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Original article

Synthesis, HIV-RT inhibitory activity and SAR of 1-benzyl-1*H*-1,2,3-triazole derivatives of carbohydrates

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Abstract

This paper describes the synthesis of several 1-benzyl-1*H*-1,2,3-triazoles attached to different carbohydrate templates and their *in vitro* inhibitory profile against HIV-1 reverse transcriptase. In addition a theoretical comparison of the most active compounds with other classical antivirals was also performed. Our results showed **2a**, **2d** and **2g** as the most active compounds that inhibited the HIV-1 reverse transcriptase catalytic activity with cytotoxicity lower than AZT and SI higher than DDC and DDI. The overall theoretical analysis of the molecular descriptors of **2a**, **2d** and **2g** revealed that their HOMO energy is similar to other antivirals in use (AZT, DDC, DDI and 3TC) and together with the volume may contribute for the biological profile as they may allow new interactions with the target. In fact the 1,2,3-triazole compounds presented more lipophilicity and higher molecular volume and weight than the antivirals studied, which suggested that these features might not only contribute for new interactions with the HIV-RT but also influence the specificity and consequently the low cytoxicity profile of these compounds. Thus these data point them as promising leading compounds for generating new anti-HIV-RT compounds.

Keywords: HIV-RT; 1,2,3-Triazole; Anti-HIV; Antiviral

1. Introduction

Acquired immune deficiency syndrome (AIDS) is the first pandemic disease of the molecular biology era, which killed over 20 million people worldwide [1,2]. The human immunodeficiency virus (HIV) is the AIDS causative agent and its reverse transcriptase (HIV-TR) is the main target of AIDS treatment [3,4]. This enzyme plays an essential and multifunctional role in the virus replication. It allows the transcription of HIV-single-stranded RNA genome into a DNA double helix capable of integration into host cell chromosomes [5–9].

The combination of HIV reverse transcriptase and protease inhibitors in the highly active antiretroviral therapies (HAART) against HIV infection led to a decline in morbidity and mortality [5–9]. Nonetheless, viral replication is still persisting as lymphatic system and central nervous system acts as reservoir for the virus [5], and wherein some antivirals, more particularly the protease inhibitors (PIs), do not penetrate at an efficient inhibitory level [10]. In addition the emergence of drug-resistant

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virus strains due to high HIV mutation rate continues to restrain the long-term clinical efficacy of these molecules [11–14] whereas side effects limit the use of some of these antivirals (*i.e.* AZT) demanding new options [14–21]. Therefore despite the advances in therapy, the progression of AIDS in HIV-positive individuals remains a major health problem in the world today.

1,2,3-Triazoles are an important class of heterocyclic compounds due to their wide range of applications as pharmaceutical agents [22]. Among the pharmaceutical uses, 1,2,3-triazoles are known as antiplatelets [23], dopamine D2 receptor ligands (related to Schizophrenia) [24], β-lactamase inhibitors [25], anticonvulsants [26], antimicrobials [27–29], and antiinflammatory [30,31] agents, but the potential of this class as antivirals against HIV is poorly described in literature. Alvarez and coworkers described the series of [2,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-spiro-5′-(4′-amino- and 4′-(*N*-acetylamino)-l′,2′-oxathiole 2′,2′-dioxide) (TSAO) derivatives in which the pyrimidinelpurine moiety of the molecule was replaced by a 1,2,3-triazole ring with various substitutions at C-4 or C-5 [32]. These authors showed several members of this class of compounds with potent anti-HIV-1 activities, pointing the promising profile of 1,2,3-triazoles for generating leading compounds [32].

In order to identify a 1,2,3-triazole lead compound against HIV-RT, our research efforts have been directed towards the discovery of new chemical entities that are effective antivirals with low cytotoxicity. This paper describes the synthesis and *in vitro* activity against HIV-RT of several 1-benzyl-1*H*-1,2,3-triazoles attached to different carbohydrate templates. A theoretical comparison of the most active 1,2,3-triazole compounds and some of the current antivirals in use was also performed.

2. Chemistry

The diversity of chemical structures of the 1,2,3-triazole family and their useful biological activities made these compounds attractive targets in synthetic organic chemistry. Many studies have been reported on the synthesis of the 1,2,3-triazole ring system [33–42]. The potential of this heterocycle led us to develop a synthetic route for preparing glycoconjugate-triazoles and examine their antiviral activity (Fig. 1). The syntheses of compounds **3a–g** were started by the preparation of enamines **1a–g** by the procedure previously described by us [43]. The preparation of triazoles **2a–g** was performed by the [2N + 1N] method, as early described by Arnold and our group [44–47], performing the transference of the diazo group to enamines **1a–g**.

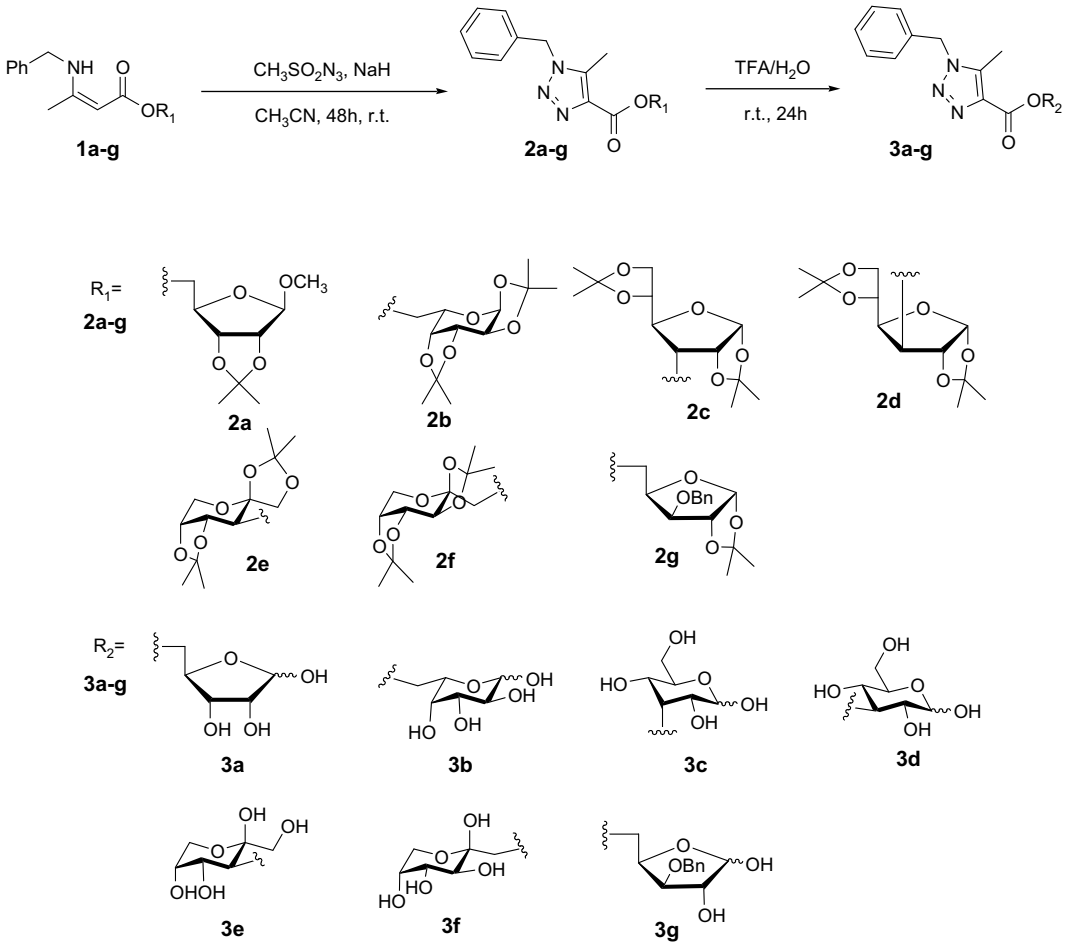


Fig. 1. Structures of 1*H*-1,2,3-Triazole derivatives **2a–g** and **3a–g**.

Thus, the isopropylidene group from carbohydrate moiety was hydrolyzed through the reaction with a solution of TFA/H_2O (1:1) at room temperature during 24 h [48] producing the corresponding D-glycoconjugated 1,2,3-triazoles **3a–g** containing as carbohydrate units ribose, galactose, fructose, alose, glucose, xylose in their native form, in excellent yields (Fig. 1). Triazoles **3a–g** were obtained as a mixture of α and β anomers for **3c** and **3f** (1,2,3-triazoles from D-glucose and D-alose, respectively, were obtained in the α and β-pyranoside forms). All the triazoles were fully characterized by ^{1}H NMR, ^{13}C NMR, IR spectroscopy and HRMS.

3. Pharmacology

3.1. Anti-HIV-RT activity assay

The inhibitory effect of triazoles at 50 μM or different concentrations (0.01–50 μM) was evaluated on the HIV-RT polymerase activity using recombinant HIV-1 enzyme. The polymerization reactions (50 μL) contained 50 mM Tris HCl (pH 7.8), 6 mM $MgCl_2$, 1 mM dithiotreitol, 50 mM KCl, 20 μM dTTP, 10 μM of [3H] dTTP (47 Ci/mmol), and 150 μg poly(rA)·oligo(dT) template primer (Pharmacia) and 1 U of enzyme. The reaction mixture was incubated at 37 °C for 30 min, and the incubation was stopped by adding ice-cold 5% trichloroacetic acid (TCA) containing 20 mM of sodium pyrophosphate. The precipitates were collected on Whatman GF/C filters and washed with sodium phosphate 0.1 M. The incorporated triphosphate was measured by assaying for ^{3}H in a liquid scintillation counter. AZT was used as antiviral control. The selective indexes were calculated based on our experimental results (1,2,3-triazole compounds and AZT) and based on literature (didadosine-DDI and zalcitabine-DDC and 3TC).

