Med Chem Res (2010) 19:1025–1035
DOI 10.1007/s00044-009-9248-y

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis and preliminary antiviral screening of new *N*-phenylpyrazole and dihydroisoxazole derivatives

Adel A. Rashad · Osama I. El-Sabbagh · Mohamed M. Baraka · Samy M. Ibrahim · Christophe Pannecouque · Graciela Andrei · Robert Snoeck · Jan Balzarini · Ahmed Mostafa

Received: 21 March 2009 / Accepted: 26 August 2009 / Published online: 14 October 2009

Abstract A new series of *N*-phenylpyrazoles and dihydroisoxazles was synthesized starting from α,β-unsaturated ketones in basic media using phenyl hydrazine and hydroxylamine HCl, respectively. Antiviral evaluation of the target compounds revealed that the dihydroisoxazole derivatives have promising antiviral activity against hepatitis A virus and herpes simplex virus type 1.

Keywords Synthesis · Antiviral activity · *N*-phenylpyrazoles · Dihydroisoxazole · Herpes simplex virus type 1 · Hepatitis A virus

Introduction

Herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) belong to the herpes virus family. HSV-1 infects mucocutaneous epithelial cells and establishes latency in sensory ganglions (Whitley and Roizman, 2001). HSV-2 causes genital lesions. Much research effort has been focused on HSV-1 and HSV-2, as these viruses have a high incidence rate and a high prevalence (Gudmundsson *et al.,* 2008). Vesicular stomatitis virus (VSV) is a single-strand RNA virus of the Rhabdoviridae family. VSV has been isolated from a variety of animals and causes nonfatal disease of significant economic importance in cattle and swine (Rose and

A. A. Rashad (✉) · O. I. El-Sabbagh · M. M. Baraka · S. M. Ibrahim
Department of Medicinal Chemistry, Faculty of Pharmacy, Zagazig University, 44511 Zagazig, Egypt
e-mail: adelrashadzu@yahoo.com

C. Pannecouque · G. Andrei · R. Snoeck · J. Balzarini
Rega Institute for Medical Research, Faculty of Medicine, K.U. Leuven, Leuven, Belgium

A. Mostafa
Environmental Virology Laboratory, Department of Water Pollution, National Research Centre, Dokki, Egypt

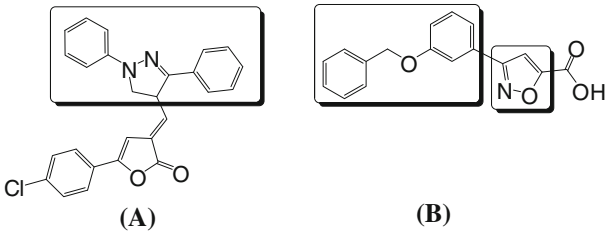


Fig. 1 Antiviral compounds used as models for the synthesis of the target compounds

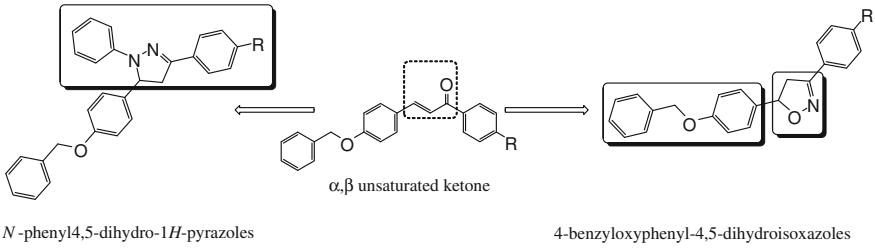


Fig. 2 Synthesis of the target compounds

Whitt, 2007). Hepatitis A is an acute infectious disease of the liver caused by hepatitis A virus (HAV), which is most commonly transmitted by the fecal-oral route via contaminated food or drinking water. Every year, approximately 10 million people worldwide are infected with the virus (Vento, 2000).

In the search for antiviral agents, several nonnucleoside derivatives have been developed (Pauwels, 2004; Rashad *et al.,* 2008), such as 5-(4-chlorophenyl)-3-(1,3-diphenylpyrazol-4-yl)-methylene-2(3H)-furanone (**A**), which exerts high antiviral activities toward HAV (Hashem *et al.,* 2007), while 3-(3-(benzyloxy)phenyl)isoxazole-5-carboxylic acid (B) shows HIV-1 integrase (IN) inhibitory effect and good antiviral activity (Zeng *et al.,* 2008) (Fig. 1).

