Chemistry of Heterocyclic Compounds, Vol. 47, No. 10, January, 2012 (Russian Original Vol. 47, No. 10, October, 2011)

SYNTHESIS AND ANTI-HEPATITIS B VIRUS ACTIVITY OF NEW PYRIMIDINE PEPTIDE NUCLEIC ACID ANALOGS

R. A. K. Al-Harbi[1] and A. A.-H. Abdel-Rahman[2]*

A series of 4-methylsulfanylpyrimidin-2(1H)-one peptide nucleic acid analogs were synthesized and tested for their antiviral activity against hepatitis B virus. Plaque reduction infectivity assay was used to determine the virus count reduction as a result of treatment with tested compounds.

Keywords: peptide nucleic acid analogs, pyrimidine nucleobases, anti-hepatitis B virus activity.

Synthetic compounds that specifically recognize and bind to a specific DNA or RNA sequence of interest are potentially useful as antisense and antigene drugs or molecular probes, which have numerous applications in the field of molecular and experimental medicine [1-3]. Particularly successful DNA binding agents are found in a recently developed class of DNA analogs, the peptide nucleic acids (PNA) [4-9]. PNA are oligonucleotide analogs, in which the phosphodiester pentose backbone of DNA or RNA is replaced by a polyamide or peptide backbone [10]. For example, the phosphoribose backbone of natural oligonucleotide has been replaced by *N*-(2-aminoethyl)glycine whereby the purine/pirimidine base pair is attached to the glycine nitrogen through a methylene carbonyl linker [11]. The complete replacement of the ribose phosphate backbone with an artificial pseudopeptide backbone results in a remarkably improved binding to complementary nucleic acid sequences occurring with both high affinity and selectivity [12-19]. The hybridization properties of PNA have attracted widespread interest in this class of compounds [20-22]. Several reviews have covered the literature concerning new chemically modified PNA [23]. Their biological and chemical stability and their superior hybridization properties relative to natural oligonucleotides make them attracttive as potential therapeutic and biomolecular tools. Owing to the described significance of PNA and in connection with our work on the synthesis of new α-amino acid derivatives [24–28] and investigating their antiviral potential [29], we report here the synthesis and anti-hepatitis B virus (HBV) activity of new PNA with 4-methylsulfanyl-pyrimidin-2(1*H*)-one as the heterocyclic nucleobase.

The coupling reaction at one of the nitrogen atoms of the heterocyclic base is the most effective method for introducing certain substituents with desired functionalities attached to the heterocycle. Thus, reaction of 4-(methylsulfanyl)uracils **1a-c** [30] with ethyl chloroacetate in the presence of potassium carbonate afforded the corresponding nucleobase-substituted acetates **2a-c** in 71-75% yields. The ^{1}H NMR spectra of compounds **2a-c** showed the characteristic signals of the ester ethyl group and a singlet peak for the remaining CH_2 group at

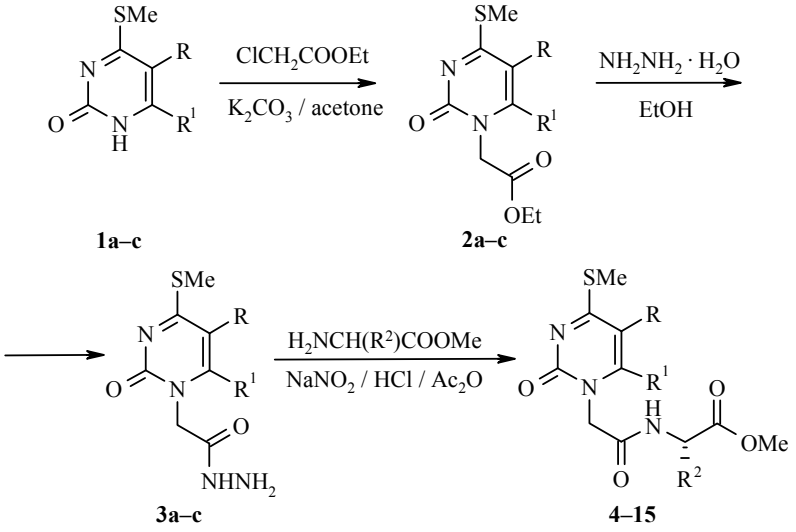
*To whom correspondence should be addressed, e-mail: adelnassar63@yahoo.com.

[1]Department of Applied Chemistry, Faculty of Applied Science, Taibah University, Madinah P.O. 3193, Saudi Arabia; Remal94@hotmail.com.
[2]Department of Chemistry, Faculty of Science, Northern Border University, P.O. 1321 Arar, Saudi Arabia.

Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 10, pp. 1560-1567, October, 2011. Original article submitted March 3, 2011.

 0009-3122/12/4710-1290

4.96−4.99 ppm. Treatment of compounds **2a–c** with hydrazine hydrate gave the corresponding acid hydrazides **3a–c** in quantitative yields. The structures were proved by means of IR, ^{1}H NMR, and mass spectra, which all agreed with the assigned structure. These hydrazides were selected as starting materials for the coupling reaction with the appropriate acylated amino acids *via* the azide coupling method [31]. Thus, treatment of compounds **3a–c** at -5 °C in AcOH and 1 N HC1 with $NaNO_2$ afforded the corresponding azide derivatives as an inseparable mixture. The yellow syrupy azide compound was then treated, *in situ*, with the corresponding amino acid methyl esters in ethyl acetate containing Et_3N at 0°C to give, after neutralization, the desired peptides **4-15** in 80–90% yields. For this study, the amino acids were selected according to the amino acid side chain: a) glycine (without side chain, peptides **4**, **8**, **12**); b) L-valine (branched side chain, peptides **5**, **9**, **13**); c) L-leucine (long side chain, peptides **6**, **10**, **14**); d) L-phenylglycine (aromatic side chain, peptides **7**, **11**, **15**). The structures of **4**−**15** were assigned from their ^{1}H NMR and mass spectra.



