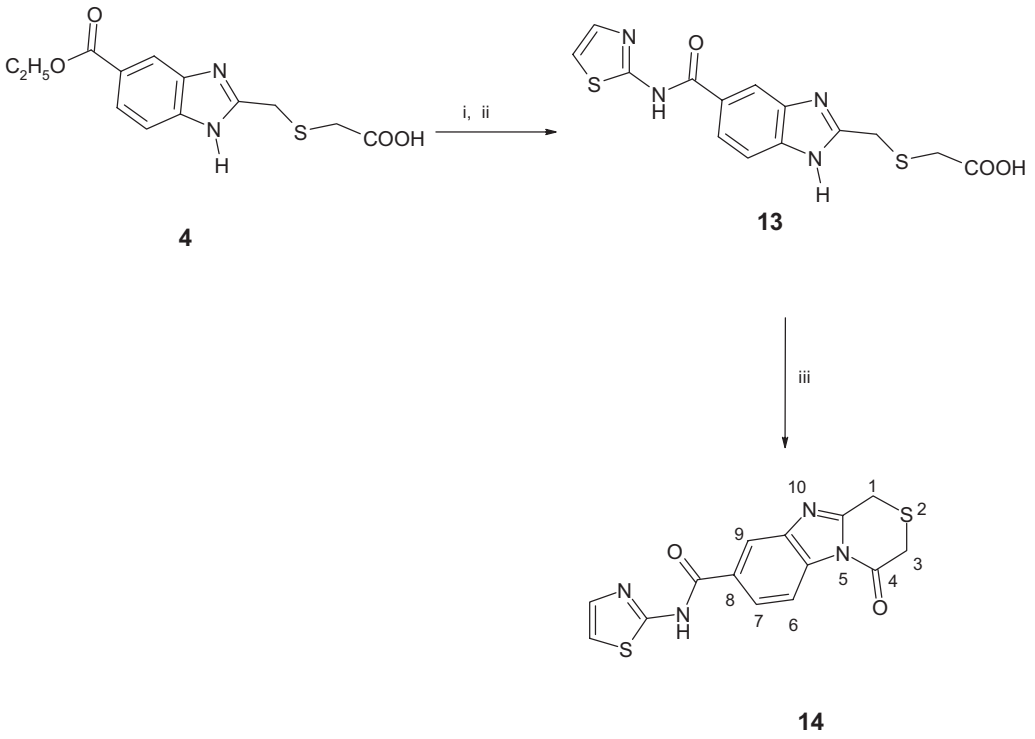
### Antiviral activity

The antiviral activity against herpes simplex virus 1 of the synthesized compounds **1–14** was tested using the plaque reduction infectivity assay in vero cell line (Table 1). For comparison Acyclovir was used as control [19-21]. The test was performed at concentrations of 20 μg and 50 μg. The starting compound, 2-chloromethyl benzimidazole derivative **1** showed high activity at a concentration of 20 μg whereas it was toxic at the concentration of 50 μg. Esterification of compound **1** and/or formation of [(carboxymethylthio)methyl] benzimidazole derivatives **3** and **4** led to a decrease in the activity with respect to compound **1** at a concentration of 20 μg. Interestingly, formation of the tricyclic moiety benzimidazothiazine, was the key for the high activity of compound **5**, which showed striking activities at both concentrations. On the other hand, decreasing the hydrophilicity of compound **5** by ester formation to compound **6** had a negative effect on potency. Also, the presence of the sulfonyl group in the thiazine ring of compounds **7** or **8**, instead of sulfur as in compound **5** or **6**, respectively, decreases the activity. Furthermore, substitutions at position 1 in compounds **9–11** or positions 1 and 3 of compound **12** by heteromethylene moieties lowered the activity compared to **5**. On the other hand, amide formation to compound **13** had a positive effect on the anti-HSV-1 activity at both studied concentrations with respect to the free acid **3** or the ester **4**. Furthermore, cyclization of compound **13** to yield the tricyclic compound **14** enhanced the activity. In general, the activity is greatly affected by substitution on thiazinone moiety and this may be due to steric factor. Also the activity is enhanced by the presence of hydrogen bond donor groups, as free carboxylic or amide, at position 8 of the benzimidazothiazinone nucleus. Compounds **5** and **14** were found to be more effective than Acyclovir at concentrations of 20 μg and 50 μg showing that compounds **5** and **14** have higher activity (see Fig. 2).

