Bioorganic & Medicinal Chemistry Letters 24 (2014) 4227–4230

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of {[5-(adenin-9-yl)-2-furyl]methoxy}methyl phosphonic acid and evaluations against human adenylate kinases

Malika Kaci [a], Jean-Pierre Uttaro [a], Valérie Lefort [b], Christophe Mathé [a,*], Chahrazade El Amri [b], Christian Périgaud [a]

[a] Institut des Biomolécules Max Mousseron (IBMM), UMR 5247 CNRS-UM 1-UM 2, cc 1705, Université Montpellier 2, Place Eugène Bataillon, 34095 Montpellier cedex 5, France
[b] Enzymologie Moléculaire et Fonctionnelle, UMR 8256 UPMC-CNRS, ERL INSERM U1164, Université Pierre et Marie Curie, 7 Quai St-Bernard, 75252 Paris cedex 5, France

ARTICLE INFO

Article history:
Received 27 May 2014
Revised 11 July 2014
Accepted 12 July 2014
Available online 17 July 2014

Keywords:
Adenosine
Analogue/AMP mimics
Phosphotransfer
Nucleoside
Phosphonate
Kinase

ABSTRACT

AMP mimics constitute an important class of therapeutic derivatives to treat diseases where the pool of ATP is involved. A new phosphonate derivative of 9-(5-hydroxymethylfuran-2-yl)adenine was synthesized in a multi-step sequence from commercially available adenosine. Its ability to behave as a substrate of human adenylate kinases 1 and 2 was assessed. The phosphonate was shown to be a moderate but selective substrate of the mitochondrial human AK2, better than well-known antiviral acyclic phosphonates 9-(2-phosphonomethoxyethyl)adenine (PMEA, Adefovir) and (*R*)-9-(2-phosphonomethoxypropyl)adenine (PMPA, Tenofovir). Putative binding mode within adenylate kinase NMP site revealed by molecular docking in comparison to AMP native substrate allowed to illustrate this selective behavior.

9-(5-Hydroxymethylfuran-2-yl)adenine derivative (**5**) (Fig. 1) has been known since 1974 when Robins et al. described its formation as a side-product following treatment of the parent 1′,2′-dide-hydro-2′-deoxynucleoside adenosine under mild acidic conditions.[1]

Chemical modifications and hydrogenation of this derivative provided di- and trideoxy nucleosides as a racemic mixture.[2] Afterwards, improved procedures of the preparation have been reported in the literature.[3,4] However, data can be found neither on the biological evaluations of this mimic nucleoside of adenosine nor on any of the phosphorylated forms. The discovery of new analogues of adenosine can be of interest in the drug discovery process. Adenosine mimics represent an important class of therapeutical molecules, due to the importance of the regulation of ATP levels in metabolic processes that are deregulated in various diseases, including cancer,[5] type 2 diabetes,[6] and viral infections.[7] Particularly, AMP signaling also plays an important role in hypoxic response, immune function and taste reception.[8] These nucleotide forms are usually obtained from adenosine via phosphorylation steps by cellular kinases.[9,10]

Phosphorus-modified nucleoside analogues, bearing a phosphonate group, display interesting biological properties, mainly as antiviral agents. As examples, Adefovir dipivoxil and Tenofovir disoproxil fumarate, the prodrugs of Adefovir (PMEA) and Tenofovir (PMPA), acyclic nucleoside phosphonate analogues have been approved as anti-HBV, and anti-HIV/HBV agents, respectively.[11] Indeed, phosphonates are structurally and electronically similar to phosphates and have been successfully applied as phosphate mimics.[12] Additionally, the phosphonate group has the advantage of being more stable than its phosphate counterpart owing to the resistance of phosphorus–carbon bond to hydrolytic cleavage.[13] Finally, the presence of a phosphonate group allows bypassing the first phosphorylation step required for nucleoside activation.

