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Short communication

Synthesis and anti-HSV-1 evaluation of some pyrazoles and fused pyrazolopyrimidines

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ABSTRACT

5-Amino-1-substituted-1*H*-pyrazole-4-carbonitrile derivative **1** was used as a precursor for preparation of some novel substituted pyrazole and pyrazolo[3,4-*d*]pyrimidine derivatives **2**–**10**. Furthermore, the preparation of sugar hydrazone derivatives **11a**,**b**, **12a**,**b** and their annelated *C*-nucleosides **13a**,**b** was described. Some of the prepared products revealed promising antiviral activity against herpes simplex virus type-1 (HSV-1) in comparison to Acyclovir as a control.

1. Introduction

Several pyrazole derivatives received great attention due to their biological activities as potential HIV-1 inhibitors [1], insecticides [2], fungicides [2], antiviral agents [3] and due to their anticancer activity [4].

Also, the chemistry of fused pyrazolo[3,4-*d*]pyrimidine derivatives has drawn great attention due to the pharmacological importance and their structural resemblance to purines [5]. Several substituted pyrazolo[3,4-*d*]pyrimidine derivatives demonstrated significant antimicrobial [6] and antiviral activities [3,7].

On the other hand, nucleoside analogues as antiviral chemotherapeutic agents have been comprehensively described in the literature [8–10], and many of these analogues were synthesized recently as antiviral agents [10–12]. Moreover, *C*-nucleosides were reported to be of great value from the biological point of view [13,14], and many of their derivatives have been synthesized as potential antibacterial [15], antifungal agents [16,17] and were tested also against herpes simplex viruses (HSV-1 and HSV-2) [13].

In the literature [17,18], it was reported that replacement of 1H of the pyrazole ring of pyrazolo[3,4-*d*]pyrimidine derivatives by some other bioactive moieties drastically alters its pharmacological properties. Keeping this in mind, it was aimed in this report to synthesize pyrazolo[3,4-*d*]pyrimidine derivatives bearing a heterocyclic moiety namely: 5,6-dihydronaphtho[1′,2′:4,5]thieno[2,3-*d*]pyrimidine moiety which is known to exhibit pronounced biological activities.

2. Results and discussion

2.1. Chemistry

The starting material 5-amino-1-(5,6-dihydronaphtho[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-11-yl)-1*H*-pyrazole-4-carbonitrile (**1**) was prepared [17], and then heated with formic acid to afford pyrazolo[3,4-*d*]pyrimidinone derivative **2**. The structure of compound **2** was confirmed with spectral data, where its IR spectrum showed bands at (ν, cm^{-1}): 3200 (NH), 1674 (C=O) and ^1H NMR spectrum showed signals at δ 7.40 and 8.40 ppm for NH and C_6-H, respectively. Also, MS gave the molecular ion peak at m/z 372 (M^+, 27%) (Section 3).

Some reports [19,20] stated that the reaction of compounds analogous to **1** with acetic anhydride gave the corresponding

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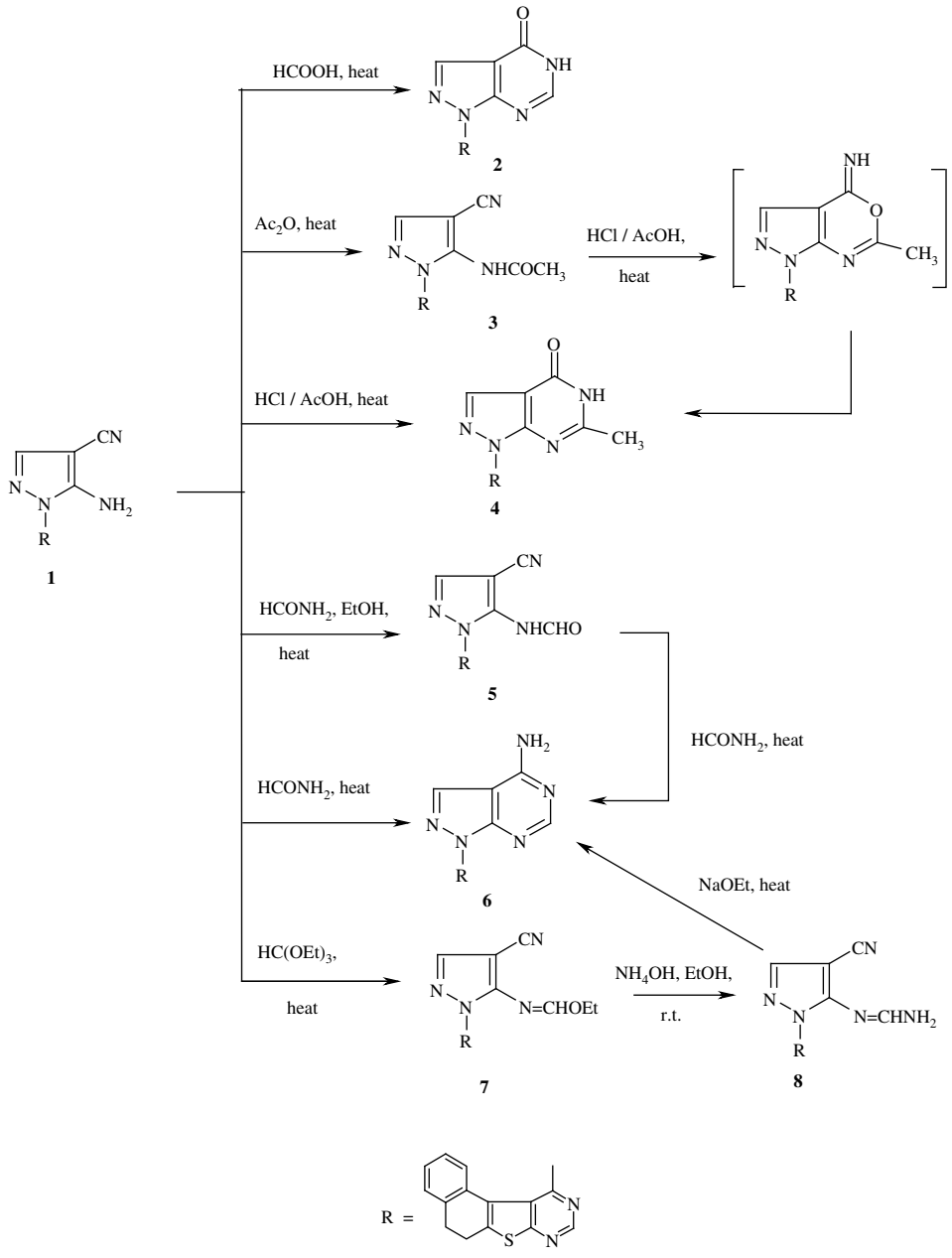
pyrimidine derivatives similar to compound **4**, however our attempts to cyclize compound **1** to compound **4** by refluxing with acetic anhydride gave the *N*-4-cyano-1-(5,6-dihydronaphtho[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-11-yl)-5-acetamide (**3**) and not compound **4** (Scheme 1, Section 3). The structure of compound **3** was confirmed on the basis of its spectral data, where its IR spectrum showed bands at (ν, cm^{-1}): 3421 (NH), 2218 (C≡N), 1651 (C=O) and MS spectrum gave the molecular ion peak at *m*/*z* 386 (M^+, 84%) (Section 3). Meanwhile, treatment of compounds **1** or **3** with a mixture of hydrochloric acid/acetic acid (3 mL:9 mL) under reflux, gave in both cases one and the same product assigned the structure of compound **4** (Scheme 1, Section 3). The ^{1}H NMR spectrum of the latter compound showed the presence of the methyl and NH groups at δ 2.50, 8.10 ppm in addition to the peaks corresponding to the methyl and C=O groups at δ 20.1, 160 ppm in its ^{13}C NMR spectrum (Section 3). The pyrimidinone derivative **4** could be formed *via* the formation of the oxazine intermediate, which was not isolated and suffered Dimroth rearrangement to pyrazolo[3,4-*d*]pyrimidinone derivative **4** under the condition of the reaction [21] (Scheme 1).

