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Synthesis of substituted thieno[2,3-*d*]pyrimidine-2,4-dithiones and their *S*-glycoside analogues as potential antiviral and antibacterial agents

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ABSTRACT

Previously, we synthesized and evaluated several thienopyrimidine derivatives containing heterocyclic ring substituents linked to the pyrimidine-2-thione nucleus at C-2 by a two- to four-atom spacer as potential anti-HIV-1 and antimicrobial agents. Also, from the literature, *S*-substituted pyrimidin-4-ones **A** and **B** exhibited interesting anti-HIV-1 activity. To further investigate the synthesis, tools and biological activities, we synthesized several new thienopyrimidine derivatives derived from thieno[2,3-*d*]-pyrimidine-2,4-dithione (**3a**,**b**) The compounds were designed to comprise the heterocyclic substituents directly linked to the thienopyrimidines nucleus at C-2. Moreover, various related triazolo[4,3-*a*]benzothieno[2,3-*d*]pyrimidines derived from 2-thioxothienopyrimidine were also prepared as isosteres. Among the synthesized derivatives **3**–**18**, the compounds **3a**, **8a**, **10a**, **13a** and **14a** were showing complete inhibition at 128 mg/mL or less.

1. Introduction

Acquired immunodeficiency syndrome (AIDS) was identified by Centers for Disease Control and Prevention (CDC) in June 1981. Since then, it has been widely spread in the world and seriously affects healthy conditions of human being, HIV-1 reverse transcriptase (RT) [1] played a critical role in the process of HIV replication and has been identified as one of the main targets for anti-HIV drug discovery [2].

Two classifications of RT inhibitors have been studied: nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside transcriptase inhibitors (NNRTIs). NNRTIs, being less toxic than NRTIs, had received great attention [3–5]. Three NNRTIs nevirapine [6], delavirdine [7], and efavirenz [8] have been approved by FDA. Not only being effective RT inhibitors, these three NNRTIs also acted as the key components of the combination therapy [9–11]. However, NNRTIs can easily induce drug-resistant variants of HIV-1, which have also been seen in the treatment with other chemotherapeutic agents. New anti-HIV agents with novel structures or mechanism(s) of action are still in demand [12,13].

A previous study [14] described a variety of series of 6-naphthylmethyl substituted 2-(alkylthio)-5-alkyl-6-arylmethyl-3,4-dihydro-4-oxopyrimidines (*S*-dihydro-(alkyloxy)-benzyl-oxypyrimidines) (**A**) that received a great deal of attention for their anti-HIV-1, characterized by the presence of a β-carbonyl group on the C-2 side chain (Fig. 1) [15,16]. These compounds exhibited significant anti-HIV activity and more interesting, they might interfere with a target that differed from HIV RT, or act on RT in a way that is different from typical NNRTIs. To further optimize the structure of *S*-DABO derivatives and improve the anti-HIV functionality, we designed two series of thienopyrimidine and its *S*-glycoside derivatives based on previously investigated scaffolds **A** and **B** (Fig. 1) [17]. These novel chemical entities were characterized by (i) introduction of a heteroaromatic ring at C-6, C-5 fused pyrimidines; (ii) introduction of a triazole ring at C-2, C-3 fused pyrimidines; (iii) the presence of *S*-glycosides substituent at the end of C-2 side chain to investigate the importance of the glycosyl moiety, which might favor the enhance of intact molecular structure to the binding pocket of the target due to the increasing flexibility. Herein, we report the synthesis, antiviral and antimicrobial evaluation, and the structure–activity relationship (SAR) of these compounds.

On the basis of the above considerations, original nucleoside analogues directed upon reverse transcriptase still arouse considerable interest [18]. In the hope of elucidating and/or finding better therapeutic agents, *S*-glycosidothieno[2,3-*d*]-pyrimidine analogues seem to be one of the recommended targets and also an extension of our work on pyrimidines [19], *C*-nucleosides [20,21] and thieno

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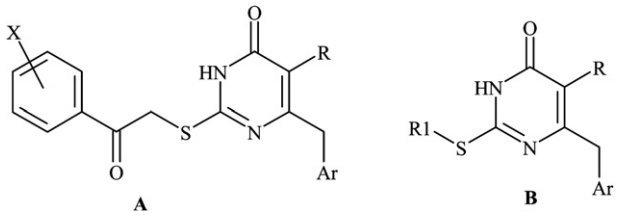


Fig. 1. Structure of *S*-DABSs.

[2,3-*d*]pyrimidines [22–24]. Since the data of *S*-glycosides are relatively few, also promoted us to make research and developed in the synthesis of these types of nucleosides.

2. Results and discussion

With respect to the previous studies carried out in our laboratory, 3-(2-amino-thiophene)-carbonitrile derivative precursors [25] have been regarded as promising intermediates to produce thieno[2,3-*d*]pyrimidine-2,4-dithione derivatives **3a**,**b** with some structural analogies with natural nucleobases. The interaction of acyclic ketone with malononitrile and sulfur element in absolute ethanol in the presence of diethylamine led to 3-(2-amino-thiophene)-carbonitrile derivatives **1a**,**b**. The pyrimidine-2,4-dithione derivatives **3a**,**b** were obtained by refluxing of **1a**,**b** with carbon disulfide in dry pyridine (85–99% yield) as shown in Scheme 1.

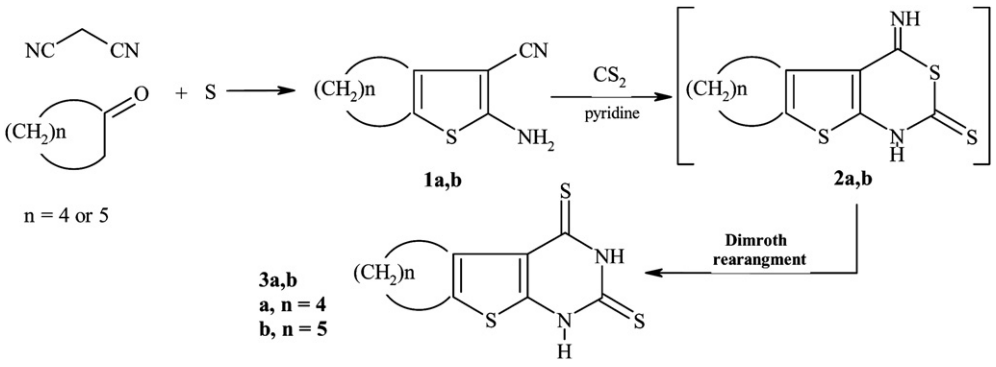
The 1,3-dipolar cycloaddition of the nitrileimines (generated *in situ* from hydrazonoyl chlorides **4a**–**d** and triethylamine in dry chloroform) to thienopyrimidine-2,4-dithione derivatives **3a**, **b** afforded novel 1,3-disubstituted triazolo[1,2,4]thieno[2,3-*d*] pyrimidine derivatives **8a**–**d** in good yields (70–82%) under dry conditions (Scheme 2). This cycloaddition is chemoselective as it occurs only on C=S at C-2 of compound **3** furnishing exclusively the triazol[1,2,4]thieno[2,3-*d*]pyrimidines **8** after H_2S elimination. The mechanism of the reaction may proceed *via* the cycloaddition of one mole of hydrazonoyl chloride **4** (via electron reach nitrogen of the dipole) to the C-2 of compound **3** (Scheme 2). The spectroscopic analyses (IR, NMR, MS) conformed the structure **8** and not structure **5**.

Compounds **3a**,**b** were found to be useful for the syntheses of the interesting *S*-glycosides. As a model experiment the alkylation of **3a** was carried out by the reaction of 1 mol equiv of methyl iodide with the potassium salt **9a** (generated *in situ* by the reaction of **3a** with alcoholic potassium hydroxide). Structure of the new 2-methylthiothieno[2,3-*d*]pyrimidines **10** was confirmed by spectroscopic data. The ^{13}C NMR spectrum as an example, revealed that the signal of the *C*-2 (C–SCH_3) appeared at δ 159 ppm. The ^{13}C NMR spectrum of **3a** and 2-methylthiothieno[2,3-*d*]pyrimidine (**10a**) indicated that the site of the alkylation is the sulfur atom at C-2 and not the nitrogen atom (Fig. 2).

