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Synthesis and anti-HBV activity of 2-(methylthio)thieno[3,2-*d*]pyrimidin-4(1*H*)-one analogues of ACV

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ABSTRACT

*Amino thiophenecarboxylates **1a-d** were heated with thiourea to afford 2-thioxothieno[3,2-d]pyrimidin-4(1H)-ones **2a-d**. 2-(Methylthio)thieno[3,2-d]pyrimidin-4(1H)-ones **3a-d** were synthesized by alkylation of substituted 2-thioxo derivatives **2a-d** with methyl iodide. Compounds **3a-d** were treated with (2-acetoxyethoxy)methyl bromide to afford **4a-d** which were deacetylated to afford **5a-d**. The anti-HBV activity of selected compounds was studied.*

Keywords: Acyclic nucleosides, thienopyrimidines, acyclovir, anti-hepatitis activity.

INTRODUCTION

Nucleoside analogues play an important role in pharmacology, mainly as antitumoral or antiviral drugs [1-7]. The wide prevalence of Hepatitis B virus (HBV) infection and the lack of an ideal drug to treat the virus, has a great degree of prominence. Vaccination is not an effective therapy in chronic infections that ended by cirrhosis of the liver and/or hepatocellular carcinoma. In this respect alpha interferon has demonstrated some promise [8]. Thiated pyrimidinones, and their nucleosides, are of considerable biological importance [9]. Various analogues of thiopyrimidines possess effective antibacterial, antifungal, antiviral, insecticidal, and miticidal activities [10]. They are components of the tRNA of virous microorganism [11], yeasts [12], and mammalian cells [13] as a result of post-transcriptional modifications at the level of precursor tRNA, and splay a significant role in translation and its control [11, 14]. A comprehensive review covering the chemistry and antiviral activities of acyclonucleosides is available [15, 16], as reviews covering the biochemical properties of the potent antiviral agent acyclovir and nucleosides acting as inhibitors of HIV replication [17]. Acyclovir 9-(2-hydroxyethoxymethyl)guanine (ACV) (Zovirax) [18-21] (Fig. 1) has played a key role as a lead compound in this class of nucleosides. Due to the clinical efficancy of acyclovir, a number of purine and pyrimidine acyclic nucleosides were prepared.

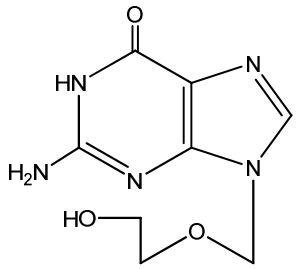


Fig. 1. Acyclovir (ACV)

MATERIALS AND METHODS

Synthetic methods, analytical and spectral data

The melting points were measured on a Büchi melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian NMR spectrometer at 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR with TMS as an internal standard. EIMS and FABMS spectra were recorded with a Finnigen MAT 312/AMD. Chemical shifts were reported in δ scale (ppm) relative to TMS as a standard, and the coupling constants (*J* values) are given in Hz. The progress of the reactions was monitored by TLC using aluminum silica gel plates 60 F_{245}. The microanalyses were performed at the microanalytical unit, Cairo University, Egypt, and were found to agree favorably with the calculated values. Antiviral activity against HBV was tested at the Liver Institute, Menoufia University, Shebin El-Koam Egypt.

Chemistry

7-Aryl-2-thioxothieno[3,2-d]pyrimidin-4(1H)-ones 2a-d

Thiophene derivatives **1a-d** (0.1 mole) and thiourea (30.4 g, 0.4 mole) in DMF (50 mL) were heated at 200 ^{o}C for 2 h. The residue was poured into water and the resulting precipitate was washed with acetic acid, water and diethyl ether respectively to give **2a-d**.

