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Thymidine- and AZT-linked 5-(1,3-dioxoalkyl)tetrazoles and 4-(1,3-dioxoalkyl)-1,2,3-triazoles

Lluís Bosch[a], Olivier Delelis[b], Frédéric Subra[b], Eric Deprez[b], Myriam Witvrow[c], Jaume Vilarrasa[a,*]

[a] *Departament de Química Orgànica, Facultat de Química, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Catalonia, Spain*
[b] *LBPA, CNRS, École Normale Supérieure de Cachan, 94235 Cachan, France*
[c] *Molecular Medicine, Katholieke Universiteit Leuven, Kapucijnenvoer 33, B-3000 Leuven, Flanders, Belgium*

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ABSTRACT

N3 of thymidine and of 3'-azido-3'-deoxythymidine (AZT) has been linked to a tetrazole ring by condensation of nucleoside-derived 2-oxonitriles with the lithium salt of 5-acetyl-1-(4-fluorobenzyl)tetrazole (obtained by a "click" reaction). 4-Acetyl-1,2,3-triazole, also prepared by a Cu-catalysed cycloaddition, has been similarly linked. A route for the conjugation of NRTIs with pharmacophoric elements of integrase inhibitors (INIs) has thus been disclosed.

The HIV-1 integrase (IN) is a well-established target in the long-lasting fight against AIDS. Many integrase inhibitors (INIs) have been developed since Shionogi and Merck scientists disclosed years ago that derivatives of 2,4-dioxobutanoic acids (so-called diketo acids) blocked the strand transfer capability of the viral integrase.[1] Other small molecules sharing the same pharmacophoric elements, such as bioisosteric 5-(1,3-dioxopropyl)-tetrazoles or heteroaromatic substrates were soon designed and synthesised. Famous INIs are depicted in Figure 1 in their Mg^{2+}-complexing anionic forms;[2] the outstanding one is yet raltegravir (Isentress, approved by FDA in 2007).[3] Nevertheless, as expected owing to the fast HIV-1 mutations, resistance against raltegravir has appeared as well.[4] Alternative INIs with independent resistance profiles and drug candidates tackling other steps of the HIV life cycle are always a must.

Conjugation of the pharmacophore—a Mg^{2+}-chelating moiety with a hydrophobic 4-fluorobenzyl group—with nucleosides could favour the trans-membrane transport of the new chemical entities and might "simplify" their catabolism affording less toxic metabolites. We also believed that conjugation of the pharmacophore with a nucleoside reverse transcriptase inhibitor (NRTI) could give rise to molecules of potentially synergic, dual effect (both NRTI and INI).[5]

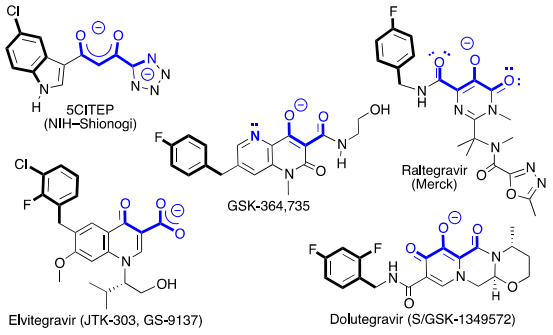


Figure 1. Common pharmacophoric elements of representative INIs.

We planned to link tetrazole or 1,2,3-triazole rings, via the formation of a 1,3-dioxo moiety, to nucleosides, without disturbing the key features of the latter. We chose thymidine

* Corresponding author. Tel.: +34 93 4021258
E-mail address: jvilarrasa@ub.edu

and 3'-azido-3'-deoxythymidine (AZT, zidovudine) as working models (Figure 2). If we were able to generate such conjugates, we could apply the concept to a number of cases by linking other nucleosides and NRTIs to related INI moieties (e.g., to the 5-hydroxypyrimidinone substructure of raltegravir and its congeners). We report here our first approach: the synthesis of molecules such as those at the left side of Figure 2, with the nucleoside and the hydrophobic group at opposite sites of the chelating group.