1–3a, 4–7 R = R^1 = H; **1–3b, 8–11** R = H, R^1 = Me; **1–3c, 12–15** R = Me, R^1 = H; **4, 8, 12** R^2 = H; **5, 9, 13** R^2 = $CHMe_2$; **6, 10, 14** R^2 = CH_2CHMe_2; **7, 11, 15** R^2 = Ph

TABLE 1. Antiviral Activity and Cytotoxicity of Compounds **4–15**

Compound	HBV DNA in supernatant, μM	Hep G2 viable cells *	HBV DNA IC_{50}, μM	SI
Lamivudine	0.25	1.00	0.1	1000.0
4	0.28	0.35	0.7	142.8
5	0.17	0.56	0.6	166.6
6	0.13	0.19	0.5	200.0
7	0.28	0.35	0.7	142.8
8	0.19	0.33	0.6	166.6
9	0.19	0.33	0.6	166.6
10	0.13	0.19	0.5	200.0
11	0.83	0.90	0.2	500.0
12	0.19	0.33	0.6	166.6
13	0.21	0.63	0.3	333.3
14	0.29	0.44	0.2	500.0
15	0.19	0.33	0.6	166.6

* At 0.1 μM concentration of the test compounds.

The synthesized compounds were tested for their antiviral activity and cytotoxicity against HBV using the HepG2.2.2.15 cell line [32], a human hepatoma cell line producing HBV viral particles [31, 32]. The drug lamivudine (4-amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one), which is a potent selective inhibitor of HBV replication [33], has been used as a standard positive control. The 50% inhibitory concentration (IC_{50}) of the antiviral drug was determined by plotting the DNA content in the cell culture supernatant versus the concentration of the test compound. The 50% cytotoxic effect (CC_{50}) was calculated from the average viability of the cells in proportion to the concentration of the drug; for all the tested compounds its value was 100 μM. The selectivity index (SI) was calculated as CC_{50}/IC_{50} [34].

The results of the antiviral activity measurements against HBV are shown in Table 1. L-valine derivative **5**, L-phenylglycine derivative **11**, and L-valine derivative **13** showed the highest activity against HBV and mild cytotoxicity with effective concentration of 0.2 μM and selectivity index 166.6−500.0. L-Leucine derivative **14** showed the highest antiviral activity against HBV and high cytotoxicity.

EXPERIMENTAL

IR spectra were recorded with a Bruker Vector 22 instrument in KBr. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Gemini spectrometer at 300 and 75 MHz, respectively, in DMSO-d_6 with TMS as internal standard. EI MS spectra were recorded with a Hewlett-Packard D5988 A instrument. Elemental analyses were carried out at the Microanalytical Center of Cairo University, Giza, Egypt. Melting points were determined using a Buchi apparatus. Antiviral activity against HBV was tested at the Liver Institute, Menoufia University, Egypt. The progress of the reactions was monitored by TLC using aluminum silica gel plates.

Ethyl [4-(Methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetates 2a–c (General Method). A mixture of an corresponding 4-methylsulfanylpyrimidin-2(1*H*)-one **1a–c** [30] (0.1 mol), ethyl chloroacetate (14.7 g, 0.12 mol), and anhydrous K_2CO_3 (13.8 g, 0.1 mol) in dry acetone (50 ml) was refluxed for 4-5 h (TLC). The solvent was removed *in vacuo*, and the residue was diluted with water and extracted with dichloromethane (3×50 ml). The combined organic layers were dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by silica gel column chromatography (dichloromethane–methanol, 98:2) to afford compounds **2a-c**.

Ethyl [4-(Methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetate (2a). White needles, yield 75%; mp 89−91°C. R_f = 0.66 (3% MeOH in CH_2Cl_2). IR spectrum, ν, cm^{-1}: 1755 (CO_2Et). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.18 (3H, t, *J* = 8.0, C<u>H</u>$_3$CH$_2$); 2.46 (3H, s, SCH$_3$); 4.15 (2H, *J* = 8.0 Hz, CH$_3$C<u>H</u>$_2$); 4.96 (2H, s, CH$_2$); 6.78 (1H, d, *J* = 5.5, H-5); 8.40 (1H, d, *J* = 5.5, H-6). ^{13}C NMR spectrum, δ, ppm: 12.5 (SCH$_3$); 13.9 (<u>C</u>H$_3$CH$_2$O); 50.4 (NCH$_2$); 61.5 (CH$_3$<u>C</u>H$_2$O); 103.5 (C-5); 138.3 (C-6); 153.9 (C-2); 167.9 (C=O); 178.0 (C-4). Mass spectrum, *m/z* (I_{rel}, %): 228 [M]$^+$ (40), 189 (17), 155 (66), 141 (100), 126 (39). Found, %: C 47.22; H 5.12; N 12.17. $C_9H_{12}N_2O_3S$. Calculated, %: C 47.35; H 5.30; N 12.27.