7-Phenyl-2-thioxothieno[3,2-d]pyrimidin-4(1H)-one (**2a**)

Yield 62%, mp 290-291 ^{o}C, R_f 0.27 (MeOH/$CHCl_3$, 9:1). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.50 (br s, 5H, phenyl protons), 8.10 (s, 1H, H-6), 11.18 (s, 1H, NH), 11.71 (s, 1H, NH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ 112.60 (C-4_a), 128.01, 128.30, 128.50, 132.00 (Ph), 131.68 (C-7), 133.00 (C-6), 143.90 (C-7_a), 160.89 (C-4), 176.02 (C-2) ppm. EI-MS: *m/z* 260 [M^+]. Anal. Calcd. For $C_{12}H_8N_2OS_2$; C, 55.36; H, 3.10; N, 10.76. Found: C, 55.22; H, 3.00; N 10.55.

7-(2-Methylphenyl)-2-thioxothieno[3,2-d]pyrimidin-4(1H)-one (**2b**)

Yield 58%, mp 277-278 ^{o}C, R_f 0.26 (MeOH/$CHCl_3$, 9:1). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.40 (s, 3H, CH_3), 7.50-7.59 (m, 4H, phenyl protons), 8.15 (s, 1H, H-6), 11.22 (s, 1H, NH), 11.88 (s, 1H, NH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ 20.20 (CH_3), 112.89 (C-4_a), 128.33, 128.39, 129.80, 135.99 (Ph), 131.77 (C-7), 133.32 (C-6), 144.14 (C-7_a), 160.98 (C-4), 176.44 (C-2) ppm. EI-MS: *m/z* 274 [M^+]. Anal. Calcd. For $C_{13}H_{10}N_2OS_2$; C, 56.91; H, 3.67; N, 10.21. Found: C, 56.80; H, 3.55; N 10.12.

7-(4-Methylphenyl)-2-thioxothieno[3,2-d]pyrimidin-4(1H)-one (**2c**)

Yield 61%, mp 280-281 ^{o}C, R_f 0.29 (MeOH/$CHCl_3$, 9:1). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.37 (s, 3H, CH_3), 7.25 (d, 2H, *J* = 7.8 Hz, phenyl protons), 7.55 (d, 2H, *J* = 7.8 Hz, phenyl protons), 8.19 (s, 1H, H-6), 11.77 (s, 1H, NH), 11.99 (s, 1H, NH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ 20.70 (CH_3), 113.11 (C-4_a), 128.44, 128.90, 129.66, 134.09 (Ph), 132.22 (C-7), 133.68 (C-6), 144.14 (C-7_a), 161.19 (C-4), 177.12 (C-2) ppm. EI-MS: *m/z* 274 [M^+]. Anal. Calcd. For $C_{13}H_{10}N_2OS_2$; C, 56.91; H, 3.67; N 10.21. Found: C, 56.73; H, 3.57; N 10.10.

7-(4-Methoxyphenyl)-2-thioxothieno[3,2-d]pyrimidin-4(1H)-one (**2d**)

Yield 60%, mp 300-302 ^{o}C, R_f 0.28 (MeOH/$CHCl_3$, 9:1). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.78 (s, 3H, OCH_3), 7.35 (d, 2H, *J* = 8.3 Hz, phenyl protons), 7.57 (d, 2H, *J* = 8.3 Hz, phenyl protons), 8.34 (s, 1H, H-6), 11.79 (s, 1H, NH), 11.98 (s, 1H, NH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ 55.00 (OCH_3), 113.33 (C-4_a), 128.67, 128.97,

129.34, 133.06 (Ph), 132.38 (C-7), 133.17 (C-6), 144.33 (C-7_a), 161.55 (C-4), 177.18 (C-2) ppm. EI-MS: *m/z* 290 [M^+]. Anal. Calcd. For $C_{13}H_{10}N_2O_2S_2$; C, 53.77; H, 3.47; N 9.65. Found: C, 53.60; H, 3.21; N 9.43.

