Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 2277–2281
Bioorganic & Medicinal Chemistry Letters

Synthesis, anti-HIV-1 activity, and modeling studies of *N*-3 Boc TSAO compound

Cyrille Tomassi,[a] Albert Nguyen Van Nhien,[a] José Marco-Contelles,[b] Jan Balzarini,[c] Christophe Pannecouque,[c] Erik De Clercq,[c] Elena Soriano[d] and Denis Postel[a,*]

[a]*Laboratoire des Glucides (UMR6219), Université de Picardie Jules Verne, 33 rue Saint Leu, 80039 Amiens, France*
[b]*Laboratorio de Radicales Libres (IQOG, CSIC), C/Juan de la Cierva, 3, 28006 Madrid, Spain*
[c]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium*
[d]*Laboratorio de Resonancia Magnetica (IIB, CSIC), C/Arturo Duperier 4, 28029 Madrid, Spain*

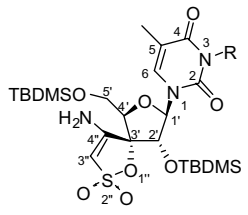
Received 4 February 2008; revised 3 March 2008; accepted 3 March 2008
Available online 7 March 2008

Abstract—The synthesis and the biological evaluation of the anti-HIV-1 activity of TSAO-Boc^{3}T (**8**) are described. The computational analysis showed that the *N*-3 Boc group promotes new interactions in the binding site of the enzyme leading to a good inhibitory activity.

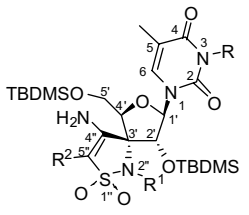
Non-nucleoside reverse transcriptase inhibitors (NNRTIs) represent a particular group of compounds which bind to a hydrophobic pocket near to, but not at the HIV-1 polymerase active site of p66, resulting in an inactive conformation of the enzyme.[1–3] TSAO compounds represent a peculiar family of HIV-1 RT inhibitors since they are the first non-peptide molecules that interact with amino acids at both HIV-1 RT subunits (p66 and p51), at the dimer interface, and thus can interfere with enzyme dimerization.[4] Since 1992, TSAO compounds have been subjected to extensive structure–activity studies (SAR) to assess the structural requirements for their optimal interaction with HIV-1 RT, a prerequisite for antiviral activity. The *ribo* configured sugar plays an essential role in the interaction as well as the presence of both a 3′-spiro-5″-(4″-amino-1″,2″-oxathiole- 2″,2″-dioxide) moiety and OTBDMS groups at positions 2′ and 5′ of the sugar moiety. Modifications either at positions 2′ or 5′,[5] on the 3′-spiro moiety,[6] or on the base part,[7] have been performed. Like for NRTIs and NNRTIs, TSAOs provoke resistance of HIV-1 RT due to the Glu-138 → Lys mutation, suggesting that they may interact with the Glu-138 residue located in the β7–β8 loop in p51.[8] The phenomenon of resistance and eagerness for complete elucidation of the precise binding mode of this drug class to this RT has prompted the exploration of further structural modifications of TSAO analogs for SAR study.

Camarasa et al. reported the synthesis and biological evaluation of several compounds bearing a variety of polar, lipophilic, or aromatic groups linked through flexible polymethylene linkers. The TSAO derivative with an *N*-methylcarboxamide group at the *N*-3 position was found to be 5- to 6-fold more active than the TSAO-T.[9]

Recently, we reported the first synthesis of ATSAOs in which the 3′-spiro-oxathiazole ring is substituted with a spiro-isothiazole moiety (Fig. 1).[10a] The evaluation



TSAO-T, R= H
TSAO-m^{3}T, R= CH_3



ATSAO-T derivatives
R= H, Boc, CH_3
R^1= H, CH_3
R^2= H, Ph

Figure 1. Aza analogs of TSAO.

