Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 5 pp. 917–925, 2012 ISSN 0001-6837
Polish Pharmaceutical Society

SYNTHESIS AND ANTIVIRAL EVALUATION OF α-AMINO ACID ESTERS BEARING N^6-BENZYLADENINE SIDE CHAIN

REEM A. K. AL-HARBI[1] and ADEL A.-H. ABDEL-RAHMAN[2*]

[1] Department of Applied Chemistry, Faculty of Applied Science, Taibah University, Madinah, Saudi Arabia
[2] Department of Chemistry, Faculty of Science, Northern Border University, Arar, Saudi Arabia

Abstract: A series of peptide derivatives conjugated with a purine residue were synthesized. The prepared compounds were tested for antiviral activity against Hepatitis B Virus (HBV) displaying different degrees of antiviral activities or inhibitory actions.

Keywords: adenine, purine, amino acids, dipeptides, antiviral activity

Peptide nucleic acids (PNAs) are oligonucleotide analogues, in which the entire phosphodiester pentose backbone of DNA or RNA is replaced by a polyamide or peptide backbone (Fig. 1) (1). The complete replacement of the ribose phosphate backbone with an artificial pseudopeptide backbone resulted in the remarkably improved binding to complementary nucleic acid sequences occurring with both high affinity and high selectivity. The most widely known PNAs are based on a *N*-(2-aminoethyl)glycine backbone, which recognize and bind strongly to specific DNA or RNA sequences (2, 3). These characteristics make them potentially useful as antisense and antigene drugs or molecular probes, which have numerous applications in the field of molecular and experimental medicine (4–10). The hybridization properties of peptide nucleic acids (PNAs) have attracted widespread interest to this class of compounds (11–13). In connection with our work in synthesis of new α-amino acid derivatives (14–22) and due to the pharmacological properties of PNAs and amino acid derivatives we were prompted to prepare new N^6-benzyladenine bearing amino acid derivatives to study their antiviral activity.

EXPERIMENTAL

Chemistry

All melting points were uncorrected and were taken in open capillary tubes using silicon oil on Gallenkamp apparatus. Elemental microanalyses were performed on Elementar, Vario EL, Microanalytical Unit, National Research Centre, Cairo, Egypt. Infrared spectra were recorded on Jasco FT/IR-330E, Fourier Transform Infrared Spectrometer at cm^{-1} scale using KBr discs.

^{1}H-NMR spectra were determined using JEOL EX-270 or JEOL ACA500 NMR spectrometers and measured in ä ppm scale using TMS as an internal

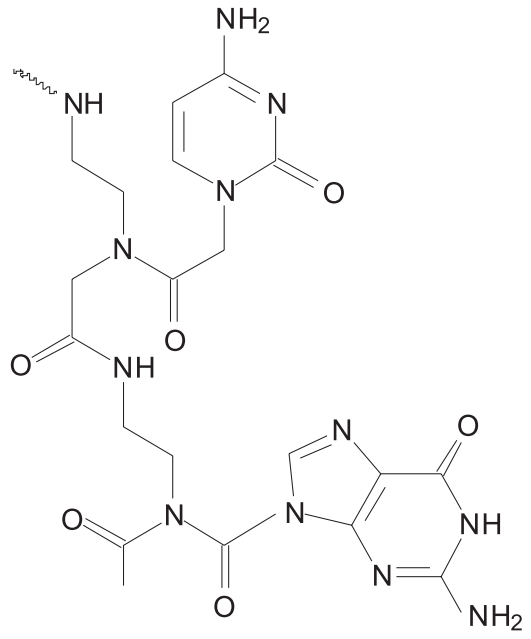


Figure 1. Structure of peptide nucleic acid (PNA)

* Corresponding author: e-mail: adelnassar63@hotmail.com

standard. Mass spectra were measured using mass spectrometer Finnigan MAT SSQ-7000 and GCMS-QP 1000EX Shimadzu Gas Chromatography Mass Spectrometer.

All reactions were followed up by TLC (aluminum sheets) using $CHCl_3/CH_3OH$ (9:1, v/v) eluent and detection by UV lamp. The chemical names given to the prepared compounds are according to the IUPAC system.

ES mass spectra were obtained from an Esquire 3000plus iontrap mass spectrometer from Bruker Daltonics.

Antiviral activity of the synthesized compounds was conducted at the Research Unit, Univet Pharmaceutical Co., Egypt.

