Nitroimidazoles Part 7. Synthesis and Anti-HIV Activity of New 4-Nitroimidazole Derivatives

Najim A. Al-Masoudi[a], Wolfgang Pfleiderer[b] and Christophe Pannecouque[c]

[a] Department of Chemistry, College of Science, University of Basrah, Basrah, Iraq
[b] Fachbereich Chemie, Universität Konstanz, 78457 Konstanz, Germany
[c] Rega Institute for Medical Research, Katholieke Universiteit, B-3000 Leuven, Belgium

Reprint requests to Prof. Dr. N. A. Al-Masoudi. E-mail: najim.al-masoudi@gmx.de

Z. Naturforsch. **2012**, *67b*, 835 – 842 / DOI: 10.5560/ZNB.2012-0122
Received May 6, 2012

Reverse transcriptase enzyme (RT) is an attractive target for the development of new drugs useful in AIDS therapy and HIV non-nucleoside reverse transcriptase inhibitors (NNRTIs), and offers the possibility of generating structures of increased potency. On this basis, a series of 4-oxo-3-phenyl-2-(phenylimino)thiazolidin-5-ylidene, 3-hydroxypropyl, 3-azidopropyl, and 3-aminopropyl derivatives of 1-benzyl-2-ethyl-4-nitroimidazoles **6**–**8**, as well as the substituted 1,2,3-triazolo analogs **12**–**14**, the diazepam **15** and carboxamide derivatives **16** and **17** were synthesized. All compounds have been evaluated for their anti-HIV activity.

Key words: Anti-HIV Activity, Nitroimidazoles, NNRTIs, Piperazine Derivatives

Introduction

Since the time of their discovery, non-nucleoside reverse transcriptase inhibitors (NNRTIs) have been particularly attractive targets due to their low cytotoxicity and high selectivity. Three NNRTIs, nevirapine [1], delaviridine [2, 3] and efavirenz [4], have been approved by the Food and Drug Administration (FDA) for the treatment of HIV infection. However, the rapid emergence of resistant mutants against the NNRTIs allowed only a few compounds to reach the stage of clinical trials [5]. For this reason RT remains a central target in the development of anti-HIV-1 drugs and new classes of NNRTIs having high potency as well as being effective against resistant mutants [6, 7].

Several potent heterocyclic NNRTIs have been synthesized with high anti-HIV inhibitory activity, some of which have an imidazole scaffold (for example, capravirine; S-1153, **1**) (Fig. 1) [8]. Moreover, numerous examples of nitroimidazoles have been reported with a range of biological activities, including antibacterial agents [9 – 12], potential radiosensitizers [13], anticancer agents [14] such as Dacarbazine® (DTIC) [15] and misonidazole [16], fungicides and/or antiprotozoal agents such as clotrinazole [1-(2-chlorotrityl)-1*H*-imidazole] [17], metronidazole (Flagyl) [18, 19] and 2-styryl-5-nitroimidazoles [20, 21].

Some arylpiperazine derivatives possess enterovirus activity [22, 23], such as atevirdine (**2**) (Fig. 1) [24] and vicriviroc, which are currently in Phase II clinical trials [25].

This work is a continuation of our investigation in the field of 4-nitroimidazoles [26 – 31], some of which represent a prototype with remarkable anti-HIV activity [27, 31]. Other efforts have focused on the development of new inhibitors based on novel scaffolds by synthesis of new 4-nitroimidazoles bearing spacer-piperazine residues at C-5 as new templates that might lead to the opimization of HIV-1 RT inhibitory activity.

Results and Discussion

The potency of thiazolidine derivatives as a new family of antiviral agents acting as NNRTIs with minimal cytotoxicity [32 – 36] has prompted us to synthesize new models of nitroimidazoles carrying a 1,3-thiazolidin-4-one moiety attached to the potent piperazine molecule. In the present work, compound **3** has been selected as a starting material for the synthesis of new substituted imidazole analogs *via* the nucleophilic displacement of the bromine atom ac-

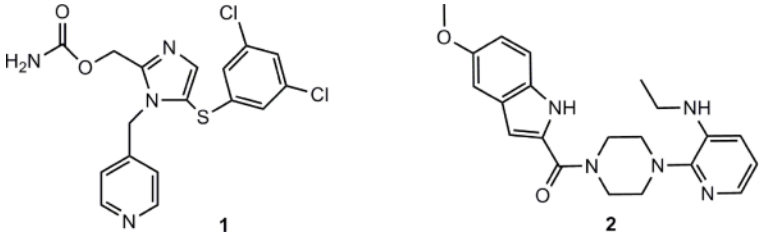
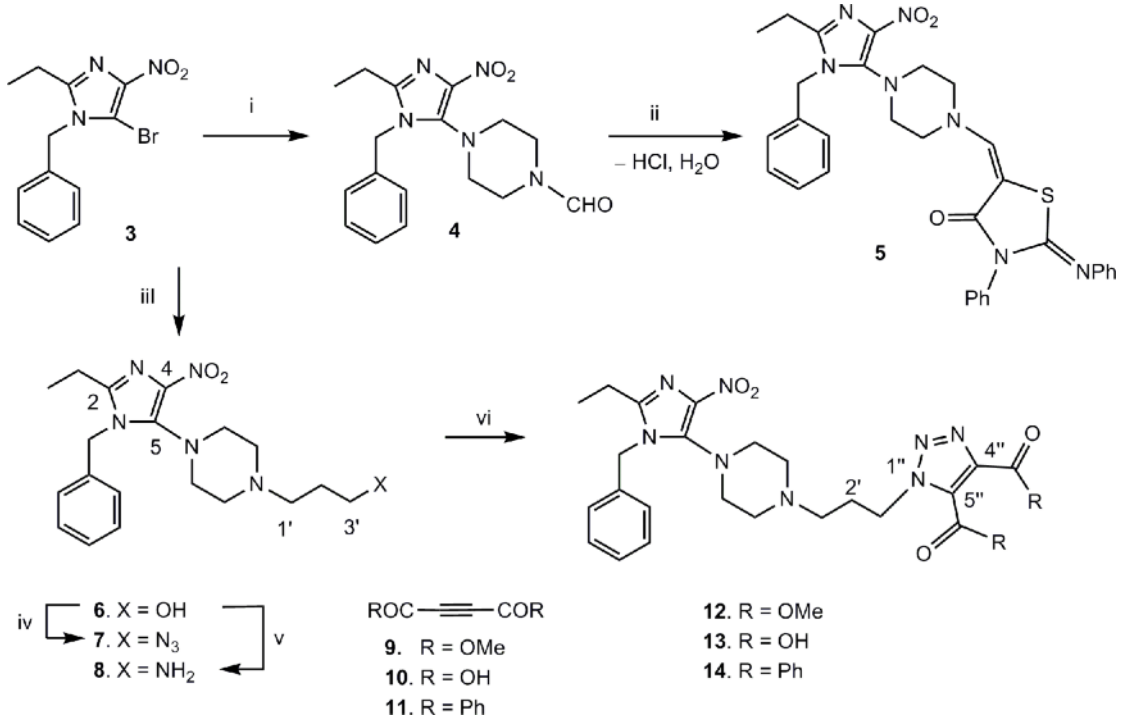


Fig. 1. Chemical structures of capravirine (**1**) and ateviridine (**2**).

tivated by an adjacent nitro function. Treatment of **3** with 1-formylpiperazine afforded the piperazine-1-carbaldehyde **4** (85 %) [29], which gave **5** in 59 % yield, on reaction with chloroacetic acid and 1,3-diphenylthiourea under microwave irradiation at 110 °C for 20 min (Scheme 1).