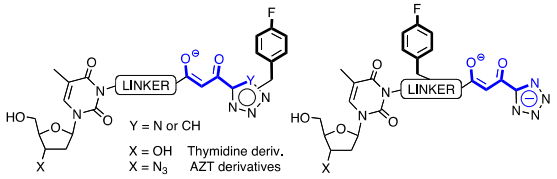


Figure 2. Examples of possible thymidine derivatives linked to Mg^{2+}-chelating moieties and to hydrophobic groups.

In this context, we evaluated first the effect of the attachment of simple (but relatively polar) substituents to appropriate positions of AZT. The activity against wild-type HIV-1 (NL4-3)[6] of the samples shown in Figure 3 (**1a–f**), prepared in our lab,[7] clearly indicated that polar organic groups attached to position 3 (N3) of the thymine ring of AZT reduced slightly or only moderately its activity (but not by a factor of let's say 10^3, which would have made our objective nonsense).

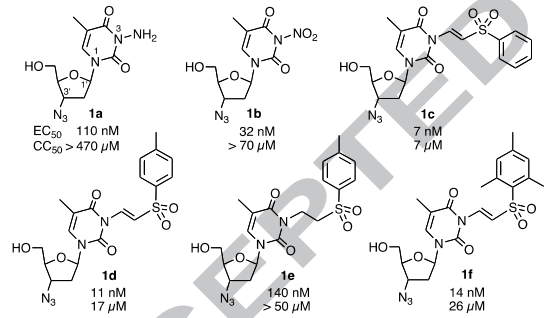
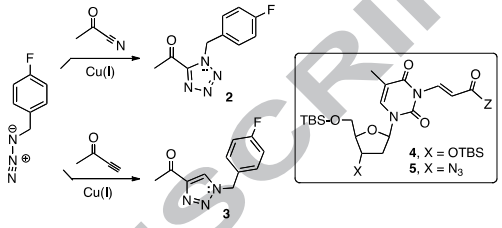


Figure 3. Anti-HIV-1 (effective concentration, EC_{50}) activities and cytotoxicities (CC_{50}) of N3-substituted AZTs. EC_{50} values for AZT are in the 2–7 nM range (mean value, 3 nm), under the conditions of Ref. 6, for different bioassays performed during three years.

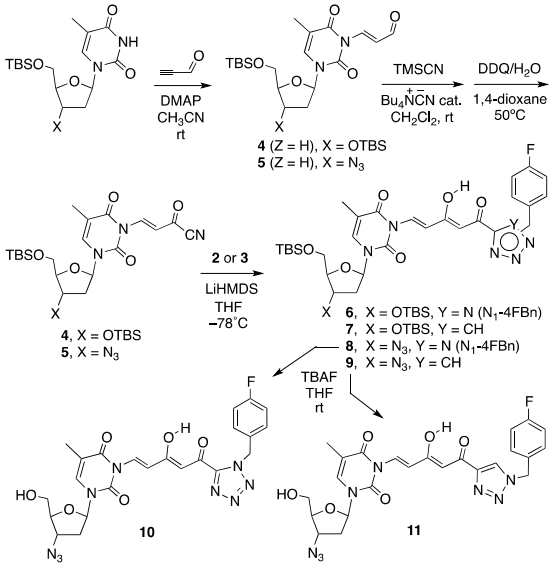
Thus, the thymidine kinase seems capable of converting these N3-substituted AZTs[8] into their phosphate esters (subsequently phosphorylated up to the triphosphate esters) and incorporate them into the nascent viral DNA, as any other chain-terminating NRTI.

The technical problem of the preparation of tetrazoles C5-substituted with $COCH_3$ or related electron-withdrawing groups (EWGs), under very mild conditions, was solved by us time ago by means of a $Cu_2(OTf)_2$-catalysed cycloaddition.[9] Moreover, the preparation of 1,2,3-triazole derivatives by Cu(I)-catalyzed alkyne–azide cycloaddition (CuAAC, Sharpless' "click chemistry") is currently a subject of great scope.[10] We applied these two "click" reactions to 4-fluorobenzyl azide (Scheme 1), to prepare **2** and **3**. Following own protocols,[7] we prepared nucleoside substrates represented by structure **4**. Among various attempted condensation reactions,[11] only the coupling of an excess of lithium enolates of **2** and **3**, at low temperature, with appropriate nucleoside derivatives **4** and **5** (with Z = CN) afforded satisfactory results.[12]



Scheme 1. Preparation of starting materials.