Ethyl [6-Methyl-4-(methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetate (2b). White needles, yield 71%; mp 99−101ºC. R_f = 0.74 (3% MeOH in CH_2Cl_2). IR spectrum, ν, cm^{-1}: 1750 (CO_2Et). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.19 (3H, t, *J* = 8.0, C<u>H</u>$_3$CH$_2$); 2.35 (3H, s, 6-CH$_3$); 2.43 (3H, s, SCH$_3$); 4.16 (2H, q, *J* = 8.0, CH$_3$C<u>H</u>$_2$); 4.97 (2H, s, CH$_2$); 6.61 (1H, s, H-5). ^{13}C NMR spectrum, δ, ppm: 13.4 (SCH$_3$); 13.8 (<u>C</u>H$_3$CH$_2$O); 23.4 (CH$_3$); 51.2 (NCH$_2$); 61.8 (CH$_3$<u>C</u>H$_2$O); 108.6 (C-5); 154.5 (C-2); 167.2 (C-6); 167.8 (C=O); 178.2 (C-4). Mass spectrum, *m/z* (I_{rel}, %): 242 [M]$^+$ (32), 227 (19), 197 (66), 169 (55), 155 (100). Found, %: C 49.44; H 5.70; N 11.45. $C_{10}H_{14}N_2O_3S$. Calculated, %: C 49.57; H 5.82; N 11.56.

Ethyl [5-Methyl-4-(methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetate (2c). White needles, yield 74%; mp 105−107ºC. R_f = 0.73 (3% MeOH in CH_2Cl_2). IR spectrum, ν, cm^{-1}: 1735 (CO_2Et). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.19 (3H, t, *J* = 8.0, C<u>H</u>$_3$CH$_2$); 2.09 (3H, s, 5-CH$_3$); 2.44 (3H, s, SCH$_3$); 4.11 (2H, q, *J* = 8.0, CH$_3$C<u>H</u>$_2$); 4.99 (2H, s, CH$_2$); 8.26 (1H, s, H-6). ^{13}C NMR spectrum, δ, ppm: 12.9 (CH$_3$); 13.6 (<u>C</u>H$_3$CH$_2$O); 14.3 (SCH$_3$); 51.6 (NCH$_2$); 61.4 (CH$_3$<u>C</u>H$_2$O); 111.8 (C-5); 136.5 (C-6); 153.9 (C-2); 167.9 (C=O); 178.9 (C-4). Mass spectrum, *m/z* (I_{rel}, %): 242 [M]$^+$ (18), 213 (33), 169 (24), 155 (38), 140 (100), 108 (29). Found, %: C 49.40; H 5.68; N 11.42. $C_{10}H_{14}N_2O_3S$. Calculated, %: C 49.57; H 5.82; N 11.56.

2-[4-(Methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetohydrazides 3a–c (General Method). A mixture of the corresponding ester **2a–c** (10 mmol) and 95% hydrazine hydrate (1.25 g, 25 mmol) in absolute ethanol (30 ml) was heated under reflux for 2 h. The excess of ethanol was removed under reduced pressure, and the resulting precipitate was filtered off, washed with ethanol, and recrystallized from ethanol to give compounds **3a–c**.

2-[4-(Methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetohydrazide (3a). White needles, yield 99%; mp 190−192ºC. R_f = 0.26 (3% MeOH in CH_2Cl_2). IR spectrum, ν, cm^{-1}: 3294 (NH_2), 3218 (NH), 1675 (CONH). ^1H NMR spectrum, δ, ppm (*J*, Hz): 2.45 (3H, s, SCH_3); 4.33 (2H, br. s, NH_2); 4.78 (2H, s, CH_2); 6.68 (1H, d, *J* = 6.0, H-5); 8.36 (1H, d, *J* = 6.0, H-6); 9.35 (1H, br. s, NH). Mass spectrum, *m/z* (I_{rel}, %): 214 $[M]^+$ (54), 198 (27), 155 (55), 141 (41), 126 (100). Found, %: C 39.08; H 4.56; N 26.00. $C_7H_{10}N_4O_2S$. Calculated, %: C 39.24; H 4.70; N 26.15.

2-[6-Methyl-4-(methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetohydrazide (3b). White needles, yield 98%; mp 170−172ºC. R_f = 0.16 (3% MeOH in CH_2Cl_2). IR spectrum, ν, cm^{-1}: 3300 (NH_2), 3200 (NH), 1670 (CONH). ^1H NMR spectrum, δ, ppm: 2.35 (3H, s, 6-CH_3); 2.46 (3H, s, SCH_3); 4.29 (2H, s, NH_2); 4.78 (2H, s, CH_2); 6.57 (1H, s, H-5); 9.35 (1H, br. s, NH). Mass spectrum, *m/z* (I_{rel}, %): 228 $[M^+]$ (46), 211 (18), 169 (37), 155 (100). Found, %: C 41.93; H 5.17; N 24.33. $C_8H_{12}N_4O_2S$. Calculated, %: C 42.09; H 5.30; N 24.54.

2-[5-Methyl-4-(methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetohydrazide (3c). White needles, yield 99%; mp 165−167ºC. R_f = 0.18 (3% MeOH in CH_2Cl_2). IR spectrum, ν, cm^{-1}: 3320 (NH), 3200 (NH_2), 1670 (CONH). ^1H NMR spectrum, δ, ppm: 2.08 (3H, s, 5-CH_3); 2.45 (3H, s, SCH_3); 4.28 (2H, s, NH_2); 4.82 (2H, s, CH_2); 8.23 (1H, s, H-6); 9.29 (1H, br. s, NH). Mass spectrum, *m/z* (I_{rel}, %): 228 $[M]^+$ (19), 212 (46), 197 (11), 169 (22), 155 (67), 140 (100). Found, %: C 41.88; H 5.11; N 24.19. $C_8H_{12}N_4O_2S$. Calculated, %: C 42.09; H 5.30; N 24.54.