The structure of **5** was identified by ^{1}H and ^{13}C NMR, and mass spectra. In the ^{1}H NMR spectrum of **5**, resonances of the piperazine moiety appeared as two broad singlets at $\delta = 3.69$ and 2.96 ppm, the signal of the benzylic protons at $\delta = 5.20$ ppm. The singlet at $\delta = 6.26$ ppm was attributed to the olefinic proton (HC=C(1)$_{\text{thiazolidine}}$). The ^{13}C NMR spectrum of **5** showed a signal at $\delta = 165.7$ ppm, attributed to a carbonyl group. The carbon atoms C-2 and C-5 of the thiazolidine ring resonated at $\delta = 159.2$ and 101.1 ppm, respectively, while the olefinic carbon (C=C(5)$_{\text{thiazolidine}}$) appeared at $\delta = 154.9$ ppm. The piperazine carbon atoms resonated at $\delta = 49.9$ and 46.0 ppm, whereas the signals of C-2, C-4 and C-5 of the imidazole ring resonated at $\delta = 141.3$, 136.1 and 138.1 ppm, respectively. In the gradient-selected HMBC [37] spectrum of **5**, the olefinic proton at $\delta = 6.26$ ppm showed a $^{2}J_{\text{C,H}}$ coupling with C(4)$_{\text{thiazolidine}}$ at $\delta = 101.1$ ppm, in addition to a $^{3}J_{\text{C,H}}$ coupling with C(4)$_{\text{thiazolidine}}$ (C=O) at $\delta = 165.7$ ppm.



Scheme 1. Conditions and reagents. (i) 1-Formylpiperazine, 80 °C, 6 h; (ii) $ClCH_2CO_2H$, 1,3-diphenylthiourea, MWI, 20 min; (iii) 1-(3-hyroxypropyl)piperazine, DMF, K_2CO_3, KI, 120 °C, 6 h; (iv) Ph_3P, bromotrichloromethane, toluene, reflux, 3 h; (v) Ph_3P, CCl_4-DMF (1 : 4), 90 °C, 5 h; (vi) **9**, **10** or **11**, toluene, 100 °C, 5 h.

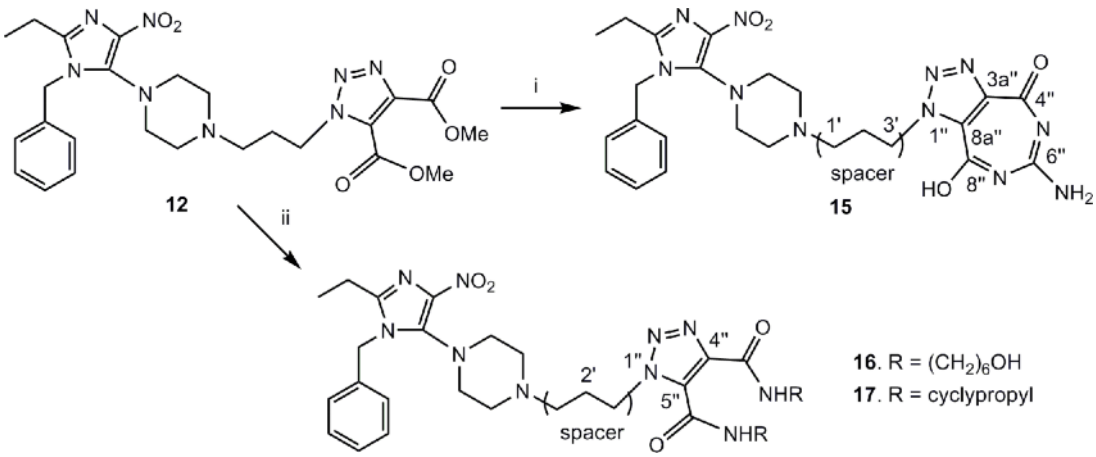
Further, other models of imidazole derivatives bearing substituted piperazines were prepared. Thus, treatment of **3** with 1-(3-hydroxypropyl)piperazine in the presence of K_2CO_3 at 120 °C furnished **6** in 67 % yield. A facile and one-pot conversion of alcohol **6** into azide **7** (65 %) was employed, following the Koziara's method [38], by treatment with Ph_3P and bromotrichloromethane. By following the one-pot methodology of Reddy *et al.* [39], the alcohol **6** afforded smoothly the amine **8** (78 %), on treatment with NaN_3 and two equivalents of PPh_3 in CCl_4-DMF (1 : 4) at 90 °C. Alternatively, amine **8** had been prepared previously in our laboratory by hydrazinolysis of the phthalimide analog (*N*-[3-[4-(1-benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-yl)piperazin-1-yl]propyl]phthalimide) [24] (Scheme 1).

The structures of **6–8** were determined from their NMR (^{1}H, ^{13}C) and mass spectra, since they showed a similar pattern of the imidazole moiety and the aromatic protons (*cf.* Experimental Section). Compound **7** has been selected for further NMR studies, and the HMBC spectrum revealed a $^2J_{C,H}$ coupling between the methylene protons (CH_2-1′) at $\delta = 2.43$ ppm and C-2′ at $\delta = 27.2$ ppm, as well as the carbons of piperazine at $\delta = 50.1$ ppm. Additionally, a $^3J_{C,H}$ coupling between C-1′ at $\delta = 47.2$ ppm and the CH_2-3′ at $\delta = 1.47$ ppm was observed.

Treatment of the azide **7** with three alkynes – dimethyl acetylenedicarboxylate (DMAD) (**9**), acetylenedicarboxylic acid (**10**) and 1,4-diphenylbut-2-yne-1,4-dione (**11**) – in refluxing toluene led to the 1,3-dipolar cycloaddition products **12–14** in 52, 49 and 65 % yield, respectively (Scheme 1).