With all this information in hand, we performed the syntheses summarised in Scheme 2. We obtained aldehydes **4** (Z = H) and **5** (Z = H), for the first time, from the corresponding nucleosides (TBS-diprotected thymidine, as a model for checking the reactions, and TBS-protected AZT) and freshly prepared propynal in the presence of DMAP, in CH_2Cl_2–CH_3CN at rt. We treated them with trimethylsilyl cyanide; the silylated cyanohydrins were oxidised in situ with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) in moist 1,4-dioxane,[13] to give **4** (Z = CN) and **5** (Z = CN) in 75–85% overall yields. In the most delicate step (the most prone to give rise to decomposition of the nucleoside substrates), these 2-oxonitrils were added drop-wise at –78ºC to **2** and **3** (220 mol%), to afford **6–9** in 44–70% yields.[14]



Scheme 2. Synthesis of the AZT derivatives **10** and **11**.

Finally, the TBS protecting groups of AZT derivatives **8** and **9** were removed with $Bu_4N^+F^-\cdot 3H_2O$ (TBAF), to give the desired AZT derivatives, **10** and **11**,[15] in 74% and 90% yields, respectively.

The integrase strand-transfer inhibitory activities of **10** and **11** (enzyme inhibitory concentrations, IC_{50})[16] are collected in Table 1, together with their effective concentrations (EC_{50}) and cytotoxic concentrations (CC_{50}) under the conditions indicated[16] (which differ from those of Ref. 6 but are useful for comparison). Compound **10** showed a low INI activity (see Table 1, entry 2, IC_{50} = 350 μM) while that of **11** (entry 3) was undetectable; an additional chelating group or atom (COO^-, OH, CONH or heterocyclic N) closer to the enol-enone moiety is probably required. On the other hand, **10** and **11** were almost as active as AZT as far as EC_{50} values[16] are concerned (compare entries 2 and 3 to entry 4), a fact that we expected (see the preceding page) but it had to be demonstrated. In this context, we should stress that **11** was more cytotoxic than AZT, so that we will discontinue the optimisation of this triazole derivative (of triazole derivatives in general); since thymidine derivatives also appeared to be cytotoxic (e.g., TBS-deprotected **7**, CC_{50} = 6 μM), we ruled out them for future improvements as well. Only tetrazole derivative **10**, among **6–11**, might become a lead.

Table 1. HIV-1 integrase inhibitory concentration (IC_{50}), antiviral activity (EC_{50}) and cytotoxicities (CC_{50}) of **10** and **11** as compared to raltegravir and AZT.[a]

entry	compound	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
1	raltegravir	0.004	0.005	1000
2	**10**	350	0.240	130
3	**11**	n.a.	0.160	8
4	AZT	—	0.150	200

[a] Under the conditions of Ref. 16; n.a. = not active (> 800 μM).

In summary, we have been fortunate to obtain for the first time representative conjugates of nucleosides and 1,3-dioxopent-4-enyl derivatives of tetrazole and 1,2,3-triazole. Condensation of 5-acetyl-1-(4-fluorobenzyl)tetrazole and 4-acetyl-1-(4-fluorobenzyl)-1,2,3-triazole with nucleoside-derived 2-oxonitriles (never reported so far in the nucleoside field, a reaction only successful with aromatic substrates) was the method of choice. The key synthetic challenges have been solved. The integrase inhibition activity of the most promising "minimalistic" candidate (**10**) is still insufficient, but we have demonstrated that it keeps the features of AZT derivatives. Syntheses of other conjugates, more advanced prototypes with one further Mg^{2+}-chelating group on **10**, are under way.