3.2. Cytotoxicity assays

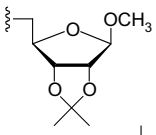
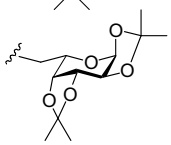
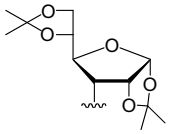
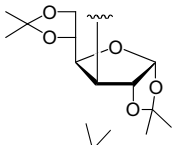
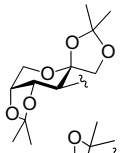
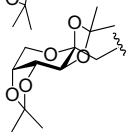
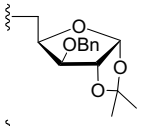
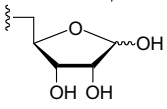
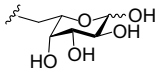
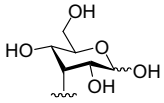
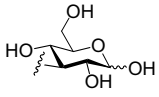
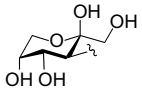
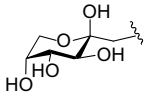
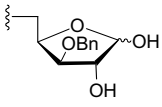
Vero cells were cultured in Dulbecco modified Eagle medium (DMEM) supplemented with 5% fetal bovine serum (FBS; HyClone, Logan, Utah), 100 U/mL penicillin and 100 μg/mL streptomycin, at 37 °C in 5% CO_2. Monolayers of about 104 Vero cells in 96-multiwell plates were treated with several concentrations of the compounds for the 72 h. Then, 50 μl of 1 mg/mL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma) was added to evaluate cell viability according to procedures described elsewhere [49]. The 50% cytotoxic concentration (CC_{50}) was calculated by linear regression analysis of the dose–response curves. All experiments were performed in duplicate at least three times.

4. Results

4.1. Anti-HIV-RT activity

Initially all compounds were tested at 50 μM and the results are shown in Table 1. All compounds inhibited HIV-RT to some degree and **2a–g** derivatives presented an inhibitory profile higher than compounds **3a–g**. Importantly, **2a**, **2d** and **2g** produced the highest inhibitory values (63–65%) at 50 μM (Table 1).

Table 1
Biological characterization of 1*H*-1,2,3-triazole derivatives **2a–g** and **3a–g** including anti-HIV-1-RT and cytotoxicity activities

Compound	R	Inhibition[a] (%)	CC_{50} (μM)
2a		63.6	837.45
2b		28.8	123.42
2c		3.0	3529.00
2d		64.7	208.37
2e		43.5	2745.47
2f		47.9	3382.47
2g		65.4	724.06
3a		33.2	1172.64
3b		45.6	25,640.55
3c		14.0	1437.50
3d		35.9	1798.60
3e		45.6	648.90
3f		35.8	800.45
3g		36.2	596.00
AZT	–	94.25	126.00

[a] Determined at 50 μM.

The determination of the IC_{50} of the most active compounds against HIV-RT (**2a** = 2.2 ± 0.8 μM, **2d** = 5.0 ± 0.5 μM and **2g** = 1.98 ± 0.4 μM) revealed values higher than AZT, but similar to ddC (zalcitabine) and 3TC (lamivudine) and lower than ddI (didadosine) (Fig. 2).

4.2. Cytotoxicity profile

In this study, we also determined the cytotoxicity profile (CC_{50}) of the 1,2,3-triazoles (Table 1). Our experimental results of the most active compounds (**2a**, **2d** and **2g**) revealed a better cytotoxicity profile (**2a** = 837.45 μM, **2d** = 208.37 μM and **2g** = 724.06 μM) compared to AZT (CC_{50} = 126 μM) (Table 1). In fact the two series **2** and **3** presented a lower cytotoxic profile than AZT (CC_{50} = 126 μM) except for the **2b** compound (CC_{50} = 123.42 μM) (Table 1). Interestingly, the selective index of the **2a** and **2g** was higher than DDC and DDI whereas **2d** was only higher than DDI (Fig. 2).

4.3. Molecular modeling evaluation

We employed molecular modeling studies for the most active triazole derivatives in the attempt of providing useful guidelines for the design of more potent antivirals comparing them with other antivirals currently in use. The minimum energy conformation of the most active compounds and antivirals current issue, obtained by the AM1 semiempirical method, were submitted to a Single-Point *ab initio* calculation with a 3-21G* basis set available on SPARTAN'04 program (Wavefunction Inc. Irvine, CA, 2000). Molecular electrostatic potential maps (MEPs), HOMO and LUMO eigen values and orbital coefficients, and the molecular dipole moments were calculated. In this work, we also studied the drug score of the compounds, which are based on topological descriptors, fingerprints of molecular druglikeness, structural keys or other properties as *c*log *P* and molecular weight [49]. In case of osiris property explorer (http://www.organic-chemistry.org/), the occurrence frequency of each fragment is determined within the collection of traded drugs and within the supposedly non-druglike collection of Fluka compounds.

By comparing the three more active drugs (**2a**, **2d** and **2g**) with other antivirals (AZT, DDC, 3TC and DDI), we observed that they presented similar HOMO and LUMO energy values. In case of triazoles, HOMO and LUMO energy ranges from −8.67 to −9.57 and 2.39 to 2.76, respectively, whereas the antivirals evaluated herein presented values from −8.37 to

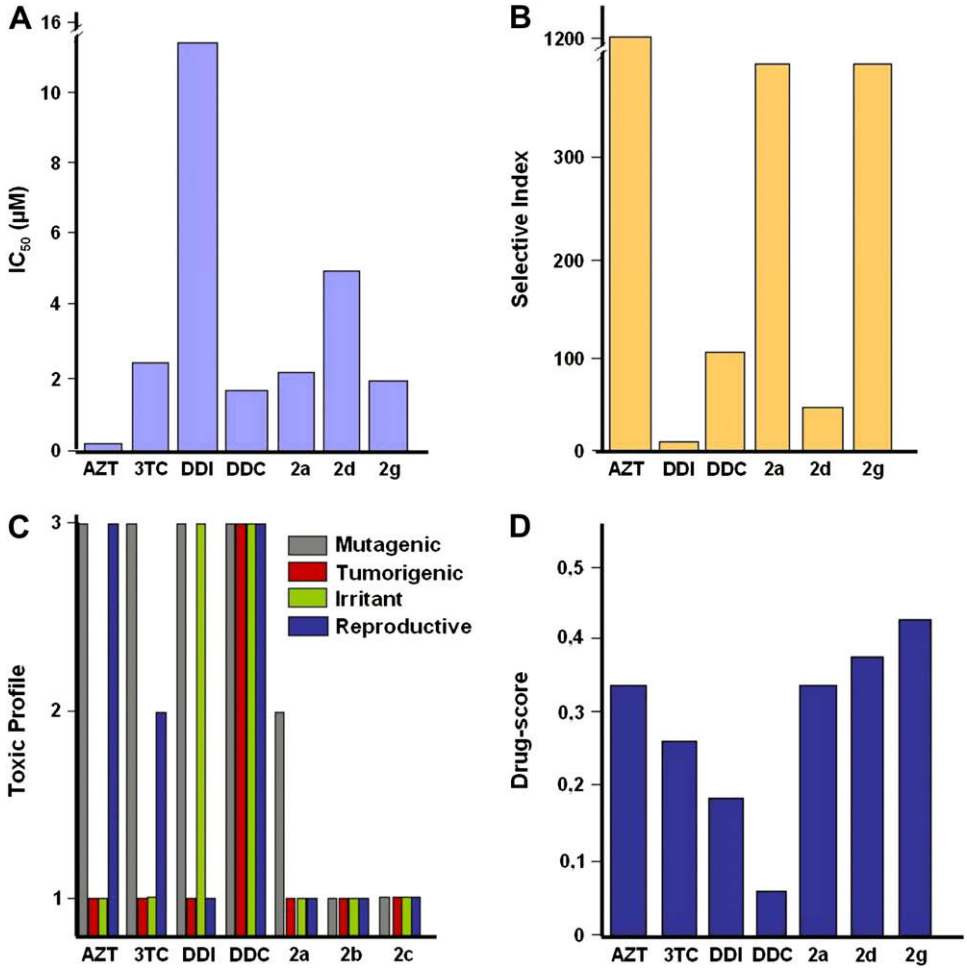


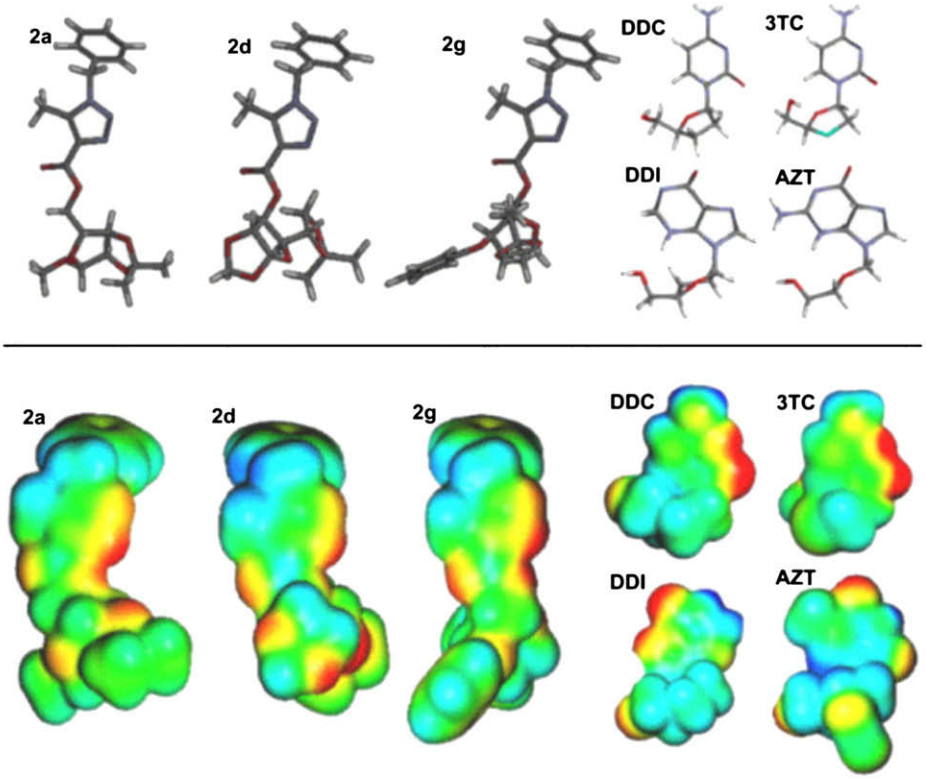
Fig. 2. Comparison of the most active 1,2,3-triazoles compounds (**2a**, **2d**, **2g**) and some anti-HIV drugs (AZT, DDC, DDI and 3TC) currently in use. Experimental IC_{50} (A), selective index (B), and theoretical toxicity risk profile and drug score calculated using Osiris program (C and D, respectively).