## Conclusion

From the screening of the antiviral results, we can deduce that formation of the tricyclic moiety, benzimidazothiazine, is crucial for activity. Also, substitution of thiazinone ring in




(i) 2-Aminothiazole, $NaOC_2H_5$, ethanol, stirring. (ii) Diluted HCl. (iii) $NaOCOCH_3$, acetic anhydride, stirring at 90°C for 1 h.

**Scheme 4.** Synthesis of compounds **13** and **14**.

positions 1 or 1and 3 is unfavored for activity. Moreover, changing the carboxylic group to amide in position 5, keeping the thiazine ring unaltered, reduces the activity. Furthermore, compounds **5** and **14** were found to be potent compounds as they reduce the virus by 94.7% and 91.3% at 50 μg, respectively. They also showed higher activity by comparing them to Acyclovir at the examined doses. The free carboxylic group and the unsubstituted thiazinone ring are necessary for activity. This work could be a starting point to screen further related substitutions at this nucleus.

**Table 1.** The % of reduction of herpes simplex virus type 1 (HSV-1) by the synthesized compounds **1–14** and Acyclovir at concentrations 20 μg/$10^5$ and 50 μg/$10^5$ cells.

| Compound no. | % of Reduction of HSV-1 | |
|---|---|---|
| | 20 μg | 50 μg |
| **1** | 66.8 | toxic |
| **2** | 16 | 26 |
| **3** | 12 | 23 |
| **4** | 0 | 4.9 |
| **5** | 89.7 | 94.8 |
| **6** | 53 | 66 |
| **7** | 60 | 79 |
| **8** | 36 | 66 |
| **9** | 56 | 66 |
| **10** | 20 | 63 |
| **11** | 46 | 70 |
| **12** | 50 | 53 |
| **13** | 23 | 59 |
| **14** | 79 | 91.3 |
| Acyclovir | 0 | 65 |

## Experimental

### Chemistry

All melting points were uncorrected. The infrared spectra were carried out in potassium bromide disk on JASCO F.T/IR Fourier Transform infrared spectrophotometer. The NMR spectra were carried out in DMSO on Jeol-EX-200 MHz NMR spectrometer using TMS as internal reference. The mass spectra were recorded on mass spectrometer MS 50 equipment of (AEI) (Kratos, FRG) and Finnegan SSQ 7000 at 70 eV. The elemental analyses were carried at Cairo University in elemental analyzer Vario EI ± 0.5%.

#### *2-(Chloromethyl)-1H-benzo[d]imidazole-5-carboxylic acid hydrochloride **(1)***

A mixture of 2-chloroacetic acid (5.67 g, 60 mmol) in 30 mL of 6 N hydrochloric acid was refluxed for 10 min, then 3,4-diaminobenzoic acid (6.08 g, 40 mmol) was added to the mixture which was refluxed for 3 h and then left to cool to room temperature. The separated solid was filtered off, washed with acetone,