Keywords: NNRTI; Aminonitrile; CSIC reaction; TSAO; ATSAO; Computational study.
* Corresponding author. Tel.: +33 22 82 75 70; fax: +33 22 82 75 68; e-mail: denis.postel@sc.u-picardie.fr

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.03.010

of the inhibitory effects on HIV-1 replication demonstrated that these ATSAO derivatives showed the same HIV-1 specific activity spectrum (EC_{50}: 0.13–0.53 μM) but significantly, showed no inhibitory activity against HIV-2 at subtoxic concentrations. A computational study revealed that the ATSAO compounds adopt a similar conformation at the p66–p51 interface as that of TSAO analogs.[11]

AzaTSAO-T derivatives bearing a substituted dihydroisothiazole dioxide ring with a phenyl group at 5″ position have also been reported. Their biological evaluation revealed that the phenyl group leads to a dramatic decrease in the inhibitory effect. Continuing our work on this area, and in order to gain a deeper insight into the SAR of (A)TSAO derivatives, herein we report the synthesis of TSAO-Boc^{3}T (**8**) as well as its biological evaluation and modeling study of the most active TSAO and ATSAO compounds (with a Boc functionality at *N*-3).

In the previous work,[10a] we demonstrated that ATSAO-Boc^{3}T, which has an unsubstituted isothiazolic ring, proved to be only 2- to 7-fold less active than TSAO-T against HIV-1 replication in MT-4 and CEM cells. The inhibitory effects for ATSAO-Boc^{3}T and the unsubstituted *N*-3 analog (EC_{50}: 0.13 vs 0.53 μM) suggested that it was the Boc group at the *N*-3 position that increases the activity. In spite of the large number of compounds tested,[4] it is surprising that no TSAO analog equivalent to our ATSAO-Boc^{3}T has been synthesized so far.

In order to establish the role of a Boc group at *N*-3 on the biological activity and to further elaborate SAR studies, here we describe the synthesis, modeling studies, and the anti-HIV-1 activity of compound TSAO-Boc^{3}T (**8**).

Following the synthetic pathway described by Camarasa et al., carbamoylation of the TSAO-T was unsuccessful; consequently, the Boc group was introduced before the *Carbanion mediated Sulfonate Intramolecular Cyclization* (*CSIC*)-reaction step.[10b,c] Accordingly, compound **4**, obtained from precursor **1**, was reacted under the usual conditions to give the Boc-derivative **5**, which was submitted to the CSIC protocol to provide product **6** in 60% yield. Subsequent deprotection (NH_3/MeOH) and silylation afforded the target compound **8** in 50% yield.

The inhibitory activity of compound **8** against HIV-1 (IIIB) and HIV-2 (ROD) was evaluated in CEM cell cultures. In a previous work,[10a] we demonstrated that the ATSAO derivatives lacking a substituent (methyl) on the endocyclic nitrogen atom of the spiro moiety were potent and selective inhibitors of HIV-1 in CEM cell cultures, as none of them showed inhibitory activity against HIV-2 replication, at subtoxic concentrations.

However, for compound **8**, which has an oxathiole spiro ring, the concentration required to protect CEM cells against the cytopathogenicity of HIV by 50% (EC_{50}: 0.023 ± 0.010 μM) clearly demonstrates that introduction of a Boc group at the *N*-3 position improves the antiviral activity over that of the lead TSAO-T compound (EC_{50} = 0.06 ± 0.010 μM). We hypothesized that this might probably be due to the establishment of additional interactions with the dimer interface near the β7–β8 loop. Moreover, *N*-3 carbamoylation decreased the cytotoxic concentration compared with TSAO-T (CC_{50} = 26.2 ± 1.12 μM vs 16 ± 1.0 μM for TSAO-T). No antiviral activity was observed against HIV-2. This result is in accordance with our preliminary results obtained in the ATSAO series.

In order to further elucidate the binding mode induced by this novel pharmacophore, a modeling study was undertaken. A recent computational study focused on the role of the heteroatom at the spirocycle revealed that its endocyclic moiety is mostly exposed to the solvent, and it was argued that additional electrostatic stabilization by the polar solvent might stabilize the complex formed with the oxathiole or unmethylated aza derivatives.[11] It was postulated, on the other hand, that the presence of a substituent on the endocyclic nitrogen atom constrains the ring pucker to a narrow region of the pseudorotational cycle. This leads to a fairly distorted disposition of the base and substituents of furanose, mainly the 5′-O-TBDMS group. Because of the well-known rigorous geometric requirements for the functional groups at C-5′,[5a,12] we speculated that this enhanced conformational rigidity may impede the compound's efficient adaptation to the enzyme binding site, leading to a loss of affinity.

