ACS chemical biology

Letters

pubs.acs.org/acschemicalbiology

Functional and Structural Characterization of 2-Amino-4-phenylthiazole Inhibitors of the HIV-1 Nucleocapsid Protein with Antiviral Activity

Mattia Mori,[†] Alessandro Nucci,[†] Maria Chiara Dasso Lang,[†] Nicolas Humbert,[‡] Christian Boudier,[‡] Francois Debaene,[‡] Sarah Sanglier-Cianferani,[‡] Marjorie Catala,[§] Patricia Schult-Dietrich,[∥] Ursula Dietrich,[∥] Carine Tisné,[§] Yves Mely,*[,‡] and Maurizio Botta*[,†,⊥]

[†]Department of Biotechnology, Chemistry and Pharmacy, Università degli Studi di Siena, via Aldo Moro 2, I-53019 Siena, Italy

[‡]Laboratoire de Biophotonique et Pharmacologie, Faculté de Pharmacie, UMR 7213 CNRS, 74 route du Rhin, F-67401 Illkirch, France

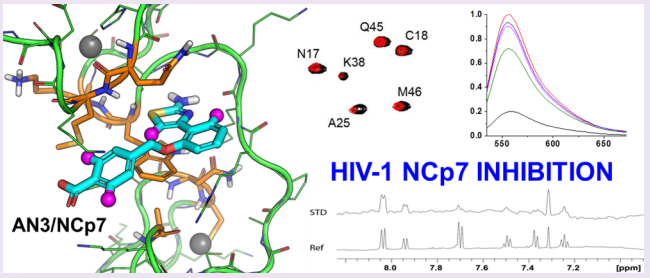
[§]Laboratoire de Cristallographie et RMN Biologiques, CNRS, Paris Sorbonne Cité, 4 avenue de l'Observatoire, F-75006 Paris, France

[∥]Georg-Speyer-Haus, Institute for Tumor Biology and Experimental Therapy, Paul-Ehrlich-Str. 42-44, D-60596 Frankfurt, Germany

[⊥]Sbarro Institute for Cancer Research and Molecular Medicine, Temple University, BioLife Science Bldg., Suite 333, 1900 N. 12th Street, Philadelphia, Pennsylvania 19122, United States

Supporting Information

ABSTRACT: The nucleocapsid protein (NC) is a highly conserved protein in diverse HIV-1 subtypes that plays a central role in virus replication, mainly by interacting with conserved nucleic acid sequences. NC is considered a highly profitable drug target to inhibit multiple steps in the HIV-1 life cycle with just one compound, a unique property not shown by any of the other antiretroviral classes. However, most of NC inhibitors developed so far act through an unspecific and potentially toxic mechanism (zinc ejection) and are mainly being investigated as topical microbicides. In an effort to provide specific NC inhibitors that compete for the binding of nucleic acids to NC, here we combined molecular modeling, organic synthesis, biophysical studies, NMR spectroscopy, and antiviral assays to design, synthesize, and characterize an efficient NC inhibitor endowed with antiviral activity *in vitro*, a desirable property for the development of efficient antiretroviral lead compounds.



During the past 25 years, the arsenal of drugs to combat HIV-1 infection has increased continuously, even though most anti-HIV-1 drugs experienced clinical failure due to antiretroviral drug resistance resulting from mutations in HIV-1 protein targets as a consequence of the error-prone nature of HIV-1 reverse transcriptase (RT) and its lack of a proofreading function. The most common and realistic strategy adopted so far to overcome this issue is the design of more potent and selective inhibitors of entry/fusion, RT, Integrase (IN), and Protease (PR), but these compounds often lack full activity against drug-resistant virus strains selected by the same drug classes.[1] Therefore, a medical need in this field is still felt, and additional conserved drug targets need to be explored to achieve virus suppression in drug-experienced patients or patients infected with drug-resistant viruses. In this context, the HIV-1 nucleocapsid protein (NC), which is highly conserved throughout different HIV-1 subtypes and exerts essential functions in virus replication, is a promising anti-HIV-1 target.[2,3] NC is a 55-amino-acid small basic protein with two zinc fingers that assists RT during reverse transcription by chaperoning the annealing of the cellular primer tRNA to the primer binding site (PBS) and the two obligatory DNA strand transfers necessary for the synthesis of a complete functional double-stranded vDNA with two long terminal repeats.[4] NC is also thought to protect the nascent vDNA against nucleases[5] and to assist IN for the integration of the viral DNA into the host genome.[6] As a domain of the Gag structural polyprotein precursor, NC selects the genomic RNA and promotes its dimerization and packaging during virus assembly.[7−9] Finally, NC binding to RNA is critical for Gag processing by PR and for the subsequent formation of the condensed ribonucleoprotein architecture within the virion.[10] NC can hardly mutate, so that NC mutants are generally nonfunctional and result in noninfectious viruses.[11,12] Therefore, specific inhibition of NC is thought to generate a sustained antiretroviral activity.