9-Carbethoxymethyl-N^6-benzyladenine (2)

A mixture of N^6-benzyladenine (**1**) (23) (22.5 g, 0.1 mol), ethyl chloroacetate (14.7 g, 0.12 mol), and anhydrous potassium carbonate (13.8 g, 0.1 mol) in dry acetone (60 mL) was refluxed for 2 h (TLC). The solvent was removed *in vacuo* and the residue was diluted with water and extracted with chloroform (3 × 50 mL). The combined organic layers were dried over sodium sulfate and the solvent was evaporated. The residue was purified by silica gel column chromatography using (petroleum ether : ethyl acetate 7 : 1, v/v) to afford **2**.

9-Acetylhydrazine-N^6-benzyladenine (3)

A mixture of **2** (3.11 g, 10 mmol) and hydrazine hydrate (1.25 g, 25 mmol) in absolute ethanol (50 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 **3**.

General procedure for preparation of N^6-benzyladenine bearing amino acid esters 4–10

A solution of **3** (1.19 g, 4 mmol) in acetic acid (50 mL), 1 M hydrochloric acid (18 mL), and water (130 mL) were cooled in an ice-bath (–5°C). Sodium nitrite (4.35 g, 63 mmol) in cold water (20 mL) was added with stirring. After stirring at –5°C for 15 min, the yellow syrup was formed. The azide was taken in cold ethyl acetate (150 mL), washed with 3% sodium bicarbonate (150 mL), water (150 mL), and dried over sodium sulfate. A solution of the appropriate amino acid methyl ester hydrochloride (4.5 mmol) in ethyl acetate (100 mL) containing triethylamine (1.0 mL) was stirred at 0°C for 20 min, filtered, and the filtrate was added to the azide solution. The mixture was kept at –5°C for 12 h, then at room temperature for another 12 h, followed by washing with 0.5 M hydrochloric acid (150 mL), 3% sodium bicarbonate (150 mL), water (150 mL), and dried over sodium sulfate. The filtrate was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography using (petroleum ether : ethylacetate 7 : 1, v/v) to afford **4–10** in 70–78% yields.

General procedure for preparation of the hydrazides 11 and 12

A mixture of **4** or **5** (5 mmol) and hydrazine hydrate (0.63 g, 12.5 mmol) in absolute ethanol (20 mL) was heated under reflux for 3 h. The excess of ethanol was removed under reduced pressure and the resulting precipitate was filtered off and recrystallized from ethanol to give **11** and **12** in 95–97% yields.

General procedure for preparation of dipeptides 13–20

A solution of **11** or **12** (0.80 mmol) in acetic acid (10 mL), 1 M hydrochloric acid (5 mL), and water (30 mL) was cooled in an ice-bath (–5°C). Sodium nitrite (0.87 g, 12.6 mmol) in cold water (5 mL) was added with stirring. After stirring at –5°C for 15 min, the yellow syrup was formed. The azide was taken in cold ethyl acetate (30 mL), washed with 3% sodium bicarbonate (30 mL), water (30 mL), and dried over sodium sulfate. A solution of the appropriate amino acid methyl ester hydrochloride (0.90 mmol) in ethyl acetate (25 mL) containing triethylamine (0.3 mL) was stirred at 0°C for 20 min, filtered, and the filtrate was added to the azide solution. The mixture was kept at –5°C for 12 h, then at room temperature for another 12 h, followed by washing with 0.5 M hydrochloric acid (30 mL), 3% sodium bicarbonate (30 mL), water (30 mL), and dried over sodium sulfate. The filtrate was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography using petroleum ether : ethylacetate 5 : 1, v/v to afford **14–20** in 70–75% yields.