Therefore, in our quest for synthesis of new antiviral agents with an efficacy similar to or greater than that of the other reported compounds, a series of 4,5-dihydro-1H-pyrazoles was synthesized bearing *N*-phenylpyrazole moiety similar to that of compound **A**. Moreover, a series of 4-benzyloxyphenyl-4,5-dihydroisoxazoles was synthesized based on the aforementioned structurally similar compound (**B**), with the aim to explore their antiviral activity (Fig. 2).

Experimental

Melting points were determined with a Gallenkamp melting-point apparatus and are uncorrected. IR spectra (KBr, cm^{-1}) were recorded on a Testscan Shimadzu FT 8000 spectrometer. ^{1}H NMR (200- and 500-MHz) and ^{13}CNMR (125-MHz) spectra were recorded on a Bruker AC 200-MHz and an Avance 500-MHz spectrometer with DMSO-*d6* or $CDCl_3$ as a solvent and tetramethylsilane (TMS) as an internal

standard (chemical shift in δ, ppm). Mass spectra were determined using a GCMS-QP1000EX Shimadzu spectrometer with an ionization energy of 70 eV. Elemental analyses were determined using Heraeus and Vario EL-III (Elemntar; Germany) CHNS analyzers at the Microanalytical Center, Faculty of Science, University of Cairo. All results of elemental analyses corresponded to the calculated values within experimental error. TLC was performed on silica gel G (Fluka) and spots were visualized by iodine vapors or irradiation with UV light (254 nm). All chemicals were purchased from Sigma Aldrich.

3-(4-(Benzyloxy)phenyl)-1-(4-substituted phenyl)prop-2-en-1-ones (**1–4**)

The key chalcone intermediates **(1–4)** were synthesized through base-catalyzed Claisen–Schmidt condensation of equimolar amounts of acetophenone and its derivatives with 4-benzyloxybenzaldehyde by stirring the reactants in an aqueous ethanolic solution containing 20% NaOH at room temperature for 24 h in accordance with the method described in the literature (Li *et al.,* 2005; Robinson *et al.,* 2005).

General procedure for synthesis of 5-(4-(benzyloxy)phenyl)-3-(4-substituted phenyl)-1-phenyl-4,5-dihydro-1H-pyrazoles (**5–8**)

A mixture of the corresponding chalcone (**1–4**; 0.01 mol), phenyl hydrazine (0.01 mol), and NaOH (0.025 mol) was refluxed in ethanol for 4 h. The mixture was cooled and the precipitated product was filtered, washed, and crystallized from dioxane/H_2O

*5-(4-(Benzyloxy)phenyl)-1,3-diphenyl-4,5-dihydro-1H-pyrazole (**5**)*

Yield, 50%; m.p., 135–136°C. ^{1}H NMR (500 MHz, DMSO-d6): $\delta = 3.05$–3.10 (two d, $J = 6.3$, 6.35 Hz, 1H, C_4–H of pyrazole), 3.85–3.91 (two d, $J = 12.15$, 12.15 Hz, 1H, C_4–H of pyrazole), 5.05 (s, 2H, benzylic H), 5.41–5.45 (two d, $J = 6.3$, 6.25 Hz, 1H, C_5–H of pyrazole), 6.70–7.76 (m, 19H, ArH) ppm. ^{13}C NMR (DMSO): $\delta = 157.61$ (C–O), 147.14 (C = N, pyrazole), 144.24 (N–C) 136.99–112.99 (phenyl-C), 69.19 (OCH_2), 62.63 (CH of pyrazole), 42.95(CH_2 of pyrazole) ppm. Analysis for $C_{28}H_{24}N_2O$. Calcd: C, 83.14; H, 5.98; N, 6.93. Found: C, 83.29; H, 5.81; N, 7.00.