Received: April 29, 2014
Accepted: July 2, 2014
Published: July 2, 2014

Inhibition of NC has been achieved by different strategies leading to antiretroviral effects *in vitro* and *in vivo*,[13] although generally through unspecific mechanisms and thus causing significant cytotoxicity. Indeed, there is a critical deficiency of small molecule NC inhibitors (NCIs) endowed with a clear and safe mechanism of action as well as with antiviral activity *in vitro*, which may be developed as effective and safe antiretroviral agents.

Targeting protein/nucleic acids interaction by small molecules is a highly promising strategy in anti-HIV therapy, even if generally considered as a tough challenge due to several difficulties, most often related to the intrinsic structural disorder and highly basic nature of nucleic acids-binding proteins. In continuation of our interest in discovering specific NCIs competing with nucleic acids for the binding to NC and exhibiting antiviral activity, we established a multidisciplinary strategy based on three pioneer works in the field. Indeed, Shvadchak et al. have developed a high-throughput screening assay based on targeting the NC chaperone activities, which led to the identification of five molecular fragments that efficiently inhibit NC but do not show antiviral activity.[14] Recently, Goudreau and co-workers have provided the first solution structure of NC in complex with an NCI that mimics the guanosine found in many NC/RNA complexes.[15] Moreover, as NC is a highly flexible protein, it was essential to use molecular dynamics (MD) simulations to study the conformational behavior of NC in complex with nucleic acids and to develop a new strategy based on virtual screening to design small molecule NCIs showing antiretroviral activity.[16,17]

On the basis of a combination of molecular simulations, biophysical studies, NMR analysis, and antiviral assays, here we elucidate the NC inhibitory activity and structural requirements for NC binding of **AN3**, a new NCI targeting efficiently NC nucleic acid chaperone activity and endowed with antiviral activity in cells. Notably, **AN3** was designed starting from NCI fragments identified by Shvadchak,[14] whose complexes with NC have been already investigated by molecular docking simulations and GRID-based analysis.[16] Starting from docking complexes, here we performed preliminary MD simulations of 25 ns each, showing that fragment A10 had the highest conformational stability within the hydrophobic pocket of NC located at the top of the folded zinc fingers (Supporting Information, Figure S1), which play a key role in NC chaperoning activities.[18−20] For this reason, A10 was selected for further studies aimed at corroborating the validity of the 2-amino-4-phenylthiazole scaffold for NC inhibition as well as to develop NCI with antiviral activity in cells.

In agreement with our previous observations,[16] MD analysis showed that the aminothiazole moiety nicely fits the hydrophobic pocket of NC and may behave as a guanine-mimetic. The catechol phenyl ring is stacked with the Trp37 side chain, whereas hydroxyl groups are projected toward the solvent but do not bind to basic residues that have been shown to play a key role in binding to nucleic acids.[21] Moreover, catechol species are particularly sensitive to oxidation, giving quinone or semiquinone radicals, which may interfere with the functional characterization of the mechanism of action through binding to proteins or nucleic acids. Indeed, a number of catechols of polyhydroxylated compounds have been previously shown to inhibit NC functions by an unclear mechanism, but without showing antiviral activity *in vitro*.[22] Therefore, our strategy focused on the structure-guided replacement of the catechol moiety in A10 with functional groups that may eventually improve membrane permeability. To this end, compounds sharing the common 2-amino-4-phenylthiazole substructure and bearing substitutions on the phenyl ring were downloaded from the ZINC database (about 950 molecules) and submitted to the virtual screening protocol already described.[16] In parallel, these molecules were also docked toward the NMR structure of NC in complex with an NCI.[15] Compounds **AN1**, **AN2**, and **AN3** (Figure 1A and Supporting Information, Table S1, for basic properties) were prioritized by virtual screening and further studied by means of 0.3 μs of MD for checking their conformational stability (Supporting Information). Then, these molecules were purchased and tested *in vitro*.