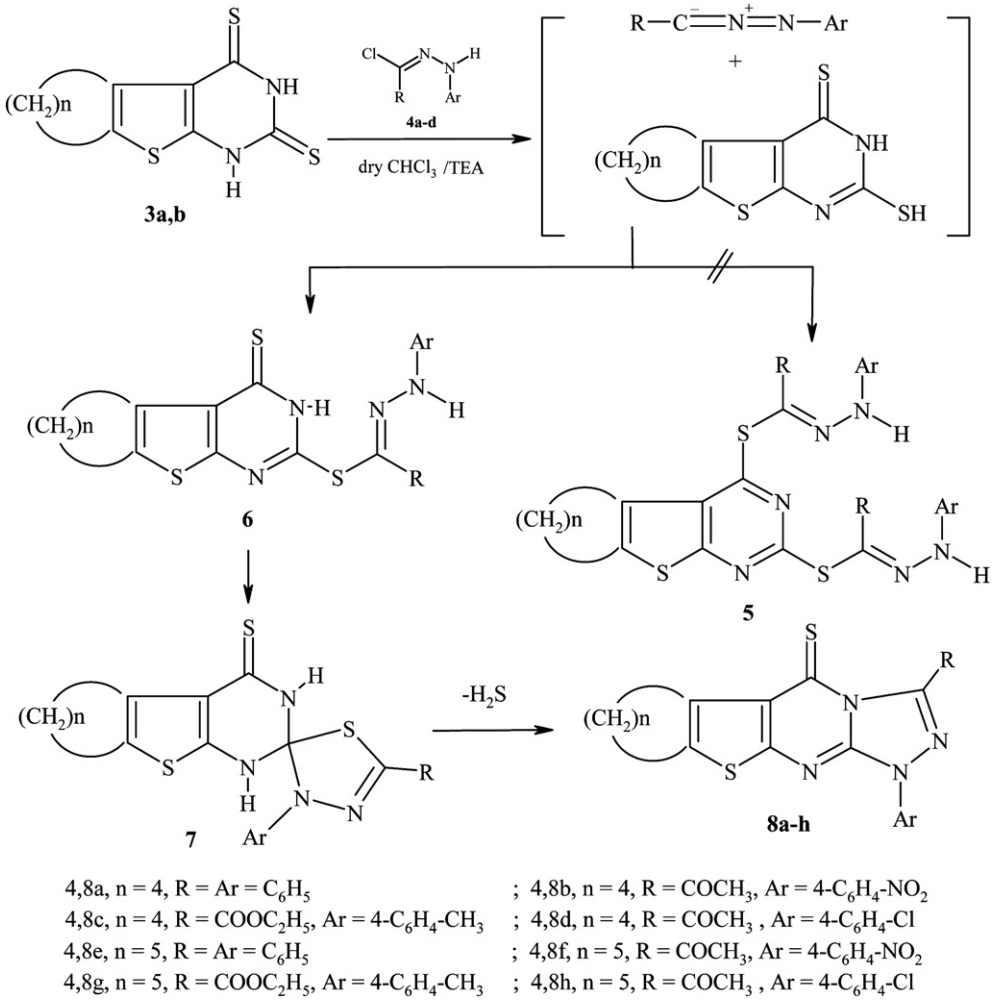
The synthetic route we used for the preparation of 2-*S*-(β-D-glycopyranosyl/or furanosyl)-thieno[2,3-*d*]pyrimidine is outlined in Scheme 3. The thieno[2,3-*d*]pyrimidines **3a**,**b** were converted into their potassium salts **9a**,**b** by using of KOH in acetone and stirring at room temperature for long time with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**11b**) or 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**11c**), yielding the *S*-glycosylated nucleosides **17a**–**d** in good yields. Thin layer chromatography (chloroform:methanol, 7:3) indicated the purity of the compounds. Structures of the *S*-glycosides were confirmed by elemental analyses and spectral data (IR, ^{1}H, ^{13}C NMR). The ^{1}H NMR spectrum of compound **17a** as an example, showed the anomeric proton of the glucose moiety as a doublet at δ 5.94 ppm with a coupling constant $J = 10.72$ Hz indicating β-configuration of the anomeric center. Other protons of the glucopyranose ring resonated at δ 3.97–5.13 ppm, while the four acetoxy groups appeared as four singlets at δ 1.94–2.14 ppm. The ^{13}C NMR spectrum revealed the absence of the thione C-2 atom at about 174.2 ppm and a resonance of –N=C–N– carbon atom (C-2) at δ 158 ppm (Fig. 2). The signals in the region δ 168.3–170.9 ppm are due to the four acetoxy carbonyl atoms (4C=O), and the four signals around δ 22.19–22.59 ppm are assigned to the acetate methyl carbon atoms. Also, the five signals at δ 60.23, 65.22, 67.68, 69.32, 75.63, 87.59 ppm were assigned to C-6′, C-3′, C-2′, C-4′, C-5′ and C-1′, respectively. Moreover, the IR spectra of compounds **13** revealed the absence of the vibration band of a thione group. Similarly, the reaction of heterocycle base **3a**,**b** with 1-bromo-2,3,5-tri-*O*-acetyl-α-D-arabinofuranose (**11a**) furnished the *S*-glycosated product **13a**, **b**. The structure assignments of this product are based on its elemental analysis and the spectral data (see Experimental).

In addition to the above synthetic methods a so called "one-pot" protocol of the silyl-Hilbert–Johnson reaction was described [26]. This 'one-pot' protocol was used by Wolfe for the synthesis of β-D-ribonucleosides [27]. In this procedure, the nucleobase was silylated with HDMS in presence of $(NH_4)_2SO_4$ in MeCN and glycosylated with 1,2,3,4,6-penta-*O*-acetyl-α-D-glycopyranose in presence of $SnCl_4$. Here, MeCN was used as a solvent and HMDS (hexamethyldisilane) as glycosylation catalyst (Vorbrüggen conditions). The nucleobases were silylated and directly glycosylated in one step. Townsend applied this procedure for the synthesis of toyocamycin [28].

We used the 'one-pot' method for the synthesis of the (2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glycopyranosylthio)-thieno[2,3-*d*]pyrimidin-4-one analogues **17a**,**b**. The nucleobases **3a**,**b** were silylated with HMDS in anhydrous MeCN at room temperature in presence of



Scheme 1.



Scheme 2.

ammonium sulfate and then reacted with 1,2,3,4,6-penta-*O*-acetyl-α-D-glycopyranose (Scheme 3). This afforded the glycosylated compounds **17a**,**b** (in 54% yields) after working up.

Deacetylation of *S*-nucleosides **13a**,**b** and **17a**–**d** proceeded smoothly *via* methanolic ammonia treatment to afford the free nucleosides **14a**,**b** and **18a**–**d** in good to excellent yields (Scheme 3). The ^{1}H NMR data of the compounds **14** and **18** revealed the absence of the acetyl protons in the region δ 1.90–2.20 and appearance of the D_2O-exchangeable OH protons in the region δ 4.60–5.56. The IR data of the compound **14a** as a typical example showed also the absence of the acetyl carbonyl function around 1700 cm^{-1} and the appearance of the characteristic OH band at 3500 (br) cm^{-1}.