The structures of **12–14** were assigned on the basis of their ^{1}H, ^{13}C NMR and mass spectra which showed a similar pattern of the imidazole unit and the aromatic protons. In the ^{1}H NMR spectra of **12–14**, CH_2-1′ and CH_2-2′ protons appeared as a multiplet in the region $\delta = 2.38 - 2.45$ ppm, while the CH_2-3′ protons resonated at $\delta = 4.32 - 5.12$ ppm. The ^{13}C NMR spectra of **12–14** revealed signals at $\delta = 53.2$–53.9, 27.0–27.2 and 45.2 – 46.3 ppm, which were attributed to C-1′, C-3′ and C-2′, respectively. The proton system ($N_{triazole}CH_2CH_2CH_2$) was further identified from the DFQ-COSY spectra [40].

Next, the triazole **12** was selected for further treatment leading to a new cyclized product having a spacer. In the present work, we have selected a spacer of three carbon atoms due to the fact that the mutual position of two pharmacophoric elements in flexible biologically active molecules depends on the spacer conformation. This is true even for a two-atomic chain as a spacer [41]. Thus, reaction of **12** with guanidine, generated freely in the presence of NaOMe, at room temperature followed by neutralization with 1 N HCl gave the crude product, which was purified by chromatography to give pure 1,2,3-triazolo-diazepam **15** in 42 % yield (Scheme 2). The structure of **15** was established by ^{1}H, ^{13}C, and HMBC NMR and mass spectral data. In the ^{13}C NMR spectrum, carbon atoms C=O, C-6 and C-8 of the diazepam ring resonated at $\delta =$



Scheme 2. Conditions and reagents. (i) Guanidine·HCl, NaOMe, then stirring at r. t., 48 h; (ii) 6-amino-1-hexanol or cyclopropylamine, r. t., 18 h.

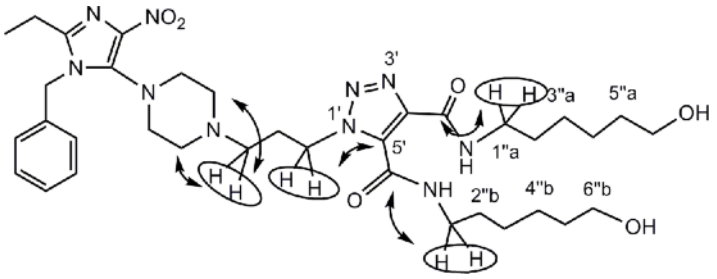


Fig. 2. $J_{C,H}$ correlations in the HMBC NMR spectrum of **16**.

190.2, 164.1 and 158.1 ppm, respectively, while C-3a″ and C-8a″ of the diazepam ring appeared at $\delta = 133.9$ and 139.4 ppm, respectively. In the gradient-selected HMBC spectrum [34] of **15**, the CH_2-3′ methylene protons at $\delta = 4.39$ ppm showed a $^3J_{C,H}$ coupling to C-5″ of the triazole ring at $\delta = 139.2$ ppm. It is expected that several tautomeric structures are possible for **15**, but only one of them is shown in Scheme 2.

Other amide derivatives having a spacer were prepared. Compound **12** was converted to the carboxamide derivatives **16** and **17** in 78 and 63 % yield, respectively, by direct treatment at room temperature with 1-amino-6-hexanol and cyclopropylamine (Scheme 2).

The structures of **16** and **17** were determined on the basis of their ^{1}H, ^{13}C NMR and mass spectra, since they showed similar patterns of the imidazole unit and the aromatic protons (*cf.* Experimental Section). Compound **16** was selected for further NMR (DFQ-COSY) [40] spectroscopic studies. The multiplets of $2\times CH_2$OH and $2\times$ NCH_2-1″ of the bis(6-hydroxyhexyl)carboxamide substituents at $\delta = 3.26-3.36$ ppm were correlated to the singlets at $\delta = 38.5$ and 60.2 ppm, repectively. The signals of (CH_2-2″a,b), and (CH_2-5″a,b) were found as multiplets at $\delta = 1.28$ and $1.40-1.53$ ppm, respectively, and correlated with the singlets at $\delta_C = 25.7$, 24.8, 28.4, and 32.0 ppm, respectively. The gradient HMBC spectrum of **16** showed a $^3J_{CH}$ coupling beween C=O at $\delta = 160.6$ and 156.8 ppm with NCH_2-1″ at $\delta = 3.26$ ppm. Additionally, a $^3J_{C,H}$ coupling between the methylene protons (CH_2-3′) at $\delta = 4.36$ ppm and C-5″ of the triazole ring at $\delta = 147.4$ ppm was observed (Fig. 2).

In-vitro anti-HIV assay

Compounds **5–7** and **12–17** were tested for their *in vitro* anti-HIV-1 (strain IIIB) and HIV-2 (strain ROD) activity in human T-lymphocyte (MT-4) cells. based on an MTT assay [42]. The results are summarized in Table 1, in which the data for nevirapine (BOE/BIRG587) [43] and azidothymidine (DDN/AZT) [44] were included for comparison. Compounds **13** and **15** were found to be the only compounds in the series inhibiting HIV-1 replication in cell cultures with EC_{50} of $> 4.11\ \mu g\,mL^{-1}$ and > 2.26 of a CC_{50} of 4.11 and 31.85 $\mu g\,mL^{-1}$, resulting in a selectivity index of < 1 and 14, respectively.

Table 1. *In-vitro* anti-HIV-1[a] and HIV-2[b] activity of new 4-nitroimidazoles **5–7** and **12–17**.

Compound	Virus strain	EC_{50} ($\mu g\,mL^{-1}$)[c]	CC_{50} ($\mu g\,mL^{-1}$)[d]	SI[e]
5	III_B	> 27.23	27.23	< 1
	ROD	> 27.23	27.23	< 1
6	III_B	> 66.41	66.41	< 1
	ROD	> 66.41	66.41	< 1
7	III_B	> 54.38	54.38	< 1
	ROD	> 54.38	54.38	< 1
12	III_B	> 79.45	79.45	< 1
	ROD	> 79.45	79.45	< 1
13	III_B	> 2.11	2.11	< 1
	ROD	> 2.11	2.11	< 1
14	III_B	> 60.26	60.26	< 1
	ROD	> 60.26	60.26	< 1
15	III_B	> 2.26	31.85	14
	ROD	> 2.26	2.26	< 1
16	III_B	> 8.02	8.02	< 1
	ROD	> 8.02	8.02	< 1
17	III_B	> 75.22	75.22	< 1
	ROD	> 75.22	75.22	< 1
AZT	III_B	0.0022	> 25	> 11 363
	ROD	0.00094	> 25	> 26 596
Nevirapine	III_B	0.050	> 4.00	> 80
	ROD	> 4.00	> 4.00	< 1

[a] Anti-HIV-1 activity measured with strain III_B; [b] anti-HIV-2 activity measured with strain ROD; [c] compound concentration required to achieve 50 % protection of MT-4 cells from the HIV-1- and 2-induced cytopathogenic effect; [d] compound concentration that reduces the viability of mock-infected MT-4 cells by 50 %; [e] SI: selectivity index (CC_{50}/EC_{50}).