*5-(4-(Benzyloxy)phenyl)-3-(4-bromophenyl)-1-phenyl-4,5-dihydro-1H-pyrazole (**6**)*

Yield, 65%; m.p., 158–159°C. ^{1}H NMR (500 MHz,DMSO-d6): $\delta = 3.08$–3.13 (two d, 1H, $J = 7.2$, 7.25 Hz, C_4–H of pyrazole), 3.77–3.83 (two d, $J = 12.35$, 12.35 Hz, 1H, C_4–H of pyrazole), 5.05 (s, 2H, benzylic H), 5.25–5.29 (two d, $J = 7.2$, 7.15 Hz, 1H, C_5–H of pyrazole), 6.84–7.61 (m, 18H, ArH) ppm. EI (70 eV) m/z (%): (M^++1, 485, 17.4), (M^+, 484, 70.6), 391 (15.6), 298 (13.6), 91 (100.0). Analysis for $C_{28}H_{23}BrN_2O$. Calcd: C, 69.57; H, 4.80; N, 5.80. Found: C, 69.74; H, 4.82; N, 5.58.

*5-(4-(Benzyloxy)phenyl)-3-(4-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazole (**7**)*

Yield, 59%; m.p., 148–149°C. ^{1}H NMR (500 MHz,$CDCl_3$): $\delta = 3.08$–3.13 (two d, $J = 7.2, 7.2$ Hz, 1H, C_4–H of pyrazole), 3.77–3.83 (two d, $J = 12.3, 12.35$ Hz, 1H, C_4–H of pyrazole), 5.05 (s, 2H, benzylic H), 5.26–5.30 (two d, $J = 7.2, 7.15$ Hz, 1H, 5-pyrazole H), 6.76–7.88 (m, 18H, ArH) ppm. ^{13}C NMR ($CDC1_3$): $\delta = 158.28$ (C–O), 145.54 (C = N, pyrazole), 144.62 (N–C) 136.86–104.62 (phenyl–C), 70.07 (OCH_2), 64.11 (CH of pyrazole), 43.46 (CH_2 of pyrazole) ppm. Analysis for $C_{28}H_{23}ClN_2O$. Calcd: C, 76.61; H, 5.28; N, 6.38. Found: C, 76.74; H, 5.14; N, 6.18.

5-(4-(Benzyloxy)phenyl)-1-phenyl-3-(4-methylphenyl)-4,5-dihydro-1H-pyrazole (8)

Yield, 60%; m.p., 154–155°C. IR (KBr, cm^{-1}): $\upsilon = 3026$ (CH, aromatic), 2916 (CH, aliphatic), 1596 (C = N), 1510 (C = C) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d6): $\delta = 2.34$ (s, 3H, CH_3), 3.03–3.08 (two d, $J = 6.3, 6.35$ Hz, 1H, C_4–H of pyrazole), 3.83–3.88 (two d, $J = 12.05, 12.15$ Hz, 1H, C_4–H of pyrazole), 5.05 (s, 2H, benzylic H), 5.37–5.41(two d, $J = 6.35, 6.3$ Hz, 1H, C_5–H of pyrazole), 6.69–7.65 (m, 18H, ArH) ppm. ^{13}C NMR (DMSO) $\delta = 157.59$ (C–O), 147.27 (C = N, pyrazole), 144.39 (N–C) 138.24–112.93 (phenyl–C), 69.19 (OCH_2), 62.57 (CH of pyrazole), 43.07 (CH_2 of pyrazole), 20.91 (CH_3) ppm. Analysis for $C_{29}H_{26}N_2O$. Calcd: C, 83.22; H, 6.26; N, 6.69. Found: C, 83.79; H, 6.36; N, 6.43.

General procedure for synthesis of 5-(4-(benzyloxy)phenyl)-3-(4-substituted phenyl)-4,5-dihydroisoxazole (**9–12**)

A mixture of the corresponding chalcone (**1–4**; 0.01 mol), hydroxylamine HCl (0.01 mol), and NaOH (0.025 mol) was refluxed in ethanol for 8 h. The mixture was cooled and the precipitated product was filtered, washed, and crystallized from an appropriate solvent.

5-(4-(Benzyloxy)phenyl)-3-phenyl-4,5-dihydroisoxazole (9)

Yield, 56%; m.p., 143–144°C; crystallized from ethanol. ^{1}H NMR (200 MHz, DMSO-d6): $\delta = 3.31$–3.39 (two d, $J = 3.2, 3.25$ Hz, 1H, C_4–H of dihydroisoxazole), 3.71–3.79 (two d, $J = 6, 6.12$ Hz, 1H, C_4–H of dihydroisoxazole), 5.10 (s, 2H, benzylic H), 5.38–5.48 (trip, 1H, C_5–H of dihydroisoxazole), 7.00–7.64 (m, 14H, ArH) ppm. EI (70 eV) m/z (%): (M^++1, 330, 2.24), (M^+, 329, 8.59), 281 (2.6), 256 (6.85), 57 (100). Analysis for $C_{22}H_{19}NO_2$. Calcd: C, 80.22; H, 5.81; N, 4.25. Found: C, 80.05; H, 6.20; N, 4.15.