2.1. In vitro anti-herpes simplex-1 virus (HSV-1) evaluation

The synthesized compounds were tested for their *in vitro* antiviral activity against herpes simplex-1 virus (HSV-1) grown on Vero African green monkey kidney cells. The antiviral, antimitotic and antibiotic aphidicolin was used as a control [29]. Antiviral activity is defined as confluent, relatively unaltered monolayers of stained Vero cell treated with HSV-1. The cytotoxic activity of the tested compounds was performed using Vero cell culture [29]. Cytotoxicity was estimated as the concentration that caused approximately 50% loss of the monolayer present around the plaques caused by HSV-1 (Table 1). An improved plague reduction assay for antiviral activity was used to test the compounds. Plague reduction assay typically

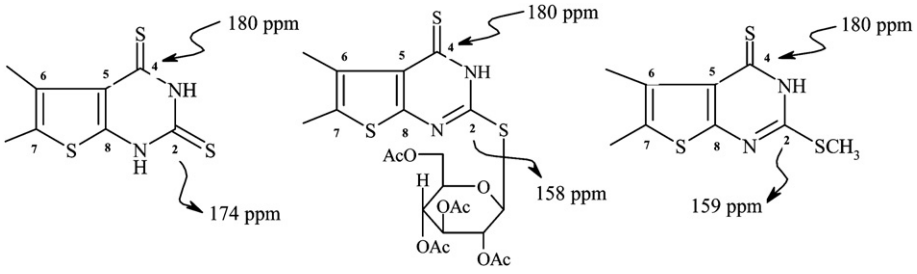
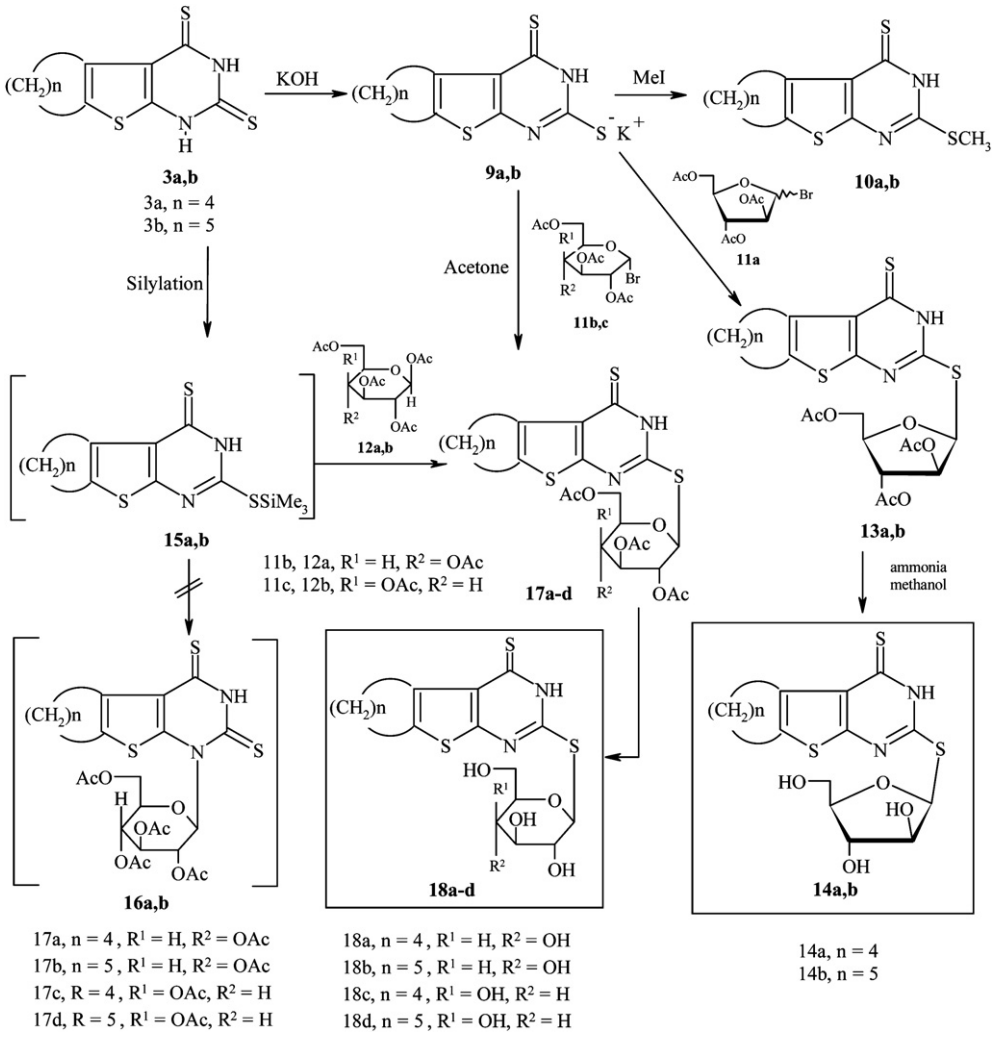


Fig. 2. ^{13}C NMR for some C=S and C–S analogues.



Scheme 3.

used a monolayer of cultured host-cells which were allowed to bind virus, then overplayed with a layer of medium thickened with agar or another thickener. Samples to be tested were either incorporated into the thickened layer or absorbed in a paper disc laid on the thickened layer. Abou-Karam and Shier [29] modified this approach to allow the production of acceptable HSV-1 plaques without the use of a thickening. A serial dilution of samples in 96-plates was used to estimate the end-point concentration for antiviral agents [30]. In the same time, this assay retains the ability to estimate the cytotoxicity which is reflected as reduction in the size or number of viral plaques in a cell monolayer. Among the 13 tested compounds, compounds **14a** and **14b** showed marginal activity as it reduced the number of the plagues by 30% and 24% at a minimum antiviral concentration (MAC) of 0.29 and 0.21 μM/L with lowest cytotoxicity effect (IC_{50} >1.50 μM/L). The rest of the compounds did not show any inhibitory effect against HSV-1 (Table 1). The results also revealed that the synthesized compounds showed different levels of cytotoxicity. The highest cytotoxicity was observed in compounds **3a**, **3b**, **8d** and **8h** (IC_{50} <0.1 μM/L), which containing dithions (2C=S) in positions 2 and 4 in compounds **3a** and **3b**, also compounds **8d** and **8h** which are halogen containing derivatives. On the other hand, the deprotected *S*-glycoside, specially *S*-β-D-arabinofuranosyl thienopyrimidine derivatives **14a**, **14b** where found to possess the lowest cytotoxic effect (IC_{50} >1.5 μM/L).

Table 1
The cytotoxic and anti-HSV-1 activities of the synthesized compounds and the antiviral antibiotic aphidicolin.

Compound	% Reduction in number in plaques	Minimum antiviral conc (MAC) μM/L	Cytotoxicity (IC_{50}) μM/L[a]
Aphidicolin	100	0.02	0.58
3a	B	B	0.07
3b	B	B	0.09
8d	B	B	0.09
8h	B	B	0.07
10a	B	B	0.64
10b	B	B	0.45
13a	B	B	1.39
13b	B	B	1.15
14a	30	0.29	>1.70
14b	24	0.21	>1.46
17a	B	B	0.56
18a	B	B	0.61
18d	B	B	0.38

B: 0% reduction in number of viral plaques.
[a] IC_{50}: The concentration of drug that caused 50% loss of the monolayer present around the plaques.

2.2. In vitro anti-human immunodeficiency virus-1 (HIV-1) evaluation

The procedure used to evaluate the anti-HIV-1 potency is designed to detect agents acting at any stage of the virus

reproductive cycle [31]. The assay involves the killing of T4 lymphocytes by HIV-1; compounds that interfere with viral activities will protect cells from cytolysis. The median effective concentration (EC_{50}) of the tested compounds using infected cells was compared with their cytotoxic effect (IC_{50}) in uninfected cultures. Infected and uninfected cultures were incubated without test compounds to serve as controls. Zidovudine (AZT) treated cultures were also used as positive controls. The screening results (Table 2) revealed that the 2,4-dithiones derivatives **3a** and **3b** exhibited marginal activity against HIV-1 with EC_{50} 6.5 and 5.25 μM/L, respectively. Although the effective concentrations of compounds **3a** and **3b** are greatly higher than that of AZT, they seemed to be less cytotoxic. The obtained results proved that non-substituted of the thieno[2,3-*d*]pyrimidines nucleus are essential for antiviral activity. The weak activity of compounds **3a** and **3b** and the absence of activity in the other compounds may be attributed to the lack of substitution in position 2 of the thieno[2,3-*d*]-pyrimidines nucleus as compared with the active HEPT derivatives.

2.3. Antibacterial activity

The results of antibacterial studies are given in Table 3. Among the synthesized derivatives **3**–**18**, compounds **3a**, **8a**, **10a**, **13a** and **14a** were showing complete inhibition at 128 mg/mL or less. The rest of the compounds showed incomplete inhibition.