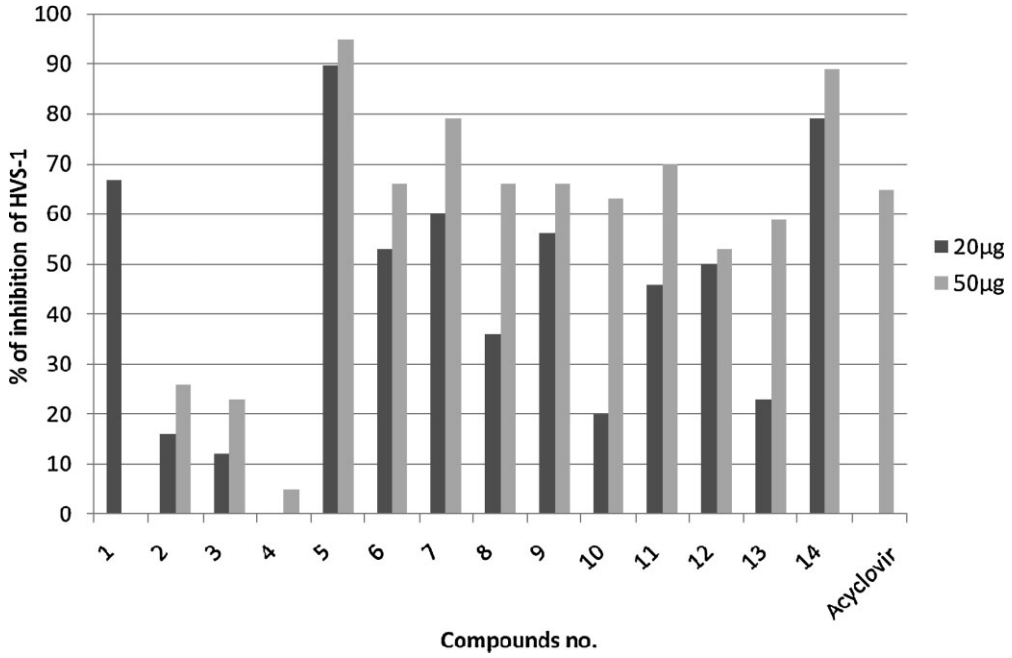



**Figure 2.** The antiviral activity of the tested compounds **1-14** against herpes simplex virus HSV-1.

chloroform, and recrystallized from ethanol to give compound **1** as gray crystals.

Yield (85%), mp > 300°C. IR ($cm^{-1}$) 3520–3320 (OH, NH), OH bonded centered at 2800, 1695 (C=O, acid), 1637 (C=N). $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 5.15 (s, 2H, $CH_2Cl$), 7.75 (d, 1H, $J$ = 10 Hz, H-7), 7.95 (d, $J$ = 10 Hz, H-6), 8.25 (s, 1H, H-4), 11.5 (br., NH, $D_2O$ exchangeable), 12.9 (br., OH, $D_2O$ exchangeable). $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 35.8, 114.6, 116.6, 125.5, 127.0, 134.2, 137.0, 151.3, 166.9. MS (*m*/*z*, %): 246 ($M^+$, 5), 212 ($M^{2+}$ – HCl, 11.07%), 210 ($M^+$ – HCl, 34. %), 175 ($M^+$ – (HCl + Cl), 100%). Calcd. for $C_9H_8Cl_2N_2O_2$: C, 43.75; H, 3.26; Cl, 28.70; N, 11.34. Found: C, 43.45; H, 3.09; Cl, 28.96; N, 11.48.

### *Ethyl 2-(chloromethyl)-1H-benzo[d]imidazole-5-carboxylate* ***(2)***

A mixture of compound **1** (2.53 g, 12 mmol) in dry ethanol (30 mL) and conc. sulfuric acid (2 mL), was heated under reflux for 4 h. The reaction mixture was added to saturated solution of sodium bicarbonate (50 mL). The resulting precipitate was filtered off and recrystallized from petroleum ether (80–100°C) to give white crystals of compound **2**.

Yield (80%), mp 135–137°C. IR ($cm^{-1}$): 3480–3400 (NH), 1720 (C=O, ester), 1623 (C=N). $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 1.38 (t, 3H, $J$ = 7 Hz, $CH_3$), 4.36 (q, 2H, $J$ = 7 Hz, $CH_2$), 4.89 (s, 2H, $CH_2Cl$), 7.61 (d, 1H, $J$ = 8 Hz, H-7), 8.01 (d, 1H, $J$ = 8 Hz, H-6), 8.36 (s, 1H, H-4), 8.59 (bs, NH, $D_2O$ exchangeable). $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 14.5, 35.8, 61.3, 114.4, 116.6, 124.5, 126.5, 138.3, 143.7, 151.6, 165.6. MS (*m*/*z*, %): 240 ($M^{2+}$, 20), 238 ($M^+$, 59), 195 ($M^{2+}$ – $OC_2H_5$, 36), 193 ($M^+$ – $OC_2H_5$, 100). Calcd. for $C_{11}H_{11}ClN_2O_2$ (238.68): C, 55.36; H, 4.65; Cl, 14.85; N, 11.74. Found: C, 55.35; H, 4.67; Cl, 14.84; N, 11.75.

### *2-((Carboxymethylthio)methyl)-1H-benzo[d]imidazole-5-carboxylic acid* ***(3)***

A mixture of mercaptoacetic acid (1.10 g, 12 mmol) in 10 mL of dimethylformamide and few drops of triethylamine was stirred for 10 min, and then a solution of compound **2** (2.52 g, 12 mmol) in DMF (10 mL) was added. The reaction mixture was stirred for 1 h at room temperature, heated at 90°C for 4 h, evaporated under reduced pressure, washed with water, dichloromethane, chloroform and the resulting solid was recrystallized from acetone as white crystals.