5-(4-(Benzyloxy)phenyl)-3-(4-bromophenyl)-4,5-dihydroisoxazole (10)

Yield, 63%; m.p., 197–198°C; crystallized from dioxane/H_2O. ^{1}H NMR (200 MHz, DMSO-d6): $\delta = 3.32$–3.41 (two d, $J = 4.2, 4$ Hz, 1H, C_4–H of dihydroisoxazole), 3.72–3.86 (two d, $J = 6, 6.15$ Hz, 1H, C_4–H of dihydroisoxazole), 5.11 (s, 2H,

benzylic H), 5.41–5.49 (trip, 1H, C_5–H of dihydroisoxazole), 7.01–7.66 (m, 13H, ArH) ppm. Analysis for $C_{22}H_{18}BrNO_2$. Calcd: C, 64.72; H, 4.44; N, 3.43. Found: C, 64.88; H, 4.94; N, 3.19.

5-(4-(Benzyloxy)phenyl)-3-(4-chlorophenyl)-4,5-dihydroisoxazole (11)

Yield, 62%; m.p., >300°C; crystallized from dioxane/H_2O. ^{1}H NMR (200 MHz, DMSO-d6): $\delta = 3.32$–3.41 (two d, $J = 4.15$, 4.2 Hz, 1H, C_4–H of dihydroisoxazole), 3.72–3.86 (two d, $J = 6$, 6.2 Hz, 1H, C_4–H of dihydroisoxazole), 5.11 (s, 2H, benzylic H), 5.59–5.72 (trip, 1H, C_5–H of dihydroisoxazole), 7.01–7.74 (m, 13H, ArH) ppm. Analysis for $C_{22}H_{18}ClNO_2$, Calcd: C, 72.62; H, 4.99; N, 3.85. Found: C, 73.00; H, 4.50; N, 3.43.

5-(4-(Benzyloxy)phenyl)-3-(4-methylphenyl)-4,5-dihydroisoxazole (12)

Yield, 48%; m.p., 154-155°C; crystallized from ethanol; ^{1}H NMR (200 MHz, DMSO-d6): $\delta = 2.35$ (s, 3H, CH_3), 3.30–3.37 (two d, $J = 3.3$, 3.35 Hz, 1H, C_4–H of dihydroisoxazole), 3.72–3.81 (two d, $J = 6.2$, 6.25 Hz, 1H, C_4–H of dihydroisoxazole), 5.12 (s, 2H, benzylic H), 5.39–5.47 (trip, 1H, C_5–H of dihydroisoxazole), 7.02–7.66 (m, 13H, ArH) ppm. MS EI (70 eV) m/z (%): (M^++1, 344, 0.0), (M^+, 343, 3.83), 284 (2.65), 256 (7.82), 55 (100). Analysis for $C_{23}H_{21}NO_2$. Calcd: C, 80.44; H, 6.16. N, 4.08; Found: C, 80.00; H, 6.36; N, 3.88.

Antiviral activity evaluation

Antiviral assays for compounds ***5–8***

The antiviral activity of the new compounds (**5–8**) was determined using cytopathicity (CPE) assay (Gazivoda *et al.,* 2007; Pauwels *et al.,* 1988) against HSV-1 (KOS), HSV-2 (G), vaccinia virus (VV), VSV, HSV-1 TK^- KOS ACV^r in HEL cell cultures; VSV, coxsackie virus B4, respiratory syncytial virus in HeLa cell cultures; parainfluenza-3 virus, reovirus-1, sindbis virus, coxsackie virus B4, punta toro virus in Vero cell cultures. Stock solutions of the test compounds were prepared in DMSO at a concentration of 10 mg/ml. Cells, grown to confluency in 96-well plates, were infected with 100 $CCID_{50}$ of virus, one $CCID_{50}$ being the 50% cell culture infective dose. After an adsorption period of 2 h at 37°C, virus was removed and serial dilutions of the compounds were added. Cultures were further incubated at 37°C for 3 days, until complete CPE was observed in the infected and untreated virus control. Determination of the anti-HIV activity of the compounds was based on virus-induced cytopathicity (destruction) of HIV-infected MT-4 cells, measured at day 5 post virus infection by the MTT colorimetric method (Pauwels *et al.,* 1988). Results are expressed as the 50% effective concentration (EC_{50}) as reported in Tables 1, 2 and 3. The 50% effective antiviral concentration (EC_{50}) was defined as the compound concentration required to protect 50% of the virus-infected cells against viral cytopathogenicity. The symbol ">" is used to indicate the highest

