Electronic Supplementary Information

Bisimidazoline arylamides binding to the DNA minor groove. *N*1-hydroxylation enhances binding affinity and selectivity to AATT sites

Carlos H. Ríos Martínez[a†], Laura Lagartera[a], Cristina Trujillo[c], and Christophe Dardonville[a*]

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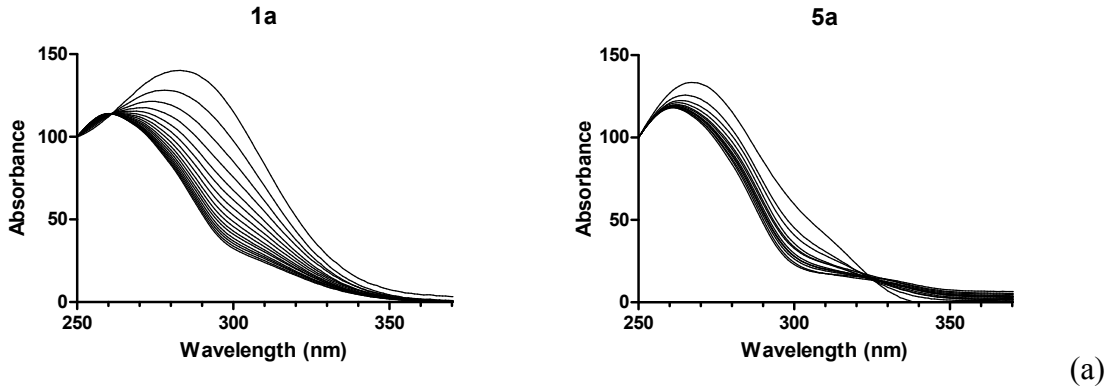
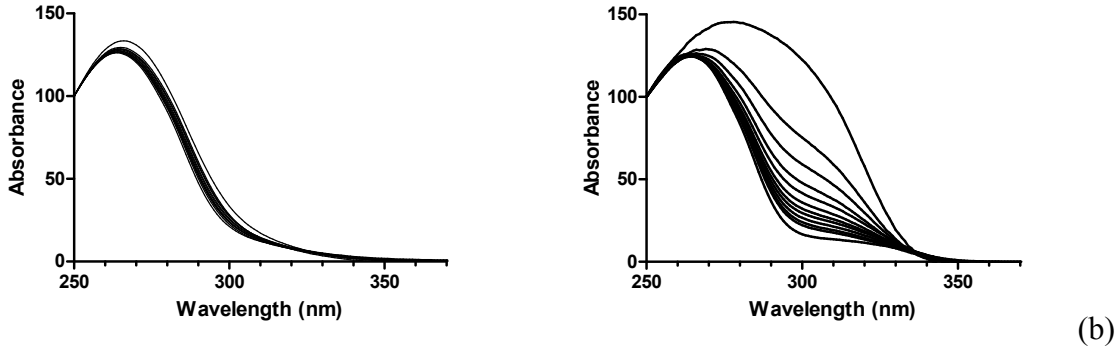
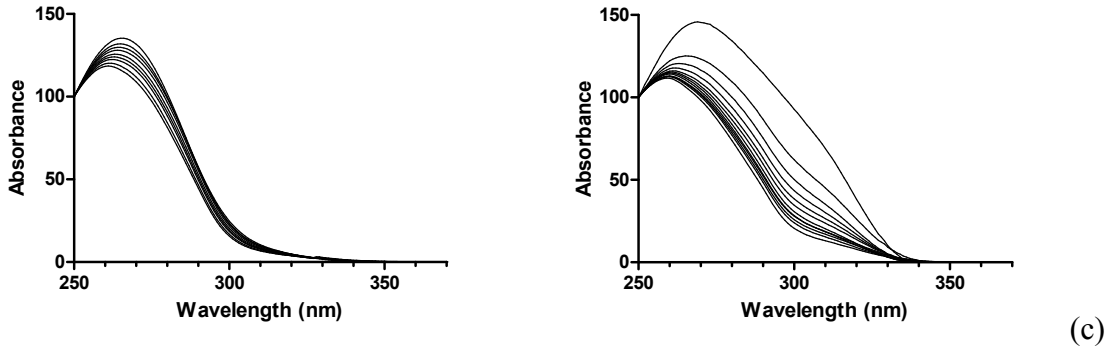
Figure S1. UV titration of **1a** (left panels) and **5a** (right panels) with AATT (top), $(AT)_4$ (middle), and $(CG)_4$ (bottom) hairpin oligonucleotides