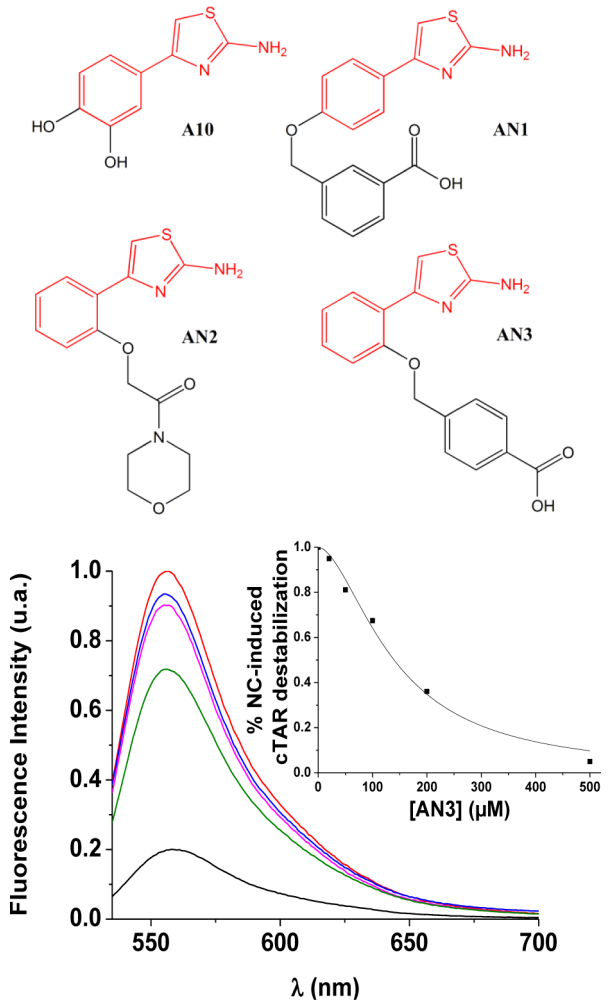
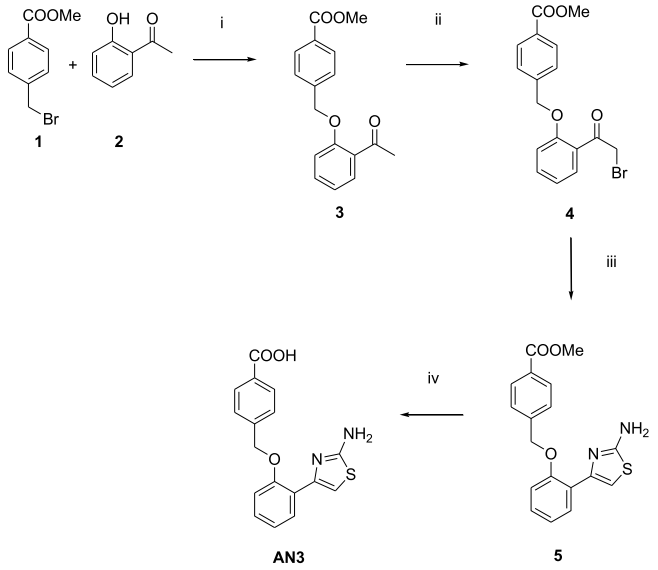


Figure 1. AN compounds and their effects on the NC(11-55)-induced cTAR destabilization. (A) Chemical structure of A10 and the three compounds selected by virtual screening. (B) Emission spectra of cTAR (0.1 μM) were recorded in the absence (black) and in the presence of 1 μM NC(11-55) before (red) and after addition of 100 μM **AN1** (blue), **AN2** (magenta), or **AN3** (green). Excitation wavelength was at 520 nm. The concentration of DMSO was less than 2% by volume. Insert: Inhibition of NC(11-55)-induced cTAR melting by **AN3**. The percentage of NC(11-55) activity is plotted as a function of the **AN3** concentration. An IC_{50} value of 140 ± 90 μM was calculated for **AN3** from the fit of the experimental data (squares) to the following equation: % NC-induced cTAR destabilization = $1/(1 + (IC_{50}/[\mathbf{AN3}])^p)$, where p = 1.8(±1) is the Hill coefficient.