PNA Methyl Esters 4–15 (General Method). A solution of an corresponding hydrazide **3a–c** (4 mmol) in acetic acid (30 ml), 1 N HCl (15 ml), and water (125 ml) was cooled in an ice-bath (-5ºC). Sodium nitrite (4.35 g, 63 mmol) in cold water (15 ml) was added with stirring. After stirring at -5ºC for 15 min, a yellow syrup was formed, which was taken up in cold ethyl acetate (150 ml), washed with $NaHCO_3$ (3%, 150 ml) and water (150 ml), and dried over Na_2SO_4. A solution of the corresponding amino acid methyl ester hydrochloride (4.5 mmol) in ethyl acetate (100 ml) containing triethylamine (1.0 ml) was stirred at 0ºC for 20 min and filtered, and the filtrate was added to the hydrazide solution. The mixture was kept at -5ºC for 12 h, then at room temperature for another 12 h, followed by washing with 0.5 N HC1 (150 ml), 3% $NaHCO_3$ (150 ml), and water (150 ml), and drying over Na_2SO_4. The filtrate was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (petroleum ether–ethylacetate, 7:1) to afford the corresponding product **4–15**.

***N*-{[4-(Methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetyl}glycine Methyl Ester (4)**. White crystals, yield 85%; mp 173−175ºC. R_f = 0.57 (5% MeOH in CH_2Cl_2). ^1H NMR spectrum, δ, ppm (*J*, Hz): 2.45 (3H, s, SCH_3); 3.60 (3H, s, OCH_3); 4.85 (4H, s, $2CH_2$); 6.75 (1H, d, *J* = 5.5, H-5); 8.31 (1H, d, *J* = 5.5, H-6); 8.34 (1H, s, NH). ^{13}C NMR spectrum, δ, ppm: 12.6 (SCH_3); 51.2 (NCH_2); 52.8 (CH_2); 54.1 (OCH_3); 103.7 (C-5); 139.0 (C-6); 154.1 (C-2); 167.8 and 172.9 (2C=O); 178.2 (C-4). Mass spectrum, *m/z* (I_{rel}, %): 271 $[M]^+$ (21), 240 (44), 214 (39), 183 (49), 141 (100). Found, %: C 44.13; H 4.66; N 15.33. $C_{10}H_{13}N_3O_4S$. Calculated, %: C 44.27; H 4.83; N 15.49.

***N*-{[4-(Methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetyl}-L-valine Methyl Ester (5)**. White crystals, yield 83%; mp 119−121 ºC. R_f = 0.56 (5% MeOH in CH_2Cl_2). ^1H NMR spectrum, δ, ppm (*J*, Hz): 0.87 (6H, d, *J* = 7.2, $(C\underline{H}_3)_2CH$); 2.02 (1H, m, $C\underline{H}(CH_3)_2$); 2.43 (3H, s, SCH_3); 3.63 (3H, s, OCH_3); 4.21 (1H, t, *J* = 6.6, $NHC\underline{H}$); 4.88 (2H, d, *J* = 3.6, $C\underline{H}_2N$); 6.67 (1H, d, *J* = 5.5, H-5); 8.34 (1H, d, *J* = 5.5, H-6); 8.41 (1H d, *J* = 5.9, NH). ^{13}C NMR spectrum, δ, ppm: 12.4 (SCH_3); 16.5 and 18.6 ($2CH_3$); 31.6 (CH); 51.0 (CH); 52.8 (NCH_2); 54.5 (OCH_3); 103.8 (C-5); 138.9 (C-6); 153.8 (C-2); 167.4 and 175.1 (2C=O); 177.8 (C-4). Mass spectrum, *m/z* (I_{rel}, %): 313 $[M]^+$ (18), 283 (19), 252 (63), 211 (54), 141 (100), 126 (42). Found, %: C 49.77; H 6.00; N 13.32. $C_{13}H_{19}N_3O_4S$. Calculated, %: C 49.83; H 6.11; N 13.41.

***N*-{[4-(Methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetyl}-L-leucine Methyl Ester (6)**. Pale-yellow oil, yield 86%. R_f = 0.59 (5% MeOH in CH_2Cl_2). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.84 (6H, d, *J* = 5.4, (C<u>H</u>$_3$)$_2$CH); 1.45 (1H, m, C<u>H</u>(CH$_3$)$_2$); 1.57 (2H, m, CHC<u>H</u>$_2$CH); 2.45 (3H, s, SCH$_3$); 3.62 (3H, s, OCH$_3$); 4.39 (1H, m, NHC<u>H</u>); 4.85 (2H, s, C<u>H</u>$_2$N); 6.65 (1H, d, *J* = 5.5, H-5); 8.32 (1H, d, *J* = 5.5, H-6); 8.43 (1H, s, NH). ^{13}C NMR spectrum, δ, ppm: 12.8 (SCH$_3$); 21.6 (2CH$_3$); 25.8 (CH); 39.6 (CH$_2$); 51.6 (CH); 53.7 (NCH$_2$); 54.3 (OCH$_3$); 104.3 (C-5); 139.7 (C-6); 154.4 (C-2); 167.5 and 172.5 (2C=O); 177.7 (C-4). Mass spectrum, *m/z* (I_{rel}, %): 327 [M]$^+$ (11), 284 (19), 270 (37), 239 (39), 141 (100). Found, %: C 51.22; H 6.33; N 12.67. $C_{14}H_{21}N_3O_4S$. Calculated, %: C 51.36; H 6.47; N 12.83.