In this work, we report on the synthesis of (**8**) designed as a structurally related analog of adenosine 5′-monophosphate in order to test its ability to behave as substrate of human adenylate kinases. Adenylate kinases (AK, EC 2.7.4.4.3) that catalyze the nucleotide phosphoryl exchange reaction (2ADP → ATP + AMP) maintain the consistent concentration and fixed ratio of adenine

Abbreviations: ADP, adenosine diphosphate; AK, adenylate kinase; ANP, acyclic nucleotide phosphonate; AMP, adenosine monophosphate; AMPK, AMP-activated kinase; ATP, adenosine triphosphate; Ap4P, P^1,P^4-di(adenosine-5′) tetraphosphate; Ap5P, P^1,P^5-di(adenosine-5′)pentaphosphate; GSK3β, glycogen synthase kinase 3 beta; HBV, hepatitis B virus; HIV, human immunodeficiency virus; NMP, nucleoside monophosphate kinase; PMEA, 9-(2-phosphonomethoxyethyl)adenine; PMPA, (*R*)-9-(2-phosphonomethoxypropyl)adenine; TEAB, triethylammonium hydrogen carbonate buffer; THF, tetrahydrofuran; DMAP, dimethylamino pyridine; DBN, 1,5-diazabicyclo[4.3.0]non-5-ene; $TIPSCl_2$, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane; TMSBr, trimethylsilyl bromide.

* Corresponding author. Tel.: +33 (0)4 67 14 47 76; fax: +33 (0)4 67 04 20 29.
E-mail address: christophe.mathe@univ-montp2.fr (C. Mathé).

http://dx.doi.org/10.1016/j.bmcl.2014.07.036
0960-894X/

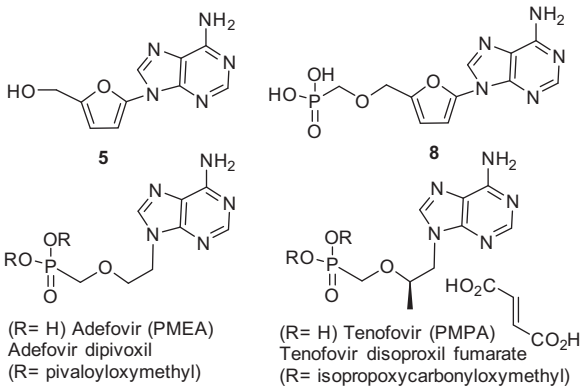


Figure 1.