We first monitored the effect of AN compounds on the NC(11-55)-induced destabilization of the secondary structure

dx.doi.org/10.1021/cb500316h | *ACS Chem. Biol.* 2014, 9, 1950−1955

Scheme 1. Synthesis of AN3[a]



[a]Reaction and conditions: (i) K_2CO_3, DMF, rt, overnight; (ii) $CuBr_2$, $CHCl_3$/AcOEt (1:1), reflux, overnight; (iii) thiourea, DMF, rt, 1 h; (iv) LiOH·H_2O, THF/MeOH/H_2O (3:1:1), rt 5 h.

of cTAR DNA, which is exquisitely dependent on the hydrophobic plateau at the top of the properly folded zinc finger motifs,[19] by using the double-labeled Rh6G-5′-cTAR-3′-Dabcyl derivative. In the absence of NC, cTAR is mainly in a nonfluorescent closed form, where the Rh6G and Dabcyl labels at the 5′ and 3′ termini of the cTAR stem are close together. Addition of a 10-fold excess of NC(11-55) led to melting of the bottom of the cTAR stem, partially restoring the Rh6G fluorescence.[23] Therefore, NCIs designed to interfere with NC/nucleic acid interactions are expected to reverse the NC-promoted increase in Rh6G fluorescence. Addition of 100 μM concentration of **AN1**, **AN2**, and **AN3** to the NC(11-55)/cTAR complex was found to induce 7%, 10%, and 28% decrease of the Rh6G emission, respectively (Figure 1B). A control experiment performed in the absence of NC excluded any direct effect of the compounds on Rh6G emission (data not shown). Thus, all three compounds are able to partially inhibit the NC(11-55)-induced destabilization of cTAR, **AN3** being the most efficient NCI with an IC_{50} of 140(±90) μM (Figure 1B, insert). The inhibition constant K_i was then calculated according to the Cheng and Prussof equation:

$$K_i = \frac{IC_{50}}{1 + \frac{[cTAR]}{K_d}} \quad (1)$$

with [cTAR] = 100 nM and the dissociation constant ($K_{d(NC\text{-}cTAR)}$ = 5.9 × 10^{-8} M) of NC(11-55) for cTAR.[19] A K_i value of 50(±30) μM was obtained for **AN3**.

Since **AN3** showed the most efficient inhibition of NC chaperone activity, it was selected for further characterization and synthesized in larger quantities as outlined in Scheme 1. Compound **3** was obtained by nucleophilic substitution of commercially available methyl 4-(bromomethyl)benzoate **1** with 2′-hydroxyacetophenone **2** in a very good yield. Bromination at the α position of the ketone with copper(II) bromide afforded the monohalogenated compound **4** in moderate yield (60%); this latter was cyclized with thiourea in *N*,*N*-dimethylformamide to afford the 2-aminothiazole **5** quantitatively.[24] Finally the methyl ester was hydrolyzed with lithium hydroxide to afford **AN3**.

To confirm the direct binding of **AN3** to NC as well as to rule out possible zinc ejection, we analyzed by mass spectrometry the changes in the mass of NC(11-55) after addition of **AN3** to NC(11-55) at 1:1, 2:1, 5:1, and 10:1 molar ratio. While no peak indicates that **AN3** could eject zinc, a peak corresponding to the sum of the masses of NC(11-55) and **AN3** indicates the formation of a 1:1 complex with NC(11-55) (Supporting Information, Figure S2). Notably, the adduct between the reference fragment A10 and NC(11-55) was not detected by MS analysis.[14]

In a next step, the binding constant of **AN3** to NC(11-55) was measured by monitoring the changes in the fluorescence anisotropy of **AN3**, which is intrinsically fluorescent, during its titration by NC(11-55) (Supporting Information, Figure S3). Assuming a 1:1 stoichiometry, we found an association constant $K_{NC\text{-}AN3}$ of (1.0 ± 0.2) × 10^4 M^{-1}, which is well consistent with the K_i value determined in Figure 1B. We further characterized the binding properties of **AN3** to NC(11-55) by isothermal titration calorimetry (ITC). Since marginal heat exchange accompanied the binding of **AN3** to NC(11-55) even at high concentrations of both partners, indicating a mainly entropy-driven interaction, we performed a competition titration in which **AN3** competed with cTAR for binding to NC(11-55). By monitoring the heat of reaction associated with the binding of NC to cTAR (Supporting Information, Figure S4), we obtained an association constant $K_{NC\text{-}AN3}$ = (1.4 ± 0.3) × 10^4 M^{-1}, in good agreement with the above value.

Moreover, the ITC experiment confirmed that **AN3** is a competitive inhibitor of NC/nucleic acids interaction. Taken together, our experiments consistently indicated that **AN3** binds to NC(11-55) with a K_d value of ∼100 μM.