In conclusion, the structure-activity relationship (SAR) suggested that the compounds with the 5-

piperazino-nitroimidazole backbone bearing spacer carbon atoms and carrying a diazepam moiety showed higher activity than those of the corresponding substituted derivatives bearing a 1,2,4-triazole ring alone. However, the anti-HIV activity and the selectivity of these compounds are too limited to perform extensive mode-of-action studies. Compound **15** might be considered as a new lead in the development of antiviral agents as a non-nucleoside reverse transcriptase inhibitor.

Experimental Section

General

Melting points were measured on a Büchi melting point apparatus B-545 (Büchi Labortechnik AG, Switzerland) and are uncorrected. Microanalytical data were obtained with a Vario Elemental apparatus (Shimadzu, Japan). NMR spectra were recorded on 400 and 600 MHz (^{1}H) and on 150.91 MHz (^{13}C) spectrometers (Bruker, Germany) with TMS as internal standard and on the δ scale in ppm. Signal assignments for protons were performed by selective proton decoupling or by COSY spectra. Heteronuclear assignments were verified by ^{1}H-^{13}C COSY, or HMBC experiments. Mass spectra (EI, 70 eV, and FAB) were recorded on MAT 8200 spectrometers (Finnegan MAT, USA). TLC plates 60 F254 were purchased from Merck, Germany.

*(4-(1-Benzyl)-2-ethyl-4-nitro-1H-imidazol-5-yl)piperazine-1-carbaldehyde (**4**)*

This compound was prepared according to the procedure previously described [29] from **3** (618 mg, 2.00 mmol) and 1-formylpiperazine (274 mg, 2.40 mmol). Yield: 570 mg (83 %); m. p. and mixed m. p. 296–299 °C.

*(5-((4-(1-Benzyl)-2-ethyl-4-nitro-1H-imidazol-5-yl)piperazin-1-yl)methylene)-3-phenyl-2-(phenylimino)thiazolidin-4-one (**5**)*

Aldehyde **4** (344 mg, 1.00 mmol), chloroacetic acid (151 mg, 1.6 mmol), and 1,3-diphenylthiourea (228 mg, 1.00 mmol) were placed in a cylindrical quartz tube (∅ = 1.5 cm). Then the tube was introduced into the Synthwave® 402 Prolabo microwave reactor and irradiated at 110 °C for 20 min. The microwave reactor was monitored by a computer which allowed the temperature of the reaction mixture to be adjusted. After cooling, the mixture was extracted with CH_2Cl_2, filtered, and evaporated to dryness. Recrystallization from EtOH gave **5** (350 mg, 59 %); m. p. 231–234 °C (dec.). – ^{1}H NMR ($CDCl_3$): δ = 7.47–7.04 (m, 15H, Ar-H), 6.26 (s, 1H, *H*C=C$_{thiazolidine}$), 5.20 (s, 2H, *CH*$_2$Ph), 3.69, 2.96 (2 × br s., 8H, H$_{piperazine}$), 2.56 (q, 2H, J = 7.4 Hz, *CH*$_2$CH$_3$), 1.13 (t, 3H, CH$_2$*CH*$_3$). – ^{13}C NMR ($CDCl_3$): δ = 165.7 (C(4)$_{thiazolidine}$ =O), 159.2 (C(2)$_{thiazolidine}$), 154.9 (*C*=C(5)$_{thiazolidine}$), 146.3 (=N-C)(1)$_{arom}$); 141.3 (C-2); 138.1 (C-5), 136.1 (C-4 + CH$_2$-*C(1)*$_{arom}$), 132.0–122.3 (16 × C$_{arom}$), 101.1 (C(5)$_{thiazolidine}$), 49.9, 46.0 (C$_{piperazine}$), 44.1 (*C*H$_2$Ph), 20.8 (*C*H$_2$CH$_3$), 11.7 (CH$_2$*C*H$_3$). – MS ((+)-FAB): m/z = 593 [M]$^+$. – $C_{32}H_{31}N_7O_3S$ (593.70): calcd. C 64.74, H 5.26, N 16.51; found C 64.49, H 5.11, N 16.68.

*(3-(4-(1-Benzyl)-2-ethyl-4-nitro-1H-imidazol-5-yl)piperazin-1-yl)propanol (**6**)*

A mixture of **3** (309 mg, 1.00 mmol), 1-(3-hydroxypropyl)piperazine (2.00 mmol), anhydrous K_2CO_3 (415 mg, 3.00 mmol), and a catalytic amount of KI in DMF (15 mL) was heated, with stirring, at 120 °C for 6 h. The solvent was evaporated, and the residue was purified by column chromatography (eluting with $CHCl_3$-MeOH 95:5) to give **6** (208 mg, 67 %); m. p. 135–138 °C. – ^{1}H NMR ($CDCl_3$): δ = 7.36–7.18 (m, 5H, Ar-H), 5.18 (s, 2H, *CH*$_2$Ph), 3.69, 3.01 (2 × br s., 8H, H$_{piperazine}$), 2.58 (q, 2H, J = 7.0 Hz, *CH*$_2$CH$_3$), 3.42 (t, 2H, J = 7.0 Hz, CH$_2$-3′), 2.54 (q, 2H, J = 7.4 Hz, *CH*$_2$CH$_3$), 2.29 (d, 2H, J = 7.0 Hz, CH$_2$-1′), 1.51 (m, 2H, CH$_2$-2′), 1.14 (t, 3H, CH$_2$*CH*$_3$). – ^{13}C NMR ($CDCl_3$): δ = 146.2 (C-2); 138.4 (C-5), 136.8 (C-4); 136.1 (CH$_2$-*C(1)*$_{arom}$), 129.1, 128.5, 127.2, 125.2 (C$_{arom}$), 59.2 (C-3′); 56.8 (C-1′)); 46.8 (C$_{piperazine}$), 46.0 (*C*H$_2$Ph); 29.4 (C-2′), 20.9 (*C*H$_2$CH$_3$),11.8 (CH$_2$*C*H$_3$). – MS ((+)-FAB): m/z = 374 [M+H]$^+$. – $C_{19}H_{27}N_5O_3$ (373.45): calcd. C 61.11, H 7.29, N 18.75; found C 60.83, H 7.11, N 18.55.