Antiviral activity

Anti-hepatitis C virus (HCV) activity

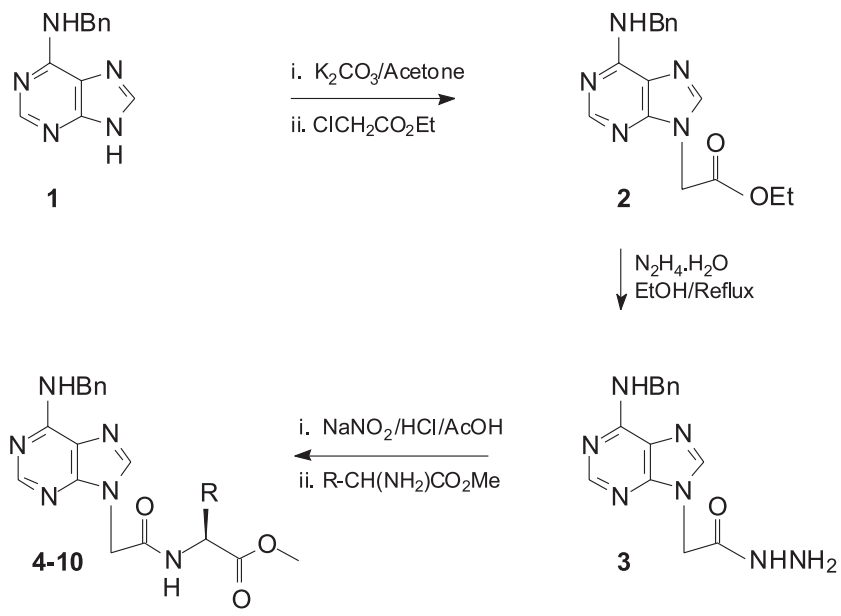
Determination of anti-HCV activity

Screening of compounds and determination of their minimum inhibitory concentration (MIC) in HCV replicon cells was performed as following: Briefly, 1×10^4 replicon cells per well were plated in 96-well plates. On the following day, replicon cells were incubated at 37°C for the indicated period of time with antiviral agents serially diluted in DMEM

plus 2% FBS and 0.5% dimethyl sulfoxide (DMSO). Total cellular RNA was extracted using an RNeasy-96 kit (QIAGEN, Valencia, CA), and the copy number of HCV RNA was determined using a quantitative RTPCR (QRT-PCR) assay. Each datum point represents the average of five replicates in cell culture. The cytotoxicity of tested compound was measured under the same experimental settings using a tetrazolium (MTS)-based cell viability assay (Promega, Madison, WI). For the cytotoxicity assay with human hepatocyte cell lines, 1×10^4 parental Huh-7 cells per well or 4×10^4 HepG2 cells per well were used.

MIC of tested compounds and their combination in hamster brains were conducted for antiviral chemotherapy for Subacute Sclerosing Panencephalitis (SSPE).

Under ether anesthesia, 50 mL of either tested compounds (or the newly synthesized compound) or their different combinations solutions at dosages of 5, 10, and 20 mg/kg/day were injected for 10 days intracranially to a depth of 2 mm by using a 27-gauge needle placed within the subarachnoid space. At 1, 2, 3, 5, 7, 10, 12, 15, and 20 days after the initial injection, four hamsters from each group were sacrificed. The brains were aseptically removed, washed twice with phosphate-buffered saline (PBS), homogenized, and suspended in PBS. The suspension was centrifuged at 1,600 × g for 10 min. The supernatant was collected, ethanol was added to remove proteins, and the mixture was heated at 90°C to evaporate ethanol. The protein-free samples were used to evaluate the MIC in brain tissue by HPLC and bioassay.



No.	R	amino acid
4	H	glycine
5	Me	L-alanine
6	CH_2OH	L-serine
7	$CHMe_2$	L-valine
8	CH_2CHMe_2	L-leucine
9	CH_2CH_2SMe	L-methionine
10	Ph	L-phenylglycine

Scheme 1.

Table 1. Physical and analytical data of all new compounds.

Comp. no.	M.p. (°C)	Yield (%)	Mol. Formula (M. w.)	Analysis (%) Calcd. / Found		
				C	H	N
2	160–162	94	$C_{16}H_{17}N_5O_2$ (311.34)	61.72 61.57	5.50 5.33	22.49 22.19
3	245–247	98	$C_{14}H_{15}N_7O$ (297.32)	56.56 56.34	5.09 5.00	32.98 32.87
4	White foam	73	$C_{17}H_{18}N_6O_3$ (354.36)	57.62 57.55	5.12 5.03	23.72 23.67
5	White foam	77	$C_{18}H_{20}N_6O_3$ (368.39)	58.69 58.44	5.47 5.37	22.81 22.69
6	White foam	71	$C_{18}H_{20}N_6O_4$ (384.39)	56.24 56.06	5.24 5.13	21.86 21.69
7	White foam	75	$C_{20}H_{24}N_6O_3$ (396.44)	60.59 60.43	6.10 6.00	21.20 21.12
8	White foam	72	$C_{21}H_{26}N_6O_3$ (410.47)	61.45 61.22	6.38 6.21	20.47 20.29
9	Pale yellow foam	70	$C_{20}H_{24}N_6O_3S$ (428.51)	56.06 55.90	5.65 5.55	19.61 19.49
10	133–135	78	$C_{23}H_{22}N_6O_3$ (430.46)	64.17 64.03	5.15 5.01	19.52 19.33
11	209–211	95	$C_{16}H_{18}N_8O_2$ (334.16)	54.23 54.13	5.12 5.03	31.62 31.55
12	237–239	97	$C_{17}H_{20}N_8O_2$ (368.39)	55.43 55.22	5.47 5.28	30.42 30.33
13	White foam	72	$C_{19}H_{21}N_7O_4$ (411.41)	55.47 55.40	5.14 5.07	23.83 23.66
14	White foam	75	$C_{20}H_{23}N_7O_4$ (425.44)	56.46 56.30	5.45 5.33	23.05 22.91
15	White foam	71	$C_{20}H_{23}N_7O_5$ (441.44)	54.42 54.29	5.25 5.13	22.21 22.11
16	White foam	73	$C_{22}H_{27}N_7O_4$ (453.49)	58.27 58.12	6.00 5.85	21.62 21.53
17	White foam	71	$C_{20}H_{23}N_7O_4$ (425.44)	56.46 56.33	5.45 5.30	23.05 22.88
18	White foam	74	$C_{21}H_{25}N_7O_4$ (439.47)	57.39 57.22	5.73 5.53	22.31 22.17
19	White foam	70	$C_{21}H_{25}N_7O_4$ (455.47)	55.38 55.20	5.53 5.35	21.53 21.42
20	White foam	75	$C_{23}H_{29}N_7O_4$ (467.52)	59.09 58.91	6.25 6.12	20.97 20.88