To check whether **AN3** may directly interact with the Trp37 residue, which has been shown to be crucial for NC activity,[4,19,21,25] we monitored the intrinsic fluorescence emission of the Trp37 residue in NC(11-55) upon the addition of 100 μM **AN3**, a concentration close to its K_d value.[26,27] **AN3** binding was found to decrease by 22% the fluorescence

dx.doi.org/10.1021/cb500316h | *ACS Chem. Biol.* 2014, 9, 1950−1955

emission of Trp37, in line with a direct binding of **AN3** to the Trp37 residue (Supporting Information, Figure S5). This direct interaction was also observed by molecular modeling, as the phenyl-aminothiazole moiety was found to be persistently over the Trp37 side chain along MD trajectories (Figure 2A). This

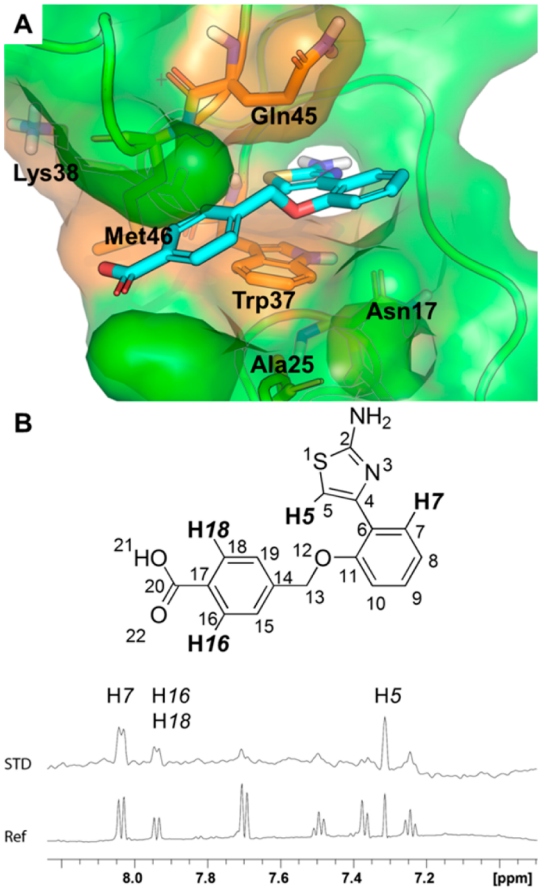


Figure 2. Binding of **AN3** to NC. (A) Representative MD structure: **AN3** is shown as cyan sticks, NC as cartoon and transparent surface. Residues highlighted by NMR and biophysical studies are shown as sticks and colored orange. (B) **AN3** protons involved in the interaction with NC by STD NMR experiment are shown on the STD spectrum. **AN3** concentration was 1 mM, whereas NC concentration was 30 μM. The STD experiment was recorded with selective saturation of the methyl group resonances of NC at 1 ppm, and with selective saturation in an empty spectral region (off-resonance). The subtraction of the spectra was performed internally via phase cycling after every scan to minimize the subtraction artifacts. The resulting subtracted spectrum contains only the signals of **AN3** interacting with NC. The signals of NC were suppressed with the use of a transverse relaxation filter applied before the acquisition.

nearly stacked conformation may well explain the decrease of Trp37 fluorescence emission, since Trp37 fluorescence was shown to be highly sensitive to stacking with oligonucleotide bases.[26,28]

NMR saturation-transfer difference (STD)[29] and chemical shift mapping experiments further confirmed the interaction of **AN3** with NC. STD experiment (Figure 2B) clearly indicates that H5, H7, and the equivalent H16 and H18 protons of **AN3** are involved in the interaction with NC. Moreover, NMR chemical shift mapping performed on N^{15}-labeled NC suggests that residues Ala25, Asn17, and Met46 (as well as Gly4, Gln45, Cys18, Lys38, albeit to a lesser extent) of NC are involved in the binding with **AN3** (Supporting Information, Figure S6). These findings nicely match with the binding mode of **AN3** addressed by MD simulations (Figure 2A), thus facilitating further structure-based computer-aided ligand optimization studies. In particular, chemical optimization of **AN3** should concentrate on improving intermolecular interactions with the NC to enhance its inhibitory potency, as well as optimizing the druggability of the small molecule. Concentrations of DMSO (50%) necessary for solubilizing **AN3** during the NMR chemical shift mapping experiment prevented us from observing the interaction of **AN3** with Trp37. Most probably, DMSO competed with **AN3** for hydrophobic interactions with NC.