Among the 2,4-thieno[2,3-*d*]pyrimidine-dithiones (**3a**,**b**), 2-*S*-methyl-thieno-[2,3-*d*]pyrimidine-4-thione (**10a**,**b**) and triazolothienopyrimidenes (**8a**,**e**) compound **3a** completely inhibited *Escherichia coli* at 64 mg/mL but it could inhibit *Staphylococcus aureus* and *Pseudomonas putida* at 128 mg/mL. Introducing a diphenyl-triazolo group at C-2–C-3 ring (**8a**) made it relatively ineffective towards *E. coli* but found to be active against other two strains. Compound **10a** with an *S*-methyl group at C-2 in pyrimidine ring exhibited activity similar to compound **8a**. Any other substitution at the positions C-2–N-3 of the pyrimidine ring and C-4–C-5 of the thiophene ring system retarded the efficiency of the resulting compounds. The acetylated glycosides **17a**,**b** resulted in decreased activity when compared to **3a** but placing a acetylated arabinofuranosyl group at C-2 in pyrimidine ring on thieno[2,3-*d*]pyrimidine resulted in potent compounds **13a**,**b** when compared to all other compounds. De-acetylated *S*-glycosides compounds in thienopyrimidine resulted in **14a**,**b** and **18a**,**b**, which were active towards *P. putida* and the activity is enhanced in case of arabinofuranosyl group at position of C-2. Further, the MIC of **14a**,**b** towards *P. putida* is 64 mg/mL whereas ampicillin was inactive up to 256 mg/mL.

Table 2
The cytotoxic and anti-HIV-1 activities of some compounds **3**–**18** and the antiviral drug zidovudine (AZT).

Compound	IC_{50} (μM/L)	EC_{50} (μM/L)	TI_{50} (IC_{50}/EC_{50})
3a	>100	6.5	>15.38
3b	>100	5.25	>19.05
8d	>100	>100	>1.00
8h	>100	>100	>1.00
10a	>100	>100	>1.00
10b	>100	>100	>1.00
13a	>100	>100	>1.00
13b	>100	>100	>1.00
14a	>100	>100	>1.00
14b	>100	>100	>1.00
17a	>100	>100	>1.00
18a	>100	>100	>1.00
18d	>100	>100	>1.00
AZT	35.6	0.7	50.86

IC_{50}: 50% inhibitory concentration (the molar concentration of drug that caused 50% inhibition of cell growth). EC_{50}: 50% effective concentration (the molar concentration of drug that caused 50% protection against HIV cytopathic effect). TI_{50}: therapeutic index.

3. Experimental

All starting materials, solvents, and reagents were very pure grade and used as purchased. Chromatography solvents were HPLC grade and used without further purification. Flash column chromatography was performed on Merck KGaA Silica gel 60 F_{254} (particle size 0.040–0.063 mm) unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) using Merck KGaA precoated glass plates (silica gel 60 F_{254}). Melting points were determined on the Electrothermal 9100 melting point apparatus (Electrothermal, UK) and are uncorrected. The IR spectra (KBr) were recorded on an FT-IR NEXCES spectrophotometer (Shimadzu, Japan). The ^{1}H NMR spectra were measured with a Jeol ECA 500 MHz (Japan) in DMSO-d_6 or $CDCl_3$ and chemical shifts were recorded in δ ppm relative to TMS. Mass spectra (EI) were run at 70 eV with a Finnigan SSQ 7000 spectrometer (Thermo-Instrument System Incorporation, USA). The biological medium components were obtained from Sigma Chem. Co., St. Louis, MO, USA. The pharmacological evaluations of the products were carried out in Pharmacological Unit Pharmacology department, (NCI, Cairo University, Egypt). The starting materials, 2-amino-4,5,6,7-tetrahydrobenzo-/and or 4,5,6,7,8-pentahydrocyclohepta-thiophen-3-carbonitrile (**1a**,**b**) were prepared according to the literature procedures [32,33]. Table 4 shows the characterization data of compounds **3**–**18.**

3.1. Synthesis of substituted thieno[2,3-d]pyrimidine-2,4(1H,3H)-dithiones (*3a*,*b*)

General procedure. To a solution of *o*-aminonitrile **1a** or **1b** (0.01 mol) in pyridine (10 mL), carbon disulfide (0.05 mol) was added and the mixture was heated on a water-bath for 6–8 h. After cooling, ethanol was added and the separated solid was collected by filtration, washed with ether to give **3a**,**b** in good yield.

3.1.1. Synthesis of *3a*

From **1a**, as yellow crystals; IR (cm^{-1}, ν): 3424 (br, NH), 2923 (CH alkyl), 1230, 1235 (2CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.67–1.69 (m, 4H, $2CH_2$), 2.96 (m, 2H, CH_2), 3.34 (m, 2H, CH_2), 13.0, 14.0 (2br, 2H, 2NH, D_2O-exchangeable); ^{13}C NMR: 22.23, 22.49, 25.43, 26.59 ($4CH_2$), 126.5, 130.8, 135.8, 148.3 (carbon of the thiophene ring), 174.2 (C=S), 180.0 (C=S); Its MS (*m*/*z*), 254 (M^+, 100).

3.1.2. Synthesis of *3b*

From **1b**, as yellow crystals; IR (cm^{-1}, ν): 3424 (br, NH), 2931 (CH alkyl), 1235, 1242 (2CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.56 (m, 4H, $2CH_2$), 1.75 (m, 2H, CH_2), 2.64 (m, 2H, CH_2), 3.21 (m, 2H, CH_2), 12.60, 13.20 (2br, 2H, 2NH, D_2O-exchangeable); Its MS (*m*/*z*), 268 (M^+, 100).

3.2. Synthesis of 1,3-disubstituted-6,7,8,9-tetrahydro[1,2,4]triazolo [4,3-a]substituted thieno[2,3-d]pyrimidine-5-thiones (*8a*–*h*)

General procedure. A mixture of **3a**,**b** (0.01 mol) and hydrazonoyl chlorides **4a**–**d** (0.01 mol) was stirred under reflux in dry chloroform (30 mL) and 4 drops of triethylamine for 12 h. The solvent was evaporated under reduced pressure. The solid produced was washed three times by 30 mL methanol, and crystallized to produce (**8a**–**h**).

Table 3
MIC (mg/mL) and the zone of inhibition (in mm) values of various thieno-, triazolothieno- and *S*-glycosido-thieno[2,3-*d*]pyrimidines in gram positive and gram negative bacteria.

Compound	*E. coli*			*S. aureus*			*P. putida*		
	Zone of inhibition (mm)			Zone of inhibition (mm)			Zone of inhibition (mm)		
	MIC (mg/mL)	128 mg/mL	64 mg/mL	MIC (mg/mL)	128 mg/mL	64 mg/mL	MIC (mg/mL)	128 mg/mL	64 mg/mL
3a	64	>3	>3	128	>3	<1	128	>3	<1
3b	64	>3	>3	>128	>2	>2	128	>3	>2
8a	128	>3	<1	128	>3	<1	128	>3	<1
8b	64	>3	>3	>128	>2	>2	128	>3	>2
8c	128	>3	<1	128	>3	>2	128	>3	<1
10a	128	>3	<1	128	>3	<1	128	>3	<1
10b	128	>3	<1	128	>3	>2	128	>3	<1
13a	64	>3	>3	64	>3	>3	128	>3	<1
13b	>128	<1	<1	>128	<1	<1	>128	<1	<1
14a	128	>3	>2	>128	>2	>2	64	>3	>3
14b	128	>3	>2	128	>3	>2	128	>3	>2
17a	>128	>2	<1	>128	<1	<1	>128	>2	>2
17b	>128	>2	>2	>128	>2	>2	128	>3	>2
18a	>128	<1	<1	>128	<1	<1	128	>3	<1
18b	>128	<1	<1	>128	<1	<1	>128	<1	<1
Ampicillin	16	>5	>5	16	>5	>5	>256	0	0

3.2.1. 1,3-Diphenyl-6,7,8,9-tetrahydro[1,2,4]triazolo[4,3-a] benzothieno[2,3-d]pyrimidine-5-thione (**8a**)

It was obtained from **3a** and *N*-phenylbenzenecarbohydrazonoyl chloride **4a** as white crystals; IR (cm^{-1}, ν): 3039 (CH aryl), 2921 (CH alkyl), 1645 (C=N), 1238 (CS); ^1H NMR (DMSO-d_6, δ, ppm): 1.52 (m, 4H, $2CH_2$), 1.69 (m, 2H, CH_2), 2.62 (m, 2H, CH_2), 6.96–7.11 (m, 4H, phenyl), 7.37–7.49 (m, 6H, phenyl); Its MS (*m*/*z*), 414 (M^+, 39).