According to the proposed model, the *N*-3 substituent would be mostly exposed to the solvent and run parallel to the subunit interface. Thus additional interactions with other interface residues may help destabilize the RT dimer by disrupting key p51/p66 interface interactions.[8] In fact, it has been demonstrated that modifications at the *N*-3 position are well tolerated,[13] and in some cases are even better than the prototype TSAO-T at disrupting the inter-subunit interactions. Thus, they are more potent inhibitors of the enzyme's DNA polymerase activity, and also exhibit potent antiviral activity.[14]

We have now conducted a molecular modeling analysis (see Supplementary Data) on the effect of a Boc substituent on *N*-3 to identify the structural features that are important for the improved ability, relative to the lead TSAO compound, to inhibit HIV-1 RT dimerization.

Docking studies[15] (see Supplementary Data) were performed on the proposed binding site using the compounds shown in Figure 2. The number of clusters found for each compound, the proportion of highly populated clusters, and the corresponding binding and docking energies of each complex were calculated (see Supplementary Data) (Scheme 1).

Our data indicate that the Boc-derivatives can pose in the presumed binding site mainly in two different modes, each differing in orientation of the Boc group at *N*-3: complexes **a** and **b**. Figure 3 shows both conformations for compound **8**. As can be seen, the Boc carbonyl oxy-

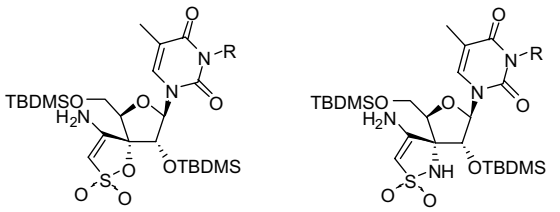
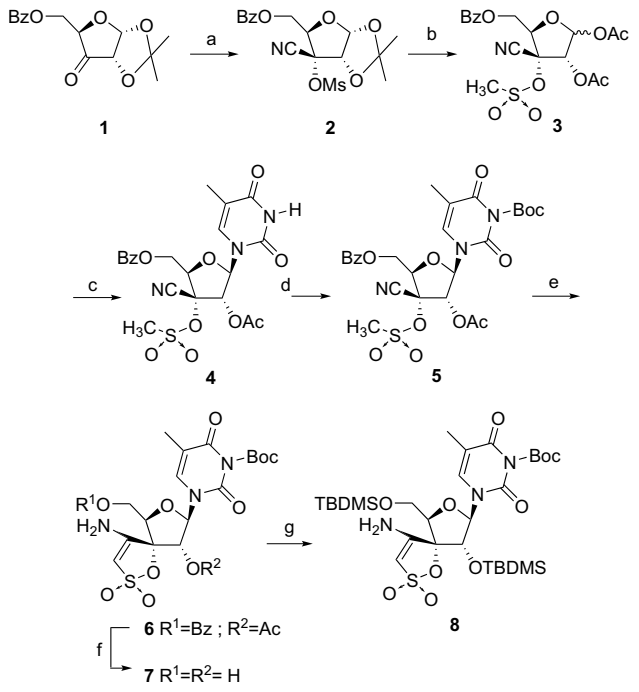


Figure 2.



Scheme 1. Reagents and condition: (a) i—NaCN, $NaHCO_3$, Et_2O–H_2O; ii—MsCl, py (78%); (b) i—TFA–H_2O; ii—Ac_2O, py (95%); (c) silylated thymine, TMSOTf (77%); (d) $(Boc)_2O$, CH_2Cl_2/py (4/1; v/v) (85%); (e) Cs_2CO_3, CH_3CN (60%); (f) NH_3/MeOH (70%); (g) TBDMSCl, CH_3CN (50%).

gen may hydrogen bond to the hydroxy group of the side chain of Thr-B139 (complexes of type **a**) or with Lys-A172 (complexes of type **b**). Such stabilizing interactions might be responsible for the improved activity seen for the Boc-derivatives relative to unsubstituted ones. At first sight, the *tert*-butyl group seems to be unhampered, well accommodated in both conformations, which provides an ambiguous identity of the relevant amino acid involved in this stabilizing interaction.

To take into account protein flexibility, the behavior of the predicted complexes was studied in a dynamic context (see Supplementary Data). The lowest energy docked structure for each conformation was selected, and for each binding mode, a molecular dynamics (MD) simulation.[16] The whole system was partitioned into a frozen and a mobile region, where positional restraints on both the ligand and the protein were progressively reduced and finally removed.