On the other hand, the interaction of compound **1** with formamide in ethanol under reflux caused the formation of *N*-formyl derivative **5**. The latter compound was cyclized to the aminopyrimidine derivative **6** by fusion with formamide. The IR and ^{1}H NMR spectra of compound **6** showed the absence of the CN group as well as the presence of NH_2 (Section 3).

When compound **1** was refluxed with triethyl orthoformate, it afforded the ethyl-4-cyano-1-(5,6-dihydronaphtho[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-11-yl)-1*H*-pyrazol-5-yl-imidoformate (**7**). Inspection of the ^{1}H NMR and ^{13}C NMR spectra of product **7** revealed the presence of N=CHOCH$_2$CH$_3$ signals. Also, MS gave the molecular ion peak at *m*/*z* 400 (M^+, 58%) (Section 3). Attempts of



Scheme 1.

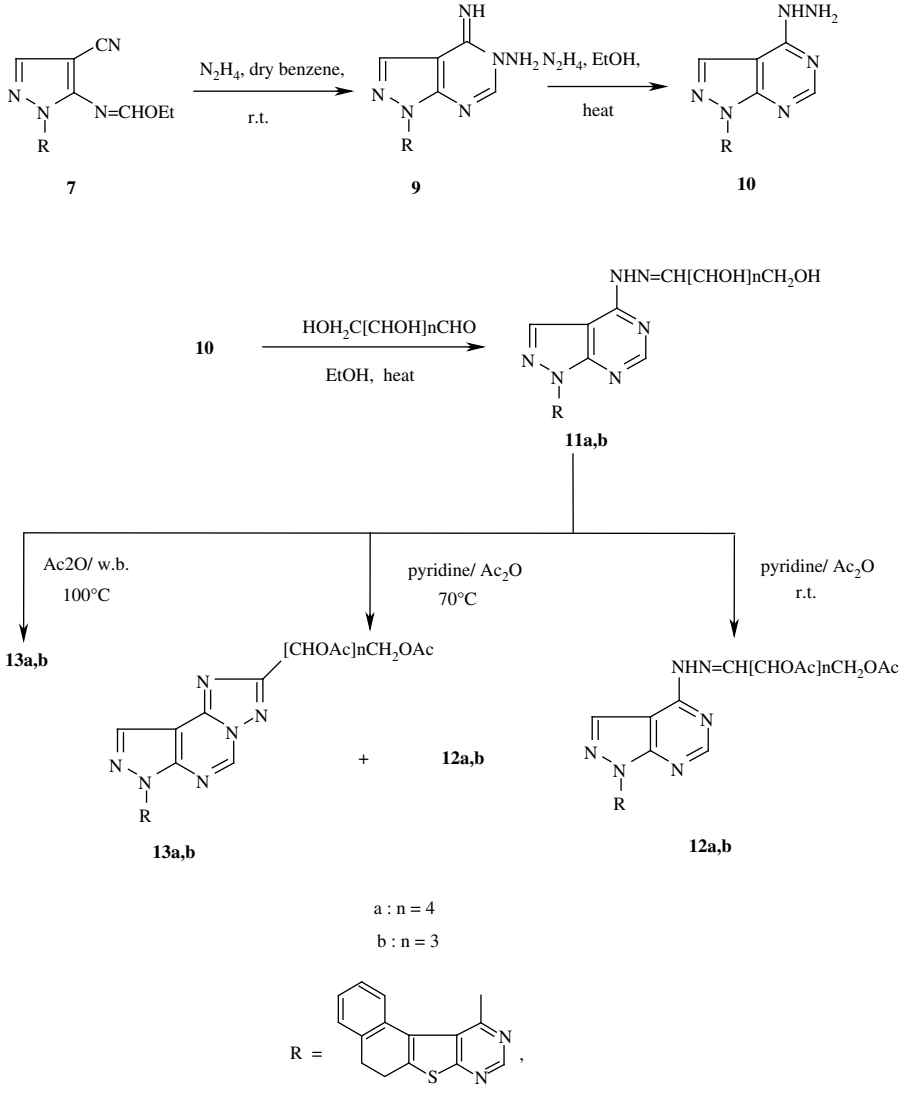
cyclization of compound **7** to the aminopyrimidine derivative **6** by stirring with ammonium hydroxide solution (25%) at room temperature, gave the *N*-[4-cyano-1-(5,6-dihydronaphtho[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-11-yl)-1*H*-pyrazol-5-yl]formamidine (**8**) instead of compound **6**. The aminopyrimidine derivative **6** could be obtained by refluxing compound **8** with sodium ethoxide or directly by heating compounds **1** or **5** (as mentioned above) with formamide.