7-Aryl-2-(Methylthio)thieno[3,2-d]pyrimidin-4(1H)-ones 3a-d

A solution of substituted 2-thiothieno[3,2-*d*]pyrimidin-4(1*H*)-ones **2a-d** (0.05 mole), methyl iodide (3.1 mL, 0.05 mole) and sodium hydroxide (2.0 g, 0.05 mole) in water (50 mL) and ethanol (100 mL) was stirred at 60°C for 2 h. A white solid began to precipitate by cooling. The solid was filtered off, washed with water, dried and recrystallized from ethanol to give **3a-d**.

2-(Methylthio)-7-phenylthieno[3,2-d]pyrimidin-4(1H)-one (**3a**)

Yield 87%, mp 180-181 °C, R_f 0.54 (MeOH/$CHCl_3$, 9:1). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.55 (s, 3H, SCH_3), 7.34-7.50 (m, 5H, phenyl protons), 8.50 (s, 1H, H-6), 11.34 (s, 1H, NH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ 13.60 (SCH_3), 112.06 (C-4_a), 128.21, 128.80, 128.90, 132.66 (Ph), 131.90 (C-7), 133.12 (C-6), 143.80 (C-7_a), 159.66 (C-2), 162.22 (C-4) ppm. EI-MS: *m/z* 274 [M^+]. Anal. Calcd. For $C_{13}H_{10}N_2OS_2$; C, 56.91; H, 3.67; N, 10.21. Found: C, 56.79; H, 3.49; N, 10.11.

7-(2-Methylphenyl)-2-(methylthio)thieno[3,2-d]pyrimidin-4(1H)-one (**3b**)

Yield 79%, mp 193-194 °C, R_f 0.52 (MeOH/$CHCl_3$, 9:1). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.38 (s, 3H, CH_3), 2.54 (s, 3H, SCH_3), 7.44-7.57 (m, 4H, phenyl protons), 8.15 (s, 1H, H-6), 11.22 (s, 1H, NH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ 13.55 (SCH_3), 20.28 (CH_3), 114.12 (C-4_a), 128.39, 128.44, 129.82, 135.80 (Ph), 132.55 (C-7), 133.45 (C-6), 144.33 (C-7_a), 159.44 (C-2), 162.34 (C-4) ppm. EI-MS: *m/z* 288 [M^+]. Anal. Calcd. For $C_{14}H_{12}N_2OS_2$; C, 58.31; H, 4.19; N, 9.71. Found: C, 58.20; H, 4.10; N, 9.53.

7-(4-Methylphenyl)-2-(methylthio)thieno[3,2-d]pyrimidin-4(1H)-one (**3c**)

Yield 87%, mp 211-212 °C, R_f 0.54 (MeOH/$CHCl_3$, 9:1). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.35 (s, 3H, CH_3), 2.53 (s, 3H, SCH_3), 7.33 (d, 2H, *J* = 7.8 Hz, phenyl protons), 7.56 (d, 2H, *J* = 7.8 Hz, phenyl protons), 8.33 (s, 1H, H-6), 11.87 (s, 1H, NH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ 13.54 (SCH_3), 20.72 (CH_3), 113.41 (C-4_a), 128.49, 129.11, 129.88, 134.12 (Ph), 132.44 (C-7), 133.77 (C-6), 144.45 (C-7_a), 159.12 (C-2), 161.99 (C-4) ppm. EI-MS: *m/z* 288 [M^+]. Anal. Calcd. For $C_{14}H_{12}N_2OS_2$; C, 58.31; H, 4.19; N, 9.71. Found: C, 58.17; H, 4.13; N, 9.54.