***N*-{[4-(Methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetyl}-L-phenylglycine Methyl Ester (7)**. White foam, yield 88%. R_f = 0.5 (5% MeOH in CH_2Cl_2). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.34 (3H, s, SCH$_3$); 3.63 (3H, s, OCH$_3$); 4.81 (2H, d, *J* = 5.7, C<u>H</u>$_2$N); 5.57 (1H, d, *J* = 7.2, CHPh); 6.44 (1H, d, *J* = 5.5, H-5); 7.30 (5H, m, H Ar); 7.33 (1H, d, *J* = 7.2, NH); 8.19 (1H, d, *J* = 5.5, H-6). ^{13}C NMR spectrum, δ, ppm: 12.9 (SCH$_3$); 52.4 (NCH$_2$); 53.9 (OCH$_3$); 56.5 (CH); 104.6 (C-5); 125.5, 127.9, 134.6, and 137.9 (Ph); 139.0 (C-6); 154.8 (C-2); 167.4 and 172.4 (2C=O); 179.2 (C-4). Mass spectrum, *m/z* (I_{rel}, %): 347 [M]$^+$ (33), 316 (17), 288 (34), 211 (22), 183 (27), 141 (100). Found, %: C 55.17; H 4.88; N 12.01. $C_{16}H_{17}N_3O_4S$. Calculated, %: C 55.32; H 4.93; N 12.10.

***N*-{[6-Methyl-4-(methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetyl}glycine Methyl Ester (8)**. Pale-yellow oil, yield 82%. R_f = 0.7 (5% MeOH in CH_2Cl_2). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.32 (3H, s, 6-CH$_3$); 2.44 (3H, s, SCH$_3$); 3.69 (3H, s, OCH$_3$); 4.03 (2H, d, *J* = 5.1, NHC<u>H</u>$_2$); 4.84 (2H, s, C<u>H</u>$_2$CO); 6.29 (1H, s, H-5); 6.82 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 13.5 (SCH$_3$); 23.5 (CH$_3$); 51.5 (NCH$_2$); 52.9 (CH$_2$); 53.9 (OCH$_3$); 109.1 (C-5); 154.9 (C-6); 167.0 (C-6); 167.6 and 173.2 (2C=O); 179.3 (C4). Mass spectrum, *m/z* (I_{rel}, %): 285 [M]$^+$ (32), 270 (22), 226 (44), 197 (27), 169 (43), 155 (100), 140 (23). Found, %: C 46.22; H 5.09; N 14.66. $C_{11}H_{15}N_3O_4S$. Calculated, %: C 46.31; H 5.30; N 14.73.

***N*-{[6-Methyl-4-(methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetyl}-L-valine Methyl Ester (9)**. Yellow oil, yield 82%. R_f = 0.48 (5% MeOH in CH_2Cl_2). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.80 (3H, d, *J* = 6.9) and 0.85 (3H, d, *J* = 6.9, (C<u>H</u>$_3$)$_2$CH); 2.07 (1H, m, C<u>H</u>(CH$_3$)$_2$); 2.33 (3H, s, 6-CH$_3$); 2.43 (3H, s, SCH$_3$); 3.66 (3H, s, OCH$_3$); 4.53 (1H, dd, *J* = 5.1, *J* = 1.5, NHC<u>H</u>); 4.75 (1H, d, *J* = 15.3) and 4.88 (1H, d, *J* = 15.3, CH$_2$N); 6.32 (1H, s, H-5); 6.63 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 13.7 (SCH$_3$); 16.8 and 18.9 (2CH$_3$); 23.9 (CH$_3$); 32.3 (CH); 51.3 (CH); 52.9 (NCH$_2$); 54.9 (OCH$_3$); 109.3 (C5); 155.5 (C-2); 167.1 (C-6); 167.9 and 175.8 (2C=O); 179.5 (C-4). Mass spectrum, *m/z* (I_{rel}, %): 327 [M]$^+$ (29), 284 (45), 253 (15), 212 (40), 155 (100), 140 (16). Found, %: C 51.23; H 6.33; N 12.62. $C_{14}H_{21}N_3O_4S$. Calculated, %: C 51.36; H 6.47; N 12.83.

***N*-{[6-Methyl-4-(methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetyl}-L-leucine Methyl Ester (10)**. Pale-yellow oil, yield 80%. R_f = 0.6 (5% MeOH in CH_2Cl_2). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.82 (3H, d, *J* = 6.0) and 0.87 (3H, d, *J* = 6.0, (C<u>H</u>$_3$)$_2$CH); 1.52–1.59 (3H, m, C<u>H</u>(CH$_3$)$_2$, CHC<u>H</u>$_2$CH); 2.31 (3H, s, 6-CH$_3$); 2.44 (3H, s, SCH$_3$); 3.35 (3H, s, OCH$_3$); 4.30 (1H, m, NHC<u>H</u>); 4.82 (2H, s, C<u>H</u>$_2$N); 6.52 (1H, s, H-5); 8.42 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 13.8 (SCH$_3$); 21.8 (2CH$_3$); 23.8 (CH$_3$); 25.5 (CH); 39.2 (CH$_2$); 51.3 (CH); 53.4 (NCH$_2$); 54.0 (OCH$_3$); 110.1 (C-5); 155.7 (C-2); 166.9 (C-6); 167.7 and 172.9 (2C=O); 179.9 (C-4). Mass spectrum, *m/z* (I_{rel}, %): 341 [M]$^+$ (43), 326 (22), 282 (42), 252 (23), 212 (54), 155 (100). Found, %: C 52.50; H 6.63; N 12.12. $C_{15}H_{23}N_3O_4S$. Calculated, %: C 52.77; H 6.79; N 12.31.