Anti –HIV activity

Cells and viruses

The established human cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, penicillin (100 U/mL), and streptomycin (100 µg/mL). Fresh human cells were obtained from the American Red Cross (Baltimore, Md.).

Antiviral and cross-resistance assays

The inhibitory activities of the compounds against HIV were evaluated by microtiter anti-HIV assays with CEM-SS cells or fresh human peripher-

Table 2. Spectral data of the newly synthesized compounds.

Comp. no.	IR (KBr) cm^{-1}, ^1H NMR (DMSO-d_6, δ, ppm), MS (m/z (%))
2	**IR:** 1730 (C=O), 3425 (NH). **^1H NMR:** 1.25 (t, *J* = 6.1 Hz, CH_3CH_2O), 4.21 (q, *J* = 6.1 Hz, CH_3CH_2O), 4.40 (d, *J* = 5.5 Hz, CH_2), 4.70 (s, N^9-CH_2), 6.90 (br, s, NH), 7.18–7.36 (m, Ar-H), 8.10 (s, H-2), 8.60 (s, H-8). **MS:** 334 [(M^+ + Na), 36].
3	**IR:** 1666 (C=O), 3558 (NH_2), 3338 (NH). **^1H NMR** 4.15 (br, s, $NHNH_2$), 4.33 (d, *J* = 5.5 Hz, CH_2), 4.68 (s, N^9-CH_2), 6.70 (br, s, NH), 7.22–7.35 (m, Ar-H), 8.12 (s, H-2), 8.53 (s, H-8), 9.30 (br, s, $NHNH_2$). **MS:** 320 [(M^+ + Na), 23].
4	**^1H NMR:** 3.50 (s, OCH_3), 4.15 (s, CH_2), 4.37 (d, *J* = 5.5 Hz, CH_2), 4.66 (s, N^9-CH_2), 6.79 (br, s, NH), 7.22–7.35 (m, Ar-H), 8.15 (s, H-2), 8.44 (s, H-8), 9.05 (br, s, NH). **MS:** 377 [(M^+ + Na), 18].
5	**^1H NMR:** 1.44 (d, *J* = 5.0 Hz, CH_3), 3.53 (s, OCH_3), 4.39 (d, *J* = 5.5 Hz, CH_2), 4.65–4.71 (m, CH, N^9-CH_2), 6.79 (br, s, NH), 7.25–7.44 (m, Ar-H), 8.13 (s, H-2), 8.43 (s, H-8), 9.03 (br, s, NH). **MS:** 391 [(M^+ + Na), 29].
6	**^1H NMR:** 3.30 (m, CH_2), 3.59 (s, OCH_3), 4.41 (d, *J* = 5.5 Hz, CH_2), 4.55–4.69 (m, CH, N^9-CH_2), 6.80 (br, s, NH, OH), 7.23–7.47 (m, Ar-H), 8.10 (s, H-2), 8.37 (s, H-8), 9.01 (br, s, NH). **MS:** 407 [(M^+ + Na), 34].
7	**^1H NMR:** 0.99 (dd, *J* = 1.9, 7.2 Hz, 2×CH_3), 2.28 (m, CH), 3.59 (s, OCH_3), 4.37 (d, *J* = 5.5 Hz, CH_2), 4.45 (m, CH), 4.67 (s, N^9-CH_2), 6.72 (br, s, NH), 7.25–7.47 (m, Ar-H), 8.08 (s, H-2), 8.37 (s, H-8), 9.04 (br, s, NH). **MS:** 419 [(M^+ + Na), 23].
8	**^1H NMR:** 0.93 (dd, *J* = 1.9, 7.2 Hz, 2×CH_3), 1.45–1.65 (m, CH_2, CH), 3.59 (s, OCH_3), 4.29–4.63 (m, CH_2, CH, N^9-CH_2), 6.65 (br, s, NH), 7.22–7.47 (m, Ar-H), 8.10 (s, H-2), 8.36 (s, H-8), 9.00 (br, s, NH). **MS:** 433 [(M^+ + Na), 19].