Yield (78%), mp 248–250°C. IR ($cm^{-1}$): 3500–3360 (OH, NH), 1700 (C=O), 1685 (C=O), 1617 (C=N). $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 3.41 (s, 2H, $CH_2CO$), 4.07 (s, 2H, $CH_2S$), 7.57 (d, 1H, $J$ = 8 Hz, H-7), 7.81 (d, 1H, $J$ = 8. Hz, H-6), 8.14 (s, 1H, H-4), 11.55 (br., NH, $D_2O$ exchangeable), 12.31 (br., OH, $D_2O$ exchangeable). 12.89 (br., OH, $D_2O$ exchangeable). $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 28.6, 33.4, 114.9, 117.6, 123.3, 124.2, 133.2, 137.0, 154.1, 167.8, 170.9. MS (*m*/*z*, %): 266 ($M^+$, 30), 248 ($M^+$ – $H_2O$, 100). Calcd. for $C_{11}H_{10}N_2O_4S$ (266.28): C, 49.62; H, 3.79; N, 10.52; S, 12.04. Found: C, 49.43; H, 3.68; N, 10.44; S, 12.13.

### *2-((5-(Ethoxycarbonyl)-1H-benzo[d]imidazol-2-yl)-methylthio)acetic acid* ***(4)***

A mixture of mercaptoacetic acid (1.10 g, 12 mmol) in 10 mL acetone and few drops of triethyl amine were heated under reflux temperature for 10 min. The solution of compound **2** (2.86 g, 12 mmol) in 10 mL acetone was added and the reaction mixture was heated under reflux temperature for 4 h. The mixture was evaporated under reduced pressure. Water (10 mL) was added and the mixture was extracted with ethyl acetate (3 × 50),

the extracts was dried over sodium sulfate and evaporated. The resulting solid was recrystallized from ethanol.

Yield (48%), mp 195–197°C. IR ($cm^{-1}$): 3440 (NH), bonded (OH) centered at 3200, 1705 (C=O, ester), 1690 (C=O, acid), 1622 (C=N). $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 1.32 (t, 3H, $J = 7$ Hz, $CH_3$), 3.42 (s, 2H, $CH_2CO$), 4.07 (s, 2H, $CH_2S$), 4.29 (q, 2H, $J = 7$ Hz, $CH_2$), 7.63 (d, 1H, $J = 8$ Hz, H-7), 7.85 (d, 1H, $J = 8$ Hz, H-6), 8.15 (s, 1H, H-4), 8.95 (bs, NH, $D_2O$ exchangeable), 12.62 (OH, $D_2O$ exchangeable). $^{13}$C-NMR (DMSO-$d_6$, δ ppm): 15.3, 48.4, 60.4, 115.3, 117.6, 123.6, 124.8, 133.2, 137.6, 143.6, 152.3, 165.6, 173.9. MS (*m*/*z*, %): 294 ($M^+$, 20), 276 ($M^+ - H_2O$, 40), 204 ($M^+ - SCH_2CO_2$, 100). Calcd. for $C_{13}H_{14}N_2O_4S$ (294.33): C, 53.05; H, 4.79; N, 9.52; S, 10.89. Found: C, 53.23; H, 4.77; N, 9.54; S, 10.88.

### *General procedure for the preparation of compounds **5** and **6***

A mixture of compound **3** or **4** (19 mmol) in acetic anhydride (10 mL) and pyridine (5 mL) was heated at 90°C for 0.5 h. The reaction mixture was evaporated under reduced pressure. The resulting residue was column chromatographed from petroleum ether (40–60°C)/chloroform (2:1) and recrystallized from acetone as buff crystals.