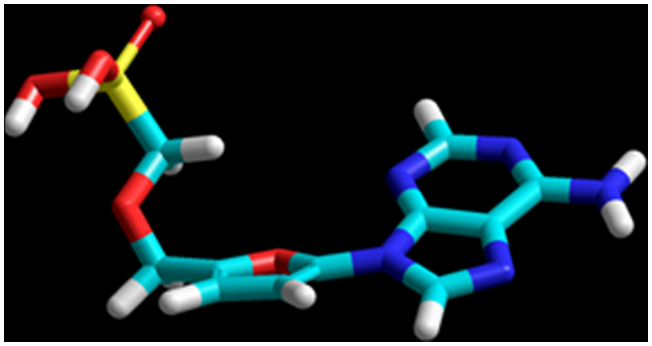


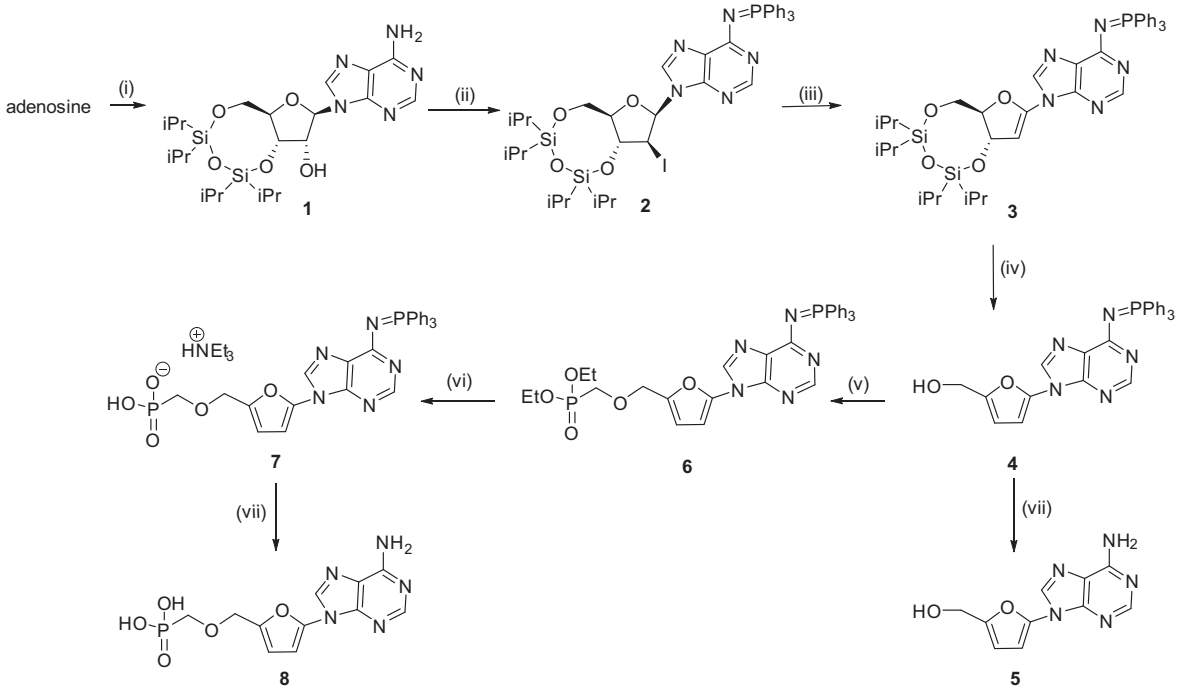
Figure 2. Geometry optimization of compound **8**.

nucleotides. AKs function as a sensitive reporter of cellular energy state by regulating small changes in the ADP/ATP balance into relatively larger changes in AMP levels.[8] Indeed, dysfunction of AKs is associated with various diseases, including genetic reticular dysgenesis disease implying mutations in the AK2 gene,[14] Alzheimer disease through the neuropathogenic role of AK1 in Aβ-mediated tau phosphorylation via AMPK and GSK3β.[15] Furthermore, AK2 plays an essential role in neutrophil differentiation. A possible causative link between AK2 deficiency and neutropenia in reticular dysgenesis has also been recently established.[16] Thus, the development of modulators of AKs' activity could be of a great interest to treat these latter diseases.

As a starting material, commercially available adenosine was converted into 3′,5′-O-TIPS-adenosine (**1**) (Scheme 1). Application from **1** of the methodology described by Gimisis et al.[4] provided the silylated 1′,2′-unsaturated N^6-triphenylphosphoranylidene nucleoside **3**. Thermal reaction and desilylation afforded the protected furanyl nucleoside **4**.[3] Cleavage of the N^6 protecting group gave 9-(furan-2-yl)adenine derivative (**5**). The N^6-triphenylphosphoranylidene group of **5** was used as a transient protection of the exocyclic amino function in order to avoid N-alkylation during installation of the phosphonate.

Nucleoside analogue **4** was treated by LiOtBu and coupled with diethyl *p*-toluene sulfonyloxymethyl phosphonate in dry THF at 0 °C yielding the phosphonate derivative **6**.[17] Phosphonate diester protecting groups of **6** were removed with TMSBr in anhydrous acetonitrile to give **7** as triethyl ammonium salt after TEAB treatment. Finally, treatment of **7** with an acetic acid/EtOH solution afforded the target phosphonate **8** as an acid form.

Conformational analysis was performed using a geometry optimization with MM + as implemented in the HyperChem 7.5 software. A root-mean-square gradient termination cutoff of 0.1 kcal $Å^{-1}$ mol^{-1} was used for geometry optimization with the Polak–Ribiere conjugate gradient algorithm. The calculations provided a geometry optimized structure (Fig. 2). Within this structure, both heterocyclic aromatic moieties are almost coplanar with a syn orientation[18] of adenine and an angle χ (dihedral angle O_4'–C_1'–N_9–C_4) with a value of 25.85°.