Acknowledgments

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6. Evaluated with the MTT method [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] in MT-4 cells. Stock solutions of the compounds were prepared in DMSO (10 mg/mL). After 4 days of incubation at 37 ºC, the number of viable cells was determined with MTT. Standard parallel tests for cytotoxic effects in uninfected MT-4 cells were always performed. See, e.g.: Moncunill, G.; Armand-Ugon, M.; Clotet-Codina, I.; Pauls, E.; Ballana, E.; Llano, A.; Romagnoli, B.; Vrijbloed, J. W.; Gombert, F. O.; Clotet, B.; De Marco, S.; Este, J. A. *Mol. Pharmacol*. **2008**, *73*, 1264.
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14. Experimental procedure. The methyl ketone (**2** or **3**, 2.20 mmol) was solved in anhyd. THF (10 mL) and cooled to –78ºC. This solution was added dropwise, under Ar, to a LiHMDS solution (0.2 M, 11 mL) at –78ºC; stirring was maintained for further 5 min. Acyl cyanide (**4** or **5**, 1.00 mmol), solved in anhyd. THF (5 mL) at –78ºC was then added dropwise to the enolate solution. The reaction mixture was stirred for 2 h while the temperature was allowed to rise up to –10ºC. The resulting dark-red solution was quenched with cold aqueous HCl (0.05 M, 4.5 mmol), to give a yellowish solution. The mixture was diluted with EtOAc (50 mL) and water (20 mL), the layers were separated and the aqueous one extracted twice with further EtOAc (20 mL). The organic extracts were treated with brine (25 mL), dried over Na_2SO_4, filtered and evaporated to dryness. Purification of the crude product by silica gel chromatography (hexanes/EtOAc/AcOH, 70:30:1) afforded the desired compounds (**6–9**) in 44%, 65%, 54%, and 70% yields, respectively.

Spectral data of **6**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.09 (s, 3H), 0.10 (s, 3H), 0.12 (s, 3H), 0.12 (s, 3H), 0.91 (s, 9H), 0.93 (s, 9H), 1.94 (m, 1H), 1.97 (d, *J* = 0.7, 3H), 2.02 (ddd, *J* = 13.3, 7.6, 6.1, 1H), 2.33 (ddd, *J* = 13.1, 5.8, 2.6, 1H), 3.78 (dd, *J* = 11.4, 2.4, 1H), 3.90 (dd, *J* = 11.5, 2.6, 1H), 3.99 (m, 1H), 4.41 (dt, *J* = 5.3, 2.5, 1H), 5.95 (s, 2H), 6.34 (dd, *J* = 7.7, 5.8, 1H), 6.78 (s, 1H), 7.04 (t, *J* = 8.6, 2H), 7.39 (d, *J* = 14.6, 1H), 7.44 (dd, *J* = 8.5, 5.2, 2H), 7.57 (m, 1H), 8.43 (d, *J* = 14.5, 1H), 14.72 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ –5.4 (CH_3), –5.4 (CH_3), –4.8 (CH_3), –4.6 (CH_3), 17.4 (CH_3), 18.0 (C), 18.4 (C), 25.7 (CH_3), 25.9 (CH_3), 41.6 (CH_2), 52.1 (CH_2), 62.9 (CH_2), 72.2 (CH), 86.1 (CH), 88.2 (CH), 101.0 (CH), 109.6 (C), 115.9 (d, *J* = 21.9, CH), 116.1 (CH), 129.8 (d, *J* = 3.4, C), 130.7 (d, *J* = 8.4, CH), 133.0 (CH), 134.2 (CH), 149.1 (C), 149.5 (C), 162.1 (C), 163.4 (d, *J* = 239.2, C), 178.5 (C), 180.1 (C).