7-(4-Methoxyphenyl)-2-(methylthio)thieno[3,2-d]pyrimidin-4(1H)-one (**3d**)

Yield 85%, mp 230-232 °C, R_f 0.54 (MeOH/$CHCl_3$, 9:1). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.55 (s, 3H, SCH_3), 3.74 (s, 3H, OCH_3), 7.38 (d, 2H, *J* = 8.3 Hz, phenyl protons), 7.55 (d, 2H, *J* = 8.3 Hz, phenyl protons), 8.55 (s, 1H, H-6), 11.89 (s, 1H, NH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ 13.52 (SCH_3), 55.11 (OCH_3), 113.36 (C-4_a), 128.88, 128.99, 129.74, 133.45 (Ph), 132.50 (C-7), 133.50 (C-6), 144.60 (C-7_a), 159.18 (C-2), 161.95 (C-4) ppm. EI-MS: *m/z* 304 [M^+]. Anal. Calcd. For $C_{14}H_{12}N_2O_2S_2$; C, 55.24; H, 3.97; N, 9.20. Found: C, 55.13; H, 3.78; N, 9.06.

1-[(2-Acetoxyethoxy)methyl]-7-aryl-2-(methylthio)thieno[3,2-d]pyrimidin-4(1H)-ones 4a-d

To a stirred dispersion of 2-methylthio derivatives **3a-d** (5 mmol) in dry DMF (20 ml) was added NaH (0.12 g, 5 mmol) and after almost complete evolving of hydrogen, the mixture was heated to 80 °C for 1 h. Then, (2-acetoxyethoxy)methyl bromide [22] (1.0 g, 5 mmol) was added, the reaction mixture stirred for additional 2-3 h at 80-90 °C, cooled to room temperature and filtered. The mixture was evaporated to dryness at reduced pressure and purified on silica gel column chromatography using 5 % MeOH in $CHCl_3$ to give **4a-d** in 89-91% yields.

1-[(2-Acetoxyethoxy)methyl]-2-(methylthio)-7-phenylthieno[3,2-d]pyrimidin-4(1H)-one (**4a**)

Yield 91%, mp 177-178 °C, R_f 0.75 (MeOH/$CHCl_3$, 9:1). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.96 (s, 3H, $COCH_3$), 2.75 (s, 3H, SCH_3), 3.64 and 3.80 (2m, 4H, OCH_2CH_2O), 5.70 (s, 2H, NCH_2O), 7.45-7.53 (m, 5H, phenyl protons), 8.30 (s, 1H, H-6) ppm. FABMS (DMSO + 3-nitrobenzyl alcohol): *m/z* = 391 [M + H^+]. Anal. Calcd. For $C_{18}H_{18}N_2O_4S_2$; C, 55.37; H, 4.65; N, 7.17. Found: C, 55.20; H, 4.39; N, 7.00.

1-[(2-Acetoxyethoxy)methyl]-7-(2-methylphenyl)-2-(methylthio)thieno[3,2-d]pyrimidin-4(1H)-one (**4b**)

Yield 89%, mp 198-199 °C, R_f 0.72 (MeOH/$CHCl_3$, 9:1). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.97 (s, 3H, $COCH_3$), 2.37 (s, 3H, CH_3), 2.74 (s, 3H, SCH_3), 3.60 and 3.89 (2m, 4H, OCH_2CH_2O), 5.69 (s, 2H, NCH_2O), 7.42-7.55 (m, 4H, phenyl protons), 8.18 (s, 1H, H-6) ppm. FABMS (DMSO + 3-nitrobenzyl alcohol): *m/z* = 405 [M + H^+]. Anal. Calcd. For $C_{19}H_{20}N_2O_4S_2$; C, 56.42; H, 4.98; N, 6.93. Found: C, 56.33; H, 4.87; N, 6.69.