The methanimidate **7** was cyclized with hydrazine hydrate, in dry benzene, at room temperature to give compound **9**. The elemental analysis and spectral data of compound **9** confirmed its structure (Scheme 2, Section 3). Thus, its IR spectrum showed characteristic absorption bands at 3404, 3362, and 3312 corresponding to the ($NH_2 + NH$). Moreover, the ^1H NMR spectrum showed signals exchangeable with D_2O at δ 4.70 and 7.70 ppm for NH_2 and NH, respectively (Section 3).

Compound **9** was isomerized by Dimroth rearrangement, upon refluxing in ethanol, in the presence of few drops of hydrazine hydrate to the corresponding more thermodynamically stable 1-(5,6-dihydronaphtho[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-11-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylhydrazine (**10**). The IR spectrum of compound **10** showed characteristic absorption bands at 3300, 3191 and 3119 corresponding to the ($NH_2 + NH$) and its ^1H NMR spectrum showed signals exchangeable with D_2O at δ 4.20 and 6.50 ppm for NH_2 and NH, respectively (Section 3). This result is in agreement with reported results for analogous compounds with hydrazines [22,23] (Scheme 2).

In this part, the syntheses of some *C*-nucleosides related to the pyrazolo[3,4-*d*]pyrimidine derivatives are described. Thus, the condensation of the 4-hydrazino derivative **10** with some monosaccharides: namely, D-glucose or D-ribose in the presence of a catalytic amount of glacial acetic acid (2 drops) under reflux gave the corresponding aldehydo sugar hydrazones **11a** and **11b**, respectively. The latter products revealed absorption bands for ($OH + NH$) and (C=N) in IR spectra and their ^1H NMR spectra showed the presence of the sugar protons, NH, and azo-methine protons (*CH*=N) (Section 3).

Acetylation of the hydrazone derivatives **11a** and **11b** with acetic anhydride, in dry pyridine at room temperature, gave the *O*-acetylated sugar derivatives **12a** and **12b**, respectively. The IR spectra of compounds **12a** and **12b** revealed the absence of hydroxyl groups and showed absorption bands due to (NH) and (C=O). Also, the ^1H NMR and ^{13}C NMR spectra showed the presence of OAc groups (Scheme 2, Section 3).



Scheme 2.

Meanwhile, stirring of compounds **11a** and **11b** with acetic anhydride, in dry pyridine at 70 °C, gave two sets of products: the *O*-acetylated sugar hydrazone derivatives **12a** (42%) and **12b** (39%) and the *O*-acetylated cyclic *C*-nucleosides **13a** (54%) and **13b**, (56%), respectively (Scheme 2, Section 3). However, heating of derivatives **11a** and **11b** with acetic anhydride using water bath, gave products assigned to the structures of compounds **13a** and **13b**, respectively. The IR spectra of the latter compounds revealed the absence of NH and hydroxyl groups and showed absorption bands due to C=O; while their ^{1}H NMR and ^{13}C NMR spectra showed the presence of OAc groups (Scheme 2, Section 3).

This could be explained *via* acetylation of the hydrazone derivatives **11a** and **11b** to give their corresponding *O*-acetylated sugar hydrazone derivatives **12a** and **12b**, and then followed by oxidative cyclization [13,15,17] to give the *O*-acetylated cyclic *C*-nucleosides **13a** and **13b**, respectively.

The formation of triazolo[1,5-*c*]pyrimidine acyclic *C*-nucleosides **13a** and **13b** took place presumably *via* the formation of their corresponding isomeric triazolo[4,3-*c*]pyrimidine acyclic *C*-nucleosides, which underwent a Dimroth rearrangement under the conditions of the reaction [13,17].

2.2. Antiviral screening

Plaque infectivity assay was carried out to test Compounds **1**–**6**, **10**, **11a**, **12a** and **13a** for antiviral activity. The test was performed to include the three possibilities for antiviral activity; virucidal effect, virus adsorption and effect on virus replication for HSV-1. It was obvious that, at concentration of 10 μg/10^5 cells, compound **10** revealed the highest anti-HSV-1 activity in comparison with the other tested compounds and the control, while, at concentration of 20 μg/10^5 cells, compound **11a** revealed the highest anti-HSV-1 activity in comparison with the other tested compounds and the control, where its antiviral activity increased from 43% at concentration of 10 μg/10^5 cells to 99% at concentration of 20 μg/10^5 cells (Fig. 1, Table 1).

2.3. Conclusion

Structural activities correlation of the obtained results revealed that, at concentration of 10 μg/10^5 cells addition of fused pyrimidine to the pyrazole ring (compounds **2**, **4**, **6**, and **10**) slightly increases the anti-HSV-1 activity than substituted pyrazole derivatives (compounds **3** and **5**) (Fig. 1, Table 1). At concentration of 20 μg/10^5 cells this phenomena was reversed and the substituted pyrazole derivatives (compounds **3** and **5**) revealed higher anti-HSV-1 activity than the fused pyrazolopyrimidine derivatives (**2, 4**, **6**, and **10**) (Fig. 1, Table 1). Moreover, addition of sugar moieties to the pyrazolopyrimidin-4-ylhydrazine derivative **10** increases the antiviral activity (% of HSV-1 reduction; compounds **11a**, **12a** and **13a**). In general, at both concentrations the sugar hydrazone derivative **11a** revealed more anti-HSV-1 activity than the *O*-acetylated sugar hydrazone **12a** and the *O*-acetylated cyclic *C*-nucleoside **13a** (Fig. 1, Table 1).

3. Experimental

3.1. Chemistry

All melting points are uncorrected and measured using Electro-thermal IA 9100 apparatus, (Shimadzu, Tokyo, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin–Elmer 1650 spectrophotometer (Perkin–Elmer, Norwalk, CT, USA). ^{1}H and ^{13}C NMR spectra were determined on a Jeol-Ex-300 NMR spectrometer (JEOL, Tokyo, Japan) and chemical shifts were expressed as parts per million; ppm (δ values) against TMS as internal reference. Mass spectra were recorded on VG 2AM-3F mass spectrometer (Thermo Electron Corporation, USA). Microanalyses were performed using Mario El Mentar apparatus, Organic Microanalysis Unit, and the results were within the accepted range (±0.40) of the calculated values. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminum sheets (Type 60 F254, Merck, Darmstadt, Germany). Compound **1** was prepared according to a reported method [17].