Scheme 1. Reagents and conditions: (i) $TIPSCl_2$, DMAP, pyridine, rt, 94%; (ii) PPh_3, I_2, imidazole, toluene, reflux, 88%; (iii) DBN, pyridine, rt, 85%; (iv) (1) xylene, reflux; (2) NH_4F, MeOH, reflux, 45% overall yield; (v) (1) LiOtBu, THF, 0 °C; (2) $(EtO)_2POCH_2OTs$, THF, 0 °C, 62% overall yield; (vi) (1) TMSBr, CH_3CN, 0 °C; (2) TEAB (pH 7, 1 M), 28% (vii) MeOH, AcOH, 92% for **5**, 86% for **8**.

Table 1
Catalytic properties of AK1 and AK2 with compounds **5** and **8** obtained at 37 °C

Compounds	AK1			AK2		
	k_{cat} (s^{-1})	K_M (mM)	k_{cat}/KM ($M^{-1}\,s^{-1}$) × 10^6	k_{cat} (s^{-1})	K_M (mM)	k_{cat}/K_M ($M^{-1}\,s^{-1}$) × 10^6
AMP	500	0.175 ± 0.05	2.85	80	0.38 ± 0.11	0.21
dAMP	240	1.5 ± 0.3	0.16	110	0.21 ± 0.05	0.5
5	ns[a]	ns	ns	ns	ns	ns
8	ns	ns	ns	1.06	0.42 ± 0.013	0.025
PMPA	0.22	3 ± 0.3	75	2.4	3 ± 0.3	800
PMEA	0.08	6 ± 1	14	1.5	6 ± 0.5	250

[a] ns: non substrate. The assay mix contained 50 mM Tris–HCl pH 7.4, 5 mM $MgCl_2$, 1 mM ATP, 0.2 mM NADH, 10 mM DTT, 1 mM phosphoenolpyruvate and auxiliary enzymes: pyruvate kinase (4 Units) and lactate dehydrogenase (4 Units) $MgCl_2$ concentration in the AK2 experiments was 2 mM as slight inhibition were observed above 3 mM. Experiments were performed at least three times. Pyruvate kinase (404 Units/mg at 37 °C) was purchased from Sigma and lactate dehydrogenase (1100 U/mg at 37 °C) from Roche.