*(1-(3-Azidopropyl)-4-(1-benzyl)-2-ethyl-4-nitro-1H-imidazol-5-yl)piperazine (**7**)*

A mixture of **6** (373 mg, 1.00 mmol), triphenylphosphane (281 mg, 1.10 mmol), bromotrichloromethane (210 mg, 1.10 mmol), and toluene (20 mL) was refluxed gently with stirring for 3 h. After cooling, NaN_3 (143 mg, 2.20 mmol), tetrabutylammonium bromide (25 mg) and DMF (2 mL) were added, and the mixture was refluxed again with stirring for 6 h. The mixture was then poured into water (40 mL) and extracted with toluene (2 × 10 mL). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated to dryness. The residue was placed on a short silica gel column and eluted, in gradient, with MeOH (0 % – 5 %) and $CHCl_3$ to give **7** (259 mg, 65 %) as an oil. – ^{1}H NMR ($CDCl_3$): δ = 7.32–7.21 (m, 5H, Ar-H), 5.22 (s, 2H, *CH*$_2$Ph), 3.64, 3.20 (2× br s., 8H, H$_{piperazine}$), 2.56 (q, 2H, J = 7.4 Hz, *CH*$_2$CH$_3$), 2.43 (m, 2H, J = 6.4 Hz, CH$_2$-3′), 1.47 (m, 4H, CH$_2$-2′ + CH$_2$-3′); 1.13 (t, 3H, CH$_2$*CH*$_3$). – ^{13}C NMR ($CDCl_3$): δ = 146.7 (C-2); 139.1 (C-4), 137.2 (C-5), 136.8 (CH$_2$-*C(1)*$_{arom}$), 129.2, 128.3, 126.8, 125.8 (C$_{arom}$), 52.8

(C-1′), 50.1 ($C_{piperazine}$), 47.2 (C-3′), 45.5 (CH_2Ph); 27.2 (C-2′), 20.6 (CH_2CH_3), 11.4 (CH_2CH_3). – HRMS ((+)-ESI): $m/z = 398.2169$ (calcd. 398.2187 for $C_{19}H_{26}N_8O_2$, $[M]^+$).

*(3-(1-(Benzyl)-2-ethyl-4-nitro-1H-imidazol-5-yl)piperazin-1-yl)propan-1-amine (**8**)*

A mixture of **6** (373 mg, 1.00 mmol), NaN_3 (78 mg, 1.20 mmol) and PPh_3 (550 mg, 2.10 mmol) in 10 mL of CCl_4-DMF (1:4) was warmed at 90 °C with stirring. After 5 h, the reaction mixture was cooled and quenched by adding 5 mL of water. After stirring for 10 min, the reaction mixture was partitioned between $CHCl_3$ (3 × 20 mL) and water. The combined organic layer was dried (Na_2SO_4), filtered, and the filtrate was concentrated to afford the crude amine, which was purified on a short column of silica gel (eluting with $CHCl_3$-MeOH 95:5) to give the pure amine **8** (291 mg, 78 %) as an oil. All spectroscopic data were identical with those of the same compound prepared previously in our laboratory, *via* an alternative method in 57 % yield [24].

*General procedure for the preparation of 4,5-diacyl-1,2,3-triazoles **12–14***

To a stirred solution of **7** (373 mg, 1.00 mmol) in toluene was added an alkyne **9–11** (1.00 mmol), and the mixture was heated under reflux for 5 h. After cooling, the mixture was filtered and evaporated to dryness. The residue was placed on a short silica gel column (5 g) and eluted, in gradient, with MeOH (0 – 2 %) and $CHCl_3$ to give the pure product.

*Dimethyl 1-(3(4-(1-benzyl-2-ethyl-4-nitro-1H-imidazol-5-yl)piperazin-1-yl)propyl)-1,2,3-triazole-4,5-dicarboxylate (**12**)*

From dimethyl acetylenedicarboxylate (DMAD) (**9**) (142 mg). Yield: 281 mg (52 %); m. p. 164 – 167 °C. – ^{1}H NMR ($CDCl_3$): δ = 7.31–7.19 (m, 5H, Ar-H), 5.27 (s, 2H, CH_2Ph), 4.32 (m, 4H, CH_2-3′), 3.70, 3.41 (2 × s, 6H, 2 × OMe), 3.60, 3.18 (2 × br s., 8H, $H_{piperazine}$), 2.59 (q, 2H, J = 7.4 Hz, CH_2CH_3), 2.44–2.38 (m, 4H, CH_2-1′ + CH_2-2′), 1.13 (t, 3H, CH_2CH_3). – ^{13}C NMR ($CDCl_3$): δ = 159.8 (C=O), 147.1 (C-2), 139.0 (C-5), 137.3 (C-4), 136.6 (CH_2-$C(1)_{arom}$), 129.0, 128.1, 127.0, 126.1 (C_{arom}), 118.8 ($C(4)_{triazole}$), 117.2 ($C(5)_{triazole}$), 53.9 (C-1′), 50.9 ($C_{piperazine}$), 46.3 (C-3′), 45.8 (CH_2Ph), 27.1 (C-2′), 20.4 (CH_2CH_3), 11.3 (CH_2CH_3). – MS ((+)-FAB): m/z = 541 $[M+H]^+$. – $C_{25}H_{32}N_8O_6$ (540.57): calcd. C 55.55, H 5.97, N 20.73; found C 55.29, H 5.79, N 20.85.

*1-(3(4-(1-Benzyl-2-ethyl-4-nitro-1H-imidazol-5-yl)piperazin-1-yl)propyl)-1,2,3-triazole-4,5-dicarboxylic acid (**13**)*

From but-2-ynedioic acid (**10**) (142 mg). Yield: 255 mg (49 %); m. p. 184–187 °C. – ^{1}H NMR ($CDCl_3$): δ = 10.58, 10.38 (2 × br s., 2H, CO_2H),7.29–7.20 (m, 5H, Ar-H), 5.30 (s, 2H, CH_2Ph), 4.38 (t, 2H, J = 7.0 Hz, CH_2-3′), 3.56, 3.21 (2 × br s., 8H, $H_{piperazine}$), 2.62 (q, 2H, J = 7.5 Hz, CH_2CH_3), 2.42–2.38 (m, 4H, CH_2-1′ + CH_2-2′), 1.14 (t, 3H, CH_2CH_3). – ^{13}C NMR ($CDCl_3$): δ = 170.6 (CO_2H), 147.5 (C-2), 140.4 ($C(5)_{triazole}$), 139.1 (C-4), 137.1 (C-5), 136.5 (CH_2-$C(1)_{arom}$), 131.4 ($C(4)_{triazole}$), 128.7, 128.2, 126.7, 125.6 (C_{arom}), 53.2 (C-1′); 50.0 ($C_{piperazine}$), 45.9 (C-3′); 45.4 (CH_2Ph), 27.2 (C-2′), 20.3 (CH_2CH_3), 11.5 (CH_2CH_3). – MS ((+)-FAB): m/z = 512 $[M]^+$. – $C_{23}H_{28}N_8O_6$ (512.52): calcd. C 53.90, H 5.51, N 21.86; found C 53.68, H 5.43, N 21.58.