3.1.1. *1-(5,6-Dihydronaphtho[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-11-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(*3H*)-one (**2**)*

Compound **1** (3.44 g, 0.01 mol) was refluxed in formic acid (30 mL, 85%) for 5 h. The reaction mixture was cooled and poured into water. The formed solid was filtered off, dried, and recrystallized from dioxane to give compound **2**. Yield 75%, m.p. 265–267 °C.

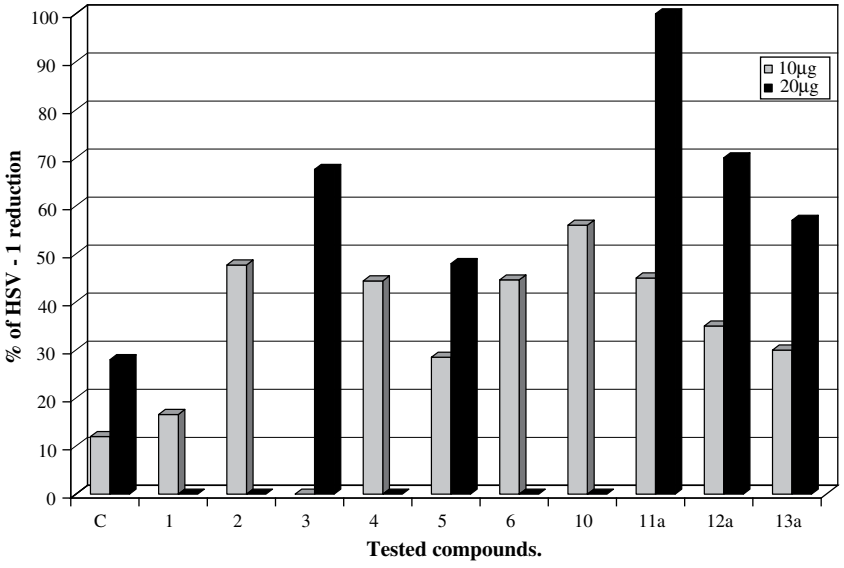


Fig. 1. Effect of some novel pyrazole and fused pyrazolopyrimidine derivatives on HSV-1 (MBB cell culture strain) in comparison with Acyclovir (C) as a control.

Table 1
Percentage of HSV-1 reduction of the tested compounds.

Compound no.	% of HSV-1 reduction at 10 μg/10^5 cells	% of HSV-1 reduction at 20 μg/10^5 cells
Acyclovir	12	28
1	16.6	0
2	47.7	0
3	0	67.6
4	44.4	0
5	28.5	48
6	44.6	0
10	56	0
11a	43	99
12a	34	69
13a	29	57

IR (KBr) ν_{max}: cm^{-1}: 3200 (NH), 1674 (C=O), 1587 (C=N).

^{1}H NMR (DMSO-d_6) δ: 2.90–3.00 (m, 4H, $C_{5'}$-CH_2 + $C_{6'}$-CH_2), 7.20–7.30 (m, 4H, 3Ar-H + C_3-H), 7.40–7.50 (m, 2H, Ar-H + NH, D_2O exchangeable), 8.40 (s, 1H, C_6-H), 9.20 (s, 1H, $C_{9'}$-H).

^{13}C NMR (DMSO-d_6) δ: 23.50 (C-5′), 29.30 (C-6′), 119, 122, 125.20, 126, 126.50, 126.90, 132, 138, 140.30, 158.40, 167.10 (sp^2 carbon atoms), 165 (C=O).

MS *m*/*z* (%): 372 (M^+, 27%), 344 (M^+ − CO, 10%), 316 (16%), 292 (42%).

Anal. calcd. for $C_{19}H_{12}N_6OS$ (372.41): C 61.28, H 3.25, N 22.57, S 8.61. Found: C 61.05, H 3.37, N 22.41, S 8.57.

3.1.2. N-*[4-Cyano-1-(5,6-dihydronaphtho[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-11-yl)-1*H*-pyrazol-5-yl]acetamide (**3**)*

Compound **1** (3.44 g, 0.01 mol) was refluxed in acetic anhydride (20 mL) for 5 h. The reaction mixture was cooled, poured into water, filtered, dried, and recrystallized from dioxane (20 mL)/ethanol (20 mL) to give compound **3**. Yield 45%, m.p. 150–152 °C.

IR (KBr) ν_{max}: cm^{-1}: 3421 (NH), 2218 (C≡N), 1651 (C=O).

^{1}H NMR (DMSO-d_6) δ: 2.50 ppm (s, 3H, $COCH_3$), 2.90–3.00 (m, 4H, $C_{5'}$-CH_2 + $C_{6'}$-CH_2), 7.20–7.40 (m, 5H, 3Ar-H + C_3-H, NH, D_2O, exchangeable), 8.70 (s, 1H, $C_{9'}$-H), 9.10 (d, J = 10.80 Hz, 1H, Ar-H).

MS *m*/*z* (%): 386 (M^+, 84%), 360 (45%), 342 (32%).

Anal. calcd. for $C_{20}H_{14}N_6OS$ (386.43): C 62.61, H 3.65, N 21.75, S 8.30. Found: C 62.32, H 3.81, N 21.64, S 8.12.

3.1.3. *6-Methyl-1-(5,6-dihydronaphtho[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-11-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(*3H*)-one (**4**)*

Method A. Compound **1** (3.44 g, 0.01 mol) was refluxed in a mixture of hydrochloric acid (3 mL) and acetic acid (9 mL) for 3 h. The reaction mixture was cooled, poured into water and the solid formed was filtered off, dried and recrystallized from dioxane to give compound **4** in 75% yield.

Method B. To a solution of hydrochloric acid (3 mL) and acetic acid (9 mL) compound **3** (3.86 g, 0.01 mol) was added, and then the reaction mixture was refluxed for 2 h. The reaction mixture was cooled, poured into water and the solid formed was filtered off, dried and recrystallized from dioxane to give compound **4** in 85% yield; m.p. 185–187 °C.

IR (KBr) ν_{max}: cm^{-1}: 3200 (NH), 1677 (C=O), 1587 (C=N).

^{1}H NMR (DMSO-d_6) δ: 2.50 ppm (s, 3H, C_6-CH_3), 2.80–3.00 (m, 4H, $C_{5'}$-CH_2 + $C_{6'}$-CH_2), 7.10–7.40 (m, 4H, 3Ar-H + C_3-H), 8.10 (s, NH, D_2O exchangeable), 8.20 (s, 1H, $C_{9'}$-H), 8.30 (d, J = 8.90 Hz, 1H, Ar-H).