Antiretroviral activity against HIV-1 and cytotoxicity of **AN3** were preliminarily evaluated as previously described.[17,30] Briefly, HeLa P4.R5MAGI cells (expressing CD4, CCR5, and β-gal under the control of HIV-1 LTR) were infected by HIV-1Lai (MOI = 0.3) in the presence of serial dilutions of **AN3** in medium for 24 h. After extensive washing, fresh medium with the respective **AN3** concentrations was added. Viral supernatants were collected 24 h later, and viral titers were quantified on TZM-bl cells expressing firefly luciferase from an HIV-1 LTR promoter. Luciferase activity in cell lysates was measured by luminometry 44 h after infection. Compared to the DMSO control, which was set to 100% infection, **AN3** clearly showed concentration-dependent antiviral activity with EC_{50} = 95 μM (Figure 3). This value is highly comparable to the K_d value

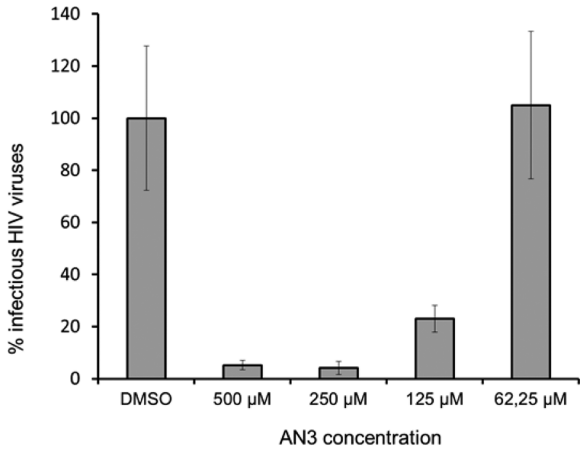


Figure 3. AN3 showed a concentration-dependent antiviral activity *in vitro*. Infectious viruses produced by P4.R5MAGI cells in the presence of **AN3** were quantified by luminometry on Tzm-bl cells. The mean of triplicates is shown with standard deviations. Data were normalized with respect to DMSO (100%). One of two representative experiments is shown.

calculated by ITC or fluorescence anisotropy, as well as to the IC_{50} value determined for the inhibition of NC(11-55)-induced cTAR destabilization, strongly suggesting that NC may be the primary target of the antiviral activity of **AN3.** Notably, complete inhibition was always achieved at 250 μM, a concentration where toxic effects were still low (25%) (Supporting Information, Figure S7).

In conclusion, starting from the previously identified NCI fragment A10, here we designed **AN3** as an efficient inhibitor of the HIV-1 NC chaperone functions. Although the NC inhibitory activities of A10 and **AN3** are comparable, **AN3** showed antiviral activity in infected cells with a mechanism of

dx.doi.org/10.1021/cb500316h | *ACS Chem. Biol.* 2014, 9, 1950−1955

action clearly indicating NC as the primary target. Structural requirements for **AN3** binding to NC were elucidated by NMR, biophysical studies, and MD simulations, showing that **AN3** interacts within the hydrophobic pocket of NC and competes with nucleic acids for this binding site. In agreement with MD simulations, a direct interaction of **AN3** to the key residue Trp37 was observed, whereas NMR chemical shift mapping experiments further highlighted the involvement of Met46, Ala25, and Asn17, thus clearly identifying the hydrophobic pocket of NC as the binding site of **AN3**. Our multidisciplinary strategy demonstrates that the challenging goal of targeting the NC with specific small molecules is truly feasible, thus paving the way for the structure-based design of effective NCI. Overall, **AN3** fulfills all expectations for an effective NCI, thus becoming a valuable starting point for further medicinal chemistry-oriented optimization.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Molecular dynamics, materials, mass spectrometry, general biophysics directions, isothermal titration calorimetry, general NMR directions, chemistry, and details of the antiretroviral and cytotoxicity assays. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: yves.mely@unistra.fr.

*E-mail: botta.maurizio@gmail.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the European Project THINPAD "Targeting the HIV-1 Nucleocapsid Protein to fight Anti-retroviral Drug Resistance" (FP7−Grant Agreement 601969).

■ REFERENCES

(1) Tang, M. W., and Shafer, R. W. (2012) HIV-1 antiretroviral resistance: scientific principles and clinical applications. *Drugs 72*, e1−25.