−9.42 and 2.29 to 2.90, respectively (Fig. 3). In contrast, the triazole derivatives presented more lipophilicity (*c*log *P*) and higher molecular volume (MV) and molecular weight (MW) (Fig. 3). Despite their structural differences, the electrostatic potential map of **2a**, **2d** and **2g** revealed an analogous negative charge distribution pattern in the right of the molecule, similar to DDC and 3TC (Fig. 3).

The comparison of the most active compound (**2g**) with the less active one (**2c**) showed a different distribution of the density of the orbital HOMO, which is concentrated in different moieties of their structures. In the most active compound (**2g**), HOMO is concentrated at the benzyloxy substituent from the furanose moiety, in contrast to the less active compound (**2c**) where it remains on the benzyl substituent of the triazolic moiety (Fig. 4). In addition compound **2g** also presented a higher volume (MV = 465.66 Å^3) than compound **2c** (MV = 417.05 Å^3) (not shown).

The three 1,2,3-triazoles (**2a**, **2d** and **2g**) were submitted to an *in silico* ADMET screening (http://www.organic-chemistry.org/prog/peo/) to analyze their overall drug score potential compared to the commercial antiviral drugs, AZT, DDC, 3TC and DDI. Therefore we determined for the most active compounds and antivirals currently in use: (a) all parameters for the fulfilment of Lipinski rule of 5; (b) the predicted toxicity risks (mutagenic, irritant, tumorigenic, and reproductive effects), and (c) the drug score that combines druglikeness, *c*log *P*, log *S*, molecular weight and toxicity risks, and theoretically may be used to evaluate the drug potential of a compound (Fig. 3).

Our results revealed that the lipophilicity (*c*log *P*) of these 1,2,3,triazoles is higher than that observed for commercial drugs but is not greater than 5.0, fulfilling one of the Lipinski rule of 5 [50]. The molecular weight of 1,2,3-triazole derivatives (403 < MW > 465) is greater than other drugs (211



Compound	Lipinski Rule of 5*					HOMO (eV)	LUMO (eV)	Dipole (Debye)
	Hydrogen donnors	Hydrogen acceptors	clogP	MW	MV (A^3)			
2a	0	9	1.48	403.44	400.79	-9.44	2.69	4.72
2d	0	10	1.94	431.45	417.04	-9.57	2.39	7.65
2g	0	9	2.91	465.51	465.66	-8.67	2.76	5.30
AZT	2	9	-0.86	267.25	240.38	-9.42	2.29	1.25
DDC	3	6	-0.86	211,22	203.53	-9.12	2.65	4.29
3TC	3	6	-0.22	229.26	203.19	-9.02	2.90	5.33
DDI	2	7	-1.42	236.23	220.91	-8.37	2.44	8.06

*Lipinski rule of 5 (number of hydrogen bond acceptors <10 and donors <5, clogP<5, molecular weight <500)

Fig. 3. Comparison of theoretical parameters of **2a**, **2d** and **2g** compounds and of some anti-HIV compounds currently in use. The most stable conformers, electrostatic potential maps, fulfilment of Lipinsky rule of 5 (number of hydrogen donors and acceptors, *c*log *P* and molecular weights − MW, molecular volumes − MV), and molecular electronic properties (E_{HOMO}, E_{LUMO}, dipole moment).

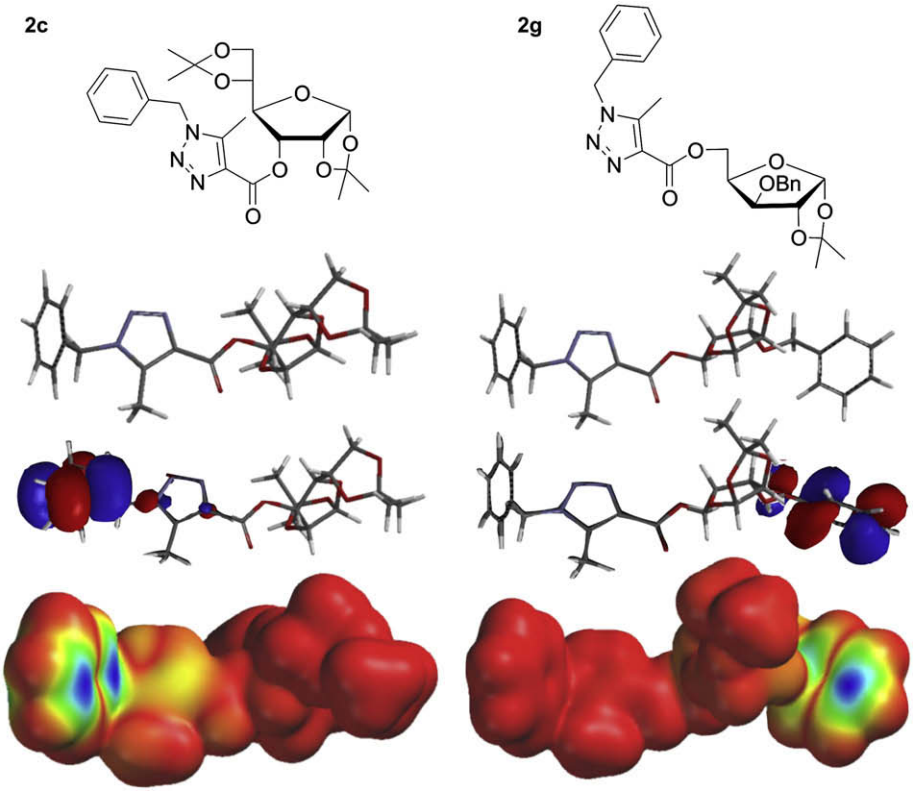


Fig. 4. Comparison of the 2D-structure, the most stable conformer, the HOMO density and the HOMO density encoded onto a van der Waals surface of 1,2,3-triazole compounds presenting the lower (**2c**) and the higher (**2g**) inhibitory profile against HIV-RT. In HOMO density encoded onto a van der Waals surface (isodensity 0.002 e/au^3), the HOMO absolute density coefficient was mapped from deepest red (0.00) to deepest blue (0.01) (for interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$<$ MW $>$ 267) but close to more than 80% of all Fluka traded drugs (MW $>$ 450), and the number of hydrogen bond acceptors (HA) and donors (HD) are also within the Lipinski rule of 5 (HA $<$ 10 and HD $<$ 5 respectively) (Fig. 3).

Interestingly the 1,2,3-triazole derivatives (**2a**, **2d** and **2g**) showed equivalent or higher drug score values than the antivirals AZT, DDC, 3TC and DDI (Fig. 2). Importantly the most active derivative (**2g**) presented a higher value than that observed for DDC, 3TC and DDI (Fig. 2). In agreement to our cytotoxicity experimental results, our theoretical study showed **2a**, **2d** and **2g** with a better profile for low tumorigenicity, mutagenicity and reproductive effects than the antivirals currently in use studied herein (Fig. 2).

5. Discussion

Two 1,2,3-triazole series were synthesized and tested against HIV-RT, a key enzyme for HIV treatment. Interestingly, all compounds inhibited HIV-RT at some degree, which reinforced the potential profile of 1,2,3-triazoles not only for those biological activities already described in the literature (*i.e.* antiplatelets [23]) but also for an antiviral profile.

Importantly, our biological data suggested that hydroxyl blockage in **2** is important for interacting with HIV-RT as these compounds were the most active molecules. The different biological profiles observed for compounds **2a**, **2d** and **2g** derived from acetonide carbohydrates and for analogues **3a**, **3d** and **3g** from native carbohydrates reinforced that the protective group is an important feature for the anti-HIV activity observed.

In a promising way, the biological activity of **2a**, **2d** and **2g** was analogous to DDC, DDI and 3TC but with a higher selective index, and a safer experimental cytotoxicity profile as their CC_{50} increased at least two-fold compared to AZT. In addition, as the two 1,2,3-triazole series (**2** and **3**) presented both low theoretical and experimental cytotoxic results, these data may indicate the potential safer profile of this heterocycle class compared to other groups currently in use.

The overall theoretical analysis of the molecular descriptors of **2a**, **2d** and **2g** revealed that their HOMO energy is similar to other antivirals in use (AZT, DDC, DDI and 3TC), which suggested that the nucleophilicity might be an important feature for the biological activity. In addition, considering the importance of stereoelectronic complementarity to the drug–receptor interaction, the comparison between the most active (**2g**) and the less active (**2c**) compounds revealed that features such as HOMO density (concentrated at the benzyloxy substituent of **2g**) and volume (related to the benzyl substituent on the **2g** furanose moiety) may together contribute for the biological profile as they may allow new interactions with the target (*i.e.* hydrophobic). In fact the 1,2,3-triazole compounds presented more lipophilicity and higher molecular volume and

molecular weight than the antivirals studied, which suggested that these features might not only contribute for new interactions with the HIV-RT but also influence the specificity and consequently the low cytoxicity profile of these compounds. In addition the evaluation of the electrostatic potential map of **2a**, **2d** and **2g** revealed an analogous negative electronic distribution to DDC and 3TC that may help to orientate them on binding to the HIV-RT.