^{13}C NMR (DMSO-d_6) δ: 20.1 (CH_3), 24.10 (C-5′), 29.30 (C-6′), 119, 122, 125.20, 126, 126.50, 126.90, 132, 138, 140.30, 158.40, 167.10 (sp^2 carbon atoms), 160 (C=O).

MS *m*/*z* (%): 386 (M^+, 100%), 371 (65%), 328 (60%).

Anal. calcd. for $C_{20}H_{14}N_6OS$ (386.43): C 62.16, H 3.65, N 21.75, S 8.30. Found: C 62.02, H 3.41, N 21.54, S 8.61.

3.1.4. N-*[4-Cyano-1-(5,6-dihydronaphtho[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-11-yl)-1*H*-pyrazol-5-yl]formamide (**5**)*

A solution of compound **1** (3.44 g, 0.01 mol) in dry ethanol (20 mL) was treated with formamide (0.55 g, 0.01 mol). The reaction mixture was refluxed for 1 h, and the formed solid was collected by filtration on hot, dried and recrystallized from dioxane to give compound **5**.

Yield 76%; m.p. 198–200 °C.

IR (KBr) ν_{max}: cm^{-1}: 3325 (NH), 2216 (C≡N), 1687 (C=O).

^{1}H NMR (DMSO-d_6) δ: 2.90–3.10 (m, 4H, $C_{5'}$-CH_2 + $C_{6'}$-CH_2), 6.20 (s, 1H, NH, D_2O exchangeable), 6.90–7.30 (m, 4H, 3Ar-H + C_3-H), 8.10 (d, J = 9.50 Hz, 1H, Ar-H), 8.80 (s, 1H, CHO), 9.20 (s, 1H, $C_{9'}$-H).

^{13}C NMR (DMSO-d_6) δ: 23.50 (C-5′), 29.30 (C-6′), 116.20 (C≡N), 119, 122, 125.20, 126, 126.50, 126.90, 132, 138, 140.30, 158.40, 167.10 (sp^2 carbon atoms), 165 (HC=O).

MS *m*/*z* (%): 372 (M^+, 43%), 344 (M^+ − CO, 30%), 328 (80%).

Anal. calcd. for $C_{19}H_{12}N_6OS$ (372.40): C 61.28, H 3.25, N 22.57, S 8.61. Found: C 61.66, H 3.13, N 22.78, S 8.32.

3.1.5. *1-(5,6-Dihydronaphtho[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-11-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (**6**)*

Method A. A mixture of compound **8** (3.71 g, 0.01 mol) and sodium ethoxide (0.23 g Na in 5 mL dry ethanol, 0.01 mol) was refluxed for 5 h. The formed precipitate was filtered on hot, dried and recrystallized from dioxane to give compound **6** in 35% yield.

Method B. Compound **1** (3.44 g, 0.01 mol) was refluxed in formamide (20 mL) for 3 h. The reaction mixture was cooled and poured into water. The solid that formed was filtered off, dried and recrystallized from dioxane to give compound **6** in 47% yield.

Method C. Compound **5** (3.71 g, 0.01 mol) was refluxed in formamide (20 mL) for 2 h. The reaction mixture was cooled and poured into water. The formed solid was filtered off, dried and recrystallized from dioxane to afford compound **6** in 45% yield; m.p. 255–257 °C.

IR (KBr) ν_{max}: cm^{-1}: 3400, 3114 (NH_2).

^{1}H NMR (DMSO-d_6) δ: 2.80–3.00 ppm (m, 4H, $C_{5'}$-CH_2 + $C_{6'}$-CH_2), 5.50 (s, 2H, NH_2, D_2O exchangeable), 7.20 (m, 2H, Ar-H + C_3-H), 7.20–7.40 (m, 3H, 2Ar-H + C_6-H), 7.50 (d, J = 7.50 Hz, 1H, Ar-H), 8.30 (s, 1H, $C_{9'}$-H).

^{13}C NMR (DMSO-d_6) δ: 24.10 (C-5′), 29.30 (C-6′), 119, 122, 125.20, 126, 126.50, 126.90, 132, 138, 140.30, 148.25, 156.30, 158.40, 167.10 (sp^2 carbon atoms).

MS *m*/*z* (%): 371 (M^+, 100%), 355 (50%), 329 (25%), 252 (25%).

Anal. calcd. for $C_{19}H_{13}N_7S$ (371.42): C 61.44, H 3.53, N 26.40, S 8.63. Found: C 61.12, H 3.84, N 26.76, S 8.36.

3.1.6. *Ethyl-4-cyano-1-(5,6-dihydronaphtho[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-11-yl)-1*H*-pyrazol-5-yl-imidoformate (**7**)*

Compound **1** (3.44 g, 0.01 mol) was refluxed in a mixture of triethyl orthoformate (20 mL) and acetic anhydride (5 mL) for 6 h. The solvent was removed under reduced pressure, and the remaining solid was recrystallized from dry dioxane to give compound **7**.

Yield 77%; m.p. 270–272 °C.

IR (KBr) ν_{max}: cm^{-1}: 2220 (C≡N), 1556 (C=N).

^{1}H NMR (DMSO-d_6) δ: 1.00 ppm (t, J = 8.50 Hz, 3H, OCH_2CH_3), 2.70–3.10 (m, 4H, $C_{5'}$-CH_2 + $C_{6'}$-CH_2), 4.10 (q, J = 8.20 Hz, 2H, OCH_2CH_3), 6.10 (d, 1H, J = 10.00 Hz, Ar-H), 6.90–7.00 (m, 2H, 2Ar-H), 7.20–7.40 (m, 2H, Ar-H + C_3-H), 7.40 (s, 1H, N=CH), 9.20 (s, 1H, $C_{9'}$-H).

^{13}C NMR (DMSO-d_6) δ: 14.50 (CH_3), 23.50 (C-5′), 29.30 (C-6′), 58.60 (CH_2), 116.20 (C≡N), 119, 122, 125.20, 126, 126.50, 126.90, 132, 138, 140.30, 158.40, 167.10 (sp^2 carbon atoms).

MS *m*/*z* (%): 400 (M^+, 58%), 371 (10%), 355 (7%), 328 (20%).