(2) Darlix, J. L., Godet, J., Ivanyi-Nagy, R., Fosse, P., Mauffret, O., and Mely, Y. (2011) Flexible nature and specific functions of the HIV-1 nucleocapsid protein. *J. Mol. Biol. 410*, 565−581.

(3) Thomas, J. A., and Gorelick, R. J. (2008) Nucleocapsid protein function in early infection processes. *Virus Res. 134*, 39−63.

(4) Godet, J., and Mely, Y. (2010) Biophysical studies of the nucleic acid chaperone properties of the HIV-1 nucleocapsid protein. *RNA Biol. 7*, 687−699.

(5) Krishnamoorthy, G., Roques, B., Darlix, J. L., and Mely, Y. (2003) DNA condensation by the nucleocapsid protein of HIV-1: a mechanism ensuring DNA protection. *Nucleic Acids Res. 31*, 5425−5432.

(6) Poljak, L., Batson, S. M., Ficheux, D., Roques, B. P., Darlix, J. L., and Kas, E. (2003) Analysis of NCp7-dependent activation of HIV-1 cDNA integration and its conservation among retroviral nucleocapsid proteins. *J. Mol. Biol. 329*, 411−421.

(7) Zhang, Y., Qian, H., Love, Z., and Barklis, E. (1998) Analysis of the assembly function of the human immunodeficiency virus type 1 gag protein nucleocapsid domain. *J. Virol. 72*, 1782−1789.

(8) Muriaux, D., Mirro, J., Harvin, D., and Rein, A. (2001) RNA is a structural element in retrovirus particles. *Proc. Natl. Acad. Sci. U.S.A. 98*, 5246−5251.

(9) Clever, J., Sassetti, C., and Parslow, T. G. (1995) RNA secondary structure and binding sites for gag gene products in the 5′ packaging signal of human immunodeficiency virus type 1. *J. Virol. 69*, 2101−2109.

(10) Mirambeau, G., Lyonnais, S., Coulaud, D., Hameau, L., Lafosse, S., Jeusset, J., Borde, I., Reboud-Ravaux, M., Restle, T., Gorelick, R. J., and Le Cam, E. (2007) HIV-1 protease and reverse transcriptase control the architecture of their nucleocapsid partner. *PLoS One 2*, e669.

(11) Demene, H., Dong, C. Z., Ottmann, M., Rouyez, M. C., Jullian, N., Morellet, N., Mely, Y., Darlix, J. L., Fournie-Zaluski, M. C., Saragosti, S., et al. (1994) 1H NMR structure and biological studies of the His23—>Cys mutant nucleocapsid protein of HIV-1 indicate that the conformation of the first zinc finger is critical for virus infectivity. *Biochemistry 33*, 11707−11716.

(12) Gorelick, R. J., Gagliardi, T. D., Bosche, W. J., Wiltrout, T. A., Coren, L. V., Chabot, D. J., Lifson, J. D., Henderson, L. E., and Arthur, L. O. (1999) Strict conservation of the retroviral nucleocapsid protein zinc finger is strongly influenced by its role in viral infection processes: characterization of HIV-1 particles containing mutant nucleocapsid zinc-coordinating sequences. *Virology 256*, 92−104.

(13) Mori, M., Manetti, F., and Botta, M. (2011) Targeting protein-protein and protein-nucleic acid interactions for anti-HIV therapy. *Curr. Pharm. Des. 17*, 3713−3728.

(14) Shvadchak, V., Sanglier, S., Rocle, S., Villa, P., Haiech, J., Hibert, M., Van Dorsselaer, A., Mely, Y., and de Rocquigny, H. (2009) Identification by high throughput screening of small compounds inhibiting the nucleic acid destabilization activity of the HIV-1 nucleocapsid protein. *Biochimie 91*, 916−923.

(15) Goudreau, N., Hucke, O., Faucher, A. M., Grand-Maitre, C., Lepage, O., Bonneau, P. R., Mason, S. W., and Titolo, S. (2013) Discovery and structural characterization of a new inhibitor series of HIV-1 nucleocapsid function: NMR solution structure determination of a ternary complex involving a 2:1 inhibitor/NC stoichiometry. *J. Mol. Biol. 425*, 1982−1998.

(16) Mori, M., Manetti, F., and Botta, M. (2011) Predicting the binding mode of known NCp7 inhibitors to facilitate the design of novel modulators. *J. Chem. Inf. Model. 51*, 446−454.