All active compounds (**2a**, **2d** and **2g**) fulfilled the Lipinski rule of 5 (number of hydrogen bond acceptors < 10 and donors < 5, *c*log $P < 5$, molecular weight < 500) despite their chemical structural differences from the antivirals studied herein. The theoretical parameters such as the *c*log *P* that according to Lipinski rule of 5 is important for drug good absorption and permeation are kept under the best values [50].

All drug score values and theoretical toxicity evaluations of the most active compounds were better than that observed for commercial antivirals. These data reinforced the potential profile of these compounds but it is important to notice that the toxicity predicted herein neither is a fully reliable toxicity prediction, nor guarantees that these compounds are completely free of any toxic effect [51–53]. However, once again these theoretical results help to strengthen the promising profile of these compounds already pointed by the experimental cytotoxicity assay performed herein.

6. Conclusion

To summarize, we synthesized two new classes of triazoles (**2** and **3**) that present HIV-RT inhibition activity. Importantly the triazoles with the protected carbohydrate (**2**) are more effective than those containing unprotected carbohydrate groups (**3**). The significant activity, low cytotoxicity, and potential theoretical profile of triazoles **2a**, **2d** and **2g** suggest that these may be considered as promising lead molecules for further synthetic and biological exploration.

7. Experimental protocol

7.1. Chemistry

Melting points were determined with a Buchi Model B-545 instrument and are uncorrected. Infrared (IR) spectra were recorded on Perkin–Elmer 1420 spectrophotometer in KBr pellets. NMR spectra were recorded on a Varian Unity Plus 300 spectrometer in the indicated solvent, operating at 300 MHz (^{1}H) and 75 MHz (^{13}C) employing tetramethylsilane or the solvent as the internal reference, at room temperature. Chemical shifts (δ) are expressed in ppm and the coupling constant (J) in hertz. Purified samples were used for measuring physical constants and spectral data. The optical rotations were recorded with a Perkin–Elmer 243B Polarimeter (sodium lamp at 589 nm). Analytical grade solvents were used. Trifluoroacetic anhydride was freshly distilled before being used. Column chromatography was performed on silica gel flash from Acros. Reactions were routinely monitored by thin layer chromatography (TLC) on silica gel precoated F_{254} Merck plates. Microanalyses were performed on Perkin–Elmer Model 2400 instrument and all values were within ±0.4% of the calculated compositions. Chemicals employed were obtained from commercial supplies and used without purifications, unless otherwise stated. High-resolution electron-impact mass spectra (70 eV) were performed on Auto Spec M513 Waters instrument.

7.2. General procedures

7.2.1. General procedure for the preparation of 3-benzylamino-but-2-enoic ester derivatives

The preparation of enamines **1a–g** was achieved as described in our previous publications [43].

7.2.1.1. Spectral data of the new enamine esters

*7.2.1.1.1. 3-Benzylamino-but-2-enoic acid 3-(1,2:5,6-di-O-isopropylidene)-α-D-allofuranose ester (**1c**).* Compound **1c** was obtained as a white solid. Yield: 60%; m.p. 124–124.5 °C; $[\alpha]_D^{20}$ +160 (*c* 0.4, CH_2Cl_2); IR (KBr) ν (cm^{-1}): 1648 and 1606; ^{1}H NMR (300.00 MHz, $CDCl_3$) δ (ppm): 1.34 (3H, s, C*H*$_3$), 1.35 (3H, s, C*H*$_3$), 1.43 (3H, s, C*H*$_3$), 1.55 (3H, s, C*H*$_3$), 5.82 (1H, d, $J = 3.9$ Hz, H-1′), 4.81 (1H, dd, $J = 3.7$ and 4.6 Hz, H-2′), 4.91 (1H, dd, $J = 4.9$ and 8.8 Hz, H-3′), 4.20 (1H, dd, $J = 3.4$ and 8.8 Hz, H-4′), 4.35 (1H, ddd, $J = 3.4$, 6.6 and 6.6 Hz, H-5′), 4.04 (1H, dd, $J = 6.8$ and 8.5 Hz, H-6′), 3.92 (1H, dd, $J = 6.3$ and 8.3 Hz, H-6″), 4.61 (1H, s, H-2), 2.03 (3H, s, H-4), 4.51 (2H, d, $J = 6.3$ Hz, NHC*H*$_2$Ph), 7.29–7.39 (2H, m, H-2a), 7.29–7.39 (2H, m, H-3a), 7.29–7.39 (1H, m, H-4a), 8.89 (1H, t, $J = 6.3$ Hz, NH); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ (ppm): 25.1 (CH_3), 26.0 (CH_3), 26.5 (CH_3), 26.6 (CH_3), 103.8 (C-1′), 78.2 (C-2′), 70.2 (C-3′), 77.1 (C-4′), 74.8 (C-5′), 64.9 (C-6′), 112.8 (C-7′), 109.6 (C-8′), 46.7 (NH*C*H$_2$Ph), 82.2 (C-2), 162.7 (C-3), 19.3 (C-4), 138.2 (C-1a), 127.2 (C-2a), 128.6 (C-3a), 126.6 (C-4a), 168.8 (C=O). HRMS calcd for $C_{23}H_{31}NO_7$: 433.2101, found 433.2101.

*7.2.1.1.2. 3-Benzylamino-but-2-enoic acid 5-(3-O-benzyl-1,2-O-isopropylidene)-α-D-xylofuranose ester (**1g**).* Compound **1g** was obtained as a yellow oil. Yield: 67%; $[\alpha]_D^{20}$ −25 (*c* 1.2, CH_2Cl_2); IR (film, $CHCl_3$) ν (cm^{-1}): 1651 and 1606; ^{1}H NMR (300.00 MHz, $CDCl_3$) δ (ppm): 1.31 (3H, s, CH_3), 1.48 (3H, s, CH_3), 5.94 (1H, d, $J = 3.7$ Hz, H-1′), 4.61 (1H, d, $J = 3.9$ Hz, H-2′), 3.97 (1H, d, $J = 2.9$ Hz, H-3′), 4.39–4.42 (1H, m, H-4′), 4.33 (1H, dd, $J = 6.3$ and 11.7 Hz, H-5′), 4.39–4.42 (1H, m, H-5″), 4.66 (1H, d, $J = 12.0$ Hz, H-6′), 4.52 (1H, d, $J = 12.0$ Hz, H-6″), 4.56 (1H, s, H-2), 1.90 (3H, s, H-4), 4.42 (2H, d, $J = 6.0$ Hz, NHC*H*$_2$Ph), 7.23–7.35 (2H, m, H-2a), 7.23–7.35 (2H, m, H-3a), 7.23–7.35 (1H, m, H-4a), 7.23–7.35 (2H, m, H-2′a), 7.23–7.35 (2H, m, H-3′a), 7.23–7.35 (1H, m, H-4′a), 8.92 (1H, t, $J = 5.8$ Hz, NH); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ (ppm): 26.1 (CH_3), 26.6 (CH_3), 105.0 (C-1′), 82.1 (C-2′), 81.5 (C-3′), 78.4 (C-4′), 59.9 (C-5′), 71.8 (C-6′), 111.5 (C-7′), 46.6 (NH*C*H$_2$Ph), 82.7 (C-2), 162.0 (C-3), 19.2 (C-4), 138.4 (C-1a), 126.5 (C-2a), 128.6 (C-3a), 128.3 (C-4a), 137.2 (C-1′a), 127.2 (C-2′a), 127.7 (C-3′a), 127.5 (C-4′a),

169.7 (C=O). HRMS calcd for $C_{26}H_{31}NO_6$: 453.2151, found 453.2151.

*7.2.2. General procedure for the preparation of 1-benzyl-1H-1,2,3-triazole derivatives **2a–g***

To a stirred mixture of sodium hydride (6.67 mmol, free of oil) in anhydrous acetonitrile (4 mL), under nitrogen at room temperature, was added a solution of the β-enamino ester **1** (1.85 mmol in 4 mL of anhydrous acetonitrile). The stirring was continued for 30 min, followed by dropwise addition of mesyl azide (5 mmol in 1 mL of anhydrous acetonitrile). Additional stirring was kept for 48 h and the reaction was quenched with water. The resulting solution was extracted with methylene chloride (3 × 30 mL) and the organic layer was washed (2 × 20 mL) with aqueous sodium hydroxide solution (10%, w/v) and water (1 × 10 mL). After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure to leave the 1,2,3-triazoles (**2a–g**) as yellow oils or solids.

*7.2.2.1. Spectral data of the 1-benzyl-1H-1,2,3-triazole derivatives (**2a–g**)*

*7.2.2.1.1. 1-Benzyl-5-methyl-1H-[1,2,3]triazole-4-carboxylic acid 5-(1-methoxy-2,3-O-isopropylidene)-β-D-ribofuranose ester (**2a**).* Compound **2a** was obtained as a yellow oil. Yield: 92%; $[\alpha]_D^{20}$ −33 (*c* 1.5, $CHCl_3$); IR (film, $CHCl_3$) *ν* (cm^{-1}): 1652 and 1605; ^1H NMR (300.00 MHz, $CDCl_3$) *δ* (ppm): 1.32 (3H, s, CH_3), 1.48 (3H, s, CH_3), 4.99 (1H, s, H-1′), 4.64 (1H, d, *J* = 5.8 Hz, H-2′), 4.80 (1H, dd, *J* = 0.6 and 5.8 Hz, H-3′), 4.51 (1H, dt, *J* = 0.6 and 6.9 Hz, H-4′), 4.39 (2H, dd, *J* = 2.0 and 6.9 Hz, H-5′), 3.33 (3H, s, OCH_3), 2.46 (3H, s, C5−C*H*$_3$), 5.54 (2H, s, NCH_2Ph), 7.16−7.39 (2H, m, H-2a), 7.16−7.39 (2H, m, H-3a), 7.16−7.39 (1H, m, H-4a); ^{13}C NMR (75.0 MHz, $CDCl_3$) *δ* (ppm): 24.7 (CH_3), 26.2 (CH_3), 109.2 (C-1′), 84.9 (C-2′), 81.6 (C-3′), 83.8 (C-4′), 64.6 (C-5′), 112.3 (C-6′), 54.8 (OCH_3), 51.8 (N*C*H_2Ph), 136.3 (C-4), 138.3 (C-5), 8.9 (C5−*C*H_3), 133.6 (C-1a), 127.0 (C-2a), 128.9 (C-3a), 128.4 (C-4a), 160.9 (C=O). HRMS calcd for $C_{20}H_{25}N_3O_6$: 430.1743, found 403.1705.