### *Preparation of 1,3-dihydro-4H-benzo[4',5']imidazo[2,1-c]-[1,4]thiazine-4-one-8-carboxylic acid **(5)***

Yield (59%), mp 193–195°C. IR ($cm^{-1}$): 3500–3380 (OH), bonded (OH) centered at 2800, 1697 (C=O, acid and lactam), 1637 (C=N). $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 3.55 (s, 2H, $CH_2CO$), 4.30 (s, 2H, $CH_2S$), 7.87 (m, 2H, H-6 and H-7) 8.13 (s, 1H, H-9), 12.67 (br., OH, $D_2O$ exchangeable). $^{13}$C-NMR(DMSO-$d_6$, $\delta$ ppm): 33.2, 33.6, 115.3, 119.3, 125.0, 125.6, 135.3, 138.5, 141.7, 166.4, 198.5. MS (*m*/*z*, %): 248 ($M^+$, 100). Calcd. for $C_{11}H_8N_2O_3S$ (248.26): C, 53.22; H, 3.25; N, 11.28; S, 12.92. Found: C, 53.42; H, 3.36; N, 11.39; S, 12.81.

### *Ethyl 1,3-dihydro-4H-benzo[4',5']imidazo[2,1-c]-[1,4]thiazine-4-one-8-carboxylate **(6)***

Yield (55%), mp 183–185°C. IR ($cm^{-1}$): 1716 (C=O, ester), 1700 (C=O, lactam), 1627 (C=N). $^1$HNMR (DMSO-$d_6$, $\delta$ ppm): 1.39 (t, 3H, $J = 7$ Hz, $CH_3$), 3.43 (s, 2H, $CH_2CO$), 4.20 (s, 2H, $CH_2S$), 4.35 (q, 2H, $J = 7$ Hz, $CH_2$), 7.79 (m, 2H, H-6 and H-7), 8.14 (s, H, H-9). $^{13}$C-NMR(DMSO-$d_6$, $\delta$ ppm): 14.5, 36.2, 36.5, 60.5, 115.1, 117.9, 124.7, 126.1, 134.4, 138.8, 141.7, 165.5, 198.7. MS (*m*/*z*, %): 276 ($M^+$, 20), 231 ($M^+ - OC_2H_5$, 45), 204 ($M^+ - (OC_2H_5 + CO)$, 100). Calcd. for $C_{13}H_{12}N_2O_3S$ (276.32): C, 56.51; H, 4.38; N, 10.14; S, 11.66. Found: C, 56.47; H, 4.34; N, 10.20; S, 11.60.

### *General procedure of the preparation of compounds **7** and **8***

A mixture of compound **5** or **6** (3.8 mmol) in glacial acetic acid (10 mL) and hydrogen peroxide (15 mL, 30%) was stirred for 24 h at room temperature. The resulting solid of compounds **7** or **8** was separated, filtered, washed with acetone and recrystallized from DMF.

### *2,2-Dioxo-1,3-dihydro-4H-benzo[4',5']imidazo[2,1-c]-[1,4]thiazine-4-one-8-carboxylic acid **(7)***

Yellow solid, yield (66%), mp >300°C. IR ($cm^{-1}$): 3480 (OH), 1704 (C=O, acid), 1690 (C=O, lactam), 1629 (C=N), 1560 and 1467 ($SO_2$). $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 4.35 (s, 2H, $CH_2CO$), 4.73 (s, 2H, $CH_2S$), 7.85 (m, 2H, H-6 and H-7), 8.25 (s, 1H, H-8), 10.57 (br., OH, $D_2O$ exchangeable). $^{13}$C-NMR(DMSO-$d_6$, $\delta$ ppm): 52.2, 52.6, 115.1, 118.9, 125.2, 135.5, 138.3, 142.1, 166.7, 198.5. MS (*m*/*z*, %): 280 ($M^+$, 20), 263 ($M^+ - OH$, 50), 91 ($CH_2SO_2CH_2 - 1$, 100). Calcd. $C_{11}H_8N_2O_5S$ for (280.26): C, 47.14; H, 2.88; N, 10.00; S, 11.44. Found: C, 47.20; H, 2.80; N, 10.04; S, 11.46.