Table 1 Cytotoxicity and antiviral activity of compounds **5-8** in HEL cell cultures

Compound	Minimum cytotoxic concentration[a] (μg/ml)	EC_{50}^{b} (μg/ml)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK^- KOS ACV^r
5	100	>20	>20	>20	>20	>20
6	100	>20	>20	>20	>20	>20
7	100	>20	>20	>20	>20	>20
8	100	>20	>20	>20	>20	>20
Brivudin (μM)	>250	0.08	126	10	>250	>250
Ribavirin (μM)	>250	>250	>250	146	>250	>250
Cidofovir (μM)	>250	3	5	10	>250	5
Ganciclovir (μM)	>100	0.08	0.08	>100	>100	6

[a] Required to cause a microscopically detectable alteration of normal cell morphology

[b] Required to reduce virus-induced cytopathogenicity by 50%

Table 2 Cytotoxicity and antiviral activity of compounds **5–8** in HeLa cell cultures

Compound	Minimum cytotoxic concentration[a] (μg/ml)	EC_{50}^{b} (μg/ml)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
5	100	>20	>20	>20
6	100	>20	>20	>20
7	≥20	>20	>20	>20
8	100	>20	>20	>20
DS-5000	>100	>100	9	0.8
(*S*)-DHPA (μM)	>250	>250	>250	>250
Ribavirin (μM)	>250	29	146	10

[a] Required to cause a microscopically detectable alteration of normal cell morphology

[b] Required to reduce virus-induced cytopathogenicity by 50%

concentration at which the compounds were tested and found not to be antiviral active.

*Antiviral bioassays for compounds **5–12***

African green monkey kidney-derived cells (Vero cell culture) were propagated in Dulbecco's minimum essential medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic mixture. The pH was adjusted to 7.2–7.4 with a 7.5% sodium bicarbonate solution. HSV-1 and HAV (MBB cell culture adapted strain) were obtained from Environmental Virology Laboratory, Department of Water Pollution Research, National Research Centre, Cairo.

Table 3 Cytotoxicity and antiviral activity of compounds **5–8** in Vero cell cultures

Compound	Minimum cytotoxic concentration (μg/ml)[a]	EC_{50}^{b} (μg/ml)				
		Para-influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
5	100	>20	>20	>20	>20	>20
6	≥20	>20	>20	>20	>20	>20
7	100	>20	>20	>20	>20	>20
8	100	>20	>20	>20	>20	>20
DS-5000	>100	>100	>100	59	>100	100
(*S*)-DHPA (μM)	>250	>250	>250	>250	>250	>250
Ribavirin (μM)	>250	45	>250	>250	>250	146

[a] Required to cause a microscopically detectable alteration of normal cell morphology

[b] Required to reduce virus-induced cytopathogenicity by 50%

Plaque infectivity reduction assay

Preparation of synthetic compounds for bioassay

The tested compounds were dissolved at 100 mg each in 1 ml of 10% DMSO in water. The final concentration was 100 μg/ml (stock solution). The dissolved stock solutions were sterilized by the addition of a 50 μg/ml antibiotic-antimycotic mixture (10,000 U) penicillin G sodium, 10,000 mg streptomycin sulfate, and 250 μg amphotericin B.

Plaque reduction assay

A six-well plate was cultivated with Vero cell culture (10^5 cell/ml) and incubated overnight at 37°C. HSV-1 and HAV were diluted to give a 10^4 plaque forming unit (PFU)/ml final concentration for each virus, mixed with the tested compound at the previous concentration, and incubated overnight at 4°C. Growth medium was removed from the multiwell plate and the virus compound mixture was inoculated (100 μ1/well). After 1 h of contact, the inoculum was aspirated and 3 ml of MEM with 1% agarose was overlaid on cell sheets. Plates were left to solidify and incubated at 37°C until the development of virus plaques. Cell sheets were fixed in 10% formalin solution for 2 h, then stained with crystal violet stain. Control virus and cells were treated identically without chemical compounds. Virus plaques were counted and the percentage reduction was calculated (Tebas *et al.,* 1998).