*7.2.2.1.2. 1-Benzyl-5-methyl-1H-[1,2,3]triazole-4-carboxylic acid 6-(1,2:3,4-di-O-isopropylidene)-α-D-galactopyranose ester (**2b**).* Compound **2b** was obtained as a yellow oil. Yield: 50%; $[\alpha]_D^{20}$ - 40 (*c* 1.5, $CHCl_3$); IR (film, $CHCl_3$) *ν* (cm^{-1}): 1732, 1382 and 1373; ^1H NMR (300.00 MHz, $CDCl_3$) *δ* (ppm): 1.32 (3H, s, CH_3), 1.34 (3H, s, CH_3), 1.46 (3H, s, CH_3), 1.49 (3H, s, CH_3), 5.54 (1H, d, *J* = 4.5 Hz, H-1′), 4.33 (1H, dd, *J* = 2.4 and 4.6 Hz, H-2′), 4.63 (1H, dd, *J* = 2.4 and 7.8 Hz, H-3′), 4.34 (1H, dd, *J* = 1.9 and 7.8 Hz, H-4′), 4.18 (1H, dt, *J* = 1.7 and 6.3 Hz, H-5′), 4.49 (1H, d, *J* = 6.3 Hz, H-6′), 2.45 (3H, s, C5−C*H*$_3$), 5.52 (1H, d, *J* = 15.6 Hz, NCH_2Ph), 5.58 (1H, d, *J* = 15.6 Hz, NCH_2Ph), 7.15−7.18 (2H, m, H-2a), 7.32−7.39 (2H, m, H-3a), 7.32−7.39 (1H, m, H-4a); ^{13}C NMR (75.0 MHz, $CDCl_3$) *δ* (ppm): 24.3 (CH_3), 24.8 (CH_3), 25.8 (CH_3), 25.9 (CH_3), 96.1 (C-1′), 70.4 (C-2′), 70.5 (C-3′), 70.8 (C-4′), 65.8 (C-5′), 63.5 (C-6′), 109.5 (C-7′), 108.6 (C-8′), 51.9 (N*C*H_2Ph), 136.7 (C-4), 138.2 (C-5), 9.1 (C5−*C*H_3), 133.8 (C-1a), 127.1 (C-2a), 129.0 (C-3a), 128.5 (C-4a), 161.1 (C=O). HRMS calcd for $C_{23}H_{29}N_3O_7$: 444.1771, found 444.1633.

*7.2.2.1.3. 1-Benzyl-5-methyl-1H-[1,2,3]triazole-4-carboxylic acid 3-(1,2:5,6-di-O-isopropylidene)-α-D-allofuranose ester (**2c**).* Compound **2c** was obtained as a yellow solid. Yield: 50%; m.p. 97−98 °C; $[\alpha]_D^{20}$ +83 (*c* 1.5, $CHCl_3$); IR (KBr) *ν* (cm^{-1}): 1733, 1714, 1380 and 1372; ^1H NMR (300.00 MHz, $CDCl_3$) *δ* (ppm): 1.32 (3H, s, CH_3), 1.32 (3H, s, CH_3), 1.38 (3H, s, CH_3), 1.54 (3H, s, CH_3), 5.88 (1H, d, *J* = 3.9 Hz, H-1′), 4.93 (1H, dd, *J* = 3.9 and 5.1 Hz, H-2′), 5.11 (1H, dd, *J* = 5.1 and 8.3 Hz, H-3′), 4.36−4.43 (1H, m, H-4′), 4.36−4.43 (1H, m, H-5′), 4.03 (1H′ dd, *J* = 6.1 and 8.8 Hz, H-6′), 4.10 (1H, dd, *J* = 6.6 and 8.8 Hz, H-6″), 2.46 (3H, s, C5−C*H*$_3$), 5.50 (1H, d, *J* = 15.6 Hz, NCH_2Ph), 5.56 (1H, d, *J* = 15.6 Hz, NCH_2Ph), 7.17−7.21 (2H, m, H-2a), 7.32−7.39 (2H, m, H-3a), 7.32−7.39 (1H, m, H-4a); ^{13}C NMR (75.0 MHz, $CDCl_3$) *δ* (ppm): 25.1 (CH_3), 26.4 (CH_3), 26.9 (CH_3), 26.9 (CH_3), 104.4 (C-1′), 78.0 (C-2′), 72.8 (C-3′), 77.8 (C-4′), 75.0 (C-5′), 65.7 (C-6′), 113.5 (C-7′), 110.2 (C-8′), 52.3 (N*C*H_2Ph), 136.4 (C-4), 139.1 (C-5), 9.4 (C5−*C*H_3), 134.0 (C-1a), 127.6 (C-2a), 129.4 (C-3a), 128.9 (C-4a), 160.8 (C=O). HRMS calcd for $C_{23}H_{29}N_3O_7$: 444.1771, found 444.1765.

*7.2.2.1.4. 1-Benzyl-5-methyl-1H-[1,2,3]triazole-4-carboxylic acid 3-(1,2:5,6-di-O-isopropylidene)-α-D-glucofuranose (**2d**).* Compound **2d** was obtained as a yellow oil. Yield: 50%; $[\alpha]_D^{20}$ −30 (*c* 1.6, $CHCl_3$); IR (film, $CHCl_3$) *ν* (cm^{-1}): 1726, 1383 and 1374; ^1H NMR (300.00 MHz, $CDCl_3$) *δ* (ppm): 1.27 (3H, s, CH_3), 1.31 (3H, s, CH_3), 1.41 (3H, s, CH_3), 1.54 (3H, s, CH_3), 5.97 (1H, d, *J* = 3.7 Hz, H-1′), 4.64 (1H, d, *J* = 3.7 Hz, H-2′), 5.51 (1H, d, *J* = 2.9 Hz, H-3′), 4.33 (1H, dd, *J* = 2.9 and 7.8 Hz, H-4′), 4.41 (1H, ddd, *J* = 5.1, 5.6 and 7.8 Hz, H-5′), 4.09 (1H, dd, *J* = 5.1 and 8.8 Hz, H-6′), 4.14 (1H, dd, *J* = 5.6 and 8.8 Hz, H-6″), 2.46 (3H, s, C5−C*H*$_3$), 5.54 (2H, s, NCH_2Ph), 7.18−7.20 (2H, m, H-2a), 7.32−7.39 (2H, m, H-3a), 7.32−7.39 (1H, m, H-4a); ^{13}C NMR (75.0 MHz, $CDCl_3$) *δ* (ppm): 25.1 (CH_3), 26.1 (CH_3), 26.6 (CH_3), 26.7 (CH_3), 105.0 (C-1′), 83.3 (C-2′), 76.4 (C-3′), 79.7 (C-4′), 72.4 (C-5′), 67.0 (C-6′), 112.1 (C-7′), 109.1 (C-8′), 51.9 (N*C*H_2Ph), 136.1 (C-4), 138.8 (C-5), 8.9 (C5−*C*H_3), 133.6 (C-1a), 127.1 (C-2a), 129.0 (C-3a), 128.5 (C-4a), 160.3 (C=O). HRMS calcd for $C_{23}H_{29}N_3O_7$: 444.1771, found 444.1785.

*7.2.2.1.5. 1-Benzyl-5-methyl-1H-[1,2,3]triazole-4-carboxylic acid 3-(1,2:4,5-di-O-isopropylidene)-β-D-fructopyranose ester (**2e**).* Compound **2e** was obtained as a yellow solid. Yield: 80%; m.p. 164−165 °C; $[\alpha]_D^{20}$ −105 (*c* 1.6, $CHCl_3$); IR (KBr) *ν* (cm^{-1}): 1729, 1373 and 1385; ^1H NMR (300.00 MHz, $CDCl_3$) *δ* (ppm): 1.36 (3H, s, CH_3), 1.43 (3H, s, CH_3), 1.50 (3H, s, CH_3), 1.59 (3H, s, CH_3), 3.93 (1H, d, *J* = 9.3 Hz, H-1′), 4.01 (1H, dd, *J* = 9.3 Hz, H-1″), 5.40 (1H, d, *J* = 8.1 Hz, H-3′), 4.48 (1H, dd, *J* = 5.4 and 8.1 Hz, H-4′), 4.28 (1H, dd, *J* = 1.8 and 5.4 Hz, H-5′), 4.12 (1H, d, *J* = 13.7 Hz, H-6′), 4.20 (1H, dd, *J* = 2.4 and 13.7 Hz, H-6″), 2.46 (3H, s, C5−C*H*$_3$), 5.53 (2H, s, NCH_2Ph), 7.13−7.21 (2H, m, H-2a), 7.32−7.40 (2H, m,

H-3a), 7.32–7.40 (1H, m, H-4a); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ (ppm): 25.9 (CH_3), 26.2 (CH_3), 26.3 (CH_3), 27.6 (CH_3), 71.5 (C-1′), 103.5 (C-2′), 70.3 (C-3′), 74.7 (C-4′), 73.6 (C-5′), 60.2 (C-6′), 112.1 (C-7′), 109.5 (C-8′), 51.8 (N*C*H_2Ph), 136.0 (C-4), 138.4 (C-5), 9.1 (C5–*C*H_3), 133.6 (C-1a), 127.0 (C-2a), 128.9 (C-3a), 128.4 (C-4a), 160.9 (C=O). HRMS calcd for $C_{23}H_{29}N_3O_7$: 444.1771, found 444.1816.