1-[(2-Acetoxyethoxy)methyl]-7-(4-methylphenyl)-2-(methylthio)thieno[3,2-d]pyrimidin-4(1H)-one (**4c**)
Yield 90%, mp 183-185 ^{o}C, R_f 0.74 (MeOH/$CHCl_3$, 9:1). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.96 (s, 3H, $COCH_3$), 2.37 (s, 3H, CH_3), 2.75 (s, 3H, SCH_3), 3.65 and 3.88 (2m, 4H, OCH_2CH_2O), 5.70 (s, 2H, NCH_2O), 7.35 (d, 2H, *J* = 7.8 Hz, phenyl protons), 7.50 (d, 2H, *J* = 7.8 Hz, phenyl protons), 8.39 (s, 1H, H-6) ppm. FABMS (DMSO + 3-nitrobenzyl alcohol): *m/z* = 405 [M + H^+]. Anal. Calcd. For $C_{19}H_{20}N_2O_4S_2$; C, 56.42; H, 4.98; N, 6.93. Found: C, 56.35; H, 4.89; N, 6.68.

1-[(2-Acetoxyethoxy)methyl]-7-(4-methoxyphenyl)-2-(methylthio)thieno[3,2-d]pyrimidin-4(1H)-one (**4d**)
Yield 90%, mp 165-167 ^{o}C, R_f 0.93 (MeOH/$CHCl_3$, 9:1). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.98 (s, 3H, $COCH_3$), 2.75 (s, 3H, SCH_3), 3.67 and 3.99 (2m, 4H, OCH_2CH_2O), 3.75 (s, 3H, OCH_3), 5.61 (s, 2H, NCH_2O), 7.41 (d, 2H, *J* = 8.3 Hz, phenyl protons), 7.55 (d, 2H, *J* = 8.3 Hz, phenyl protons), 8.50 (s, 1H, H-6) ppm. FABMS (DMSO + 3-nitrobenzyl alcohol): *m/z* = 421 [M + H^+]. Anal. Calcd. For $C_{19}H_{20}N_2O_5S_2$; C, 54.27; H, 4.79; N, 6.66. Found: C, 54.09; H, 4.67; N, 6.39.

7-Aryl-1-[(2-hydroxyethoxy)methyl]-2-(methylthio)thieno[3,2-d]pyrimidin-4(1H)-ones 5a-d Compounds **4a-d** (1 mmol) in a stirred mixture of methanol (10 ml) and ammonium hydroxide (25 %) (10 ml) were stirred at room temperature for 1h. The resulting solution was evaporated till dryness under reduced pressure. The residue was chromatographed on silica gel column using 10% MeOH in $CHCl_3$ to give **5a-d** in 92-95% yields.

1-[(2-Hydroxyethoxy)methyl]-2-(methylthio)-7-phenylthieno[3,2-d]pyrimidin-4(1H)-one (**5a**)
Yield 95%, mp 200-202 ^{o}C, R_f 0.77 (MeOH/$CHCl_3$, 9:1). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.74 (s, 3H, SCH_3), 3.44 and 3.50 (2m, 4H, OCH_2CH_2O), 4.50 (brs, 1H, OH, D_2O exchangeable), 5.70 (s, 2H, NCH_2O), 7.46-7.50 (m, 5H, phenyl protons), 8.22 (s, 1H, H-6) ppm. FABMS (DMSO + 3-nitrobenzyl alcohol): *m/z* = 349 [M + H^+]. Anal. Calcd. For $C_{16}H_{16}N_2O_3S_2$; C, 55.15; H, 4.63; N, 8.04. Found: C, 55.00; H, 4.44; N, 7.88.

1-[(2-Hydroxyethoxy)methyl]-7-(2-methylphenyl)-2-(methylthio)thieno[3,2-d]pyrimidin-4(1H)-one (**5b**)
Yield 92%, mp 215-217 ^{o}C, R_f 0.73 (MeOH/$CHCl_3$, 9:1). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.35 (s, 3H, CH_3), 2.74 (s, 3H, SCH_3), 3.40 and 3.49 (2m, 4H, OCH_2CH_2O), 4.49 (brs, 1H, OH, D_2O exchangeable), 5.69 (s, 2H, NCH_2O), 7.45-7.55 (m, 4H, phenyl protons), 8.19 (s, 1H, H-6) ppm. FABMS (DMSO + 3-nitrobenzyl alcohol): *m/z* = 363 [M + H^+]. Anal. Calcd. For $C_{17}H_{18}N_2O_3S_2$; C, 56.33; H, 5.01; N, 7.73. Found: C, 56.16; H, 4.94; N, 7.68.