### *Ethyl 2,2-dioxo-1,3-dihydro-4H-benzo[4',5']imidazo[2,1-c]-[1,4]thiazine-4-one-8-carboxylate **(8)***

Yellow solid, yield (61%), mp. 295–298°C. IR ($cm^{-1}$): 1724 (C=O, ester), 1690 (C=O, lactam), 1629 (C=N), 1564 and 1461 ($SO_2$). $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 1.39 (t, 3H, $J = 7$ Hz, $CH_3$), 4.55 (s, 2H, $CH_2CO$), 4.35 (q, 2H, $J = 7$ Hz, $CH_2$), 4.73 (s, 2H, $CH_2S$), 7.87 (m, 2H, H-6 and H-7), 8.16 (s, 1H, H-8). $^{13}$C-NMR(DMSO-$d_6$, $\delta$ ppm): 14.7, 43.5, 52.5, 60.3, 115.1, 117.9, 124.4, 134.6, 137.2, 140.7, 146.1, 165.6, 198.7. MS (*m*/*z*,%): 308 ($M^+$, 50), 263 ($M^+ - OC_2H_5$, 100). Calcd. for $C_{13}H_{12}N_2O_5S$: C, 50.64; H, 3.92; N, 9.09; S, 10.40. Found: C, 50.49; H, 3.87; N, 9.12; S, 10.49.

### *General procedure of the preparation of compounds **9–12***

A mixture of compound **3** or **5** (1.9 mmol) in acetic anhydride (10 mL), pyridine (5 mL), and pyridine-3-carbaldehyde, benzo[1,3]-dioxole-5-carbaldehyde, *1H*-Indole-3-carbaldehyde or furan-2-carbaldehyde (4.0 mmol) was heated at 90°C for 0.5–1 h, allowed to cool and evaporated under reduced pressure. The resulting residue was column chromatographed from ethyl acetate/petroleum ether (40:60) (4:1) as an eluent to give the corresponding products **9-12**, respectively.

### *1-(Pyridin-3-ylmethylene)-3-hydro-4H-benzo[4',5']imidazo[2,1-c][1,4]thiazine-4-one-8-carboxylic acid **(9)***

Buff solid. $R_f = 0.33$. Yield (59%), mp >300°C. IR ($cm^{-1}$): 3480–3320 (OH), bonded (OH) centered at 2800, 1700 (C=O, acid), 1678 (C=O, lactam), 1637 (C=N). $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 3.75 (s, 2H, $CH_2CO$), 7.07 (s, 1H, =CH),7.55 (m, 1H, H-5' pyridine ring), 7.87 (m, 2H, H-6 and H-7), 7.98 (m, 1H, H-4′ pyridine ring), 8.19 (s, 1H, H-9), 8.36 (m, 1H, H-6′ pyridine ring), 8.90 (s, 1H, H-2′ pyridine), 12.70 (br., $D_2O$ exchangeable OH). $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 34.6, 115.5, 119.4, 123.4, 124.6, 125.3, 126.1, 127.1, 135.2, 137.8, 141.5, 146.4, 147.3, 148.1, 166.6, 198.4. MS (*m*/*z*, % of abundance): 337($M^+$, 18), 320($M^+$ - OH, 48). 293 ($M^+$ – COOH, 100). Anal. calcd. for $C_{17}H_{11}N_3O_3S$ (337.36): C, 60.52; H, 3.29; N, 12.46; S, 9.50. Found: C, 60.44; H, 3.44; N, 12.6 1; S, 43.

### *1-(Benzo[1,3]dioxol-5-ylmethylene)-3-hydro-4H-benzo[4',5']imidazo[2,1-c][1,4]thiazine-4-one-8-carboxylic acid **(10)***

Brown solid. $R_f = 0.26$. Yield (66%), mp >300°C. IR ($cm^{-1}$): 3480–3400 (OH), 1690 (C=O, acid), 1672 (C=O, lactam), 1635 (C=N). $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 3.59 (s, 2H, $CH_2CO$), 6.38 (s, 2H, $CH_2$), 7.05 (m, 1H, H-7′ benzo[1,3]dioxol), 7.17 (s, 1H, =CH), 7.35 (m, 1H, H-6′ benzo[1,3]dioxol), 7.52 (s, 1H, H-4′ benzo[1,3]dioxol), 7.92 (m, 2H, H-6 and H-7), 8.21 (s, 1H, H-9), 12.10 (br., OH, $D_2O$ exchangeable). $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 34.4, 102.1, 107.6, 110.5, 115.4, 121.7, 124.5, 125.1, 125.9, 128.1, 135.2, 137.8, 141.6, 142.8, 147.7, 148.2, 165.7, 198.5. Anal. calcd. for $C_{19}H_{12}N_2O_5S$: C, 59.99; H, 3.18; N, 7.36; S, 8.43. Found: C, 59.78; H, 3.29; N, 7.46; S, 8.40.