We have monitored the root-mean square deviation (rmsd) profiles for the moving part of the system, and the ligand to get insights into the changes that are experienced upon binding. For both complexes **a** and **b**, the ligand remained docked in the binding site, conserving the initial conformation, but the backbone torsion about C4′–C5′, evolving to the expected *ap* conformation[8] upon relaxing. In addition, for complex **a**, the ligand showed an rmsd > 1.7 Å due to additional conformational adjustments on the Boc moiety, leading to an average value of rmsd higher than for complex **b** (~1.2 Å). The rmsd profiles determined for the subset of residues that form the mobile region remained stable along the simulations at ~1.8 Å (complex **a**) and ~1.6 Å (complex **b**), thus suggesting the occurrence of soft local distortions in the protein as a consequence of the binding of the drug. The main rearrangements are induced by the *tert*-butyl moiety, since it forces the side chains of residues on the palm subdomain of p61 (Thr-A165 and Glu-A169) away. This effect is enhanced in complex **a**, where initially there were more intense steric contacts.

The analysis of the intermolecular hydrogen bonds formed upon complexation has revealed significant properties. The sulfone group hydrogen bonds to one of the anticipated lysines (Lys-A101) throughout the trajectory (occupied 66%), with a mean distance separating the Nζ atom of the residue and the closest sulfone oxygen of 2.9 ± 0.16 Å. The distance for Lys-A103 (3.6 ± 0.11 Å) and the occupancy (18%) of the pertinent H-bond are far from a true hydrogen bond during most of the simulation, although this may be considered as an electrostatic interaction which allows an optimal accommodation of the sulfone group.[11] The spiro amino group establishes two stable hydrogen bonds with carboxylate oxygen atoms of Glu-B138, as can be deduced from the distances (2.8 ± 0.15 and 3.0 ± 0.18 Å, respectively) and occupancy along the simulation (78% and 59%). These values are very similar for both complexes **a** and **b**, with absolute differences on occupancy and distance <3% and <0.1 Å, respectively.

The hydrogen bond formed by carbonyl oxygen on Boc shows a contrasting behavior. For complex **a**, the hydrogen bond between Thr-B139 and the carbonyl was progressively weakened during the MD simulation, owing to the *tert*-butyl group of the Boc substituent, which hampers an effective H-bond with the secondary hydroxy group at the amino acid. This results in a long average distance (3.5 ± 0.68) and low occupancy (29%) of the H-bond, pointing to a weak interaction. For the complex **b**, the carbonyl oxygen is engaged in a H-bond with Lys-A172 all over the simulation (2.7 ± 0.19; occupancy 87%). The amino acid side chain can freely rotate since the terminal amino group is not initially hydrogen-bonded to other residues, and the new H-bond is sterically unhindered, so it appears stable along the simulation.

The energetic analysis reveals that both drug conformations give rise to favorable enzyme–ligand complexes (−10.4 and −15.1 kcal mol^{-1}, for complex **a** and **b**, respectively). The greater stability of the binding mode **b** is mainly due to the electrostatic component computed

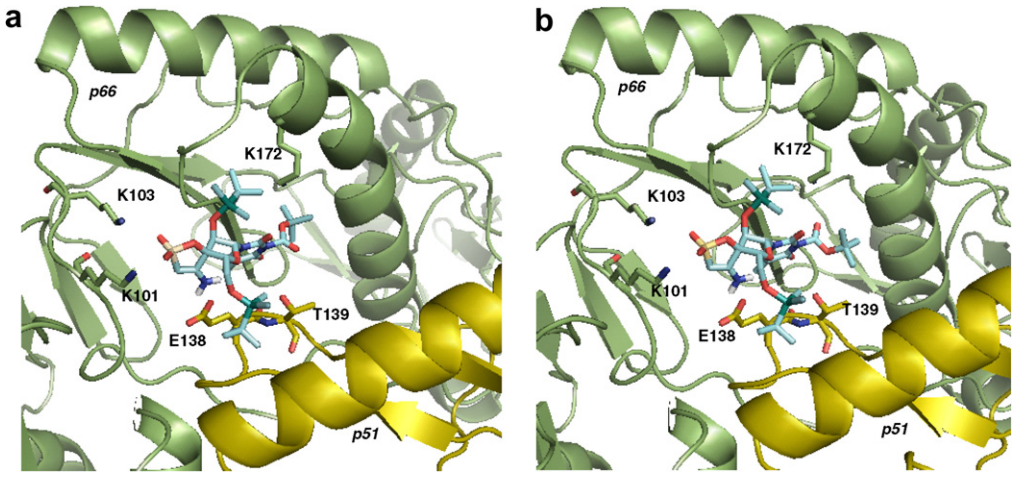


Figure 3. Schematic representation of the two binding models from docking simulations: a, left; b, right. The Cα trace of the enzyme is displayed as a ribbon, colored according to the two subunits p66 (green) and p51 (yellow). The side chains of some relevant residues (Lys-A101, Lys-A103, Lys-A172, Glu-B138, Thr-B139) are shown as sticks, with carbon atoms colored according to their subunit. Inhibitor **8** is displayed as sticks with carbon atoms in cyan.

by the MM force field, which results in a stabilization by 7.2 kcal mol^{-1} of complex **b** relative to **a**.