1-[(2-Hydroxyethoxy)methyl]-7-(4-methylphenyl)-2-(methylthio)thieno[3,2-d]pyrimidin-4(1H)-one (**5c**)
Yield 93%, mp 220-221 ^{o}C, R_f 0.73 (MeOH/$CHCl_3$, 9:1). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.36 (s, 3H, CH_3), 2.75 (s, 3H, SCH_3), 3.45 and 3.48 (2m, 4H, OCH_2CH_2O), 4.48 (brs, 1H, OH, D_2O exchangeable), 5.71 (s, 2H, NCH_2O), 7.37 (d, 2H, *J* = 7.8 Hz, phenyl protons), 7.53 (d, 2H, *J* = 7.8 Hz, phenyl protons), 8.32 (s, 1H, H-6) ppm. FABMS (DMSO + 3-nitrobenzyl alcohol): *m/z* = 363 [M + H^+]. Anal. Calcd. For $C_{17}H_{18}N_2O_3S_2$; C, 56.33; H, 5.01; N, 7.73. Found: C, 56.22; H, 4.95; N, 7.67.

1-[(2-Hydroxyethoxy)methyl]-7-(4-methoxyphenyl)-2-(methylthio)thieno[3,2-d]pyrimidin-4(1H)-one (**5d**)
Yield 94%, mp 228-229 ^{o}C, R_f 0.73 (MeOH/$CHCl_3$, 9:1). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.76 (s, 3H, SCH_3), 3.47 and 3.49 (2m, 4H, OCH_2CH_2O), 3.77 (s, 3H, OCH_3), 4.51 (brs, 1H, OH, D_2O exchangeable), 5.60 (s, 2H, NCH_2O), 7.46 (d, 2H, *J* = 8.3 Hz, phenyl protons), 7.56 (d, 2H, *J* = 8.3 Hz, phenyl protons), 8.43 (s, 1H, H-6) ppm. FABMS (DMSO + 3-nitrobenzyl alcohol): *m/z* = 379 [M + H^+]. Anal. Calcd. For $C_{17}H_{18}N_2O_4S_2$; C, 53.95; H, 4.79; N, 7.40. Found: C, 53.82; H, 4.62; N 7.28.

Preparation and culture of Hep G2 2.2.15 cells
The required cell line was made by transfection of Hep G2-cells with a plasmid containing multiple tandem copies of HBV genome (subtype ayw) [23]. The 2.2.15 cell line was maintained in RPMI-1640 (Glutamax) culture media containing 100 IU/ml nystatin and 380 µg/ml G418 (geneticin). The transferred HEP G2-2.2.15 cell line was kept in tissue culture flask at 37^{o}C + 5% CO_2. Subcultures were set up after a week by aspiration of the media from culture flask and washing the cells twice by PBS. A 10% versene/trypsin was added and the cells were incubated for 1 min. at 37^{o}C. The drug Lamivudine which is a potent selective inhibitor of HBV replication [24] has been used as a standard for the comparative studies.

DNA Extraction

HBV-DNA extraction was done by mixing 10 µl of diluted supernatant (1:5 with PBS) in reaction tube with 10 µl of 0.2 *M* NaOH and incubated at 37°C for one hour. Carefully, 9.6 µl of 0.2 *M* HCl was added followed by 90 µl of TE buffer solution.