### *1-(1H-indol-3-ylmethylene)-3-hydro-4H-benzo[4′,5′]imidazo[2,1-c][1,4]thiazine-4-one-8-carboxylic acid* ***(11)***

Brown solid. $R_f = 0.24$. Yield (67%), mp >300°C. IR ($cm^{-1}$): 3485–3250 (OH, NH), 1689 (C=O, acid and lactam), 1635 (C=N). $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 3.5 (s, 2H, $CH_2CO$), 7.02 (m, 1H, H-6′ of indole), 7.12 (m, 2H, H-5′and H-7′ of indole), 7.42 (m, 1H, H-4′ indole), 7.51 (s, 1H, =CH), 7.67 (s, 1H, H-2′ of indole), 7.85 (m, 2H, H-6 and H-7′), 8.14 (s, 1H, H-9), 11.10 (br., NH, $D_2O$ exchangeable), 12.87 (br., OH, $D_2O$ exchangeable). $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 34.1, 109.8, 112.4, 115.2, 118.5, 119.4, 120.8, 122.1, 124.6, 125.5, 126.7, 128.3, 134.6, 136.7, 137.7, 139.1, 140.6, 148.4, 165.0, 198.8. Calcd. for $C_{20}H_{13}N_3O_3S$: C, 63.99; H, 3.49; N, 11.19; S, 8.54. Found: C, 63.83; H, 3.54; N, 11.23; S, 8.44.

### *1,3-Bis-furan-2-ylmethylene-4H-benzo[4′,5′]imidazo[2,1-c]-[1,4]thiazine-4-one-8-carboxylic* ***(12)***

Yellow solid. $R_f = 0.33$. Yield (55%), mp 276–280°C. IR ($cm^{-1}$): 3480–3400 (NH), 1714 (C=O, ester), 1672 (C=O, lactam), 1635 (C=N). $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 3.5 (s, 2H, $CH_2CO$), 6.69(s, 1H, =CH), 6.85 (m, 2H, H-4′ of furan moieties), 7.23 (m, 1H, H-3′ furan moiety at position 1), 7.43 (m, 1H, H-3′ furan moiety at position 3), 7.62 (m, 1H, H-5′ of furan moiety at position 1), 7.82 (m, 2H, H-6, H-7), 7.99 (s, 1H, =CH), 8.14 (m, 1H, H-5′ furan moiety at position 3), 8.25 (s, 1H, H-9), 11.10 (bs, OH, $D_2O$ exchangeable). $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 111.8, 112.1, 115.4, 119.2, 120.4, 122.2, 125.6, 126.7, 128.3, 134.4, 136.7, 137.8, 138.6, 140.6, 148.7, 151.5, 166.1, 189.4. Calcd. for $C_{21}H_{12}N_2O_5S$: C, 62.37; H, 2.99; N, 6.93; S, 7.93. Found: C, 62.17; H, 3.19; N, 6.79; S, 7.85.

### *2-((5-(Thiazol-2-ylcarbamoyl)-1H-benzo[d]imidazol-2-yl)methylthio)acetic acid* ***(13)***

A mixture of compound **4** (6.8 mmol), 2-aminothiazole (6.8 mmol), and sodium ethoxide (6.8 mmol) in 60 mL ethanol was stirred for 4 h. Then, the reaction mixture was heated under reflux temperature for 24 h. The reaction mixture was evaporated under reduced pressure and the resulting solid was dissolved in water (100 mL) and acidified by diluted HCl. The resulting solid was filtered off and recrystallized from methanol/water (5:1).

Yellow solid. $R_f = 0.22$. Yield (86%), mp >300°C. IR ($cm^{-1}$): 3468 (NH), 3320 (NH), 1704 (C=O, acid), 1676 (C=O, amide), 1617 (C=N). $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 3.62 (s, 2H, $CH_2CO$), 4.17 (s, 2H, $CH_2S$), 6.92 (d, 1H, $J = 6.5$Hz, H-5′ of thiazole), 7.43 (d, 1H, $J = 6.5$ Hz, H-4′ of thiazole), 7.64 (d, 1H, $J = 8$ Hz, H-7), 7.82 (d, 1H, $J = 8$ Hz, H-6), 8.15 (s, 1H, H-4), 10.67 (br., NH, $D_2O$ exchangeable), 11.95 (br., NH, $D_2O$ exchangeable), 13.3 (br., OH, $D_2O$ exchangeable). $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 33.6, 44.1, 111.6, 115.1, 117.5, 122.7, 128.1, 133.3, 138.8, 141.6, 142.1, 159.6, 167.1, 175.3. Calcd. for $C_{14}H_{12}N_4O_3S_2$: C, 48.26; H, 3.47; N, 16.08; S, 18.41. Found: C, 48.29; H, 3.55; N, 16.28; S, 18.36.