***N*-{[6-Methyl-4-(methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetyl}-L-phenylglycine Methyl Ester (11)**. White foam, yield 83%. R_f = 0.66 (5% MeOH in CH_2Cl_2). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.40 (3H, s, 6-CH$_3$); 2.41 (3H, s, SCH$_3$); 3.73 (3H, s, OCH$_3$); 4.88 (2H, s, C<u>H</u>$_2$N); 5.63 (1H, d, *J* = 7.2, C<u>H</u>Ph); 6.39 (1H, s, H-5); 7.30 (5H, m, H Ar); 7.36 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 14.0 (SCH$_3$); 23.5 (CH$_3$); 52.2 (NCH$_2$); 53.7 (OCH$_3$); 56.2 (CH); 109.8 (C-5); 125.6, 127.7, 134.9, and 137.4 (Ph); 155.6 (C-2); 166.7 (C-6); 167.3 and 172.9 (2C=O); 178.5 (C-4). Mass spectrum, *m/z* (I_{rel}, %): 361 [M]$^+$ (18), 302 (15), 225 (19), 197 (39), 155 (88), 140 (100). Found, %: C 56.34; H 5.19; N 11.49. $C_{17}H_{19}N_3O_4S$. Calculated, %: C 56.50; H 5.30; N 11.63.

***N*-{[5-Methyl-4-(methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetyl}glycine Methyl Ester (12)**. White foam, yield 90%. R_f = 0.54 (5% MeOH in CH_2Cl_2). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.17 (3H, s, 5-CH_3); 2.50 (3H, s, SCH_3); 3.77 (3H, s, OCH_3); 4.11 (2H, d, *J* = 5.1, NHC<u>H</u>$_2$); 4.93 (2H, s, CH_2CO); 6.83 (1H, br. s, NH); 8.14 (1H, s, H6). ^{13}C NMR spectrum, δ, ppm: 12.8 (CH_3); 14.5 (SCH_3); 51.5 (NCH_2); 52.7 (CH_2); 54.5 (OCH_3); 112.2 (C-5); 136.8 (C-6); 154.2 (C-2); 168.1 and 172.5 (2C=O); 179.0 (C4). Mass spectrum, *m/z* (I_{rel}, %): 285 $[M]^+$ (28), 254 (17), 226 (19), 197 (23), 140 (100). Found, %: C 46.17; H 5.12; N 14.69. $C_{11}H_{15}N_3O_4S$. Calculated, %: C 46.31; H 5.30; N 14.73.

***N*-{[5-Methyl-4-(methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetyl}-L-valine Methyl Ester (13)**. Yellow oil, yield 85%. R_f = 0.47 (5% MeOH in CH_2Cl_2). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.85 (3H, d, *J* = 6.9) and 0.90 (3H, d, *J* = 6.9, (C<u>H</u>$_3$)$_2$CH); 2.13 (1H, m, C<u>H</u>(CH$_3$)$_2$); 2.17 (3H, s, 5-CH_3); 2.47 (3H, s, SCH_3); 3.71 (3H, s, OCH_3); 4.57 (1H, dd, *J* = 4.8, *J* = 1.9, NHC<u>H</u>); 4.84 (1H, d, *J* = 15.3) and 4.93 (1H, d, *J* = 15.3, CH_2N); 6.72 (1H, br. s, NH); 8.12 (1H, s, H-6). ^{13}C NMR spectrum, δ, ppm: 12.9 (CH_3); 14.1 (SCH_3); 16.1 and 18.4 ($2CH_3$); 32.0 (CH); 51.8 (CH); 52.6 (NCH_2); 54.3 (OCH_3); 111.8 (C-5); 137.4 (C-6); 153.9 (C-2); 167.4 and 175.2 (2C=O); 178.6 (C-4). Mass spectrum, *m/z* (I_{rel}, %): 327 $[M]^+$ (33), 296 (22), 268 (28), 225 (34), 155 (100), 140 (45). Found, %: C 51.20; H 6.39; N 12.60. $C_{14}H_{21}N_3O_4S$. Calculated, %: C 51.36; H 6.47; N 12.83.