3.2.2. 3-Acetyl-1-(4-nitrophenyl)-6,7,8,9-tetrahydro[1,2,4]triazolo [4,3-a]benzothieno[2,3-d]-pyrimidine-5-thione (**8b**)

It was obtained from **3a** and 2-oxo-N-(4-nitrophenyl)-propane hydrazonoyl chloride **4b** as yellow powder; IR (cm^{-1}, ν): 3022 (CH aryl), 2924 (CH alkyl), 1723 (CO), 1234 (CS), 1650 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.68 (m, 4H, $2CH_2$), 2.74 (s, 3H, CH_3), 2.89 (m, 2H, CH_2), 3.28 (m, 2H, CH_2), 7.06–7.10 (d, 2H, phenyl), 7.65–7.70 (d, 2H, phenyl); Its MS (*m*/*z*), 425 (M^+, 68).

Table 4
Characterization data of compounds **3**–**18**.

Comp. No.	M.p. [°C]	Yield [%]/solvent	Mol. formula[a] (mol.wt.)
3a	315–317	85/Dioxane	$C_{10}H_{10}N_2S_3$ (254.4)
3b	279–281	76/Ethanol	$C_{11}H_{12}N_2S_3$ (268.4)
8a	200–202	68/Ethanol	$C_{23}H_{18}N_4S_2$ (414.5)
8b	165–167	72/Ethanol	$C_{19}H_{15}N_5S_2O_2$ (425.5)
8c	170–172	70/Ethanol	$C_{21}H_{20}N_4S_2O_2$ (424.5)
8d	180–182	80/Ethanol	$C_{19}H_{15}N_4S_2OCl$ (414.9)
8e	162–164	76/Ethanol	$C_{24}H_{20}N_4S_2$ (428.9)
8f	175–177	72/Ethanol	$C_{20}H_{17}N_5O_3S_2$ (439.5)
8g	210–212	68/Ethanol	$C_{22}H_{22}N_4S_2O_2$ (438.5)
8h	150–152	73/Ethanol	$C_{20}H_{17}N_4OS_2Cl$ (428.9)
10a	148–150	86/Dioxane	$C_{11}H_{12}N_2S_3$ (268.4)
10b	239–241	79/Ethanol	$C_{12}H_{14}N_2S_3$ (282.4)
13a	250–252	65/benzene	$C_{21}H_{24}N_2S_3O_7$ (512.6)
13b	237–239	61/benzene	$C_{22}H_{26}N_2S_3O_7$ (526.6)
14a	171–173	58/n-hexane	$C_{15}H_{18}N_2S_3O_4$ (386.4)
14b	135–137	54/n-hexane	$C_{16}H_{20}N_2S_3O_4$ (400.4)
17a	188–190	64/benzene	$C_{24}H_{28}N_2S_3O_9$ (584.6)
17b	110–112	61/benzene	$C_{25}H_{30}N_2S_3O_9$ (598.7)
17c	201–203	60/benzene	$C_{24}H_{28}N_2S_3O_9$ (584.6)
17d	131–133	66/benzene	$C_{25}H_{30}N_2S_3O_9$ (598.7)
18a	211–213	58/benzene	$C_{16}H_{19}N_2S_3O_5$ (415.5)
18b	167–169	49/benzene	$C_{17}H_{21}N_2S_3O_5$ (429.5)
18c	196–198	53/benzene	$C_{16}H_{19}N_2S_3O_5$ (415.5)
18d	180–182	51/benzene	$C_{17}H_{21}N_2S_3O_5$ (429.5)

[a] Analysis for C, H, N and the results were within ±0.4% of the theoretical values.

3.2.3. 3-Ethylcarboxylate-1-(4-tolyl)-6,7,8,9-tetrahydro[1,2,4] triazolo[4,3-a]benzothieno-[2,3-d]pyrimidine-5-thione (**8c**)

It was obtained from **3a** and chloro-(4-tolylhydrazono)-ethyl-acetate **4c** as white crystals; IR (cm^{-1}, ν): 3031 (CH aryl), 2926 (CH alkyl), 1705 (CO), 1245 (CS), 1645 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.26–1.29 (t, 3H, CH_3), 1.59 (m, 4H, $2CH_2$), 2.65 (s, 3H, CH_3), 2.83 (m, 2H, CH_2), 3.31 (m, 2H, CH_2), 4.06–4.11 (q, 2H, CH_2), 7.11–7.16 (d, 2H, phenyl), 7.49–7.52 (d, 2H, phenyl); Its MS (*m*/*z*), 424 (M^+, 43).

3.2.4. 3-Acetyl-1-(4-chlorophenyl)-6,7,8,9-tetrahydro[1,2,4]triazolo [4,3-a]benzothieno[2,3-d]pyrimidine-5-thione (**8d**)

It was obtained from **3a** and 2-oxo-N-(4-chlorophenyl)-propane hydrazonoyl chloride **4d** as yellow powder; IR (cm^{-1}, ν): 2998 (CH aryl), 2920 (CH alkyl), 1711 (CO), 1654 (C=N), 1242 (CS); ^1H NMR (DMSO-d_6, δ, ppm): 1.59 (m, 4H, $2CH_2$), 2.67 (s, 3H, CH_3), 2.85 (m, 2H, CH_2), 3.27 (m, 2H, CH_2), 7.06–7.11 (d, 2H, phenyl), 7.46–7.52 (d, 2H, phenyl); Its MS (*m*/*z*), 414 (M^+, 65).

3.2.5. 1,3-Diphenyl-6,7,8,9,10-petaahydro[1,2,4]triazolo[4,3-a] cycloheptathieno[2,3-d]-pyrimidine-5-thione (**8e**)

It was obtained from **3b** and N-phenylbenzene-carbohydrazonoyl chloride **4a** as white crystals; IR (cm^{-1}, ν): 3042 (CH aryl), 2918 (CH alkyl), 1640 (C=N), 1237 (CS); ^1H NMR (DMSO-d_6, δ, ppm): 1.58 (m, 4H, $2CH_2$), 1.73 (m, 2H, CH_2), 2.68 (m, 2H, CH_2), 3.19 (m, 2H, CH_2), 6.95–7.01 (m, 4H, phenyl), 7.25–7.29 (m, 6H, phenyl); Its MS (*m*/*z*), 428 (M^+, 55).

3.2.6. 3-Acetyl-1-(4-nitrophenyl)-6,7,8,9,10-pentahydro[1,2,4] triazolo[4,3-a]cycloheptathieno[2,3-d]pyrimidine-5-thione (**8f**)

It was obtained from **3b** and 2-oxo-N-(4-nitrophenyl)-propane hydrazonoyl chloride **4b** as yellow powder; IR (cm^{-1}, ν): 3013 (CH aryl), 2919 (CH alkyl), 1703 (CO), 1638 (C=N), 1231 (CS); ^1H NMR (DMSO-d_6, δ, ppm): 1.61 (m, 4H, $2CH_2$), 1.76 (m, 2H, CH_2), 2.64 (m, 2H, CH_2), 2.81 (s, 3H, CH_3), 3.17 (m, 2H, CH_2), 6.96–6.98 (d, 2H, phenyl), 7.27–7.31 (d, 2H, phenyl); Its MS (*m*/*z*), 439 (M^+, 38).

3.2.7. 3-Ethylcarboxylate-1-(4-tolyl)-6,7,8,9,10-pentahydro[1,2,4] triazolo[4,3-a]cycloheptathieno[2,3-d]pyrimidine-5-thione (**8g**)

It was obtained from **3b** and chloro-(4-tolylhydrazono)-ethyl-acetate **4c** as white crystals; IR (cm^{-1}, ν): 3036 (CH aryl), 2920 (CH alkyl), 1720 (CO), 1619 (C=N), 1237 (CS); ^1H NMR (DMSO-d_6, δ, ppm): 1.23–1.27 (t, 3H, CH_3), 1.55 (m, 4H, $2CH_2$), 1.75 (m, 2H, CH_2),

2.66 (m, 2H, CH_2), 2.75 (s, 3H, CH_3), 3.19 (m, 2H, CH_2), 4.09–4.16 (q, 2H, CH_2), 7.13–7.19 (d, 2H, phenyl), 7.67–7.73 (d, 2H, phenyl); Its MS (*m*/*z*), 438 (M^+, 32).