9	**^1H NMR:** 2.32–2.50 (m, 2×CH_2, SCH_3), 3.60 (s, OCH_3), 4.38–4.65 (m, CH_2, CH, N^9-CH_2), 6.70 (br, s, NH), 7.25–7.43 (m, Ar-H), 8.11 (s, H-2), 8.39 (s, H-8), 9.01 (br, s, NH). **MS:** 451 [(M^+ + Na), 41].
10	**^1H NMR:** 3.61 (s, OCH_3), 4.41 (d, *J* = 5.5 Hz, CH_2), 4.50–4.69 (s, CH, N^9-CH_2), 6.73 (br, s, NH), 7.20–7.42 (m, Ar-H), 8.12 (s, H-2), 8.40 (s, H-8), 9.00 (br, s, NH). **MS:** 453 [(M^+ + Na), 27].
11	**^1H NMR:** 3.55 (s, CH_2), 4.33 (d, *J* = 5.5 Hz, CH_2), 4.61 (s, N^9-CH_2), 4.96 (br, s, $NHNH_2$), 6.76 (br, s, NH), 7.22–7.39 (m, Ar-H), 8.10 (s, H-2), 8.36 (s, H-8), 9.07 (br, s, NH), 9.40 (br, s, $NHNH_2$). **MS:** 377 [(M^+ + Na), 53].
12	**^1H NMR:** 1.40 (d, *J* = 5.0 Hz, CH_3), 4.37 (d, *J* = 5.5 Hz, CH_2), 4.60–4.86 (m, CH, N^9-CH_2, $NHNH_2$), 6.70 (br, s, NH), 7.22–7.47 (m, Ar-H), 8.10 (s, H-2), 8.32 (s, H-8), 9.09 (br, s, NH), 9.33 (br, s, $NHNH_2$). **MS:** 391 [(M^+ + Na), 24].
13	**^1H NMR:** 3.61 (s, OCH_3), 4.00 (s, CH_2), 4.18 (s, CH_2), 4.32 (d, *J* = 5.5 Hz, CH_2), 4.63 (s, N^9-CH_2), 6.81 (br, s, NH), 7.20–7.35 (m, Ar-H), 8.12 (s, H-2), 8.50 (s, H-8), 9.00 (br, s, 2×NH). **MS***:* 434 [(M^+ + Na), 22].
14	**^1H NMR:** 1.48 (d, *J* = 5.0 Hz, CH_3), 3.59 (s, OCH_3), 4.10 (s, CH_2), 4.43 (d, *J* = 5.5 Hz, CH_2), 4.60–4.69 (CH, N^9-CH_2), 6.70 (br, s, NH), 7.22–7.35 (m, Ar-H), 8.15 (s, H-2), 8.30 (br, s, NH), 8.44 (s, H-8), 9.00 (br, s, NH). **MS:** 448 [(M^+ + Na), 18].
15	**^1H NMR:** 3.62 (s, OCH_3), 4.11 (s, CH_2), 4.26 (m, CH_2), 4.34 (d, *J* = 5.5 Hz, CH_2), 4.50–4.80 (m, CH, N^9-CH_2, OH), 6.72 (br, s, NH), 7.22–7.39 (m, Ar-H), 8.15 (s, H-2), 8.29 (br, s, NH), 8.44 (s, H-8), 9.01 (br, s, NH). **MS:** 464 [(M^+ + Na), 16].
16	**^1H NMR:** 1.01 (d, *J* = 5.0 Hz, 2×CH_3), 3.09 (m, CH), 3.58 (s, OCH_3), 4.11 (s, CH_2), 4.33–4.64 (m, CH_2, CH, N^9-CH_2), 6.69 (br, s, NH), 7.22–7.38 (m, Ar-H), 8.15 (s, H-2), 8.33 (br, s, NH), 8.44 (s, H-8), 9.04 (br, s, NH). **MS:** 476 [(M^+ + Na), 29].
17	**^1H NMR:** 1.49 (d, *J* = 5.0 Hz, CH_3), 3.60 (s, OCH_3), 4.16 (s, CH_2), 4.34 (d, *J* = 5.5 Hz, CH_2), 4.60–4.70 (m, CH, N^9-CH_2), 6.71 (br, s, NH), 7.22–7.39 (m, Ar-H), 8.11 (s, H-2), 8.28 (br, s, NH), 8.40 (s, H-8), 9.05 (br, s, NH). **MS:** 448 [(M^+ + Na), 25].