(17) Mori, M., Schult-Dietrich, P., Szafarowicz, B., Humbert, N., Debaene, F., Sanglier-Cianferani, S., Dietrich, U., Mely, Y., and Botta, M. (2012) Use of virtual screening for discovering antiretroviral compounds interacting with the HIV-1 nucleocapsid protein. *Virus Res. 169*, 377−387.

(18) Avilov, S. V., Piemont, E., Shvadchak, V., de Rocquigny, H., and Mely, Y. (2008) Probing dynamics of HIV-1 nucleocapsid protein/target hexanucleotide complexes by 2-aminopurine. *Nucleic Acids Res. 36*, 885−896.

(19) Beltz, H., Clauss, C., Piemont, E., Ficheux, D., Gorelick, R. J., Roques, B., Gabus, C., Darlix, J. L., de Rocquigny, H., and Mely, Y. (2005) Structural determinants of HIV-1 nucleocapsid protein for cTAR DNA binding and destabilization, and correlation with inhibition of self-primed DNA synthesis. *J. Mol. Biol. 348*, 1113−1126.

(20) Godet, J., Ramalanjaona, N., Sharma, K. K., Richert, L., de Rocquigny, H., Darlix, J. L., Duportail, G., and Mely, Y. (2011) Specific implications of the HIV-1 nucleocapsid zinc fingers in the annealing of the primer binding site complementary sequences during the obligatory plus strand transfer. *Nucleic Acids Res. 39*, 6633−6645.

(21) Mori, M., Dietrich, U., Manetti, F., and Botta, M. (2010) Molecular dynamics and DFT study on HIV-1 nucleocapsid protein-7 in complex with viral genome. *J. Chem. Inf. Model. 50*, 638−650.

(22) Cruceanu, M., Stephen, A. G., Beuning, P. J., Gorelick, R. J., Fisher, R. J., and Williams, M. C. (2006) Single DNA molecule stretching measures the activity of chemicals that target the HIV-1 nucleocapsid protein. *Anal. Biochem. 358*, 159−170.

(23) Bernacchi, S., and Mely, Y. (2001) Exciton interaction in molecular beacons: a sensitive sensor for short range modifications of the nucleic acid structure. *Nucleic Acids Res. 29*, E62−62.

(24) Zav'yalov, S. I., Kravchenko, N. E., Ezhova, G. I., Kulikova, L. B., Zavozin, A. G., and Dorofeeva, O. V. (2007) Synthesis of 2-aminothiazole derivatives. *Pharm. Chem. J. 41*, 105−108.

(25) De Guzman, R. N., Wu, Z. R., Stalling, C. C., Pappalardo, L., Borer, P. N., and Summers, M. F. (1998) Structure of the HIV-1 nucleocapsid protein bound to the SL3 psi-RNA recognition element. *Science 279*, 384−388.
(26) Mely, Y., Piemont, E., Sorinas-Jimeno, M., de Rocquigny, H., Jullian, N., Morellet, N., Roques, B. P., and Gerard, D. (1993) Structural and dynamic characterization of the aromatic amino acids of the human immunodeficiency virus type I nucleocapsid protein zinc fingers and their involvement in heterologous tRNA(Phe) binding: a steady-state and time-resolved fluorescence study. *Biophys. J. 65*, 1513−1522.
(27) Mely, Y., De Rocquigny, H., Morellet, N., Roques, B. P., and Gerad, D. (1996) Zinc binding to the HIV-1 nucleocapsid protein: a thermodynamic investigation by fluorescence spectroscopy. *Biochemistry 35*, 5175−5182.
(28) Vuilleumier, C., Bombarda, E., Morellet, N., Gerard, D., Roques, B. P., and Mely, Y. (1999) Nucleic acid sequence discrimination by the HIV-1 nucleocapsid protein NCp7: a fluorescence study. *Biochemistry 38*, 16816−16825.
(29) Mayer, M., and Meyer, B. (2001) Group epitope mapping by saturation transfer difference NMR to identify segments of a ligand in direct contact with a protein receptor. *J. Am. Chem. Soc. 123*, 6108−6117.
(30) Dietz, J., Koch, J., Kaur, A., Raja, C., Stein, S., Grez, M., Pustowka, A., Mensch, S., Ferner, J., Möller, L., Bannert, N., Tampe, R., Divita, G., Mely, Y., Schwalbe, H., and Dietrich, U. (2008) Inhibition of HIV-1 by a peptide ligand of the genomic RNA packaging signal Psi. *ChemMedChem. 3*, 749−755.