*3.2.8. 3-Acetyl-1-(4-chlorophenyl)-6,7,8,9,10-pentahydro[1,2,4] triazolo[4,3-a]cycloheptathieno[2,3-d]pyrimidine-5-thione (**8h**)*

It was obtained from **3b** and 2-oxo-N-(4-chlorophenyl)-propane hydrazonoyl chloride **4d** as yellow powder; IR (cm^{-1}, ν): 3023 (CH aryl), 2927 (CH alkyl), 1711 (CO), 1640 (C=N), 1233 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.58 (m, 4H, $2CH_2$), 1.73 (m, 2H, CH_2), 2.65 (m, 2H, CH_2), 2.78 (s, 3H, CH_3), 3.18 (m, 2H, CH_2), 7.16–7.19 (d, 2H, phenyl), 7.36–7.40 (d, 2H, phenyl); Its MS (*m*/*z*), 428 (M^+, 82).

*3.3. Preparation of the alkylated S-methyl (**10a**,**b**)*

General procedure. To a solution of **3a**,**b** (0.01 mol) in aqueous potassium hydroxide (0.01 mol) in distilled water (5 ml) was added a solution of methyl iodide in acetone (40 ml). The reaction mixture was stirred at room temperature for 24 h (under TLC control). The solvent was evaporated under reduced pressure and the crude product was filtered off and washed with distilled water to remove KBr formed. The product was dried, and crystallized from the proper solvent.

*3.3.1. Compound **10a***

Compound **10a** was obtained from **3a**, as yellow crystals; IR (cm^{-1}, ν): 3440 (br, NH), 2918 (CH alkyl), 1230 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.62–1.65 (m, 4H, $2CH_2$), 2.23 (s, 3H, SCH_3), 2.89 (m, 2H, CH_2), 3.27 (m, 2H, CH_2), 12.00 (br, 1H, NH, D_2O-exchangeable); ^{13}C NMR: 14.38 (SCH_3), 22.36, 22.43, 25.41, 26.67 ($4CH_2$), 125.3, 131.6, 134.6, 149.5 (carbon of the thiophene ring), 158.2 (C–S), 180.0 (C=S); Its MS (*m*/*z*), 268 (M^+, 100).

*3.3.2. Compound **10b***

Compound **10b** was obtained from **3b**, as yellow crystals; IR (cm^{-1}, ν): 3420 (br, NH), 2917 (CH alkyl), 1240 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.53 (m, 4H, $2CH_2$), 1.73 (m, 2H, CH_2), 2.19 (s, 3H, SCH_3), 2.67 (m, 2H, CH_2), 3.23 (m, 2H, CH_2), 12.10 (br, 1H, NH, D_2O-exchangeable); Its MS (*m*/*z*), 282 (M^+, 100).

*3.4. Preparation of the acetylated S-nucleosides (**13a**,**b**) and (**17a**–**d**)*

Method A. To a solution of **3a**,**b** (0.01 mol) in aqueous potassium hydroxide (0.01 mol) in distilled water (5 ml) was added a solution of 1-bromo-2,3,5-tri-*O*-acetyl-α-D-arabinofuranose (**11a**) or 2,3,4,6-tetra-*O*-acetyl-α-D-gluco-/or galactopyranosyl bromide (**11b**,**c**) (0.015 mol) in acetone (40 ml). The reaction mixture was stirred at room temperature for 24 h (under TLC control). The solvent was evaporated under reduced pressure at 40 °C, and the crude product was filtered off and washed with distilled water to remove KBr formed. The product was dried, and crystallized from the proper solvent.

Method B. Compound **3a**,**b** (0.01 mol) was stirred under reflux, and dry conditions in 50 ml hexamethyldisilane (HMDS) in the presence of ammonium sulfate (0.01 mol) for 50–60 h. The clear solution formed was cooled and the solvent was evaporated in vacuo to give the silylated compound **7** as yellow oil. The latter oil was dissolved in acetonitrile (10 ml) and was added to a solution of 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (**9**) in acetonitrile (5 ml) followed by addition of $SnCl_4$ (1.8 ml). The reaction mixture was stirred at room temperature for 16–20 h (under TLC control). The mixture was poured into saturated sodium bicarbonate solution and extracted the thioglycosides by diethylether + ethylacetate (1:1, 100 ml). Evaporate the solvent under reduced pressure to furnish crude nucleosides which were purified by column chromatography (30% ethylacetate in ether) to afford the pure thioglycosides.

*3.4.1. 2-(2′,3′,5′-Tri-O-acetyl-β-D-Arabinofuranosylthio)-5,6,7,8-tetrahydrobenzothieno[2,3-d]pyrimidine-4-thione (**13a**)*

It was obtained from **3a** and (2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl)-bromide (**11a**) as yellow powder; IR (cm^{-1}, ν): 3430 (br, NH), 2985 (CH alkyl), 1752 (3CO), 1230 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.67 (m, 4H, $2CH_2$), 1.95, 1.99, 2.01 (3s, 9H, $3CH_3CO$), 2.61 (m, 2H, CH_2), 2.95 (m, 2H, CH_2), 4.07 (m, 1H, *H*-4′), 4.13 (m, 2H, *H*-5′, *H*-5″), 5.26 (m, 1H, *H*-3′), 5.34 (m, 1H, *H*-2′), 6.72 (d, 1H, $J = 3.67$ Hz, *H*-1′), 13.00 (br, 1H, NH); ^{13}C NMR: 20.83 (CH_2), 22.16, 22.20, 22.56 ($3CH_3$), 22.70, 24.90, 27.98 ($3CH_2$), 61.49 (C-5′), 66.20 (C-3′), 66.45 (C-2′), 67.32 (C-4′), 85.53 (C-1′), 125.8, 130.4, 132.7, 148.3 (carbon of the thiophene ring), 158.2 (C–S), 168.1, 169.5, 170.6 (3C=O), 180.0 (C=S); Its MS (*m*/*z*), 512 (M^+, 28%).

*3.4.2. 2-(2′,3′,5′-Tri-O-acetyl-β-D-arabinofuranosylthio)-6,7,8,9,10-pentahydrocycloheptathieno-[2,3-d]pyrimidine-4-thione (**13b**)*

It was obtained from compound **3b** and (2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl)-bromide (**11a**) as yellow powder; IR (cm^{-1}, ν): 3426 (br, NH), 2992 (CH alkyl), 1750 (3CO), 1240 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.55 (m, 4H, $2CH_2$), 1.71 (m, 2H, CH_2), 1.94, 1.98, 2.00 (3s, 9H, $3CH_3CO$), 2.65 (m, 2H, CH_2), 3.19 (m, 2H, CH_2), 4.00 (m, 1H, *H*-4′), 4.14 (m, 2H, *H*-5′, *H*-5″), 5.29 (m, 1H, *H*-3′), 5.40 (m, 1H, *H*-2′), 6.67 (d, 1H, $J = 3.67$ Hz, *H*-1′), 12.20 (br, 1H, NH); ^{13}C NMR: 19.78, 20.80 ($2CH_2$), 21.56, 22.10, 22.46 ($3CH_3$), 22.75, 24.93, 28.15 ($3CH_2$), 61.43 (C-5′), 66.29 (C-3′), 66.55 (C-2′), 67.38 (C-4′), 85.59 (C-1′), 125.7, 130.9, 133.2, 148.4 (carbon of the thiophene ring), 158.6 (C–S), 168.3, 169.1, 170.4 (3C=O), 181.0 (C=S); Its MS (*m*/*z*), 526 (M^+, 36%).