Table 2. cont.

Comp. no.	IR (KBr) cm^{-1}, ^1H NMR (DMSO-d_6, δ, ppm), MS (m/z (%))
18	**^1H NMR:** 1.49 (d, *J* = 5.0 Hz, 2×CH_3), 3.57 (s, OCH_3), 4.32 (d, *J* = 5.5 Hz, CH_2), 4.60–4.75 (m, 2×CH, N^9-CH_2), 6.71 (br, s, NH), 7.22–7.39 (m, Ar-H), 8.15 (s, H-2), 8.26 (br, s, NH), 8.44 (s, H-8), 9.05 (br, s, NH). **MS:** 462 [(M^+ + Na), 32].
19	**^1H NMR:** 1.47 (d, *J* = 5.0 Hz, CH_3), 3.58 (s, OCH_3), 4.25 (m, CH_2), 4.32 (d, *J* = 5.5 Hz, CH_2), 4.55–4.75 (m, 2×CH, N^9-CH_2, OH), 6.79 (br, s, NH), 7.22–7.35 (m, Ar-H, NH), 8.13 (s, H-2), 8.27 (br, s, NH), 8.45 (s, H-8), 9.03 (br, s, NH). **MS:** 478 [(M^+ + Na), 17].
20	**^1H NMR:** 1.00 (d, *J* = 5.0 Hz, 2×CH_3), 1.50 (d, *J* = 5.0 Hz, CH_3), 3.10 (m, CH), 3.59 (s, OCH_3), 4.37–4.44 (m, CH_2, CH), 4.62–4.74 (m, CH, N^9-CH_2), 6.73 (br, s, NH), 7.22–7.36 (m, Ar-H), 8.10 (s, H-2), 8.27 (br, s, NH), 8.41 (s, H-8), 9.05 (br, s, NH). **MS:** 490 [(M^+ + Na), 12].

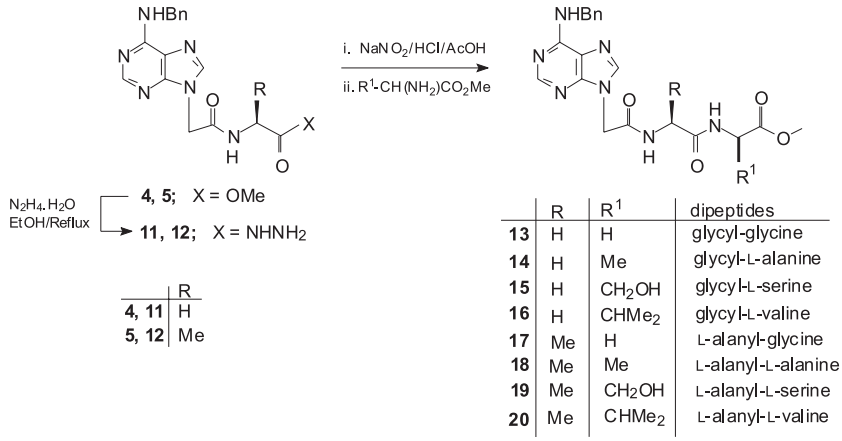
Table 3. Anti-hepatitis C virus (HCV) activity: minimum inhibitory concentration (MIC).

Comp. no.	MIC μg/mL	
	HCV	Substance sclerosing anencephalitis (SSPE)
4	8.50	
5	5.09	5.20
6	8.33	
7	5.22	5.17
8	5.11	5.10
9	1.30	4.20
10	8.44	
13	8.20	
14	8.12	
15	8.37	
16	8.41	
17	5.18	5.07
18	1.39	4.17
19	2.27	4.80
20	2.50	4.90

al blood mononuclear cells (PBMCs); these assays quantify the ability of a compound to inhibit HIV-induced cell killing or HIV replication. Quantification was performed by the tetrazolium dye XTT assay (CEM-SS, 174×CEM, MT2, and AA5 cell-based assays); the dye is metabolized to a colored formazan product by viable cells, RT assay (U937- and PBMC-based assays), and/or p24 enzyme-linked immunosorbent assay (monocyte-macrophage assays). Antiviral and toxicity data are reported as the quantity of drug required to inhibit virus-induced cell killing or virus production by 50% (EC_{50}).

In vitro assays of anti-HIV activity

Each of the newly synthesized compounds was tested for RT inhibitory activity against purified recombinant HIV-1 RT using the cell-free Quan-T-RT assay system (Amersham Corp., Arlington Heights, IL), which utilizes the scintillation proxim-



Scheme 2.