Anal. calcd. for $C_{21}H_{16}N_6OS$ (400.46): C 62.98, H 4.03, N 20.99, S 8.01. Found: C 62.62, H 3.89, N 20.78, S 8.47.

3.1.7. N-*[4-Cyano-1-(5,6-dihydronaphtho[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-11-yl)-1*H*-pyrazol-5-yl]formamidine (**8**)*

Ammonium hydroxide solution (5 mL, 25%) was added to absolute ethanol (20 mL) containing compound **7** (4.0 g, 0.01 mol), the reaction mixture was stirred at room temperature for 2 h. The formed precipitate was filtered off, dried and recrystallized from dioxane to produce compound **8**.

Yield 45%; m.p. 216–218 °C.

IR (KBr) ν_{max}: cm^{-1}: 2212 (C≡N), 3444, 3300 (NH_2).

^{1}H NMR (DMSO-d_6) δ: 2.80–3.10 ppm (m, 4H, $C_{5'}$-CH_2 + $C_{6'}$-CH_2), 6.60 (s, 2H, NH_2, D_2O exchangeable), 6.90 (m, 1H, Ar-H), 7.10 (m, 1H, Ar-H), 7.20–7.40 (m, 3H, C_3-H + 2Ar-H), 7.60 (s, 1H, N=CH), 9.00 (s, 1H, $C_{9'}$-H).

^{13}C NMR (DMSO-d_6) δ: 23.50 (C-5′), 29.30 (C-6′), 116.90 (C≡N), 119, 122, 125.20, 126, 126.50, 126.90, 132, 138, 140.30, 146.10, 149, 158.40, 167.10 (sp^2 carbon atoms).

MS *m*/*z* (%): 371 (M^+, 100%), 355 (60%), 345 (30%), 328 (45%).

Anal. calcd. for $C_{19}H_{13}N_7S$ (371.42): C 61.44, H 3.53, N 26.40, S 8.63. Found: C 61.63, H 3.27, N 26.61, S 8.92.

*3.1.8. 4-Imino-1-(5,6-dihydronaphtho[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-11-yl)-1,4-dihydropyrazolo[3,4-*d*]pyrimidin-5-ylamine (**9**)*

To a solution of compound **7** (4.0 g, 0.01 mol) in dry benzene (30 mL), hydrazine hydrate (1.50 g, 0.06 mol, 99%) was added with stirring at room temperature for 1 h, the obtained product was filtered off, dried and recrystallized from dioxane to give compound **9**.

Yield 53%; m.p. 223–225 °C.

IR (KBr) ν_{max}: cm^{-1}: 3404, 3362, 3312 (NH_2 + NH).

^{1}H NMR (DMSO-d_6) δ: 2.80–3.00 ppm (m, 4H, $C_{5'}$-CH_2 + $C_{6'}$-CH_2), 4.70 (s, 2H, NH_2, D_2O exchangeable), 6.30 (d, J = 9.50 Hz, 1H, Ar-H), 6.90 (m, 1H, Ar-H), 7.10–7.60 (m, 3H, 2Ar-H + C_3-H), 7.70 (s, 1H, NH, D_2O exchangeable), 8.40 (s, 1H, C_6-H), 9.10 (s, 1H, $C_{9'}$-H).

^{13}C NMR (DMSO-d_6) δ: 24.80 (C-5′), 29.30 (C-6′), 119, 122, 125.20, 126, 126.50, 126.90, 132, 138, 140.30, 148.25, 156.30, 158.40, 167.10 (sp^2 carbon atoms).

MS *m*/*z* (%): 386 (M^+, 64%), 250 (78%), 237 (20%).

Anal. calcd. for $C_{19}H_{14}N_8S$ (386.43): C 59.05, H 3.65, N 29.0, S 8.30. Found: C 59.42, H 3.81, N 28.87, S 8.15.

*3.1.9. 1-(5,6-Dihydronaphtho[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-11-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylhydrazine (**10**)*

Compound **9** (3.86 g, 0.01 mol) was refluxed in dry ethanol (20 mL) and hydrazine hydrate (0.30 g, 0.01 mol, 99%) for 5 h. The reaction mixture was poured onto ice water and the deposited solid was filtered off, washed several times with water, dried and recrystallized from ethanol to give compound **10**.

Yield 86%; m.p. 205–207 °C.

IR (KBr) ν_{max}: cm^{-1}: 3300, 3191, 3119 (NH_2 + NH).

^{1}H NMR (DMSO-d_6) δ: 2.80–3.00 ppm (m, 4H, $C_{5'}$-CH_2 + $C_{6'}$-CH_2), 4.20 (s, 2H, NH_2, D_2O exchangeable), 6.50 (s, 1H, NH, D_2O exchangeable), 7.20–7.50 (m, 6H, 4Ar-H + C_3-H + C_6-H), 8.60 (s, 1H, $C_{9'}$-H).

^{13}C NMR (DMSO-d_6) δ: 25.10 (C-5′), 29.30 (C-6′), 119, 122, 125.20, 126, 126.50, 126.90, 132, 138, 140.30, 148.25, 156.30, 158.40, 167.10 (sp^2 carbon atoms).

MS *m*/*z* (%): 386 (M^+, 42%), 355 (7%), 326 (10%), 299 (14%), 250 (80%).

Anal. calcd. for $C_{19}H_{14}N_8S$ (386.43): C 59.05, H 3.65, N 29.0, S 8.30. Found: C 58.88, H 3.41, N 29.38, S 8.52.

*3.1.10. General procedure for the synthesis of **11a** and **11b***

A mixture of compound **10** (3.86 g, 0.01 mol), D-glucose (1.80 g, 0.01 mol), or D-ribose (1.40 g, 0.01 mol) in ethanol (30 mL), and a catalytic amount of glacial acetic acid (3 drops) was refluxed for 2 h. The formed precipitates were filtered on hot, washed with water several times, dried and recrystallized from dioxane to give compounds **11a** and **11b**, respectively.

3.1.10.1. D-Glucose N-*1-(5,6-dihydronaphtho[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-11-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl-hydrazone (**11a**).* Yield 36%; m.p. 213–215 °C.

IR (KBr) ν_{max}: cm^{-1}: 3422–3320 (NH/OH), 1566 (C=N).