*3.4.3. 2-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-glucopyranosylthio)-5,6,7,8-tetrahydrobenzothieno-[2,3-d]pyrimidine-4-thione (**17a**)*

It was obtained from compound **3a** and (2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-bromide (**11b**) as a pale yellow powder; IR (cm^{-1}, ν): 3400 (br, NH), 2938 (CH alkyl), 1732 (CO), 1245 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.60–1.64 (m, 4H, $2CH_2$), 1.94, 2.02, 2.11, 2.14 (4s, 12H, $4CH_3CO$), 2.83 (m, 2H, CH_2), 3.19 (m, 2H, CH_2), 3.97 (m, 1H, *H*-5′), 4.20 (m, 2H, *H*-6′, *H*-6″), 4.33 (m, 1H, *H*-4′), 4.97 (t, 1H, *H*-2′), 5.13 (t, 1H, $J = 9.57$ Hz, *H*-3′), 5.94 (d, 1H, $J = 10.72$ Hz, *H*-1′), 11.00 (br, H, NH); ^{13}C NMR: 20.74 (CH_2), 22.19, 22.24, 22.37, 22.59 ($4CH_3$), 22.75, 24.93, 27.86 ($3CH_2$), 60.23 (C-6′), 65.22 (C-3′), 67.68 (C-2′), 69.32 (C-4′), 75.63 (C-5′), 87.59 (C-1′), 125.3, 129.8, 133.1, 149.6 (carbon of the thiophene ring), 158.6 (C–S), 168.3, 169.7, 170.2, 170.9 (4C=O), 180.4 (C=S); Its MS (*m*/*z*), 584 (M^+, 23%).

*3.4.4. 2-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-glucopyranosylthio)-6,7,8,9,10-pentahydrocycloheptathieno[2,3-d]pyrimidine-4-thione (**17b**)*

It was obtained from compound **3b** and (2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-bromide (**11b**) as a pale yellow powder; IR (cm^{-1}, ν): 3442 (br, NH), 2943 (CH alkyl), 1730 (CO), 1238 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.51 (m, 4H, $2CH_2$), 1.69 (m, 2H, CH_2), 1.92, 1.99, 2.02, 2.11 (4s, 12H, $4CH_3CO$), 2.65 (m, 2H, CH_2), 3.21 (m, 2H, CH_2), 3.84 (m, 1H, *H*-5′), 4.09 (m, 2H, *H*-6′, *H*-6″), 4.26 (m, 1H, *H*-4′), 4.88 (t, 1H, *H*-2′), 5.23 (t, 1H, $J = 9.61$ Hz, *H*-3′), 5.94 (d, 1H, $J = 10.59$ Hz, *H*-1′), 12.10 (br, 1H, NH, D_2O-exchangeable); Its MS (*m*/*z*), 598 (M^+, 25%).

*3.4.5. 2-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-galactopyranosylthio)-5,6,7,8-tetrahydrobenzothieno[2,3-d]pyrimidine-4-thione (**17c**)*

It was obtained from compound **3a** and (2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-bromide (**11c**) as yellow powder; IR (cm^{-1},

ν): 3996 (br, NH), 2987 (CH alkyl), 1728 (CO), 1241 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.56–1.61 (m, 4H, $2CH_2$), 1.97, 2.01, 2.13, 2.17 (4s, 12H, $4CH_3CO$), 2.81 (m, 2H, CH_2), 3.12 (m, 2H, CH_2), 3.99 (m, 1H, *H*-5′), 4.19 (m, 2H, *H*-6′, *H*-6″), 4.39 (m, 1H, *H*-4′), 4.89 (t, 1H, *H*-2′), 5.19 (t, 1H, $J = 9.62$ Hz, *H*-3′), 6.02 (d, 1H, $J = 10.68$ Hz, *H*-1′), 11.30 (br, H, NH); Its MS (*m*/*z*), 584 (M^+, 35%).

3.4.6. 2-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-galactopyranosylthio)-6,7,8,9,10-pentahydrocycloheptathieno[2,3-d]pyrimidine-4-thione (**17d**)

It was obtained from compound **3b** and (2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-bromide (**11c**) as yellow powder; IR (cm^{-1}, ν): 3425 (br, NH), 2939 (CH alkyl), 1728 (2CO), 1246 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.55 (m, 4H, $2CH_2$), 1.70 (m, 2H, CH_2), 1.96, 2.03, 2.07, 2.13 (4s, 12H, $4CH_3CO$), 2.67 (m, 2H, CH_2), 3.19 (m, 2H, CH_2), 3.92 (m, 1H, *H*-5′), 4.07 (m, 2H, *H*-6′, *H*-6″), 4.19 (m, 1H, *H*-4′), 4.87 (t, 1H, *H*-2′), 5.29 (t, 1H, $J = 9.78$ Hz, *H*-3′), 5.99 (d, 1H, $J = 10.64$ Hz, *H*-1′), 12.25 (br, 1H, NH, D_2O-exchangeable); Its MS (*m*/*z*), 598 (M^+, 31%).

3.5. Synthesis of diacetylated 2-(β-D-glycosidylthio)-thieno[2,3-d] pyrimidin-4-thiones (**14a**,**b**) and (**18a**–**d**)

General procedure. Acetylated compound **13a**,**b** or **17a**–**d** (1.0 mmol) was dissolved in methanolic ammonia (saturated with NH_3 at 0 °C, 100 ml). The reaction mixture was stirred overnight and then heated the reaction mixture for 1 h at 120–130 °C. The mixture was then cooled and the solvent was evaporated to provide the crude nucleoside. Purification by heating the crude in n-hexane (100 ml, three times) provided **14a**,**b** or **18a**–**d** as yellow solid. Crystallization from methanol gave a pale yellow powder.

3.5.1. 2-(β-D-Arabinofuranosylthio)-5,6,7,8-tetrahydrobenzothieno [2,3-d]pyrimidine-4-thione (**14a**)

It obtained from **13a**; IR (cm^{-1}, ν): 3520 (br s, OH), 3370 (br, NH), 2974 (CH alkyl), 1253 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.68 (m, 4H, $2CH_2$), 2.90 (m, 2H, CH_2), 3.23 (m, 2H, CH_2), 3.76 (m, 2H, *H*-5′, *H*-5″), 4.08 (m, 1H, *H*-4′), 4.79 (t, 1H, *H*-2′), 5.11 (t, $J = 5.40$ Hz, $J = 4.95$ Hz, OH–C(5′)), 5.18 (d, $J = 4.45$ Hz, OH-C(3′)), 5.39 (d, $J = 5.96$ Hz, OH–C (2′)), 5.63 (t, 1H, $J = 9.78$ Hz, *H*-3′), 6.94 (d, 1H, $J = 5.64$ Hz, *H*-1′), 10.20 (br, H, NH); ^{13}C NMR: 19.90, 22.76, 24.93, 27.92 ($4CH_2$), 60.89 (C-5′), 65.37 (C-3′), 67.56 (C-2′), 69.39 (C-4′), 87.47 (C-1′), 125.6, 131.2, 134.4, 149.1 (carbon of the thiophene ring), 158.5 (C–S), 179.9 (C=S); Its MS (*m*/*z*), 386 (M^+, 35%).

3.5.2. 2-(β-D-Arabinofuranosylthio)-6,7,8,9,10-pentahydrocycloheptathieno[2,3-d]-pyrimidine-4-thione (**14b**)

It obtained from **13b**; IR (cm^{-1}, ν): 3500 (br s, OH), 3410 (br, NH), 2986 (CH alkyl), 1254 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.53 (m, 4H, $2CH_2$), 1.67 (m, 2H, CH_2), 2.59 (m, 2H, CH_2), 3.15 (m, 2H, CH_2), 3.80 (m, 2H, *H*-5′, *H*-5″), 4.12 (m, 1H, *H*-4′), 4.67 (t, 1H, *H*-2′), 5.09 (t, $J = 5.42$ Hz, $J = 4.96$ Hz, OH–C(5′)), 5.19 (d, $J = 4.43$ Hz, OH–C(3′)), 5.33 (d, $J = 5.94$ Hz, OH–C(2′)), 5.66 (t, 1H, $J = 9.60$ Hz, *H*-3′), 6.89 (d, 1H, $J = 5.67$ Hz, *H*-1′), 10.80 (br, H, NH); Its MS (*m*/*z*), 400 (M^+, 42%).