Table 4. HIV inhibition activities (reverse transcriptase inhibition with therapeutic windows.

Comp. no.	EC_{50} (mM)	IC_{50} (μM)	Therapeutic index
4	1.10×10^{-5}	12.89	6.24×10^{8}
5	5.23×10^{-4}	12.44	5.78×10^{6}
6	1.15×10^{-5}	12.95	6.30×10^{8}
7	5.30×10^{-4}	12.50	5.80×10^{6}
8	2.72×10^{-3}	2.90	5.12×10^{6}
9	1.56×10^{-3}	3.11	3.45×10^{6}
10	2.75×10^{-3}	2.93	5.18×10^{6}
13	1.20×10^{-5}	3.01	6.35×10^{8}
14	5.35×10^{-4}	12.55	5.89×10^{6}
15	1.59×10^{-3}	3.14	3.49×10^{6}
16	2.79×10^{-3}	2.99	5.23×10^{6}
17	3.30×10^{-3}	1.90	2.90×10^{7}
18	3.24×10^{-3}	1.88	2.88×10^{7}
19	5.26×10^{-4}	1.44	3.15×10^{7}
20	5.28×10^{-4}	1.48	3.18×10^{7}

ity assay (SPA) principle. In the assay, a DNA/RNA template is bound to SPA beads *via* a biotin/streptavidin linkage. The primer DNA is a 16-oligomer (T), which has been annealed to a poly (rA) template. The primer-template is bound to a streptavidin-coated SPA bead. [^{3}H]TTP (thymidine 5'-triphosphate) is incorporated into the primer by reverse transcription. In brief, [^{3}H]TTP, at a final concentration of 0.5 μCi/sample, was diluted in RT assay buffer (49.5 mM Tris-HCl, pH 8.0, 80 mM KCl, 10 mM $MgCl_2$, 10 mM dithiothreitol, 2.5 mM EGTA, 0.05% Nonidet P-40) and added to annealed DNA/RNA bound to SPA beads. The compound being tested was added to the reaction mixture at 0.001–100 μM concentrations. Addition of 10 μM of recombinant HIV RT and incubation at 37°C for 1 h resulted in the extension of the primer by incorporation of [^{3}H]TTP. The reaction was stopped by addition of 0.2 mL of 120 mM EDTA. The samples were counted in an open window using a Beckman LS 7600 instrument and $IC_{50[RT]}$ values (concentration at which the compound inhibits recombinant RT by 50%) were calculated by comparing the measurements to untreated sample.

RESULTS AND DISCUSSION

Chemistry

N^6-Benzyladenine (**1**) (23) was treated with ethyl chloroacetate in dry acetone containing anhydrous potassium carbonate at reflux temperature yielding 9-carbethoxymethyl-N^6-benzyladenine (**2**) in 94% yield. Treatment of **2** with hydrazine hydrate in ethanol gave the corresponding hydrazide derivative **3** in 98% yield. This hydrazide was selected as starting material for the coupling reaction with the appropriate acylated amino acids, *via* the azide-coupling method (24). Thus, treatment of **3** at –5°C in acetic acid and 1 M hydrochloric acid with sodium nitrite afforded the inseparable azide derivative. The yellow syrupy azide compound was then treated, *in situ*, with the appropriate amino acid methyl esters in ethyl acetate containing triethylamine at 0°C to give, after neutralization, the desired peptides **4**–**10** in 70–78% yields. The structures of **4**–**10** were assigned from their ^{1}H NMR and mass spectra (Scheme 1).

Treating of **4** or **5** with hydrazine hydrate in ethanol at reflux temperature afforded the corresponding hydrazides **11** or **12** in 95–97% yields. Treatment of **11** or **12** at –5°C in acetic acid and 1 M hydrochloric acid with sodium nitrite afforded the inseparable azide derivatives. The yellow syrupy azide compounds were treated, as mentioned above, with the appropriate amino acid methyl esters in ethyl acetate containing triethylamine at 0°C to afford **13**–**20** in 70–75% yields. The structures of the dipeptide derivatives were confirmed by their ^{1}H NMR and mass spectra (Scheme 2).

Antiviral activity

Anti-hepatitis C virus (HCV) activity

The newly synthesized compounds were tested for their antiviral activity against hepatitis C virus (HCV) by using HCV replicon cells. The anti-HCV results showed that compounds **9** and **18** exhibited the highest activity with minimum inhibitory concentration (MIC) 1.30 and 1.39 μg/mL, respectively, followed by compounds **19** and **20**. Compounds **5**, **7**, **8** and **17** showed moderate inhibition activities with MIC values 5.09, 5.22, 5.11, and 5.18 μg/mL while compounds **4**, **6**, **10**, and **13**–**16** were the least active against HCV in the series of tested compounds (Table 3).