*7.2.2.1.6. 1-Benzyl-5-methyl-1H-[1,2,3]triazole-4-carboxylic acid 1-(2,3:4,5-di-O-isopropylidene)-β-fructopyranose ester (**2f**).* Compound **2f** was obtained as a yellow oil. Yield: 70%; $[\alpha]_D^{20}$ −14 (*c* 1.6, $CHCl_3$); IR (film, $CHCl_3$) ν (cm^{-1}): 1723 and 1382; ^{1}H NMR (300.00 MHz, $CDCl_3$) δ (ppm): 1.34 (3H, s, CH_3), 1.46 (3H, s, CH_3), 1.51 (3H, s, CH_3), 1.54 (3H, s, CH_3), 4.26 (1H, d, $J = 11.5$ Hz, H-1′), 4.69 (1H, dd, $J = 11.5$ Hz, H-1″), 4.59 (1H, d, $J = 2.7$ Hz, H-3′), 4.65 (1H, dd, $J = 2.7$ and 7.8 Hz, H-4′), 4.24–4.28 (1H, m, H-5′), 3.78 (1H, dd, $J = 0.5$ and 12.9 Hz, H-6′), 3.96 (1H, dd, $J = 1.7$ and 12.9 Hz, H-6″), 2.47 (3H, s, C5–CH_3), 5.51 (1H, d, $J = 15.4$ Hz, NCH_2Ph), 5.57 (1H, d, $J = 15.4$ Hz, NCH_2Ph), 7.14–7.17 (2H, m, H-2a), 7.32–7.39 (2H, m, H-3a), 7.32–7.39 (1H, m, H-4a); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ (ppm): 23.9 (CH_3), 25.2 (CH_3), 25.7 (CH_3), 26.4 (CH_3), 64.5 (C-1′), 101.4 (C-2′), 70.1 (C-3′), 69.9 (C-4′), 70.7 (C-5′), 61.1 (C-6′), 109.0 (C-7′), 108.9 (C-8′), 51.7 (N*C*H_2Ph), 136.3 (C-4), 138.7 (C-5), 8.8 (C5–*C*H_3), 133.7 (C-1a), 127.0 (C-2a), 128.9 (C-3a), 128.4 (C-4a), 160.9 (C=O). HRMS calcd for $C_{23}H_{29}N_3O_7$: 444.1771, found 444.1752.

*7.2.2.1.7. 1-Benzyl-5-methyl-1H-[1,2,3]triazole-4-carboxylic acid 5-(3-O-benzyl-1,2-O-isopropylidene)-α-D-xylofuranose ester (**2g**).* Compound **2g** was obtained as a yellow oil. Yield: 70%; $[\alpha]_D^{20}$ −25 (*c* 1.5, $CHCl_3$); IR (film, $CHCl_3$) ν (cm^{-1}): 1721, 1374 and 1360; ^{1}H NMR (300.00 MHz, $CDCl_3$) δ (ppm): 1.33 (3H, s, CH_3), 1.49 (3H, s, CH_3), 5.98 (1H, d, $J = 3.7$ Hz, H-1′), 4.65 (1H, d, $J = 3.7$ Hz, H-2′), 4.08 (1H, d, $J = 2.7$ Hz, H-3′), 4.58–4.63 (1H, m, H-4′), 4.58–4.63 (2H, m, H-5′), 4.54 (1H, d, $J = 12.0$ Hz, H-6′), 4.72 (1H, d, $J = 12.0$ Hz, H-6″), 2.42 (3H, s, C5–CH_3), 5.54 (2H, s, NCH_2Ph), 7.17–7.39 (2H, m, H-2a), 7.17–7.39 (2H, m, H-3a), 7.17–7.39 (1H, m, H-4a), 7.17–7.39 (2H, m, H-2′a), 7.17–7.39 (2H, m, H-3′a), 7.17–7.39 (1H, m, H-4′a); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ (ppm): 26.1 (CH_3), 26.6 (CH_3), 105.0 (C-1), 82.0 (C-2), 81.4 (C-3), 77.8 (C-4), 62.1 (C-5), 71.7 (C-6), 111.7 (C-7), 51.8 (N*C*H_2Ph), 137.0 (C-4), 138.3 (C-5), 8.9 (C5–*C*H_3), 133.7 (C-1a), 128.3 (C-2a), 128.9 (C-3a), 128.4 (C-4a), 136.4 (C-1′a), 127.0 (C-2′a), 127.5 (C-3′a), 127.7 (C-4′a), 161.0 (C=O). HRMS calcd for $C_{26}H_{29}N_3O_6$: 479.2056, found 479.2104.

*7.2.3. General procedure for the preparation of 1-benzyl-1H-1,2,3-triazole derivatives **3a–g***

To a bottom balloon containing 1 mmol of 1-benzyl-1*H*-1,2,3-triazole derivatives **2**, 10 mL of 50% trifluoroacetic acid in water was added. The stirring was continued for 48 h. Then the solvent was removed under reduced pressure furnishing the 1,2,3-triazoles (**3a–g**) as brown oils, in quantitative yield.

*7.2.3.1. Spectral data of the 1-benzyl-1H-1,2,3-triazole derivatives (**3a–g**)*

*7.2.3.1.1. 1-Benzyl-5-methyl-1H-[1,2,3]triazole-4-carboxylic acid 5-β-D-ribofuranose ester (**3a**).* ^{1}H NMR (300.00 MHz, DMSO-d_6) δ (ppm): 5.10 (1H, s, H-1′), 4.35 (1H, d, $J = 11.7$ Hz, H-2′) and 4.33 (1H, d, $J = 11.7$ Hz, H-2′), 4.60 (1H, dd, $J = 3.2$ and 11.7 Hz, H-3′), 4.08 (1H, dd, $J = 3.2$ and 6.6 Hz, H-4′), 3.80 (1H, d, $J = 4.4$ Hz, H-5′), 4.14 (1H, d, $J = 4.4$ and 6.6 Hz, H-5″), 2.60 (3H, s, C5-CH_3), 5.77 (2H, s, NCH_2Ph), 7.31–7.51 (2H, m, H-2a), 7.31–7.51 (2H, m, H-3a), 7.31–7.51 (1H, m, H-4a); ^{13}C NMR (75.0 MHz, DMSO-d_6) δ (ppm): 102.0 (C-1′), 79.1 (C-2′), 71.2 (C-3′), 75.4 (C-4′), 66.2 (C-5′), 51.0 (N*C*H_2Ph), 136.0 (C-4), 138.8 (C-5), 9.1 (C5–*C*H_3), 135.2 (C-1a), 127.5 (C-2a), 129.1 (C-3a), 128.3 (C-4a), 161.1 (C=O).

*7.2.3.1.2. 1-Benzyl-5-methyl-1H-[1,2,3]triazole-4-carboxylic acid 6-α-D-galactopyranose ester (**3b**).* ^{1}H NMR (300.00 MHz, DMSO-d_6) δ (ppm): 5.06 (1H, d, $J = 3.5$ Hz, H-1′) and 5.05 (1H, d, $J = 3.0$ Hz, H-1′), 3.68 (1H, dd, $J = 3.5$ and 9.5 Hz, H-2′), 3.79–3.87 (1H, m, H-3′), 3.93 (1H, dd, $J = 2.8$ and 7.0 Hz, H-4′) and 3.98 (1H, dd, $J = 5.2$ and 7.2 Hz, H-4′), 4.33–4.38 (1H, m, H-5′), 4.23–4.27 (1H, m, H-6′), 4.27–4.45 (1H, m, H-6″), 2.57 (3H, s, C5–CH_3) and 2.58 (3H, s, C5–CH_3), 5.73 (2H, s, NCH_2Ph), 7.30–7.55 (2H, m, H-2a), 7.30–7.55 (2H, m, H-3a), 7.30–7.55 (1H, m, H-4a); ^{13}C NMR (75.0 MHz, DMSO-d_6) δ (ppm): 97.5 and 92.8 (C-1′), 69.4 and 71.9 (C-2′), 73.1 and 76.0 (C-3′), 67.8 and 68.8 (C-4′), 81.5 and 82.7 (C-5′), 64.4 and 64.3 (C-6′), 50.9 (N*C*H_2Ph), 135.9 (C-4), 138.8 and 138.7 (C-5), 8.87 and 8.92 (C5–*C*H_3), 135.1 (C-1a), 127.4 (C-2a), 128.9 (C-3a), 128.2 (C-4a), 161.0 (C=O).

*7.2.3.1.3. 1-Benzyl-5-methyl-1H-[1,2,3]triazole-4-carboxylic acid 3-β-D-allopyranose ester (**3c**).* ^{1}H NMR (300.00 MHz, DMSO-d_6) δ (ppm): 5.69 (1H, d, $J = 3.2$ Hz, H-1′), 3.43 (1H, dd, $J = 3.2$ and 8.0 Hz, H-2′), 4.80 (1H, d, $J = 8.0$ Hz, H-3′), 3.68–3.76 (1H, m, H-4′), 3.49–3.58 (1H, m, H-5′), 3.49–3.58 (1H, m, H-6′), 3.75 (1H, dd, $J = 6.8$ and 11.0 Hz, H-6″), 2.58 (3H, s, C5–CH_3), 5.73 (1H, s, NCH_2Ph), 7.29–7.54 (2H, m, H-2a), 7.29–7.54 (2H, m, H-3a), 7.29–7.54 (1H, m, H-4a); ^{13}C NMR (75.0 MHz, DMSO-d_6) δ (ppm): 94.1 (C-1′), 74.6 (C-2′), 75.3 (C-3′), 65.9 (C-4′), 70.1 (C-5′), 61.2 (C-6′), 50.9 (N*C*H_2Ph), 136.8 (C-4), 137.8 (C-5), 9.4 (C5–*C*H_3), 135.2 (C-1a), 127.5 (C-2a), 129.0 (C-3a), 128.2 (C-4a), 160.6 (C=O).