Although the base part of TSAO derivatives is mostly exposed to the solvent and run parallel to the p51/p66 subunits interface, the substitution at *N*-3 may induce local distortions in the enzyme. The results summarized above suggest that the binding site of HIV-1 RT is not flexible enough as to easily accommodate ligands bearing bulky substituents, and appears as visibly sensitive to the drug conformation. For complex **a**, the *tert*-butyl unit interferes with the side chains of residues Thr-A165 and Glu-A169 on the palm subdomain of p61, thus forcing the drug to reorient inside the binding pocket (torsion of Boc substituent by 52° from the B3LYP/6-31G(d) optimized rotamer), which further inhibits the formation of an efficient hydrogen bond between the hindered carbonyl oxygen and the hydroxy group of Thr-B139. For the alternative ligand conformation (complex **b**), such an effect is less marked, since these unfavorable steric contacts are softer, which induces a minor displacement of the inhibitor and conformational torsion (28° from the B3LYP/6-31G(d) optimized rotamer). Moreover, the free and unhampered Nζ of Lys-A172 may form a stable H-bond with the carbonyl group, and is preserved along the simulation.

In summary, these results point to a better accommodation for conformation **b**,[17] and suggest that while a carbonyl group at *N*-3 position of the base is favorable for establishing binding interactions with the enzyme, the attachment of associated bulky substituents restricts the proper adjustment of the ligand inside the binding site. However, it might be speculated that compounds bearing an unhindered carbonyl group at the position *N*-3 could improve activity or resistance profiles by optimizing the H-bond interaction with binding pocket and thus reducing the unfavorable steric hindrance.

The work described herein reinforces our previous data which demonstrates that a Boc group in *N*-3 position acts as a potential novel pharmacophore for the oxo and aza TSAO derivatives. Further modifications in *N*-3 are in progress with a view to obtaining compounds with increased biological activity, and the results will be reported in due course.

Acknowledgments

C.T. thanks the Ministère Français de la Recherche (France) for a fellowship. D.P. thanks the Conseil Régional de Picardie and the Ministère Français de la Recherche for financial support. E.S. thanks MEC (Spain) for a Juan de la Cierva contract.

Supplementary data

Docking results and computational methodology for the molecular modeling can be found in the online version. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.010.

References and notes

1. Restle, T.; Muller, B.; Godoy, R. S. *J. Biol. Chem.* **1990**, *265*, 8986.
2. Wang, J.; Smerdon, S. J.; Jager, J.; Kohlstaedt, L. A.; Rice, P. A.; Friedman, J. M.; Steitz, T. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7242.
3. (a) Pandey, P. K.; Kaushik, N.; Talele, T. T.; Yadav, P. N. S.; Pandey, V. N. *Biochemistry* **2001**, *40*, 9505; (b) Pandey, P. K.; Kaushik, N.; Singh, K.; Sharma, B.; Upadhyay, A. K.; Kumar, S.; Harris, D. *BMC Biochem.* **2002**, *3*, 18.
4. (a) Camarasa, M. J.; Pérez-Pérez, M. J.; San-Félix, A.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1992**, *35*, 2721; (b) Pérez-Pérez, M. J.; San-Félix, A.; Camarasa, M. J.; Balzarini, J.; De Clercq, E. *Tetrahedron Lett.* **1992**, *33*, 3029; (c) Camarasa, M. J.; San-Félix, A.; Velázquez, S.; Pérez-Pérez, M. J.; Gago, F.; Balzarini, J. *Curr. Top. Med. Chem.* **2004**, *4*, 945.