3.5.3. 2-(β-D-Glucopyranosylthio)-5,6,7,8-tetrahydrobenzothieno [2,3-d]pyrimidine-4-thione (**18a**)

It obtained from **17a**; IR (cm^{-1}, ν): 3490 (br s, OH), 3385 (br, NH), 2987 (CH alkyl), 1250 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.64 (m, 4H, $2CH_2$), 2.87 (m, 2H, CH_2), 3.18 (m, 2H, CH_2), 3.86 (m, 1H, *H*-5′), 4.08 (m, 2H, *H*-6′, *H*-6″), 4.29 (m, 1H, *H*-4′), 4.55 (br, H, D_2O-exchangeable OH), 4.87 (t, 1H, *H*-2′), 5.02 (br s, 1H, D_2O-exchangeable OH), 5.11 (t, 1H, $J = 9.57$ Hz, *H*-3′), 5.14 (d, 1H, $J = 4.8$ Hz, D_2O-exchangeable OH), 5.52 (br, H, D_2O-exchangeable OH), 6.02 (d, 1H, $J = 10.56$ Hz, *H*-1′), 10.60 (br, H, NH); ^{13}C NMR: 19.98, 21.88, 23.79, 26.76 ($4CH_2$), 61.53 (C-6′), 66.31 (C-3′), 68.34 (C-2′), 68.94 (C-4′), 77.81 (C-5′), 89.78 (C-1′), 124.9, 130.7, 135.3, 149.8 (carbon of the thiophene ring), 159.2 (C–S), 181.4 (C=S) Its MS (*m*/*z*), 415 (M^+, 22%).

3.5.4. 2-(β-D-Glucopyranosylthio)-6,7,8,9,10-pentahydrocycloheptathieno[2,3-d]-pyrimidine-4-thione (**18b**)

It obtained from **17b**; IR (cm^{-1}, ν): 3480 (br s, OH), 3290 (br, NH), 2989 (CH alkyl), 1239 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.55 (m, 4H, $2CH_2$), 1.72 (m, 2H, CH_2), 2.62 (m, 2H, CH_2), 3.19 (m, 2H, CH_2), 3.78 (m, 1H, *H*-5′), 3.98 (m, 2H, *H*-6′, *H*-6″), 4.29 (m, 1H, *H*-4′), 4.52 (br, H, D_2O-exchangeable OH), 4.77 (t, 1H, *H*-2′), 5.00 (br s, 1H, D_2O-exchangeable OH), 5.10 (t, 1H, $J = 9.62$ Hz, *H*-3′), 5.17 (d, 1H, $J = 4.8$ Hz, D_2O-exchangeable OH), 5.60 (br, H, D_2O-exchangeable OH), 6.09 (d, 1H, $J = 10.56$ Hz, *H*-1′), 11.00 (br, H, NH); Its MS (*m*/*z*), 429 (M^+, 30%).

3.5.5. 2-(β-D-Galactopyranosylthio)-5,6,7,8-tetrahydrobenzothieno [2,3-d]pyrimidine-4-thione (**18c**)

It obtained from **17c**; IR (cm^{-1}, ν): 3510 (br s, OH), 3390 (br, NH), 2993 (CH alkyl), 1238 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.66 (m, 4H, $2CH_2$), 2.89 (m, 2H, CH_2), 3.21 (m, 2H, CH_2), 3.89 (m, 1H, *H*-5′), 4.02 (m, 2H, *H*-6′, *H*-6″), 4.32 (m, 1H, *H*-4′), 4.91 (t, 1H, *H*-2′), 4.60 (br, H, D_2O-exchangeable OH), 5.02 (br s, 1H, D_2O-exchangeable OH), 5.04 (d, 1H, $J = 4.8$ Hz, D_2O-exchangeable OH), 5.13 (t, 1H, $J = 9.54$ Hz, *H*-3′), 5.56 (br, H, D_2O-exchangeable OH), 6.11 (d, 1H, $J = 10.62$ Hz, *H*-1′), 10.30 (br, H, NH); Its MS (*m*/*z*), 415 (M^+, 27%).

3.5.6. 2-(β-D-Galactopyranosylthio)-6,7,8,9,10-pentahydrocycloheptathieno[2,3-d]-pyrimidine-4-thione (**18d**)

It obtained from **17d**; IR (cm^{-1}, ν): 3530 (br s, OH), 3315 (br, NH), 2979 (CH alkyl), 1255 (CS); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.58 (m, 4H, $2CH_2$), 1.70 (m, 2H, CH_2), 2.65 (m, 2H, CH_2), 3.20 (m, 2H, CH_2), 3.83 (m, 1H, *H*-5′), 3.89 (m, 2H, *H*-6′, *H*-6″), 4.18 (m, 1H, *H*-4′), 4.86 (t, 1H, *H*-2′), 4.63 (br, H, D_2O-exchangeable OH), 5.03 (br s, 1H, D_2O-exchangeable OH), 5.09 (d, 1H, $J = 4.8$ Hz, D_2O-exchangeable OH), 5.16 (t, 1H, $J = 9.70$ Hz, *H*-3′), 5.49 (br, H, D_2O-exchangeable OH), 6.12 (d, 1H, $J = 10.65$ Hz, *H*-1′), 10.20 (br, H, NH); Its MS (*m*/*z*), 429 (M^+, 28%).

3.6. In vitro anti-herpes simplex-1 virus (HSV-1)

Samples were prepared by dissolving in DMSO and diluting aliquots into sterile culture medium before preparing serial dilution and placed in microtiter trays. Microtiter trays with confluent monolayer cultures of Vero cells were inverted, the medium shaken out, and replaced with serial dilutions of sterile extracts in triplicate in 100 μL medium followed by tittered virus in 100 μL medium containing 10% (v/v) calf serum in each well. In each tray, the last row of wells was reserved for controls that were not treated with compounds or not treated with virus. The tray were cultured and incubated at 37 °C in 5% CO_2 atmosphere for 6 h. The trays were inverted onto a pad of paper towels, the remaining cells rinsed carefully with medium, and fixed with 3.7% (v/v) formaldehyde in saline for 20 min. The fine cells were rinsed with water, and examined visually. Antiviral activity is identified as confluent, relatively unaltered monolayers of stained *Vero* cells treated with HSV-1. Cytotoxicity was estimated as the concentration that caused approximately 50% loss of the monolayer present around the plaques caused by HSV-1 (Table 1).

3.7. In vitro anti-human immunodeficiency virus-1 (HIV-1)

Compounds were prepared for assay by dissolving in DMSO then diluted 1:100 in cell culture medium before preparing serial dilution and placed in microtiter trays. T4 lymphocytes (CEM cell line) were added and after a brief interval (1 min or more) HIV-1 was added

resulting in a 1:200 final dilution of each of the tested compounds. Cultures were incubated at 37 °C in 5% CO_2 atmosphere for 6 days. Tetrazolium salt XTT was added to all cells and cultures were incubated to allow formazan color development by virally infected cells. Individual wells were analyzed spectrophotometrically to quantitative formazan production and, in addition, were viewed microscopically for detection of viable cells. Results were compared with controls and zidovudine (AZT) treated wells as a positive control and a determination about activity was made as a percentage protection of T4 cells against HIV-1 cytopathic effect (Table 2).

3.8. Antibacterial activity

The synthesized compounds were screened for their antibacterial activity against three bacterial strains, namely *E. coli* (MTCC 41), *S. aureus* (MTCC 1144) and *P. putida* (MTCC 1072). The non-pathogenic strain *P. putida* is ampicillin resistant and is closely related to the pathogenic strain *Pseudomonas aeruginosa*. The antimicrobial activity assay (MIC and the zone of inhibition) was performed for the compounds at 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0 and 128.0 mg/mL concentrations. The MIC assay is to test the sensitivity of microorganisms to an antimicrobial agent. A set of tubes with multiple concentrations of compounds was prepared in growth medium (LB broth). The tubes were then inoculated with the microorganisms, incubated for 12–16 h, and examined for growth of bacteria. Broth tubes that appear turbid are indicative of bacterial growth while tubes that remain clear indicate no growth. Growth seems to diminish as the concentration of some compounds increase, and eventually appeared to reduce at higher concentrations.

The zone of inhibition assay is to find the extent of sensitivity of microorganisms to the organic compound being tested. This antimicrobial activity assay was performed for the compounds at different concentrations. The bacterial isolate was inoculated uniformly on to the surface of an agar plate. A filter disk impregnated with a known amount of compound was applied to the surface of the plate and the compound was allowed to diffuse into the adjacent medium. A bacterial lawn appeared on the plate after incubation for 16 h. The antimicrobial activity of the compound was recorded as the size of zone inhibition. The size of the zone obtained at a particular concentration is directly proportional to the sensitivity of the organism to the compound and thus the zone of inhibition in the disk diffusion test is inversely related to the MIC.

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