*7.2.3.1.4. 1-Benzyl-5-methyl-1H-[1,2,3]triazole-4-carboxylic acid 3-α-(or β)-D-glucofuranose ester (**3d**).* ^{1}H NMR (300.00 MHz, DMSO-d_6) δ (ppm): 5.11 (1H, d, $J = 9.3$ Hz, H-1′α), 4.56 (1H, d, $J = 7.6$ Hz, H-1′β), 3.27 (1H, m, H-2′α), 3.30 (1H, dd, $J = 7.6$ and 9.5, H-2′β), 3.55–3.66 (1H, m, H-3′α), 3.50 (1H, dd, $J = 5.8$ and 9.5 Hz, H-3′β), 3.34–3.40 (1H, m, H-4′α or H-4′β), 3.55–3.66 (1H, m, H-5′α or H-5′β), 3.55–3.66 (2H, m, H-6′α or H-6′β), 2.57 (3H, s, C5–CH_3), 5.73 (2H, s, NCH_2Ph), 7.31–7.53 (2H, m, H-2a), 7.31–7.53 (2H, m, H-3a), 7.31–7.53 (1H, m, H-4a); ^{13}C

NMR (75.0 MHz, DMSO-d_6) δ (ppm): 92.3 (C-1′α), 96.8 (C-1′β), 70.4 (C-2′α), 76.2 (C-2′β), 72.8 (C-3′α), 73.5 (C-3′β), 6′.1 (C-4′α and β), 71.9 (C-5′α), 78.5 (C-5′β), 60.8 (C-6′α and β), 50.8 (N*C*H_2Ph), 136.4 (C-4α), 136.6 (C-4β), 138.2 (C-5α), 138.3 (C-5β), 9.0 (C5−*C*H_3), 135.2 (C-1a α and β), 127.4 (C-2a α and β), 128.9 (C-3a α and β), 128.1 (C-4a α and β), 160.6 (C═O α), 160.8 (C═O β).

*7.2.3.1.5. 1-Benzyl-5-methyl-1H-[1,2,3]triazole-4-carboxylic acid 3-β-D-fructopyranose ester (**3e**).* ^{1}H NMR (300.00 MHz, DMSO-d_6) δ (ppm): 3.41−3.59 (2H, m, H-1′), 5.43 (1H, d, $J = 10.0$ Hz, H-3′), 4.01 (1H, dd, $J = 3.5$ and 10.0 Hz, H-4′), 3.88−3.89 (1H, m, H-5′), 4.00 (1H, d, $J =$ 12.0 Hz, H-6′), 3.64 (1H, dd, $J = 1.2$ and 12.0 Hz, H-6″), 2.60 and 2.61 (3H, s, C5−CH_3), 5.77 and 5.78 (2H, s, NCH_2Ph), 7.33−7.53 (2H, m, H-2a), 7.33−7.53 (2H, m, H-3a), 7.33−7.53 (1H, m, H-4a); ^{13}C NMR (75.0 MHz, DMSO-d_6) δ (ppm): 64.7 (C-1′), 97.4 and 101.6 (C-2′), 68.4 (C-3′), 70.9 (C-4′), 67.8 (C-5′), 63.6 (C-6′), 51.2 (N*C*H_2Ph), 136.7 (C-4), 138.7 (C-5), 9.3 (C5−*C*H_3), 135.5 (C-1a), 127.8 (C-2a), 129.3 (C-3a), 128.5 (C-4a), 161.0 (C═O).

*7.2.3.1.6. 1-Benzyl-5-methyl-1H-[1,2,3]triazole-4-carboxylic acid 1-β-D-fructopyranose ester (**3f**).* ^{1}H NMR (300.00 MHz, DMSO-d_6) δ (ppm): 4.22 (1H, d, $J = 11.5$ Hz, H-1′), 4.34 (1H, dd, $J = 11.5$ Hz, H-1″), 4.28−4.30 (1H, m, H-3′), 4.28−4.30 (1H, m, H-4′), 3.91−3.99 (1H, m, H-5′), 3.91−3.99 (1H, m, H-6′), 3.74−3.84 (1H, m, H-6″), 2.59 and 2.60 (3H, s, C5−CH_3), 5.77 and 5.78 (1H, s, NCH_2Ph), 7.32−7.53 (2H, m, H-2a), 7.32−7.53 (2H, m, H-3a), 7.32−7.53 (1H, m, H-4a); ^{13}C NMR (75.0 MHz, DMSO-d_6) δ (ppm): 65.2 (C-1′), 97.0 and 100.2 (C-2′), 69.1 (C-3′), 67.8 (C-4′), 69.6 (C-5′), 63.5 (C-6′), 50.9 (N*C*H_2Ph), 136.2 (C-4), 138.7 (C-5), 9.1 and 9.2 (C5−*C*H_3), 135.2 (C-1a), 127.4 (C-2a), 129.0 (C-3a), 128.2 (C-4a), (C═O).

*7.2.3.1.7. 1-Benzyl-5-methyl-1H-[1,2,3]triazole-4-carboxylic acid 5-α-D-xylofuranose ester (**3g**).* ^{1}H NMR (300.00 MHz, DMSO-d_6) δ (ppm): 4.82 (1H, d, $J = 4.4$ Hz, H-1′), 4.10−4.31 (1H, m, H-2′), 4.51−4.59 (1H, m, H-3′), 4.59−4.70 (1H, m, H-4′), 4.77 (1H, dd, $J = 3.4$ and 12.4 Hz, H-5′), 4.82 (1H, d, $J = 12.4$ Hz, H-5″), 4.61 (1H, d, $J =$ 12.0 Hz, H-6′), 4.68 (1H, d, $J = 12.0$ Hz, H-6″), 2.55 and 2.56 (3H, s, C5−CH_3), 5.77 (2H, s, NCH_2Ph), 7.32−7.57 (2H, m, H-2a), 7.32−7.57 (2H, m, H-3a), 7.32−7.57 (1H, m, H-4a), 7.32−7.57 (2H, m, H-2′a), 7.32−7.57 (2H, m, H-3′a), 7.32−7.57 (1H, m, H-4′a); ^{13}C NMR (75.0 MHz, DMSO-d_6) δ (ppm): 102.1 (C-1′), 83.8 (C-2′), 82.4 (C-3′), 78.0 (C-4′), 63.0 (C-5′), 71.4 (C-6′), 50.9 (N*C*H_2Ph), 138.3 (C-4), 138.7 (C-5), 8.8 (C5−*C*H_3), 135.1 (C-1a), 127.4 (C-2a), 128.9 (C-3a), 128.2 (C-4a), 135.1 (C-1′a), 127.4 (C-2′a), 128.9 (C-3′a), 128.2 (C-4′a), 160.9 (C═O).

All triazoles synthesized were then tested in the biological assays without any other further analytical chemistry analysis.

7.2.4. Enzyme activity assays

The inhibitory effect of triazoles at a concentration of 50 μM was evaluated on the RT polymerase activity using recombinant HIV-1 enzyme. The sequence that expressed RT HIV-1 was introduced into an *Escherichia coli* expression plasmid pUC12N. This recombinant enzyme was composed of 66 kDa protein. The bacteria containing this plasmid were grown for 12−16 h with shaking at 37 °C and were collected by centrifugation for 10 min, at 10,000 rpm. The pellet was washed once with cold 100 mM NaCl, 20% (vol/vol) glycerol, 1% triton X-100, 1 mM EDTA, 2 mM dithiotreitol, 25 mM Tris chloride, pH 8.0. The lysates were kept at 4 °C for 30 min, and the insoluble material was removed by centrifugation at 10,000 rpm. The supernatant was collected and passed over Sephadex G-25 columns at 4 °C pre-equilibrated with 0.2 M NaCl, 2 mM dithiotreitol, 0.2% Triton X-100, 20% glycerol, 25 mM Tris chloride, pH 7.4. After loading, the columns were washed with the same pre-equilibration buffer, and the fractions were assayed both for reverse transcriptase activity and for protein concentration. The polymerization reactions (50 μL) contained 50 mM Tris HCl (pH 7.8), 6 mM $MgCl_2$, 1 mM dithiotreitol, 50 mM KCl, 20 μM dTTP, 10 μM of [3H] dTTP (47 Ci/mmol), and 150 μg poly(rA)·oligo(dT) template primer (Pharmacia) and 1 U of enzyme. The reaction mixture was incubated at 37 °C for 30 min, and the incubation was stopped by adding ice-cold 5% trichloroacetic acid (TCA) containing 20 mM of sodium pyrophosphate. The precipitates were collected on Whatman GF/C filters and washed with sodium phosphate 0.1 M. The incorporated triphosphate was measured by assaying for ^{3}H in a liquid scintillation counter. One unit of enzyme is defined as the amount of enzyme that incorporates 1 pmol of dTTP in 30 min at 37 °C under standard assay conditions.

7.2.5. Cytotoxicity assay

Vero cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum (FBS; HyClone, Logan, Utah), 100 U/mL penicillin and 100 μg/mL streptomycin, at 37 °C in 5% CO2. Monolayers of about 104 Vero cells in 96-multiwell plates were treated with several concentrations of the compounds for the 72 h. Then, 50 μL of a 1 mg/mL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma) was added to evaluate cell viability according to procedures described elsewhere [49]. The 50% cytotoxic concentration (CC_{50}) was calculated by linear regression analysis of the dose−response curves. All experiments were performed in duplicate at least three times.