Spectral data of **7**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.09 (s, 3H), 0.10 (s, 3H), 0.12 (s, 3H), 0.12 (s, 3H), 0.91 (s, 9H), 0.93 (s, 9H), 1.96 (m, 1H), 1.96 (d, *J* = 1.0, 3H), 2.33 (ddd, *J* = 13.2, 5.8, 2.8, 1H), 3.78 (dd, *J* = 11.4, 2.4, 1H), 3.90 (dd, *J* = 11.4, 2.5, 1H), 3.98 (dt, *J* = 2.4, 2.3, 1H), 4.41 (td, *J* = 5.4, 2.6, 1H), 5.55 (s, 2H), 6.34 (dd, *J* = 7.6, 5.8, 1H), 6.67 (s, 1H), 7.08 (t, *J* = 8.5, 2H), 7.30 (dd, *J* = 8.4, 5.1, 2H), 7.34 (d, *J* = 14.7, 1H), 7.54 (m, 1H), 7.99 (s, 1H), 8.28 (d, *J* = 14.6, 1H), 15.23 (br. s, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ –5.5 (CH_3), –5.4 (CH_3), –4.9 (CH_3), –4.7 (CH_3), 13.4 (CH_3), 18.0 (C), 18.4 (C), 25.7 (CH_3), 25.9 (CH_3), 41.6 (CH_2), 53.7 (CH_2), 62.9 (CH_2), 72.1 (CH), 86.0 (CH), 88.1 (CH), 99.0 (CH), 109.7 (C), 116.3 (d, *J* = 22.0, CH), 117.9 (CH), 125.1 (CH), 129.7 (d, *J* = 3.0, C), 130.2 (d, *J* = 8.5, CH), 130.9 (CH), 133.9 (CH), 147.0 (C), 149.5 (C), 162.2 (C), 163.0 (d, *J* = 249.2, C), 178.4 (C), 183.2 (C).

Spectral data of **8**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.14 (s, 6H), 0.94 (s, 9H), 1.97 (d, *J* = 1.2, 3H), 2.26 (td, *J* = 13.7, 6.9, 1H), 2.51 (ddd, *J* = 13.7, 6.2, 4.5, 1H), 3.82 (dd, *J* = 11.3, 2.1, 1H), 3.99 (m, 2H), 4.24 (td, *J* = 7.1, 4.4, 1H), 5.55 (s, 2H), 6.22 (t, *J* = 6.3, 1H), 6.68 (s, 1H), 7.09 (t, *J* = 8.6, 2H), 7.30 (dd, *J* = 8.6, 5.2, 2H), 7.33 (d, *J* = 14.6, 1H), 7.51 (q, *J* = 1.1, 1H), 7.97 (s, 1H), 8.26 (d, *J* = 14.6, 1H), 15.21 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ –5.4 (CH_3), –5.3 (CH_3), 13.5 (CH_3), 18.4 (C), 25.9 (CH_3), 38.2 (CH_2), 53.8 (CH_2), 60.3 (CH), 62.8 (CH_2), 84.7 (CH), 85.7 (CH), 99.2 (CH), 109.9 (C), 116.3 (d, *J* = 22.0, CH), 118.6 (CH), 125.1 (CH), 129.7 (d, *J* = 3.0, C), 130.2 (d, *J* = 8.5, CH), 130.5 (CH), 133.3 (CH), 147.0 (C), 149.5 (C), 162.1 (C), 163.0 (d, *J* = 249.2, C), 178.1 (C), 183.2 (C).

Spectral data of **9**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.14 (s, 6H), 0.94 (s, 9H), 1.97 (d, *J* = 1.2, 3H), 2.27 (td, *J* = 13.6, 6.8, 1H), 2.52 (ddd, *J* = 13.6, 5.8, 4.7, 1H), 3.83 (dd, *J* = 11.5, 2.1, 1H), 4.00 (m, 2H), 4.25 (td, *J* = 7.2, 4.3, 1H), 5.95 (s, 2H), 6.22 (t, *J* = 6.3, 1H), 6.78 (s, 1H), 7.04 (t, *J* = 8.6, 2H), 7.37 (d, *J* = 14.5, 1H), 7.44 (dd, *J* = 8.7, 5.3, 2H), 7.54 (q, *J* = 1.2, 1H), 8.39 (d, *J* = 14.6, 1H), 14.70 (br. s, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ –5.4 (CH_3), –5.3 (CH_3), 13.5 (CH_3), 18.5 (C), 25.8 (CH_3), 38.2 (CH_2), 52.3 (CH_2), 60.4 (CH), 62.8 (CH_2), 84.7 (CH), 85.7 (CH), 101.2 (CH), 110.0 (C), 115.8 (d, *J* = 21.8, CH), 118.8 (CH), 128.9 (d, *J* = 3.2,

C), 130.8 (d, J = 8.5, CH), 130.5 (CH), 133.3 (CH), 149.2 (C), 149.6 (C), 161.9 (C), 163.4 (d, J = 248.2, C), 177.9 (C), 180.2 (C).