PCR-Ellisa

The PCR reaction mixture contained 14 µl extracted supernatant, 4 mmol/l $MgCl_2$, 10 µmol/l DIG-11-dUTP, 190 µmol/l dTTP, 200 µmol/l dATP, dGTP, dCTP, 1.5 U Taq polymerase, 20 mmol/l HCl (pH 8.4), 50 mmol/l KCl, 1 µmol/l HCID-1 primer (5′GGA AAG AAG TCA GAA GGC A3′) and 1 µmol/l HCID-2 (5′TTG GGG GAG GAG ATT AGG TT3′), in total volume 50 µl. PCR reaction conditions were 32 cycles of 1 min. at 94°C, 30 sec. at 58°C and 30 sec. at 72°C + 3 sec. for each cycle in a thermal circler as described in literature [25].

Cytotoxicity Assay

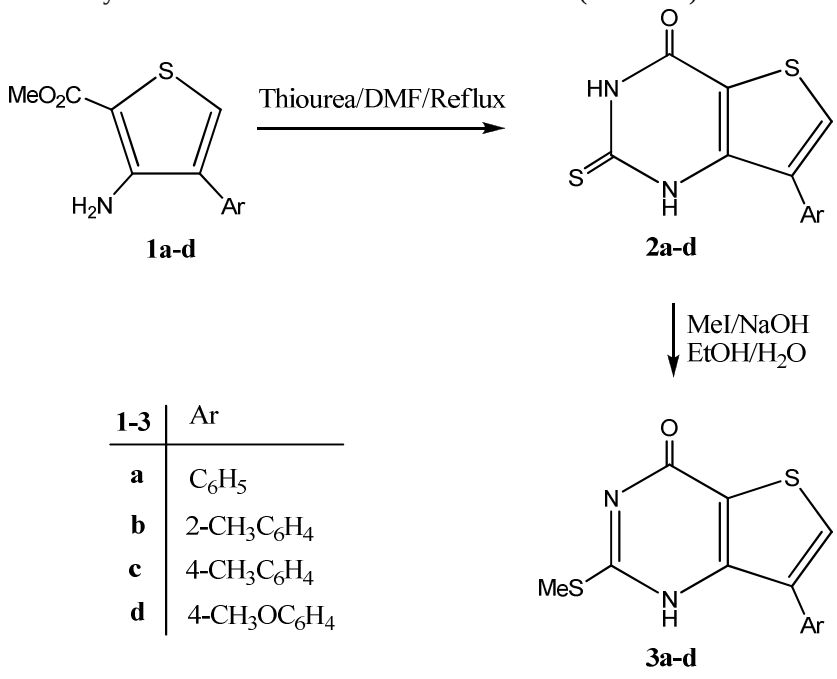
A colorimetric assay for living cells utilized the colorless substrate 3-(3,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) that is modified to colored product by any living cells, but not by dead cells or tissue culture medium. The cytotoxic effect of the compounds was accessed by culturing the Hep G2-2.2.15 cells in the presence of compounds using a MTT-assay [26].

Calculation of IC_{50}, CC_{50} and SI

The 50% inhibitory concentration of antiviral drugs (IC_{50}) was determined by interpolation from the plots of amount of DNA copies versus antiviral drug concentration. The 50% cytotoxic effect (CC_{50}) was calculated from the average viability of the cells with concentration of drugs. The selective index (SI) could be calculated as CC_{50}/IC_{50} [26].

RESULTS AND DISCUSSION

Methyl 3-amino-4-substituted-2-thiophenecarboxylates **1a-d** [27] were heated with thiourea in DMF and the resulting products precipitate at room temperature yielding **2a-d** in 58-62%. 2-(Methylthio)thieno[3,2-d]pyrimidin-4(1*H*)-ones **3a-d** were synthesized, in 79-87% yields, by treatment of substituted 2-thioxo derivatives **2a-d** with methyl iodide and sodium hydroxide in water and ethanol at 60 °C for 2h (Scheme 1).