Anti-HIV activity

The newly synthesized compounds were evaluated for their HIV inhibition activity as reverse transcriptase inhibitors by using microtiter anti-HIV assays with CEM-SS cells or fresh human peripheral blood mononuclear cells (PBMCs). The results of antiviral activity revealed that compounds **17**–**20** showed the highest activity with IC_{50} value 1.90, 1.88, 1.44, and 1.48 μM and therapeutic index 2.90×10^7, 2.88×10^7, 3.15×10^7, and 3.18×10^7 followed by compounds **8**–**10**, **15** and **16** with IC_{50} 2.90, 3.11, 2.93, 3.14 and 2.99 μM, respectively. Compounds **4**, **6**, and **13** showed moderate activities while compounds **5**, **7**, and **14** were the least active among the series of tested compounds (Table 4).

Conclusion

The amino acid methyl ester was selected according to the amino acid chain residue: a) glycine (without side chain), b) L-alanine (very short side chain), c) L-serine (short side chain with free hydroxyl group), d) L-valine (branched side chain), e) L-leucine (long side chain), f) L-methionine (long side chain containing S-atom), f) L-phenylglycine (aromatic side chain). The highest antiviral activity of the tested compounds due to the N^6-benzyladenine nucleobase when attached with amino acid ester has branched or aromatic residue.

REFERENCES

1. Egholm M., Buchardt O., Christensen L., Behrens C., Freier S.M., Driver D.A. et al.: Nature 365, 566 (1993).
2. Good L., Nielson P.E.: Antisense Nucleic Acid Drug Dev. 7, 431 (1997).
3. Nielsen P.E., Haaima G.: Chem. Soc. Rev. 2, 73 (1997).
4. Egholm M., Christensen L., Dueholm K.L., Buchardt O., Coull J., Nielson P.E.: Nucleic Acids Res. 23, 217 (1995).
5. Ray A., Norden B.: FASEB J. 14, 1041 (2000).
6. Demidov V.V.: Drug Disc. Today 7, 153 (2002).
7. Haaima G., Hansen F.H., Christensen L., Dahl O., Nielsen P.E.: Nucleic Acids Res. 25, 4639 (1997).
8. Knudsen H., Nielsen P.E.: Anticancer Drugs 8, 113 (1997).
9. Eriksson M., Nielsen P.E.: Q. Rev. Biophys. 29, 369 (1996).
10. Ganesh K. N., Nielsen P.E.: Curr. Org. Chem. 4, 931 (2000).
11. Nielsen P.E., Egholm M., Berg R.H., Buchardt O.: Science 254, 1497 (1991).
12. Nielsen P. E.: Acc. Chem. Res. 32, 624 (1999).
13. Nielsen P. E.: Curr. Opin. Biotechnol. 12, 16 (2001).

14. Ali I.A.I., Al-Masoudi I.A., Saeed B., Al-Masoudi N.A., La Colla P.: Heteroat. Chem. 16, 148 (2005).
15. Al-Masoudi N.A., Al-Masoudi I.A., Ali I.A. I., Al-Soud Y.A., Saeed B., La Colla P.: Heteroat. Chem. 16, 576 (2006).
16. Al-Masoudi N.A., Al-Masoudi I.A., Ali I.A.I., Al-Soud Y.A., Saeed B., La Colla P.: Acta Pharm. 56, 175 (2006).
17. Ali I.A.I., Ali O.M., Abdel-Rahman A.A.-H.: Monatsh. Chem. 138, 909 (2007).
18. Ali O.M., Abdel-Rahman A.A.-H.: Monatsh. Chem. 139, 53 (2008).
19. Abdel-Rahman A.A.-H.: Monatsh. Chem. 139, 61 (2008).
20. Abdel-Rahman A.A.-H., El-Sayed W.A., Abdel-Bary H.M., Abdel-Maged A.E.S., Morsy E.M.I.: Monatsh. Chem. 139, 1095 (2008).
21. Hameurlaine A., El-Sayed W.A., Abdel-Rahman A.A.-H.: Monatsh. Chem. 139, 1507 (2008).
22. Al-Harbi R.A.K., Abdel-Rahman A.A.-H.: Chem. Heterocycl. Compd. in press.
23. Davoll J., Lowy B.A.: J. Am. Chem. Soc. 73, 1650 (1951).
24. Schwyzer R., Kappeler H.: Helv. Chim. Acta 44, 1991 (1961).

Received: 18. 06. 2011