***N*-{[5-Methyl-4-(methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetyl}-L-leucine Methyl Ester (14)**. Pale-yellow oil, yield 82%. R_f = 0.48 (5% MeOH in CH_2Cl_2). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.88 (6H, d, *J* = 6.6, (C<u>H</u>$_3$)$_2$CH); 1.51–1.66 (3H, m, C<u>H</u>(CH$_3$)$_2$, CHC<u>H</u>$_2$CH); 2.13 (3H, s, 5-CH_3); 2.45 (3H, s, SCH_3); 3.69 (3H, s, OCH_3); 4.65 (1H, m, NHC<u>H</u>); 4.82 (1H, d, *J* = 14.7) and 4.90 (1H, d, *J* = 14.7, CH_2N); 6.65 (1H, br. s, NH); 8.11 (1H, s, H-6). ^{13}C NMR spectrum, δ, ppm: 12.5 (CH_3); 14.1 (SCH_3); 21.2 ($2CH_3$); 25.1 (CH); 39.6 (CH_2); 51.5 (CH); 53.3 (NCH_2); 54.8 (OCH_3); 112.1 (C-5); 136.1 (C-6); 153.5 (C2); 167.9, 172.9 (2C=O); 178.4 (C-4). Mass spectrum, *m/z* (I_{rel}, %): 341 $[M]^+$ (16), 298 (13), 284 (19), 253 (23), 211 (29), 155 (66), 140 (100). Found, %: C 52.45; H 6.59; N 12.18. $C_{15}H_{23}N_3O_4S$. Calculated, %: C 52.77; H 6.79; N 12.31.

***N*-{[5-Methyl-4-(methylsulfanyl)-2-oxopyrimidin-1(2*H*)-yl]acetyl}-L-phenylglycine Methyl Ester (15)**. White crystals, yield 90%; mp 129−131ºC. R_f = 0.66 (5% MeOH in CH_2Cl_2). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.21 (3H, s, 5-CH_3); 2.43 (3H, s, SCH_3); 3.74 (3H, s, OCH_3); 4.91 (2H, s, C<u>H</u>$_2$N); 5.61 (1H, d, *J* = 7.2, C<u>H</u>Ph); 7.30 (5H, m, H Ar); 7.36 (1H, br. s, NH); 8.16 (1H, s, H-6). ^{13}C NMR spectrum, δ, ppm: 12.6 (CH_3); 14.0 (SCH_3); 52.1 (NCH_2); 56.2 (CH); 53.6 (OCH_3); 112.3 (C-5); 125.5, 127.4, 134.8, 136.1, and 137.5 (Ph, C-6); 167.6 and 172.8 (2C=O); 178.2 (C-4). Mass spectrum, *m/z* (I_{rel}, %): 361 $[M]^+$ (22), 346 (27), 302 (26), 212 (46), 169 (50), 155 (100). Found, %: C 56.29; H 5.21; N 11.51. $C_{17}H_{19}N_3O_4S$. Calculated, %: C 56.50; H 5.30; N 11.63.

Antiviral Activity. The HepG2.2.2.15 cell line, supplied by State Serum Institute, Denmark, was maintained in RPMI-1640 Glutamax (Gibco BRL Life technologies) [32]. The standard drug lamivudine was from GlaxoSmithKline. The cell line was maintained in RPMI-1640 (Glutamax) culture medium containing 100 IU/ml nystatin, 380 μg/ml G418 (geneticin) and 10% fetal calf serum (FCS) (Gibco BRL Life Technologies). The transferred HEPG2.2.2.15 cells were kept in a tissue culture flask at 37°C and 5% CO_2. Subcultures were set up after a week by trypsination (10% versin/trypsin (Biochrome KG)) and transferred to a 96-well tissue culture plate. Fivefold serial dilutions of the tested compounds with final concentrations ranging from 100 to 0.03 μM were added to the cell suspension; it was then incubated for 6 days at 37°C and 5% CO_2. Each compound was tested in triplicate. Cells with no compounds added to their culture were used for comparison (blank cells).

DNA extraction was done by incubating 10 μl of diluted supernatant with 10 μl of 0.2 M NaOH at 37ºC for 1 h, then carefully adding 9.6 μl of 0.2 M HCl followed by addition of 90 μl of Tris–EDTA buffer [(2-amino-2-(hydroxymethyl)-1,3-propanediol–EDTA) (Gibco BRL Life Technologies)].

PCR-ELISA Detection of HBV DNA. The DNA content in the cell culture supernatant was determined by polymerase chain reaction amplification of the HBV DNA using 1 μmol/1 of each of the following primers: HCID-1 primer (5'-GGAAAGAAGTCAGAAGGCA-3') and HCID-2 primer (5'-TTGGGGGAGGAGATTAGGTT-3'), in a reaction mixture containing 14 μl extracted supernatant, 4 mmol/l $MgCl_2$, 10 μmol/1 DIG-11-dUTP (Roche, Germany), 190 μmol/l dTTP, 200 μmol/1 dATP, dGTP, dCTP (Roche), and 1.5 U Taq polymerase (Roche), in a

total volume 50 µl. PCR reaction conditions were: 32 cycles of 10 min at 94ºC, 30 s at 58ºC, and 30 s at 72ºC with a 3 s increment for each cycle in a Perkin-Elmer 480 thermal cycler (Perkin-Elmer, USA). The PCR product was detected by DIG-ELISA assay (Roche). The optical density from DNA of the test compound was compared to that of the blank culture [34].

Cytotoxicity Assay. 3-(3,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma, USA) is a colorless substrate that is transformed to a colored product by living cells, but not by dead cells. The assay utilizes this compound to test for the viability of the cells with the test compound added compared to the viability of the blank cells [35].

The authors thank DANIDA for the support of the HBV testing of the newly synthesized compounds at National Liver Institute, Menoufia University, through the project "Development of New Drugs Against Hepatitis".