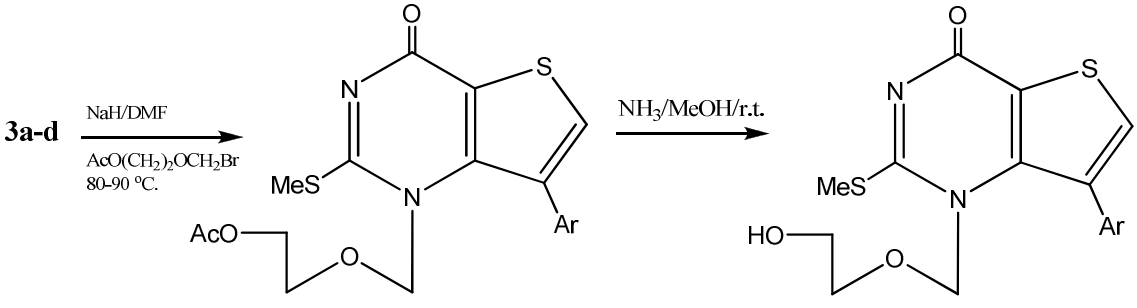
1-3	Ar
a	C_6H_5
b	2-$CH_3C_6H_4$
c	4-$CH_3C_6H_4$
d	4-$CH_3OC_6H_4$

Scheme 1. Synthesis of Thienopyrimidines **2** and **3**.

The structure of compounds **2a-d** and **3a-d** were confirmed by ^{1}H-NMR, ^{13}C-NMR, and mass spectra which agreed with the assigned structures.

2-Methylthio derivatives **3a-d** were alkylated with (2-acetoxyethoxy)methyl bromide [22] by the method of Sasaki *et al*. [28] to give the corresponding acyclic nucleosides **4a-d** in 89-91% yields after purification on silica gel column using 5% MeOH in $CHCl_3$. Deprotection of the acyclic nucleosides **4a-d** using a mixture of ammonium hydroxide (25%) and methanol at room temperature afforded **5a-d**, in 92-95% yields, after chromatographic purification using 10% MeOH in $CHCl_3$ (Scheme 2).

The structure of compounds **4a-d** and **5a-d** were confirmed by ^{1}H-NMR and mass spectra which agreed with the assigned structures.



3-5	Ar
a	C_6H_5
b	2-$CH_3C_6H_4$
c	4-$CH_3C_6H_4$
d	4-$CH_3OC_6H_4$

Scheme 2. Synthesis of ACV analogues **4** and **5**.

Preliminary viral screening against HBV of selected compounds indicated that compound **8c** was found to be active against HBV replication with IC_{50} = 0.2 µM, CC_{50} = 100 and selective index 500. Compounds **5a,b** and **5d** showed moderate viral replication inhibition and mild cytotoxicity with selective indexes 166.6 ~ 500.0. On the other hand, the compounds **2a-d** and **3a-d** showed low inhibition and high cytotoxicity with selective indexes 20.0 ~ 76.9 (Table 1).

Table 1. Inhibitory concentration (IC_{50}) and Selective index (SI) of compounds 2, 3, and 5.

Compd No.	HBV DNA IC_{50} (µM)	SI	Compd No.	HBV DNA IC_{50} (µM)	SI
Lamivudine	0.1	1000.0	**Lamivudine**	0.1	1000.0
2a	1.6	62.5	**3c**	1.6	62.5
2b	1.3	76.9	**3d**	5.0	20.0
2c	1.7	58.8	**5a**	0.7	142.8
2d	3.0	33.3	**5b**	0.6	166.6
3a	1.5	66.6	**5c**	0.2	500.0
3b	1.3	76.9	**5d**	0.6	166.6

- *Hep G2 2.2.15 CC_{50} (µM) = 100 for all tested compounds.*

CONCLUSION

New 2-(methylthio)thieno[3,2-*d*]pyrimidin-4(1*H*)-one analogues of ACV were synthesized in order to increase the number of tested compounds screened for antiviral activity. Some of them displayed promising activities.

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