### *1,3-Dihydro-4H-benzo[4′,5′]imidazo[2,1-c][1,4]thiazine-4-one-8-carboxylic acid thiazol-2-yl amide* ***(14)***

A mixture of compound **13** (2 mmol) and sodium acetate (0.19 g, 2 mmol) in 10 mL acetic anhydride was stirred for 0.5 h, then heated at 90°C for 1 h, poured onto water. The resulting residue was purified on silica gel column using ethyl acetate/petroleum ether (2:1) as an eluent, and then recrystallized from chloroform as buff crystals.

Yield (60%), mp 263–265°C. IR ($cm^{-1}$): 3327 (NH), 1691 (C=O, lactam), 1676 (C=O, amide), 1623 (C=N). $^1$H-NMR (DMSO-$d_6$, $\delta$ ppm): 3.62 (s, 2H, $CH_2CO$), 4.37 (s, 2H, $CH_2S$), 7.25 (d, 1H, $J = 6.5$ Hz, H-5′ of thiazole), 7.50 (d, 1H, $J = 6.5$ Hz, H-4′ of thiazole), 7.77 (m, 2H, H-6 and H-7), 8.15 (s, 1H, H-9), 12.15 (br., NH, $D_2O$ exchangeable). $^{13}$C-NMR (DMSO-$d_6$, $\delta$ ppm): 33.6, 37.1, 111.8, 114.8, 116.6, 121.7, 127.3, 132.4, 134.7, 140.2, 141.9, 159.6, 165.5, 196.9. Calcd. for $C_{14}H_{10}N_4O_2S_2$: C, 50.90; H, 3.05; N, 16.96; S, 19.41. Found: C, 50.76; H, 3.25; N, 16.79; S, 19.38.

## Antiviral bioassay

### Preparation of synthetic compounds for bioassay

100 mg of each tested compound were dissolved in 10% DMSO (1 mL) of in water. The final concentration was 100 μg/μL (stock solution). The dissolved stock solutions were decontaminated by addition of 50 μg/mL antibiotic–antimycotic mixture (10 000 U penicillin G sodium, 10 000 μg streptomycin sulfates, and 250 μg amphotericin B, PAA Laboratories GmbH, Austria).

### Cell culture

African green monkey kidney-derived cells (Vero) and human hepatoma cell line (HepG2) were used. Cells were propagated in Dulbeccos' minimal essential medium (DMEM) supplemented with 10% fetal bovine serum, 1% antibiotic–antimycotic mixture. The pH was adjusted at 7.2–7.4 by 7.5% sodium bicarbonate solution. The mixture was sterilized by filtration through 0.2-μm pore size nitrocellulose membrane.

### Viruses

Herpes simplex virus type-1 (HSV-1) was obtained from Environmental Virology Lab., Department of Water Pollution Research, National Research Centre, Cairo, Egypt.

### Cytotoxicity assay

To test for cytotoxicity, 100 μL of maintenance medium containing serial two-fold dilutions of the test compounds was added in 96-well culture plate in which each well contained a concentration of 2000 cells/100 μL. Control cells were incubated without test compounds but with DMSO (0.2%). After cells were cultured at 37°C in a 5%-$CO_2$ incubator for 3 days, 20 μL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (5 mg/mL in cell culture medium) were added to each well and cells were incubated for an additional 3 h at 37°C. The reaction product was determined at 570 nm with a microplate reader [19].

### Plaque reduction assay

The assay was carried out according to the method of Tebas *et al.* [21] 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 mL/well). After 1 h contact time for virus adsorption, 3 mL of Dulbecco's Modified Eagles Media (DMEM) supplemented with 2% agarose were 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 the plates were stained with crystal violet. Control wells were included where untreated virus was incubated Vero cells and finally plaques were counted and percentage reduction in plaques formation in comparison to control wells was recorded as following:

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

*The authors have declared no conflict of interest.*

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