REFERENCES

1. A. De Mesmaeker, R. Haner, and P. Martin, H. E. Moser, *Acc. Chem. Res.*, **28**, 366 (1995).
2. J. F. Milligan, M. D. Matteucci, and J. C. Martin, *J. Med. Chem.*, **36**, 1923 (1993).
3. E. Uhlmann and A. Peyman, *Chem. Rev.*, **90**, 543 (1990).
4. P. E. Nielsen, M. Egholm, R. H. Berg, and O. Buchardt, *Science*, **254**, 1497 (1991).
5. M. Egholm, O. Buchardt, P. E. Nielsen, and R. H. Berg, *J. Am. Chem. Soc.*, **114**, 1895 (1992).
6. B. Hyrup and P. E. Nielsen, *Bioorg. Med. Chem.*, **4**, 5 (1996).
7. P. E. Nielsen, *Pure Appl. Chem.*, **70**, 105 (1998).
8. E. Uhlmann, A. Peyman, G. Breipohl, and D. W. Will, *Angew. Chem. Int. Ed.*, **37**, 2796 (1998).
9. K. N. Ganesh and P. E. Nielsen, *Curr. Org. Chem.*, **4**, 931(2000).
10. M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, *Nature*, **365**, 566 (1993).
11. F. Debaene and N. Winssinger, *Org. Lett.*, **5**, 4445 (2003).
12. M. Egholm, L. Christensen, K. L. Dueholm, O. Buchardt, J. Coull, and P. E. Nielsen, *Nucleic Acids Res.*, **23**, 217 (1995).
13. A. Ray and B. Norden, *FASEB J.*, **14**, 1041 (2000).
14. P. Zhou, A. Dragulescu-Andrasi, B. Bhattacharya, H. O'Keefe, P. Vatta, J. J. Hyldig-Nielsen, and D. H. Ly, *Bioorg. Med. Chem. Lett.*, **16**, 4931 (2006).
15. S. Hyun, K. H. Lee, and J. Yu, *Bioorg. Med.Chem. Lett.*, **16**, 4757 (2006).
16. K. L. Duehohm, M. Egholm, C. Behrens, L. Christensen, H. F. Hansen, T. Vulpius, K. H. Petersen, R. H. Berg, P. E. Nielsen, and O. Buchardt, *J. Org. Chem.*, **59**, 5767 (1994).
17. J. A. Thomson, S. A. Josey, R. Cadilla, M. D. Gaul, C. F. Hassman, M. J. Luzzio, A. J. Pipe, K. L. Reed, D. J. Ricca, R. W. Wiethe, and S. A. Noble, *Tetrahedron*, **51**, 6179 (1995).
18. D. W. Will, G. Breipohl, D. Langner, J. Knolle, and E. M. Uhlmann, *Tetrahedron*, **51**, 12069 (1995).
19. A. Alahiane, M. Taourirte, A. Rochdi, N. Redwane, S. Sebti, J. W. Engels, and H. B. Lazrek, *Nucleos., Nucleot., Nucleic Acids*, **22**, 109 (2003).
20. P. Zhou, M. Wang, L. Du, G. W. Fisher, A. Waggoner, and D. H. Ly, *J. Am. Chem. Soc.*, **125**, 6878 (2003).
21. P. E. Nielsen, *Acc. Chem. Res.*, **32**, 624 (1999).
22. O. Seitz, *Angew. Chem. Int. Ed.*, **39**, 3249 (2000).
23. K. N. Ganesh and P. E. Nielsen, *Curr. Org. Chem.*, **4**, 931 (2000).
24. I. A. I. Ali, I. A. Al-Masoudi, B. Saeed, N. A. Al-Masoudi, and P. La Colla, *Heteroatom Chem.*, **16**, 148 (2005).
24. I. A. I. Ali, O. M. Ali, and A. A.-H. Abdel-Rahman, *Monatsh. Chem.*, **138**, 909 (2007).

25. O. M. Ali and A. A.-H. Abdel-Rahman, *Monatsh. Chem.*, **139**, 53 (2008).
26. A. A.-H. Abdel-Rahman, *Monatsh. Chem.*, **139**, 61 (2008).
27. A. A.-H. Abdel-Rahman, W. A. El-Sayed, H. M. Abdel-Bary, A. E.-S. Abdel-Megied, and E. M. I. Morcy, *Monatsh. Chem.*, **139**, 1095 (2008).
28. A. A.-H. Abdel-Rahman, A. E.-S. Abdel-Megied, H. M. Abdel-Bary, A. H. Abdel-Aleem, and E. M. I. Morcy, M. T. Shabaan, *Monatsh. Chem.*, **140**, 559 (2009).
29. W. A. El-Zayat, W. A. El-Sayed, and A. A.-H. Abdel-Rahman, *Z. Naturforsh.*, **64c**, 6 (2009).
30. T. H. Brown, R. C. Blakemore, G. J. Durant, J. C. Emmett, C. R. Ganellin, M. E. Parson, D. A. Rawlings, and T. F. Walker, *Eur. J. Med. Chem.*, **23**, 53 (1988).
31. R. Schwyzer and H. Kappeler, *Helv. Chim. Acta*, **44**, 1991 (1961).
32. M. A. Sells, A. Z. Zelent, M. Shvartsman, and G. Acs, *J. Virol.*, **62**, 2836 (1988).
33. B. E. Korba and J. L. Gerin, *Antiviral Res.*, **19**, 55 (1992).
34. S. L. Doong, C. H. Tsai, R. F. Chinazi, D. C. Liotta, and Y. C. Cheng, *Proc. Nat. Acad. Sci. USA*, **88**, 4895 (1991).
35. T. Fouad, C. Nielsen, L. Brunn, and E. B. Pederson, *Sc. J. Az. Med. Fac. (GIRLS)*, **19**, 1173 (1998).