Cytotoxicity assays

The cytotoxicity of the compounds was evaluated in parallel with their antiviral activity in uninfected cells and is expressed as the minimum cytotoxic concentration (MCC) that causes a microscopically detectable alteration of normal cell morphology (HEL, HeLa, and Vero cells).

Results and discussion

Chemistry

The synthetic route used to synthesize the title compounds is outlined in Scheme 1. The key α,β-unsaturated ketones 3-(4-(benzyloxy)phenyl)-1-(4-substituted phenyl)-2-propen-1-one intermediates (**1–4**) were prepared by base-catalyzed Claisen–Schmidt condensation of the acetophenone and its derivatives with 4-benzyloxy-benzaldehyde in accordance with the method described in the literature (Li *et al.,* 2005; Robinson *et al.,* 2005). In basic media, *N*-phenylpyrazole derivatives (**5–8**) were prepared by heating at reflux the corresponding α,β-unsaturated ketones (**1–4**) with phenyl hydrazine in ethanol for 4 h. 4,5-Dihydroisoxazole derivatives (**9–12**) were obtained by heating at reflux equimolar amounts of hydroxylamine HCl and the corresponding α,β-unsaturated ketones (**1–4**) in hot ethanolic NaOH solution for 8 h. Chemical structures of the target compounds were determined on the basis of spectral data analysis, such as IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry.

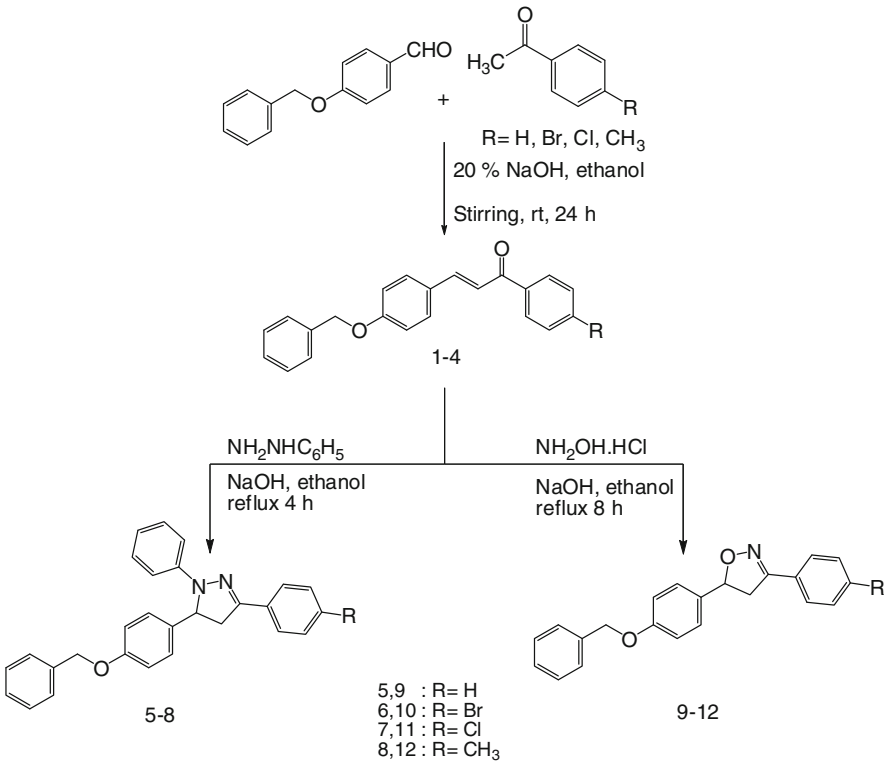
^{1}H NMR of the new 4,5-dihydro-1H-pyrazoles revealed the presence of two peaks at a chemical shift ($\delta = \sim 3.1$ and ~ 3.7) for the two magnetically nonequivalent protons at the 4-position of the pyrazoline ring. The CH proton at C_5 also appears as a doublet of doublets in the region of 5.25–5.45 ppm due to vicinal coupling with two nonmagnetically equivalent geminal protons of the C_4 carbon. The signals obtained from ^{13}C NMR spectra further confirmed the proposed structures; the C_4 and C_5 carbons of the pyrazoline ring resonate at 42.95–43.46 and 62.57–64.11 ppm, respectively. All compounds showed signals at 145–147 ppm, which was assignable to the azomethine carbon of the pyrazoline ring. ^{1}H NMR spectra of the 4,5-dihydroisoxazole derivatives revealed the presence of two peaks at a two different chemical shifts ($\delta = \sim 3.3$ and ~ 3.8) for protons at C_4 and the presence of a peak around $\delta = 5.4$ for CH proton at C_5, confirming the formation of compounds.