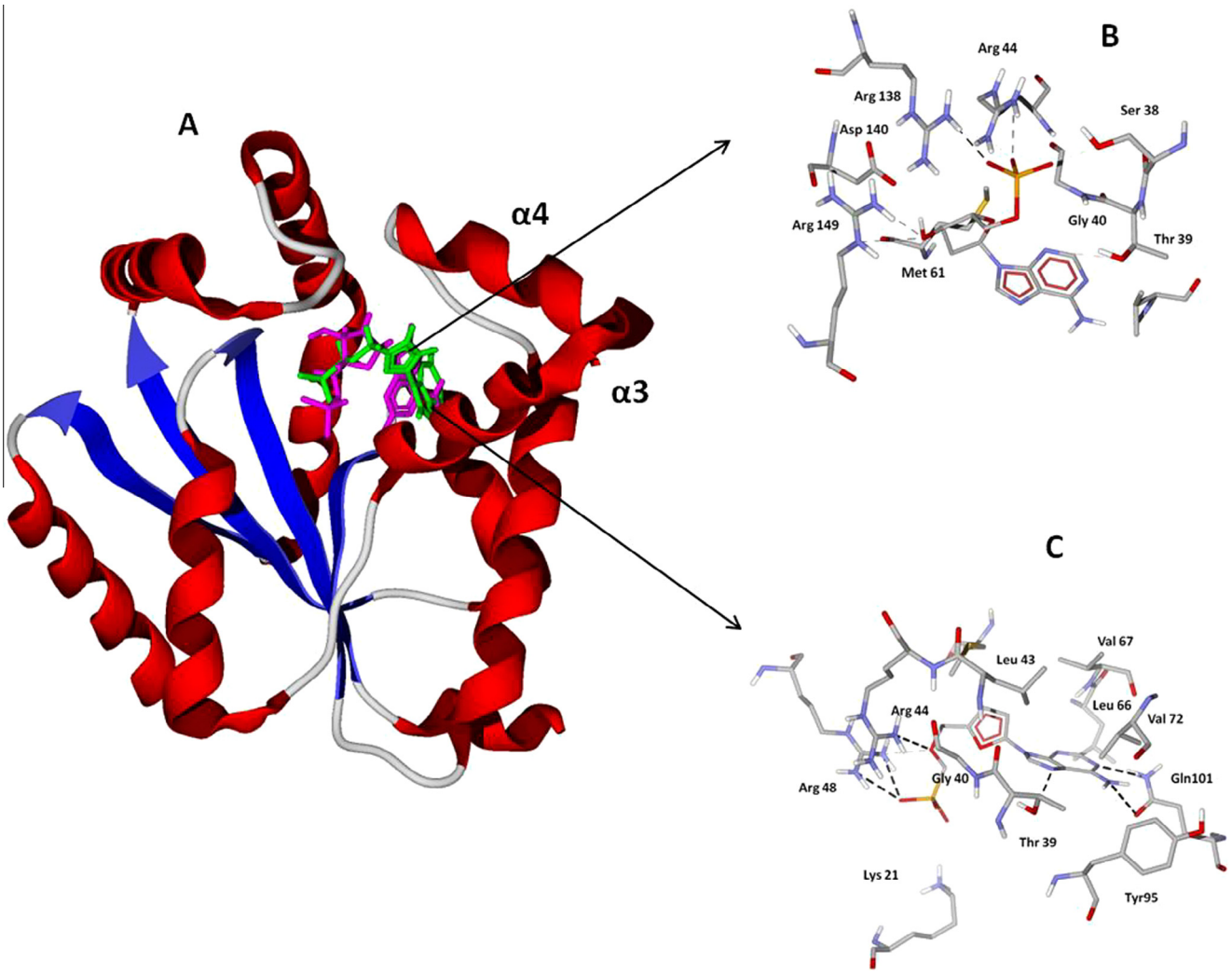


Figure 3. Predicted binding mode of compound **8** in comparison with AMP. Molecular models of docked into the NMP site of AK1 (PDB code 1Z83). (A) Overview of the binding into the NMP pocked AMP (green) versus compound **8** (magenta). (B) Interacting NMP site residues with AMP. (C) Interacting NMP site residues with compound **8**. Atom colors follow CPK nomenclature. Putative hydrogen bonds are illustrated by black dotted lines. Helices α3 and 4 that close up on acceptor molecules are indicated. Figure was prepared with Molegro Viewer software.

Compounds **8**, designed as a structurally related analog of adenosine 5′-monophosphate, as well as compound **5**, were evaluated against nucleoside monophosphate kinases (NMP kinases) involved in nucleosides metabolism in order to assess their substrate properties.

The compounds **5** and **8** were screened against human GMP kinase, AK1, AK2 and virus vaccine TMP kinase that is known to display a large repertoire of substrates including purine derivatives.[19] The recombinant enzymes were prepared as described[20] and kinetic measurements were obtained using spectroscopic Pyruvate kinase/lactate dehydrogenase coupled assay to allow measuring ADP formation.[21] The enzymatic parameters of compounds **5** and **8** in comparison with native substrates are presented in Table 1.

The results clearly underline that compound **8** is a selective substrate of AK2, no substrate properties were observed for the other screened kinases. The K_M of 0.41 mM is in the same magnitude as AMP (K_M = 0.38 mM) and dAMP (K_M = 0.21 mM) meaning that the affinity of compound **8** for AK2 is as strong as native substrates. However catalytic efficiency (k_{cat} = 1.06 s^{-1}) is roughly a hundred times lower than that of AK's biological substrates. These data contrast with those previously reported by our group with acyclic phosphonates PMPA and PMEA.[20] Indeed, we found that these latter derivatives were poorer substrates of human adenylate kinases than compound **8**.