15. **10**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.96 (d, J = 0.6, 3H), 2.49 (m, 2H), 3.88 (dd, J = 11.7, 2.4, 1H), 4.00 (td, J = 5.2, 2.5, 1H), 4.06 (dd, J = 11.8, 2.4, 1H), 4.40 (td, J = 11.9, 6.5, 1H), 5.95 (s, 2H), 6.16 (t, J = 6.2, 1H), 6.77 (s, 1H), 7.04 (t, J = 8.7, 2H), 7.35 (d, J = 14.6, 1H), 7.44 (dd, J = 8.8, 5.2, 1H), 7.60 (m, 1H), 8.38 (d, J = 14.6, 1H), 14.70 (br. s, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 13.4 (CH_3), 37.8 (CH_2), 52.2 (CH_2), 59.5 (CH), 61.8 (CH_2), 84.6 (CH), 87.0 (CH), 101.1 (CH), 110.0 (C), 115.9 (d, J = 21.7, CH), 116.4 (CH), 129.7 (d, J = 3.3, C), 130.7 (d, J = 8.4, CH), 132.7 (CH), 134.9 (CH), 149.1 (C), 149.4 (C), 161.9 (C), 162.9 (d, J = 248.7, C), 178.5 (C), 179.8 (C).
11: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.96 (d, J = 1.0, 3H), 2.99 (m, 2H), 3.86 (dd, J = 11.9, 2.6, 1H), 4.00 (td, J = 5.1, 2.4, 1H), 4.05 (dd, J = 11.9, 2.6, 1H), 4.40 (td, J = 7.1, 5.5, 1H), 5.55 (s, 2H), 6.13 (t, J = 6.3, 1H), 6.67 (s, 1H), 7.09 (t, J = 8.6, 2H), 7.30 (dd, J = 8.6, 5.2, 2H), 7.31 (d, J = 14.6, 1H), 7.52 (m, 1H), 7.98 (s, 1H), 8.23 (d, J = 14.6, 1H), 15.35 (br. s, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 13.4 (CH_3), 37.7 (CH_2), 53.8 (CH_2), 59.6 (CH), 61.8 (CH_2), 84.6 (CH), 87.2 (CH), 99.2 (CH), 110.2 (C), 116.4 (d, J = 21.7, CH), 118.2 (CH), 125.2 (CH), 128.6 (CH), 129.6 (d, J = 3.3, C), 130.2 (d, J = 8.5, CH), 130.6 (CH), 134.7 (CH), 144.4 (C), 147.0 (C), 149.5 (C), 161.9 (C), 163. 2 (d, J = 249.6, C), 178.2 (C), 183.2 (C).
16. Under the following conditions: 100 nM of HIV-1 IN (B strain), 10 mM $MgCl_2$, pH 7.0, 37 °C, according to: (a) Deprez, E.; Barbe, S.; Kolaski, M.; Leh, H.; Zouhiri, F.; Auclair, C.; Brochon, J. C.; Le Bret, M.; Mouscadet, J. F. *Mol. Pharmacol.* **2004**, *65*, 85–98; (b) Delelis, O.; Malet, I.; Na, L.; Tchertanov, L.; Calvez, V.; Marcelin, A. G., Subra, F., Deprez, E.; Mouscadet, J. F. *Nucl. Ac. Res.* **2009**, *37*, 1193–1201. Stock solutions were prepared in DMSO at concentrations of 10 mg/mL. HeLa-$CD4^+$-β-gal reporter cells were infected in triplicate with an amount of 3 ng of p24 antigen in the presence of increasing concentrations of the drug candidates. The EC_{50} values were determined 48 h post infection as the concentration of drug inhibiting β-galactosidase production by 50% in comparison to results for the untreated infected cells. Cytotoxicities were determined in parallel on uninfected cells.

Graphical abstract

Thymidine- and AZT-linked 5-(1,3-dioxoalkyl)tetrazoles and 4-(1,3-dioxoalkyl)-1,2,3-triazoles **pp xxxx–xxxy**

Lluís Bosch, Olivier Delelis, Frédéric Subra, Eric Deprez, Myriam Witvrow, Jaume Vilarrasa*