^{1}H NMR (DMSO-d_6) δ: 2.40–3.40 ppm (m, 10H, $C_{5'}$-CH_2 + $C_{6'}$-CH_2 + CH_2OH + protons of the alditol congregated with the water signal) [17], 4.40 (s, 1H, CH_2OH, D_2O exchangeable), 4.80 (s, 1H, OH, D_2O exchangeable), 4.90 (s, 1H, OH, D_2O exchangeable) 5.40 (s, 1H, OH, D_2O exchangeable), 6.20 (s, 1H, OH, D_2O exchangeable), 7.10–7.30 (m, 5H, 4Ar-H + C_3-H), 7.60 (s, 1H, N=CH), 7.70 (s, 1H, NH, D_2O exchangeable), 8.40 (s, 1H, C_6-H), 9.40 (s, 1H, $C_{9'}$-H).

^{13}C NMR (DMSO-d_6) δ: 23.60 (C-5′), 29.60 (C-6′), 61.20, 61.60, 64.70, 67.10, 73.20 (C-alditol), 119, 122, 125.20, 126, 126.50, 126.90, 132, 138, 140.30, 148.25, 156.30, 158.40, 167.10 (sp^2 carbon atoms).

Anal. calcd. for $C_{25}H_{24}N_8O_5S$ (548.57): C 54.74, H 4.41, N 20.43, S 5.85. Found: C 54.42, H 4.10, N 20.83, S 5.99.

3.1.10.2. D-Ribose N-*1-(5,6-dihydronaphtho[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-11-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl-hydrazone (**11b**).* Yield 55%; m.p. 260–262 °C.

IR (KBr) ν_{max}: cm^{-1}: 3421–3300 (NH/OH), 1570 (C=N).

^{1}H NMR (DMSO-d_6) δ: 2.20–3.80 ppm (m, 7H, $C_{5'}$-CH_2 + $C_{6'}$-CH_2 + protons of the alditol congregated with the water signal) [17], 4.30 (s, 1H, CH_2OH, D_2O exchangeable), 4.70 (s, 2H, CH_2OH), 6.10 (s, 1H, OH, D_2O exchangeable), 6.20 (s, 1H, OH, D_2O exchangeable), 6.30 (s, 1H, OH, D_2O exchangeable) 6.90 (s, 1H, NH, D_2O exchangeable), 7.20–8.40 (m, 6H, 4Ar-H + C_3-H + N=CH), 8.50 (s, 1H, C_6-H), 9.40 (s, 1H, $C_{9'}$-H).

^{13}C NMR (DMSO-d_6) δ: 23.60 (C-5′), 29.60 (C-6′), 61.20, 64.70, 67.10, 73.20 (C-alditol), 119, 122, 125.20, 126, 126.50, 126.90, 132, 138, 140.30, 148.25, 150.0, 156.30, 158.40, (sp^2 carbon atoms).

Anal. calcd. for $C_{24}H_{22}N_8O_4S$ (518.55): C 55.59, H 4.28, N 21.61, S 6.18. Found: C 55.63, H 4.07, N 21.86, S 6.01.

*3.1.11. General procedure for the synthesis of **12a** and **12b***

Compound **11a** or **11b** (0.01 mol) was stirred in a mixture of pyridine (15 mL)/acetic anhydride (5 mL) at room temperature for 3 or 4 h, respectively. The reaction mixtures were poured onto ice water with stirring and the solids that precipitated were collected by filtration, washed with water, dried and recrystallized from dioxane (20 mL)/ethanol (20 mL) to give compounds **12a** and **12b**, respectively.

*3.1.11.1. 2,3,4,5,6-Penta-*O*-acetyl-D-glucose-*N*-1-(5,6-dihydronaphtho[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-11-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl-hydrazone (**12a**).* Yield 32%; m.p. 275–276 °C.

IR (KBr) ν_{max}: cm^{-1}: 3350 (NH), 1743 (C=O), 1588 (C=N).

^{1}H NMR (DMSO-d_6) δ: 2.40–3.80 ppm (m, 19H, $C_{5'}$-CH_2 + $C_{6'}$-CH_2 + 5OAc), 4.20–4.30 (m, 2H, CH_2OAc), 4.40–5.00 (m, 4H, 4*CH*OAc), 7.20–7.90 (m, 5H, 4Ar-H + C_3-H), 8.00 (s, 1H, NH, D_2O exchangeable), 8.20 (s, 1H, N=CH), 8.40 (s, 1H, C_6-H), 8.50 (s, 1H, $C_{9'}$-H).

^{13}C NMR (DMSO-d_6) δ: 21.34, 21.46, 21.60, 21.92, 22.21 (5OCH_3), 24.0 (C-5′), 30.32 (C-6′), 61.15, 64.50, 66.0, 68.15, 72.82 (C-alditol), 119.50, 122, 125.20, 126, 126.50, 126.90, 132, 138, 140.30, 148.25, 156.30, 158.40, 167.10 (sp^2 carbon atoms), 170.86, 170.90, 171.65, 172.40 (5CO).

Anal. calcd. for $C_{35}H_{34}N_8O_{10}S$ (758.76): C 55.40, H 4.52, N 14.77, S 4.23. Found: C 55.83, H 4.21, N 14.99, S 4.52.

*3.1.11.2. 2,3,4,5-Tetra-O-acetyl-D-ribose-N-1-(5,6-dihydronaphtho [1′, 2′:4,5]thieno[2,3-d]pyrimidin-11-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl-hydrazone (**12b**).* Yield 60%; m.p. 265–267 °C.

IR (KBr) ν_{max}: cm^{-1}: 3323 (NH), 1740 (C=O), 1589 (C=N).

^1H NMR (DMSO-d_6) δ: 2.30–2.90 ppm (m, 16H, $C_{5'}$-CH_2 + $C_{6'}$-CH_2 + 4OAc), 3.80–4.40 (m, 2H, CH_2OAc), 4.60–4.70 (m, 1H, CHOAc), 5.20–5.30 (m, 1H, CHOAc), 5.80–5.90 (m, 1H, CHOAc), 7.10–8.10 (m, 6H, 4Ar-H, C_3-H + NH, D_2O exchangeable), 8.30 (s, 1H, N=CH), 8.50 (s, 1H, C_6-H), 9.00 (s, 1H, $C_{9'}$-H).

^{13}C NMR (DMSO-d_6) δ: 21.12, 21.60, 21.92, 22.21 (4OCH_3), 24.0 (C-5′), 29.40 (C-6′), 60.56, 64.50, 68.15, 71.92 (C-alditol), 119.50, 122, 125.20, 126, 126.50, 126.90, 132, 138, 140.30, 148.25, 156.30, 158.40, 167.10 (sp^2 carbon atoms), 169.80, 169.90, 170.11, 170.60 (4CO).