To achieve more insights into structural basis of the recognition of compound **8** by adenylate kinases, molecular docking was performed using Molegro Virtual Docker.[22] Although the crystallographic structure of AK2 in complex with Ap4P bivalent ligand is available (PDB code: 2C9Y), the latter did not provide information on the NMP acceptor site. While AK2 is strictly located in the mitochondrial intermembrane space, AK1 is a cytosolic enzyme expressed in high energy demanding tissues like, brain, heart, and skeletal muscles.[23] Three functional domains are important in the primary structure of nucleoside monophosphate kinases: the nucleoside triphosphate binding glycine-rich region, the nucleoside monophosphate binding site, and the lid domain that closes over the substrate upon binding.[10,23] All these domains are extremely well conserved over adenylate kinase isoforms, we thus use structure of AK1 complexed to Ap5A (PDB code: 1Z83) to perform a docking calculation.[24]

One of the best poses is presented in Figure 3. Globally, compound **8** positioning is close to the one of AMP, even though it is more extended because it is slightly bulkier. Base moiety is in interaction with same residues including the establishment of a hydrogen bond between Thr 39 hydroxyl group and N_9 of compound **8** as well as N_1 and N_6 nitrogen atoms. Compound **8** base moiety deeply accommodates the hydrophobic pocket composed of residues Leu 43, Leu 66, Val 67, Val 72.

The most distinguishable features are located in the sugar/phosphate region. Indeed, while AMP sugar-phosphate hydrogen bond network is clustered to Arg residues (44, 138, 140, 149), this of compound **8** is restricted to Arg 44 and 48. Interestingly, the sugar mimetic group seems to share hydrophobic contacts with Leu 43. Moreover, the oxygen atom of the ethoxy-phosphonate linkage shares a specific H-bond implying nitrogen atom of Arg guanidine group. Stabilizing interactions of compound **8** as well as its extended orientation with AK NMP site is in accordance with its ability to be phosphorylated by AK2. In the complex AK1-Ap5A, the phosphate group is in close contact with Arg44 belonging to helix α3 and Arg97 from the CORE domain that carries the ATP binding loop (P-loop). Both Arg residues are well conserved over NMP kinase family and play a crucial role for an efficient nucleophile attack on γ-P of ATP.[20] This configuration is disrupted for compound **8** as seen above, agreeing its lower catalytic efficiency. Adenylate kinase constitutes an archetype for the study of conformational dynamics during catalytic cycle.[25] The selective action on AK2 could thus be due to differential conformational plasticity of both enzymes. Indeed, the greatest difference between AK1 and AK2 is located in the lid domain.[23]

The lid domain of AK2 is more extended than the one of AK1 and may allow a more effective closure of the active site essential for a proper catalytic reaction.

In conclusion, we have reported here the synthesis of a new phosphonate derivative of 9-(furan-2-yl)adenine thus providing a novel nucleotide scaffold. This latter was shown to be a relatively efficient substrate selective to AK2 when compared to well-known ANPs PMEA and PMPA. Structure-based molecular docking revealed that the binding mode, although correct, presents some slight differences particularly in the positioning of phosphate group when compared to AMP, which explains its lower catalytic efficiency. Structural variations on compound **8** are ongoing as well as studies aiming at deciphering its potential anticancer/antiviral activities.

Acknowledgments

One of us M.K. is particularly grateful to the Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche (France) for a doctoral fellowship. C.E wishes to thank Pr Bertrand Friguet for laboratory facilities.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.07.036.