*1-(3-(4-(1-Benzyl-2-ethyl-4-nitro-1H-imidazol-5-yl)piperazin-1-yl)propyl)-1H-1,2,3-triazol-4,5-diyl-bis(phenylmethanone) (**14**)*

From 1,4-diphenylbut-2-yne-1,4-dione (**11**) (234 mg). Yield: 411 mg (65 %); m. p. 151–154 °C. – ^{1}H NMR ($CDCl_3$): δ = 7.87–7.47 (m, 10H, Ar-H), 7.31–7.22 (m, 5H, Ar-H), 5.35 (s, 2H, CH_2Ph), 5.12 (t, 2H, J = 7.0 Hz, CH_2-3′), 3.54, 3.23 (2 × br s., 8H, $H_{piperazine}$), 2.68 (q, 2H, J = 7.3 Hz, CH_2CH_3), 2.45–2.40 (m, 4H, CH_2-1′ + CH_2-2′), 1.13 (t, 3H, CH_2CH_3). – ^{13}C NMR ($CDCl_3$): δ = 147.1 (C-2), 145.1 ($C(4)_{triazole}$), 139.1 (C-4 + $C(5)_{triazole}$), 137.0 (C-5), 136.6 (CH_2-$C(1)_{arom}$), 131.2, 129.1, 129.0, 128.8, 128.5, 128.2, 127.8, 127.5, 127.1, 126.7, 125.3 (C_{arom}), 53.5 (C-1′), 50.3 ($C_{piperazine}$), 45.2 (C-3′), 45.9 (CH_2Ph), 27.0 (C-2′), 20.4 (CH_2CH_3), 11.3 (CH_2CH_3). – MS ((+)-FAB): m/z = 633 $[M+H]^+$. – $C_{35}H_{36}N_8O_4$ (632.71): calcd. C 66.44, H 5.73, N 17.71; found C 66.19, H 5.62, N 17.51.

*6-Amino-1-(3-(4-(1-benzyl-2-ethyl-4-nitro-1H-imidazol-5-yl)piperazin-1-yl)-8-hydroxy-[1,2,3]triazolo[4,5-e][1,3]-diazepine)-4-one (**15**)*

Guanidine hydrochloride (60 mg, 2.00 mmol) was added to a NaOMe solution (4 mL) [resulting from Na (350 mg) dissolved in abs. MeOH (7 mL)]. After stirring in an ice bath for 30 min, the precipitated NaCl was filtered off, and the filtrate was added to a solution of the triazole **12** (270 mg, 0.50 mmol) in abs. MeOH (10 mL). The mixture was stirred at room temperature for 48 h. After neutralization wih 1 N HCl, the precipitate was filtered, and the filtrate was evaporated to dryness. The residue was co-evaporated with EtOH (4 × 10 mL) to give a crude product **15**. This product was placed on a short silica gel column and eluted, in gradient, with MeOH (0 – 5 %) and $CHCl_3$ to give pure **15** (112 mg, 42 %); m. p. 197–200 °C (dec.). – ^{1}H NMR ($CDCl_3$): δ = 8.94 (br s., 2H, NH_2), 7.28–7.16 (m, 5H, Ar-H), 5.51 (br s., 1H, OH), 5.30 (s, 2H, CH_2Ph), 4.39 (t, 2H, J = 6.9 Hz, CH_2-3′), 3.57, 3.19 (2 × br s., 8H, $H_{piperazine}$), 2.63 (q, 2H, J = 7.5 Hz, CH_2CH_3), 2.43 (m, 4H, CH_2-1′ + CH_2-2′), 1.13 (t, 3H, CH_2CH_3). – ^{13}C NMR

($CDCl_3$): δ = 190.2 (C(4″)$_{diazepam}$), 164.1 (C(6″)$_{diazepam}$), 158.1 (C(8″)$_{diazepam}$), 147.0 (C-2), 138.6 (C-4), 137.2 (C-5), 139.4 (C(8a″)$_{diazepam}$), 136.6 (CH_2-*C(1)*$_{arom}$), 133.9 (C(3a″)$_{diazepam}$), 128.8, 127.5, 126.9, 125.8 (C_{arom}), 53.8 (C-1′), 50.5 ($C_{piperazine}$), 46.8 (C-3′), 45.2 (CH_2Ph), 27.2 (C-2′), 20.1 (CH_2CH_3), 11.4 (CH_2CH_3). – MS ((+)-FAB): m/z = 558 $[M+Na]^+$. – $C_{24}H_{29}N_{11}O_4$ (535.56): calcd. C 53.82, H 5.46, N 28.77; found C 53.59, H 5.33, N 28.53.

*1-(3(4-(1-Benzyl-2-ethyl-4-nitro-1H-imidazol-5-yl)piperazin-1-yl)propyl)-N^4,N^5-bis(6-hydroxy-hexyl)-1,2,3-triazole-4,5-dicarboxamide (**16**)*

A solution of **12** (250 mg, 0.46 mmol) in 1-amino-6-hexanol (269 mg, 2.30 mmol) was stirred at room temperature for 18 h, then the reaction mixture was evaporated to dryness. The residue was dissolved in $CHCl_3$ (2 mL) and placed on a short silica gel column (5 g). Elution, in gradient, with MeOH (0–5%) and $CHCl_3$ afforded pure **16** (255 mg, 78%); m. p. 158–161 °C. – ^{1}H NMR ($CDCl_3$): δ = 10.78 (t, 1H, J = 5.5 Hz, NH), 9.15 (t, 1H, J = 5.9 Hz, NH), 7.32–7.21 (m, 5H, Ar-H), 5.29 (s, 2H, CH_2Ph), 4.36 (t, 2H, J = 7.0 Hz, CH_2-3′), 4.27 (dd, 2H, J = 1.4 Hz, 2 × CH_2OH), 3.62, 3.19 (2 × br s., 8H, $H_{piperazine}$), 3.36 (m, 4H, 2 × CH_2OH), 3.26 (m, 4H, 2 × NCH_2-1″), 2.56 (q, 2H, J = 7.5 Hz, CH_2CH_3), 2.43 (m, 4H, CH_2-1′ + CH_2-2′), 1.52 (m, 4H, CH_2-4″a,b), 1.39 (m, 4H, CH_2-5″a,b), 1.31 (m, 4H, CH_2-3″a,b), 1.28 (m, 4H, CH_2-2″a,b), 1.12 (t, 3H, CH_2CH_3). – ^{13}C NMR ($CDCl_3$): δ = 160.6, 156.8 (C=O), 147.4 (C-2 + C(5)$_{triazole}$), 138.9 (C-5 + C(4)$_{triazole}$), 137.2 (C-4), 136.8 (CH_2-*C(1)*$_{arom}$), 128.7, 128.3, 127.5, 127.3, 125.7 (C_{arom}), 60.2 (2 × CH_2OH), 53.4 (C-1′), 50.1 ($C_{piperazine}$), 46.0 (C-3′); 45.4 (CH_2Ph), 38.5 (2 × C-1″a,b), 32.0 (C-5″a,b), 28.4 (C-4″a,b), 27.0 (C-2′), 25.7 (C-2″a,b), 24.8 (C-3″a,b), 20.2 (CH_2CH_3), 11.3 (CH_2CH_3). – MS ((+)-FAB): m/z = 711 $[M+H]^+$. – $C_{35}H_{54}N_{10}O_6$ (710.87): calcd. C 59.14, H 7.66, N 19.70; found C 58.93, H 7.51, N 19.89.