Anal. calcd. for $C_{32}H_{30}N_8O_8S$ (686.70): C 55.97, H 4.40, N 16.32, S 4.67. Found: C 55.62, H 4.21, N 16.07, S 4.99.

*3.1.12. General procedure for the synthesis of **13a** and **13b***

Compound **11a** or **11b** (0.01 mol) and acetic anhydride (15 mL) were stirred in a water bath for 3 or 4 h, respectively. The solid products formed were collected by filtration on hot, dried and recrystallized from ethanol to afford compounds **13a** (42%) and **13b** (48%), respectively.

*3.1.12.1. (1S)-1,2,3,4,5-Penta-O-acetyl-1-C-1-(5,6-dihydronaphtho[1′, 2′:4,5]thieno[2,3-d]pyrimidin-11-yl)-pyrazolo[4,3-e][1,2,4]triazolo[1, 5-c]pyrimidin-2-yl-D-arabinitoles (**13a**).* Yield 42%; m.p. 216–220 °C.

IR (KBr) ν_{max}: cm^{-1}: 1748 (C=O), 1590 (C=N).

^1H NMR (DMSO-d_6) δ: 1.90–2.90 ppm (m, 19H, $C_{5'}$-CH_2 + $C_{6'}$-CH_2 + 5OAc), 4.10–4.20 (m, 2H, CH_2OAc), 4.30–4.40 (m, 1H, CHOAc), 4.90–5.10 (m, 1H, CHOAc), 5.70–5.80 (m, 1H, CHOAc), 5.90–6.00 (m, 1H, CHOAc), 7.20–7.60 (m, 4H, 3Ar-H, C_9-H), 7.70–7.90 (m, 1H, Ar-H), 8.50 (s, 1H, C_5-H), 9.10 (s, 1H, $C_{9'}$-H).

^{13}C NMR (DMSO-d_6) δ: 21.40, 21.60, 21.6, 21.98, 22.21 (5OCH_3), 24.0 (C-5′), 30.32 (C-6′), 64.50, 66.0, 67.22, 68.15, 74.80 (C-alditol), 119.50, 122, 125.20, 126, 126.50, 126.90, 132, 138, 140.30, 148.25, 156.30, 158.40, 167.10 (sp^2 carbon atoms), 170.92, 170.99, 171.65, 171.9, 172.45 (5CO).

Anal. calcd. for $C_{35}H_{32}N_8O_{10}S$ (758.76): C 55.55, H 4.26, N 14.81, S 4.24. Found: C 55.22, H 4.57, N 14.97, S 4.05.

*3.1.12.2. (1S)-1,2,3,4-Tetra-O-acetyl-1-C-1-(5,6-dihydronaphtho [1′, 2′:4,5]thieno[2,3-d]pyrimidin-11-yl)-pyrazolo[4,3-e][1,2,4]triazolo [1, 5-c]pyrimidin-2-yl-D-erthritoles (**13b**).* Yield 48%; m.p. 268–270 °C.

IR (KBr) ν_{max}: cm^{-1}: 1748 (C=O), 1591 (C=N).

^1H NMR (DMSO-d_6) δ: 1.90–2.80 (m, 16H, $C_{5'}$-CH_2 + $C_{6'}$-CH_2 + 4OAc), 3.80–4.20 (m, 3H, CH_2OAc, CHOAc), 5.00–5.60 (m, 2H, 2CHOAc), 6.90–7.60 (m, 5H, 4Ar-H, C_9-H), 8.40 (s, 1H, C_5-H), 8.60 (s, 1H, $C_{9'}$-H).

^{13}C NMR (DMSO-d_6) δ: 21.40, 21.60, 21.98, 22.21 (4OCH_3), 24.0 (C-5′), 30.32 (C-6′), 64.50, 67.22, 68.15, 74.80 (C-alditol), 119.50, 122, 125.20, 126, 126.50, 126.90, 132, 138, 140.30, 148.25, 156.30, 158.40, 167.10 (sp^2 carbon atoms), 170.92, 170.99, 171.65, 172.45 (4CO).

Anal. calcd. for $C_{32}H_{28}N_8O_8S$ (686.70): C 56.14, H 4.12, N 16.37, S 4.68. Found: C 55.52, H 4.21, N 16.63, S 4.95.

*3.1.13. General procedure for the synthesis of **12a,b** and **13a,b***

Compound **11a** or **11b** (0.01 mol) was stirred at 70 °C in a mixture of pyridine (15 mL)/acetic anhydride (5 mL) for 1 h. The reaction mixtures were poured onto ice water with stirring and the solids that precipitated were collected by filtration, washed with water, dried and purified on silica gel column using petroleum ether:ethyl acetate (80 mL:20 mL) as an eluent to give compounds **12a** (42%), **12b** (39%), **13a** (54%) and **13b** (56%), respectively.

3.2. Antiviral bioassay

3.2.1. Preparation of synthetic compounds for bioassay

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

3.2.2. 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 and 1% antibiotic–antimycotic mixture. The pH was adjusted to 7.2–7.4 by 7.5% sodium bicarbonate solution. The mixture was sterilized by filtration through 0.2 μm pore size nitrocellulose membrane.

3.2.3. Viruses

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

3.2.4. Cytotoxicity assay

Cytotoxicity was assayed for both dimethyl sulfoxide (DMSO) and tested compounds. Serial dilutions were prepared and inoculated on Vero cells grown in 96-well tissue culture plates.

3.2.5. Plaque reduction infectivity assay

A 6-well plate, cell culture (10^5 cell/mL) was cultivated and incubated for 2 days at 37 °C. HSV-1 was diluted to give 10^4 PFU/mL final concentrations for each virus and mixed with the tested compound at the previous concentration [10 and 20 μg/10^5 cell] and incubated overnight at 4 °C. Growth medium was removed from the multiwell plate, and virus compound mixture was inoculated (100 μL/well). After 1 h of contact time, the inoculum was aspirated and 3 mL of MEM with 1% agarose was overlaid with cell sheets. The plates were left to solidify and incubated at 37 °C until the development of virus plaques. Cell sheets were fixed in 10% formalin solution for 2 h and stained with crystal violet stain. Control virus and cells were treated identically without any chemical compound. Virus plaques were counted, and the percentage of reduction was calculated [24].

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