Biological evaluation

Compounds **5–8** were evaluated against a group of viruses, namely, HSV-1 KOS, HSV-2 G, VV, VSV, and thymidine kinase-deficient HSV- 1 TK^{-} KOS ACV^{r} in HEL cell cultures; VSV, Coxsackie virus B4, and respiratory syncytial virus in HeLa cell cultures; para-influenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, and Punta Toro virus in Vero cell cultures; and HIV-1 (III_B) and HIV-2 (ROD) in MT-4 cell cultures.

The activities of the compounds were compared with reference antiviral drugs, namely, (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (brivudin), 1-(β-D-ribofuranosyl)-1H-1,2,4-triazole-3-carboxamide (ribavirin), 1-[(*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]-cytosine (cidofovir), 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (ganciclovir), 9-(1,3-dihydroxypropyl)adenine [(*S*)-DHPA], DS-5000, and nevirapine (Tables 1, 2 and 3).

Compounds **5–12** were further evaluated using two viruses, HAV and HSV-1 in Vero cell cultures. The plaque infectivity reduction assay was applied (Tebas *et al.,*



Scheme 1 Synthesis of *N*-phenylpyrazoles (**5–8**) and 4,5-dihydroisoxazoles (**9–12**)

1998). The two known drugs commonly utilized for therapeutic treatments of HAV and HSV-1 are amantadine and acyclovir, respectively. So, these two drugs were considered as references. Results obtained from antiviral evaluation of the tested compounds are listed in Table 4.

According to Tables 1, 2 and 3, compounds **5–8** showed appreciable antiviral activity against HSV-1 KOS, HSV-2 G, VV, VSV, and HSV-1 TK-KOS ACV^r, with an $EC_{50} > 20$ μg/ml, which was more than five times lower than its cytotoxic concentration (100 μg/ml). Regarding HeLa cell cultures, the active concentration of compound **7** equaled its cytotoxic concentration (≥20 μg/ml) therefore it is considered inactive. In Vero cell cultures compound **6** was active at a concentration which caused microscopically detectable alteration of normal cell morphology at concentrations ≥20 μg/ml, which is equal to its EC_{50}. In MT-4 cell cultures, unfortunately, none of the compounds showed activity against HIV-1 (IIIB) or HIV-2 (ROD).

The results presented in Table 4 reveal the following.

1. 4,5-Dihydroisoxazole **12** showed the best anti-HSV-1 activity compared with the other tested compounds, especially at a concentration of 20 μg/ml (79% inhibition).

Table 4 Cytotoxicity and antiviral activity of compounds **5–12** in Vero cell cultures

Compound	Minimum cytotoxic concentration (μg/ml)[a]	Percentage reduction			
		HSV-1		HAV	
		10 μg/ml	20 μg/ml	10 μg/ml	20 μg/ml
5	100	0	30	7	23
6	≥20	13.3	50	2	16
7	100	19	43	0	21
8	100	31	58	14	39
9	>100	3	15	0	2
10	≥20	26.1	**67**	7.3	21
11	>100	38	**60**	10	**58**
12	>100	45	**79**	27	46

[a] Required to cause a microscopically detectable alteration of normal cell morphology

2. 4,5-Dihydroisoxazoles **10** and **11** exhibited moderate activity toward HSV-1 (67% and 60% inhibition, respectively); compound **10** was cytotoxic at the active concentration 20 μg/ml, therefore, it is considered as inactive.
3. Compound (**11**) exerted the highest activity toward HAV (58% inhibition) compared with the other tested compounds.
4. Other compounds tested showed weak to moderate activity, especially at a concentration of 20 μg/ml.