References and notes

1. Robins, M. J.; Jones, R. A. *J. Org. Chem.* **1974**, *39*, 113.
2. Robins, M. J.; Jones, R. A.; Mengel, R. *J. Am. Chem. Soc.* **1976**, *98*, 8213.
3. Chatgilialoglu, C.; Vrantza, D.; Gimisis, T. *Tetrahedron Lett.* **2004**, *45*, 4515.
4. Gimisis, T.; Ialongo, G.; Chatgilialoglu, C. *Tetrahedron* **1998**, *54*, 573.
5. Antonioli, L.; Blandizzi, C.; Pacher, P.; Hasko, G. *Nat. Rev. Cancer* **2013**, *13*, 842.
6. Dang, Q.; Brown, B. S.; Liu, Y.; Rydzewski, R. M.; Robinson, E. D.; van Poelje, P. D.; Reddy, M. R.; Erion, M. D. *J. Med. Chem.* **2009**, *52*, 2880.
7. De Clercq, E. *Antiviral Res.* **2010**, *85*, 19.
8. Dzeja, P.; Terzic, A. *Int. J. Mol. Sci.* **2009**, *10*, 1729.
9. Boison, D. *Pharmacol. Rev.* **2013**, *65*, 906.
10. Deville-Bonne, D.; El Amri, C.; Meyer, P.; Chen, Y. X.; Agrofoglio, L. A.; Janin, J. *Antiviral Res.* **2010**, *86*, 101.
11. De Clercq, E. *Med. Res. Rev.* **2013**, *33*, 1278.
12. Thatcher, G. R. J.; Campbell, A. S. *J. Org. Chem.* **1993**, *58*, 2272.
13. Wu, T. F.; Froeyen, M.; Kempeneers, V.; Pannecouque, C.; Wang, J.; Busson, R.; De Clercq, E.; Herdewijn, P. *J. Am. Chem. Soc.* **2005**, *127*, 5056.
14. Pannicke, U.; Honig, M.; Hess, I.; Friesen, C.; Holzmann, K.; Rump, E. M.; Barth, T. F.; Rojewski, M. T.; Schulz, A.; Boehm, T.; Friedrich, W.; Schwarz, K. *Nat. Genet.* **2009**, *41*, 101.
15. Park, H.; Kam, T. I.; Kim, Y.; Choi, H.; Gwon, Y.; Kim, C.; Koh, J. Y.; Jung, Y. K. *Hum. Mol. Genet.* **2012**, *21*, 2725.
16. Tanimura, A.; Horiguchi, T.; Miyoshi, K.; Hagita, H.; Noma, T. *PLoS ONE* **2014**, *9*, e89916.
17. Uttaro, J. P.; Broussous, S.; Mathe, C.; Perigaud, C. *Tetrahedron* **2013**, *69*, 2131.
18. Mathe, C.; Perigaud, C. *Eur. J. Org. Chem.* **2008**, 1489.
19. Caillat, C.; Topalis, D.; Agrofoglio, L. A.; Pochet, S.; Balzarini, J.; Deville-Bonne, D.; Meyer, P. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 16900.
20. Topalis, D.; Alvarez, K.; Barral, K.; Munier-Lehmann, H.; Schneider, B.; Veron, M.; Guerreiro, C.; Mulard, L.; El-Amri, C.; Canard, B.; Deville-Bonne, D. *Nucleosides, Nucleotides Nucleic Acids* **2008**, *27*, 319.
21. Blondin, C.; Serina, L.; Wiesmüller, L.; Gilles, A. M.; Bârzu, O. *Anal. Biochem.* **1994**, *220*, 219.
22. Thomsen, R.; Christensen, M. H. *J. Med. Chem.* **2006**, *49*, 3315.
23. Van Rompay, A. R.; Johansson, M.; Karlsson, A. *Eur. J. Biochem.* **1999**, *261*, 509.
24. Heteroatoms (water molecules, ions) and Ap5A ligand were removed from the crystal structure and addition of hydrogen atoms was performed. The scoring function Moldock Score and the Moldock SE search algorithm were used, the resolution of the grid for estimation of the computed binding affinity was 0.3 Å with a radius of 15 Å around the catalytic triad residues. Fifteen runs were launched and up to 50 (maximum) poses subsequently analyzed.
25. Jana, B.; Adkar, B. V.; Biswas, R.; Bagchi, B. *J. Chem. Phys.* **2010**, *134*, 035101.