*1-(3(4-(1-Benzyl-2-ethyl-4-nitro-1H-imidazol-5-yl)piperazin-1-yl)propyl)-N^4,N^5-bis(cyclopropyl)-1,2,3-triazole-4,5-dicarboxamide (**17**)*

The synthesis was analogous to the preceding procedure, using cyclopropylamine (131 mg, 2.29 mmol). Yield: 171 mg (63%); m. p. 188–191 °C. – ^{1}H NMR ($CDCl_3$): δ = 10.72 (t, 1H, J = 4.0 Hz, NH), 9.17 (t, 1H, J = 4.4 Hz, NH), 7.28–7.18 (m, 5H, Ar-H), 5.29 (s, 2H, CH_2Ph), 4.38 (t, 2H, J = 7.1 Hz, CH_2-3′), 3.58, 3.17 (2 × br s., 8H, $H_{piperazine}$), 2.90–2.82 (m, 2H, $H_{cyclopropyl}$-1a,b), 2.54 (q, 2H, J = 7.3 Hz, CH_2CH_3), 2.42 (m, 4H, CH_2-1′ + CH_2-2′), 1.13 (t, 3H, CH_2CH_3), 0.76–0.52 (m, 8H, $H_{cyclopropyl}$). – ^{13}C NMR ($CDCl_3$): δ = 162.1, 157.5 (C=O), 147.5 (C-2 + C(5)$_{triazole}$),138.8 (C-5 + C(4)$_{triazole}$), 137.0 (C-4), 136.5 (CH_2-*C(1)*$_{arom}$), 128.9, 128.4, 127.2, 127.3, 125.6 (C_{arom}), 53.1 (C-1′), 50.0 ($C_{piperazine}$), 46.2 (C-3′), 45.4 (CH_2Ph), 27.2 (C-2′), 23.3, 23.0 ($C_{cyclopropyl}$-1a,b), 20.0 (CH_2CH_3), 11.2 (CH_2CH_3), 6.24, 6.10 ($C_{cyclopropyl}$-2a,b + $C_{cyclopropyl}$-3a,b). – MS ((+)-FAB): m/z = 591 $[M+H]^+$. – $C_{29}H_{38}N_{10}O_4$ (590.68): calcd. C 58.97, H 6.48, N 23.71; found C 58.69, H 6.33, N 23.50.

Acknowledgement

N. A. Al-Masoudi would like to thank Basrah University for a sabbatical leave. We thank Mr. U. Haunz and Miss A. Friemel of the Department of Chemistry, University of Konstanz, Konstanz/Germany for the NMR experiments.

[1] R. A. Koup, V. J. Merluzzi, K. D. Hargrave, J. Adams, K. Grozinger, R. J. Eckner, J. L. Sullivan, *J. Infect. Dis.* **1991**, *163*, 966–970.

[2] D. L. Romero, R. A. Morge, C. Biles, T. N. Berrios-Pena, P. D. May, J. P. Palmer, P. M. Johnson, H. W. Smith, M. Busso, C.-K. Tan, R. L. Voorman, F. Reusser, I. W. Althaus, K. M. Downey, A. G. So, L. Resnick, W. G. Tarpley, P. A. Aristoff, *J. Med. Chem.* **1994**, *37*, 999–1014.

[3] D. L. Romero, R. A. Olmsted, T. J. Poel, R. A. Morge, C. Biles, B. J. Keiser, L. A. Kopta, J. M. Friis, J. D. Hosley, K. J. Stefanski, D. G. Wishka, D. B. Evans, J. Morris, R. G. Stehle, S. K. Sharma, Y. Yagi, R. L. Voorman, W. J. Adams, W. G. Tarpley, R. C. Thomas, *J. Med. Chem.* **1996**, *39*, 3769–3789.

[4] S. D. Young, S. F. Britcher, L. O. Tran, L. S. Payne, W. C. Lumma, T. A. Lyle, J. R. Huff, P. S. Anderson, D. B. Olsen, S. S. Carroll, D. J. Pettibone, J. A. Obrien, R. G. Ball, S. K. Balani, J. H. Lin, I. W. Chen, W. A. Schleif, V. V. Sardana, W. J. Long, V. W. Byrnes, E. A. Emini, *Antimicrob. Agents Chemoth.* **1995**, *39*, 2602–2605.

[5] E. De Clercq, *Antiviral Res.* **1998**, *38*, 153–179.

[6] M. A. Wainberg, J. P. Sawyer, J. S. Montaner, R. L. Murphy, D. R. Kuritzkes, F. Raffi, *Antiviral Therapy* **2005**, *10*, 13–28.

[7] T. Imamichi, *Curr. Pharm. Design* **2004**, *10*, 4039–4053.

[8] T. Fujiwara, A. Sato, M. El-Farrash, S. Miki, K. Kabe, Y. Isaka, M. Kodama, Y. M. Wu, L. B. Chen, H. Harada, H. Sugimoto, M. M. Hatanaka, Y. Hinuma, *Antimicrob. Agents Chemoth.* **1998**, *42*, 1340–1345.

[9] T. Bergan, *Scand. J. Infec. Dis. Suppl.* **1985**, *46*, 64–71.

[10] H. Hof, J. Stroder, *Antimicrob. Agents Chemoth.* **1986**, *29*, 953 – 954.

[11] A. A. Carmine, R. N. Brogden, R. C. Heel, T. M. Speight, G. S. Avery, *Drugs* **1982**, *24*, 85 – 117.

[12] M. Castelli, M. Malagoli, L. Lupo, S. Roffia, F. Paolucci, C. Cermelli, A. Zanca, G. Baggio, *J. Antimicrob. Chemother.* **2000**, 46, 541 – 550.