7.3. Molecular modeling and SAR studies

7.3.1. Molecular modeling methods

We employed molecular modeling studies for triazole derivatives in the attempt to elucidate a structure−activity relationship (SAR) to provide useful guidelines for the design of more potent antivirals. The minimum energy conformation of the most active and the last active compounds, obtained by the AM1 semiempirical method, were submitted to a single-point *ab initio* calculation with a 3-21G* basis set available on SPARTAN'04 program (Wavefunction Inc. Irvine, CA, 2000). Molecular electrostatic potential maps (MEPs), HOMO and LUMO eigen values and orbital coefficients, and the

molecular dipole moments were calculated. In this work, we also studied the druglikeness and the drug score of the compounds, which is based on topological descriptors, fingerprints of molecular druglikeness, structural keys or other properties as *c*log *P* and molecular weights [49]. In case of Osiris Property Explorer (http://www.organic-chemistry.org/), the occurrence frequency of each fragment is determined within the collection of traded drugs and within the supposedly non-druglike collection of Fluka compounds.

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References

[1] A. Bartlett, R. Demasi, J. Quinn, C. Moxham, F. Rousseau, AIDS 15 (2001) 1369–1377.
[2] UNAIDS 2005 report, <http://www.unaids.org> (accessed, 14.11.07).
[3] F. Barre-Sinoussi, J.C. Chermann, M.T. Nugeyre, S. Chamaret, J. Gruest, C. Dauget, C. Axler-Bin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum, L. Montagnier, Science 220 (1983) 868–871.
[4] R.C. Gallo, S.Z. Salahuddin, M. Popovic, G.M. Shearer, M. Kalpana, B.F. Haynes, T.J. Palker, R. Redfield, J. Oleske, B. Safai, G. White, P. Foster, P.D. Markham, Science 224 (1984) 500–503.
[5] H.C. Castro, N.I.V. Loureiro, M. Pujol-Luz, A.M.T. Souza, M.G. Albuquerque, D.O. Santos, L.M. Cabral, I.C. Frugulhetti, C.R. Rodrigues, Curr. Med. Chem. 13 (2006) 313–324.
[6] A.S. Mehanna, in: A. Burger, D.J. Abraham, J. Andrako, J.H. Block, R.T. Borchardt, A. Abraham (Eds.), Rationale of Design of Anti-HIV Drugs, sixth ed., Burger's Medicinal Chemistry and Drug Discovery, vol. 5 John Wiley and Sons, Inc., New Jersey, 2003, pp. 4574–4584.
[7] O.S. Pedersen, E.B. Pedersen, Antivir. Chem. Chemother. 10 (1999) 285–314.
[8] E.P. Peçanha, O.A.C. Antunes, Quim. Nova 25 (2002) 1108–1116.
[9] M. Shimada, H. Hosaka, H. Takaku, J.S. Smith, M.J. Roth, S. Inouye, M. Inouye, J. Biol. Chem. 269 (1994) 3925–3927.
[10] J.N. Blankson, D. Persaud, R.F. Siliciano, Annu. Rev. Med. 53 (2002) 557–593.
[11] H. Mitsuya, K. Maeda, D. Das, A.K. Ghosh, Adv. Pharmacol. 56 (2008) 169–197.
[12] S.C. Kalichman, G. Di Berto, L. Eaton, Sex Transm. Dis. 35 (2008) 55–60.
[13] X. Duval, D. Descamps, G. Breton, S. Darmon, J.L. Ecobichon, S. Delarue, G. Peytavin, C. Leport, J.L. Vildé, F. Brun-Vézinet, Antivir Ther. 10 (2005) 197–199.
[14] R.S. Hogg, D.R. Bangsberg, V.D. Lima, C. Alexander, S. Bonner, B. Yip, E. Wood, W.W. Dong, J.S. Montaner, P.R. Harrigan, PLoS Med. 3 (2006) 356.
[15] E. De Clercq, Med. Mal. Infect. 30 (2000) 421–430.
[16] E. De Clercq, J. Clin. Virol. 22 (2001) 73–89.
[17] X.L. Li, K. Chan, Adv. Drug Deliv. Rev. 39 (1999) 81–103.
[18] E. De Clercq, Biochim. Biophys. Acta 1587 (2002) 258–275.
[19] J.R. Huff, Bioorg. Med. Chem. 7 (1999) 2667–2669.
[20] L.M. Schultze, H.H. Chapman, J.P. Dubree, R.J. Jones, K.M. Kent, T.T. Lee, M.S. Louie, M.J. Postich, E.J. Prisbe, J.C. Rodhloff, R.H. Yu, Tetrahedron Lett. 39 (1998) 1853–1856.
[21] E. De Clercq, J. Med. Chem. 48 (2005) 1297–1313.
[22] K. Kacprzak, Synlett (2005) 943–946.
[23] A.C. Cunha, J.M. Figueiredo, J.L.M. Tributino, A.L.P. Miranda, H.C. Castro, R.B. Zingali, C.A.M. Fraga, M.C.B.V. de Souza, V.F. Ferreira, E.J. Barreiro, Bioorg. Med. Chem. 11 (2003) 2051–2059.
[24] R. Menegatti, A.C. Cunha, V.F. Ferreira, E.F.R. Perreira, A. El-Nabawi, A.T. Eldefrawi, E.X. Albuquerque, G. Neves, S.M.K. Rates, C.A.M. Fraga, E.J. Barreiro, Bioorg. Med. Chem. 11 (2003) 4807–4813.
[25] R.G. Micetich, S.N. Maiti, P. Spevak, T.W. Hall, S. Yamabe, N. Ishida, M. Tanaka, T. Yamazaki, A. Nakai, K. Ogawa, J. Med. Chem. 30 (1987) 1469–1474.
[26] J.L. Kelley, C.S. Koble, R.G. Davis, E.W. McLean, F.E. Soroko, B.R. Cooper, J. Med. Chem. 38 (1995) 4131–4134.
[27] B.S. Holla, M. Mahalinga, M.S. Karthikeyan, B. Poojary, P.M. Akberali, N.S. Kumari, Eur. J. Med. Chem. 40 (2005) 1173–1178.
[28] K. Dabak, O. Sezer, A. Akar, O. Anac, Eur. J. Med. Chem. 38 (2003) 215–218.
[29] D. Willner, A.M. Jelenevsky, L.C. Cheney, J. Med. Chem. 15 (1972) 948–951.
[30] G. Biagi, G. Dell'Omodarme, M. Ferretti, I. Giorgi, O. Livi, V. Scartoni, Farmaco 45 (1990) 1181–1192.
[31] G. Biagi, O. Livi, V. Scartoni, A. Lucacchini, M.R. Mazzoni, Farmaco 41 (1986) 597–610.
[32] R. Alvarez, S. Velázquez, A. San-Félix, S. Aquaro, E. De Clercq, C.F. Perno, A. Karlsson, J. Balzarini, M.J. Camarasa, J. Med. Chem. 37 (1994) 4185–4194.
[33] W.G. Lewis, L.G. Green, F. Grynszpan, Z. Radic, P.R. Carlier, P. Taylor, M.G. Finn, K.B. Sharpless, Angew. Chem., Int. Ed. 41 (2002) 1053–1057.
[34] Q. Wang, T.R. Chan, R. Hilgraf, V.V. Fokin, K.B. Sharpless, M.G. Finn, J. Am. Chem. Soc. 125 (2003) 3192–3193.
[35] L.V. Lee, M.L. Mitchell, S.J. Huang, V.V. Fokin, K.B. Sharpless, C.H. Wong, J. Am. Chem. Soc. 125 (2003) 9588–9589.
[36] A.J. Link, D.A. Tirrell, J. Am. Chem. Soc. 125 (2003) 11164–11165.
[37] A.E. Speers, G.C. Adam, B.F. Cravatt, J. Am. Chem. Soc. 125 (2003) 4686–4687.
[38] A.E. Speers, B.F. Cravatt, Chem. Biol. 11 (2004) 535–546.
[39] T.S. Seo, Z. Li, H. Ruparel, J. Ju, J. Org. Chem. 68 (2003) 609–612.
[40] S. Lober, P. Rodriguez-Loaiza, P. Gmeiner, Org. Lett. 5 (2003) 1753–1755.
[41] C.W. Tornoe, S.J. Sanderson, J.C. Mottram, G.H. Coombs, M. Meldal, J. Comb. Chem. 6 (2004) 312–324.
[42] H. Gallardo, F. Ely, A.J. Bortoluzzi, G. Conte, Liq. Cryst. 32 (2005) 667–671.
[43] F.C. Silva, M.C.B.V. de Souza, V.F. Ferreira, S.J. Sabino, O.A.C. Antunes, Catal. Commun. 5 (2004) 151–155.
[44] F.M. Stojanovic, Z. Arnold, Collect. Czech.Chem. Commun. 32 (1967) 2155–2156.
[45] Ö. Sezer, K. Dabak, O. Anaç, A. Akar, Helv. Chim. Acta 79 (1996) 449–453.
[46] G.A. Romeiro, L.O.R. Pereira, M.C.B.V. de Souza, V.F. Ferreira, A.C. Cunha, Tetrahedron Lett. 38 (1997) 5103–5106.
[47] A.C. Cunha, L.O.R. Pereira, R.O.P. de Souza, M.C.B.V. de Souza, V.F. Ferreira, Nucleosides Nucleotides Nucleic Acids 20 (2001) 1555–1569.
[48] K.A. Winans, C.R. Bertozzi, Chem. Biol. 9 (2002) 113–129.
[49] T. Mosman, J. Immunol. Methods 65 (1983) 55–63.
[50] I.V. Tetko, Drug Discov. Today 10 (2005) 22–36.
[51] A.C. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Adv. Drug Deliv. Rev. 23 (1997) 3–25.
[52] M.S. Costa, N. Boechat, E.A. Rangel, F.de C. da Silva, A.M. de Souza, C.R. Rodrigues, H.C. Castro, I.N. Junior, M.C. Lourenço, S.M. Wardell, V.F. Ferreira, Bioorg. Med. Chem. 14 (2006) 8644–8653.
[53] L.R. Dias, M.B. Santos, S. Albuquerque, H.C. Castro, A.M. de Souza, A.C. Freitas, M.A. DiVaio, L.M. Cabral, C.R. Rodrigues, Bioorg. Med. Chem. 15 (2007) 211–219.