5. (a) Chamorro, C.; Pérez-Pérez, M. J.; Rodríguez-Barrios, F.; Gago, F.; De Clercq, E.; Balzarini, J.; San-Félix, A.; Camarasa, M. J. *Antiviral Res.* **2001**, *50*, 207; (b) Camarasa, M. J.; Pérez-Pérez, M. J.; Velázquez, S.; San-Félix, A.; Álvarez, R.; Ingate, S.; Jimeno, M. L.; De Clercq, E.; Balzarini, J. In *Antiinfectives. Recent Advances in Chemistry and Structure–Activity Relationships*; Bentley, P. H., O'Hanlon, P. J., Eds.; The Royal Society of Chemistry, 1997; p 259, Ch. 18.
6. Álvarez, R.; Jimeno, M. L.; Gago, F.; Balzarini, J.; Perez-Perez, M. J.; Camarasa, M. J. *Antiviral Chem. Chemother.* **1998**, *9*, 333.
7. (a) Velázquez, S.; San-Félix, A.; Pérez-Pérez, M. J.; Balzarini, J.; De Clercq, E.; Camarasa, M. J. *J. Med. Chem.* **1993**, *36*, 3230; (b) San-Félix, A.; Velázquez, S.; Pérez-Pérez, M. J.; Balzarini, J.; De Clercq, E.; Camarasa, M. J. *J. Med. Chem.* **1994**, *37*, 453.
8. Rodríguez-Barrios, F.; Pérez, C.; Lobatón, E.; Velázquez, S.; Chamorro, C.; San-Félix, A.; Pérez-Pérez, M. J.; Camarasa, M. J.; Pelemans, H.; Balzarini, J.; Gago, F. *J. Med. Chem.* **2001**, *44*, 1853.
9. Bonache, M. C.; Chamorro, C.; Velázquez, S.; De Clercq, E.; Balzarini, J.; Rodríguez-Barrios, F.; Gago, F.; Camarasa, M. J.; San-Félix, A. *J. Med. Chem.* **2005**, *48*, 6653.
10. (a) Nguyen Van Nhien, A.; Tomassi, C.; Len, C.; Marco-Contelles, J.; Balzarini, J.; Pannecouque, C.; De Clercq, E.; Postel, D. *J. Med. Chem.* **2005**, *48*, 4276; (b) Postel, D.; Nguyen Van Nhien, A.; Marco, J. L. *Eur. J. Org. Chem.* **2003**, 3713; (c) Domínguez, L.; Nguyen Van Nhien, A.; Tomassi, C.; Len, C.; Postel, D.; Marco-Contelles, J. *J. Org. Chem.* **2004**, *69*, 843.
11. Soriano, E.; Marco-Contelles, J.; Tomassi, C.; Nguyen Van Nhien, A.; Postel, D. *J. Chem. Inf. Model.* **2006**, *46*, 1666.
12. (a) Pérez-Pérez, M. J.; San-Félix, A.; Balzarini, J.; De Clercq, E.; Camarasa, M. J. *J. Med. Chem.* **1992**, *35*, 2988; (b) Ingate, S.; Pérez-Pérez, M. J.; De Clercq, E.; Balzarini, J.; Camarasa, M. J. *Antiviral Res.* **1995**, *27*, 281.
13. Chamorro, C.; De Clercq, E.; Balzarini, J.; Camarasa, M. J.; San-Félix, A. *Antiviral Chem. Chemother.* **2000**, *11*, 61.
14. Sluis-Cremer, N.; Hamamouch, N.; San Félix, A.; Velázquez, S.; Balzarini, J.; Camarasa, M. J. *J. Med. Chem.* **2006**, *49*, 4834.
15. *AutoDock*, Version 3.05; Morris, G. M.; Goodsell, D. S.; Huey, R.; Hart, W. E.; Halliday, S.; Belew, R.; Olson, A. J. The Scripps Research Institute, La Jolla, CA 92037-1000, 2000.
16. *AMBER 8*, Case, D. A.; Darden, T. A.; Cheatham, T. E.; Simmerling, C. L.; Wang, J.; Duke, R. E.; Luo, R.; Merz, K. M.; Wang, B.; Pearlman, D. A.; Crowley, M.; Brozell, S.; Tsui, V.; Gohlke, H.; Mongan, J.; Hornak, V.; Cui, G.; Beroza, P.; Schafmeister, C.; Caldwell, J. W.; Ross, W. S.; Kollman, P. A. University of California, San Francisco, 2004.
17. It should be noted that both drug conformers show a similar energy in the free state, being conformer **a** 0.11 kcal mol^{-1} more stable than **b** (B3LYP/6-31G*).