The structure–activity relationship of the 4,5-dihydroisoxazole derivatives reveals the following:

- Substitution at the phenyl ring at C_3 with an electron donating group increases the activity toward HSV-1 as in compound **12**.
- Substitution at the same position with electron withdrawing groups slightly decreases the activity against HSV-1 and increases the activity toward HAV as in the case of compound **11** but may produce cytotoxic compounds as in the case of 5-(4-(benzyloxy)phenyl)-3-(4-bromophenyl)-4,5-dihydroisoxazole **(10)**, which showed cytotoxicity to Vero cell cultures at a concentration ≥20 μg/ml.
- Nonsubstituted phenyl derivatives showed weak activity toward HSV-1 as well as HAV. It would therefore be of interest to further explore other substituents on the phenyl ring in the *ortho, meta*, and *para* positions to reveal whether antiviral activity can be further improved.
- The benzyloxyphenyl-substituted dihydroisoxazole ring is essential for the activity, which matches with the aforementioned compound **B** (Zeng *et al.,* 2008).

Conclusion

In summary, eight new derivatives of 4,5-dihydro-1H-pyrazole and 4,5-dihydroisoxazole were synthesized and exhibited a range of significant antiviral activities,

whereas compounds **11** [5-(4-(benzyloxy)phenyl)-3-(4-chlorophenyl)-4,5-dihydroisoxazole] and **12** [5-(4-(benzyloxy)phenyl)-3-(4-methylphenyl)-4,5-dihydroisoxazole] exhibited the best activity against HAV and HSV-1, respectively. Three derivatives, 8, 11, and 12, did not show toxicity to any of the tested cell cultures. Thus, these derivatives may possibly be used as lead compounds for developing new antiviral agents. The mechanism of antiviral activity is unknown and requires further study.

References

Gazivoda T, Raic′-Malic′ S, Marjanovic′ M, Kralj M, Pavelic′ K, Balzarini J, De Clercq E, Mintas M (2007) The novel C-5 aryl, alkenyl, and alkynyl substituted uracil derivatives of L-ascorbic acid: synthesis, cytostatic, and antiviral activity evaluations. Bioorg Med Chem 15:749

Gudmundsson KS, Johns BA, Allen SH (2008) Pyrazolopyridines with potent activity against herpesviruses: effects of C5 substituents on antiviral activity. Bioorg Med Chem Lett 18:1157

Hashem AI, Youssef ASA, Kandeel KA, Abou-Elmagd WSI (2007) Conversion of some 2(3H)-furanones bearing a pyrazolyl group into other heterocyclic systems with a study of their antiviral activity. Eur J Med Chem 42:934

Li W-J, Lin X-F, Wang J, Li G-L, Wang Y-G (2005) Palladium-catalyzed michael addition of indoles to α, β-unsaturated ketones in an ionic liquid. Synlett 13:2003

Pauwels R (2004) New non-nucleoside reverse transcriptase inhibitors (NNRTIs) in development for the treatment of HIV infections. Curr Opin Pharmacol 4:437

Pauwels R, Balzarini J, Baba M, Snoeck R, Schols D, Herdewijn P, Desmyter J, De Clercq E (1988) Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. J Virol Methods 20:309

Rashad E, Hegab MI, Abdel-Megeid RE, Micky JA, Abdel-Megeid FM (2008) Synthesis and antiviral evaluation of some new pyrazole and fused pyrazolopyrimidine derivatives. Bioorg Med Chem 16:7102

Robinson TP, Hubbard RB, Ehlers TJ, Arbiser JL, Goldsmith DJ, Bowen JP (2005) Synthesis and biological evaluation of aromatic enones related to curcumin. Bioorg Med Chem 13:4007

Rose JK, Whitt MA (2007) Rhabdoviridae: the viruses and their replication. In: Knipe DM, Howley PM, Griffin DE (eds) Field's virology, 5th edn. Lippincott Williams & Wilkins, Philadelphia

Tebas P, Scholl D, Jollick J, McHarg K, Arens M, Olivo PD (1998) A rapid assay to screen for drug-resistant herpes simplex virus. J Infect Dis 177:217

Vento S (2000) Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis. C. J Viral Hepat 7:7

Whitley RJ, Roizman B (2001) Herpes simplex virus infections. Lancet 357:1513

Zeng LF, Zhang HS, Wang YH, Sanchez T, Zheng YT, Neamati N, Long YQ (2008) Efficient synthesis and utilization of phenyl-substituted heteroaromatic carboxylic acids as aryl diketo acid isosteres in the design of novel HIV-1 integrase inhibitors. Bioorg Med Chem Lett 18:4521