[13] G. E. Adams, E. D. Clarke, I. R. Flockhart, R. S. Jacobs, D. S. Sehmi, I. J. Stratford, P. Wardman, M. E. Watts, J. Parrick, R. G. Wallace, C. E. Smithen, *Int. J. Radiat. Biol.* **1979**, *35*, 133 – 135.

[14] A. Breccia, R., Cavalleri, G. E. Adams, *Nitroimidizoles: Chemistry, Pharmacology and Clinical Application*, Vol. 42, Plenum Press, New York **1982**.

[15] J. L. Skibba, D. D. Beal, K. K. Whitnable, *Cancer Res.* **1970**, *30*, 147 – 150.

[16] J. E. Biskupiak, J. R. Grierson, J. S. Rasey, G. V. Martin, K. A. Krohn, *J. Med. Chem.* **1991**, *34*, 2165 – 2168.

[17] J. Bartroli, M. Alguero, E. Boncompte, J. Forn, *Arzneim.-Forsch.* **1992**, *42*, 832 – 835.

[18] C. Cosar, L. Julou, *Annales de l'Institut Pasteur* **1959**, *96*, 238 – 241.

[19] F. P. Tally, B. R. Goldin, N. Sullivan, J. Johnston, S. L. Gorbach, *Antimicrob. Agents Chemother.* **1978**, *13*, 460 – 465.

[20] W. J. Ross, W. B. Jamieson, M. C. McCowen, *J. Med. Chem.* **1972**, *15*, 1035 – 1040.

[21] W. J. Ross, W. B. Jamieson, M. C. McCowen, *J. Med. Chem.* **1973**, *16*, 347 – 352.

[22] J. H. Chern, K. S. Shia, S. R. Shih, T. A. Hsu, C. L. Tai, US Pat. 6815444 **2004**; *Accession No.* **2004**, 857167.

[23] J. H. Chern, K. S. Shia, T. A. Hsu, C. L. Tai, L. Chungchi, C. C. Lee, Y. C. Lee, C. S. Chang, S. N. Tseng, S. R. Shih, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2519 – 2525.

[24] D. L. Romero, R. A. Morge, M. J. Genin, C. Biles, M. Busso, L. Resnick, I. W. Althaus, F. Reusser, R. C. Thomas, W. G. Tarpley, *J. Med. Chem.* **1993**, *36*, 1505 – 1508.

[25] R. M. Gulick, Z. Su, C. Flexner, M. D. Hughes, P. R. Skolnik, T. J. Wilkin, R. Gross, A. Krambrink, E. Coakley, W. L. Greaves, A. Zolopa, R. Reichman, C. Godfrey, M. Hirsch, D. R. Kuritzkes, *J. Infect. Dis.* **2007**, *196*, 304 – 312.

[26] N. A. Al-Masoudi, Y. A. Al-Soud, A. Kalogerakis, E. De Clercq, C. Paneccoque, *Chem. Biodiver.* **2006**, *3*, 515 – 526.

[27] Y. A. Al-Soud, N. A. Al-Masoudi, E. De Clercq, C. Paneccoque, *Heteroatom Chem.* **2007**, *18*, 333 – 340.

[28] Y. A. Al-Soud, N. A. Al-Masoudi, *Synth. Commun.* **2005**, *35*, 2258 – 2264.

[29] Y. A. Al-Soud, H. Al-Sa'doni, Houssain, A. S. Amajaour, N. A. Al-Masoudi, *Z. Naturforsch.* **2007**, *62b*, 523 – 528.

[30] Y. A. Al-Soud, N. A. Al-Masoudi, H. H. Ghaffori, E. De Clercq, C. Paneccoque, *Acta Pharm.* **2007**, 57, 379 – 393.

[31] N. A. Al-Masoudi, Y. A. Al-Soud, E. De Clercq, C. Paneccoque, *Antiviral Chem. & Chemother.* **2007**, *18*, 191 – 200.

[32] M. L. Barreca, A. Chimirri, L. D. Luca, A. M. Monforte, P. Monforte, A. Rao, M. Zappala, J. Balzarini, E. De Clercq, C. Pannecouque, M. Witvrouw, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1793 – 1796.

[33] A. Rao, A. Carbone, A. Chimirri, E. De Clercq, A. M. Monforte, P. Monforte, C. Pannecouque, M. Zappala, *IL Farmaco* **2002**, *57*, 747 – 751.

[34] A. Rao, J. Balzarini, A. Carbone, A. Chimirri, E. De Clercq, A. M. Monforte, P. Monforte, C. Pannecouque, M. Zappala, *Antiviral Res.* **2004**, *63*, 79 – 84.

[35] A. Rao, J. Balzarini, A. Carbone, A. Chimirri, E. De Clercq, A. M. Monforte, P. Monforte, C. Pannecouque, M. Zappala, *IL Farmaco* **2004**, *59*, 33 – 39.

[36] A. Saeed, N. A. Al-Masoudi, A. A. Ahmed, C. Pannecouquec, *Z. Naturforsch.* **2011**, *66b*, 512 – 520.

[37] W. Willker, D. Leibfritz, R. Kerssebaum, W. Bermel, *Mag. Reson. Chem.* **1993**, *31*, 287 – 292.

[38] A. Koziara, *J. Chem. Res. (S)* **1989**, 296 – 297.

[39] V. S. Reddy, G. V. Rao, R. V. K. Subramanyam, D. S. Iyengar, *Synth. Commun.* **2000**, *30*, 2233 – 2237.

[40] N. A. Al-Masoudi, Y. A. Al-Soud, A. Geyer, *Spectroscopy Lett.* **1998**, *31*, 1031 – 1038.

[41] R. Czylkowski, J. Karolak-Wojciechowska, A. Mrozek, I. Yalcin, E. Aki-Sener, *J. Mol. Struct.* **2001**, *598*, 197 – 204.

[42] R. Pauwels, J. Balzarini, M. Baba, R. Snoeck, D. Schols, P. Herdewijn, J. Desmyter, E. De Clercq, *J. Virol. Methods* **1988**, *20*, 309 – 321.

[43] K. D. Hargrave, J. R. Proudfoot, K. G. Grozinger, E. Cullen, S. R. Kapadia, U. R. Patel, V. U. Fuchs, S. C. Mauldin, J. Vitous, M. L. Behnke, J. M. Klunder, K. Pal, J. W. Skiles, D. W. McNeil, J. M. Rose, G. C. Chow, M. T. Skoog, J. C. Wu, G. Schmidt, W. W. Engel, W. G. Eberlein, T. D. Saboe, S. J. Campbell, A. S. Rosenthal, J. Adams, *J. Med. Chem.* **1991**, *34*, 2231 – 2241.

[44] H. Mitsuya, K. J. Weinhold, P. A. Furman, M. H. St. Clair, S. N. Lehrmann, R. Gallo, D. Bolognesi, D. W. Barry, S. Broder, *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 7096 – 7100.