(a)



(b)



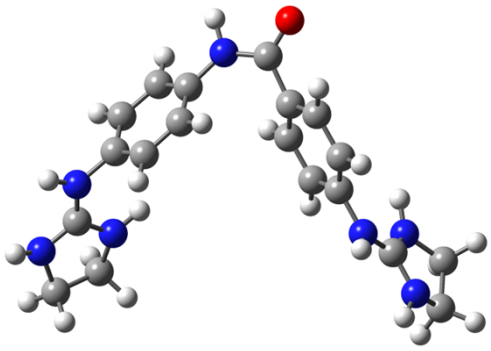
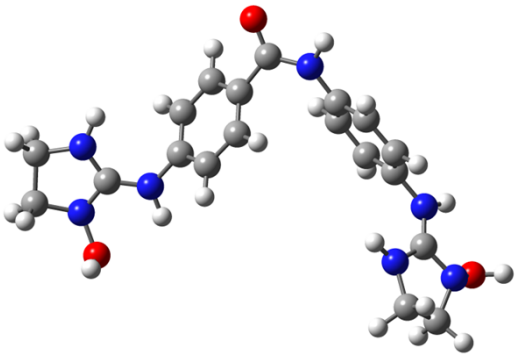
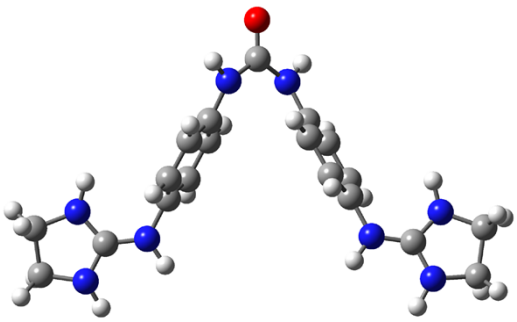
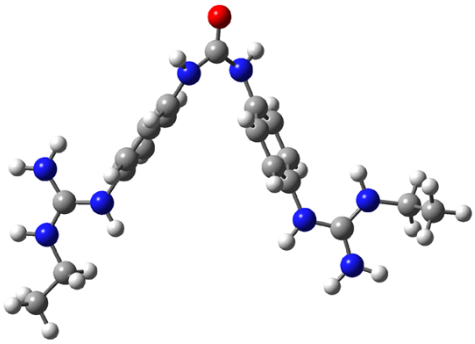
(c)

UV titration of **1a** and **5a** (2.5 μM) with AATT (top), $(AT)_4$ (middle), and $(CG)_4$ (bottom) hairpin oligonucleotides in 10 mM phosphate buffer at 25 ºC. DNA concentrations ranged from 0 to 3.92×10^{-7} M (**1a**_AATT), 0 to 7.41×10^{-7} M [**1a**_$(AT)_4$], 0 to 8.2×10^{-5} M [**1a**_$(CG)_4$)], and 0 to 7.41×10^{-7} M (**5a**_AATT, **5a**_$(AT)_4$, and **5a**_$(CG)_4$] from top to bottom.

Docking studies

1) Figure S2: optimized geometries

All compounds have been optimized using the Gaussian09 package at the B3LYP computational level with the 6–311++G(d,p) basis sets. The effect of water solvation was then accounted using the SCFR-PCM approach implemented in the Gaussian09 package including dispersing, repulsing and cavitation energy terms of the solvent in the optimization.

	
1	**1a**
	
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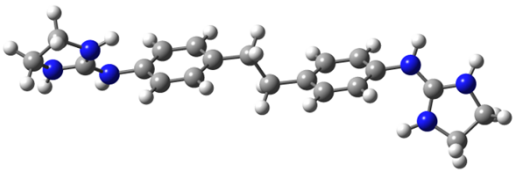
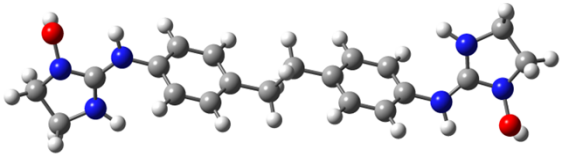
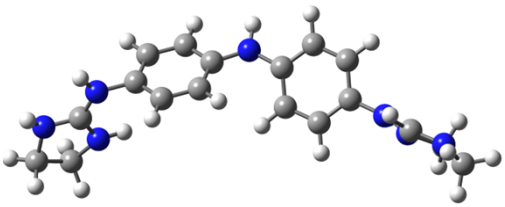
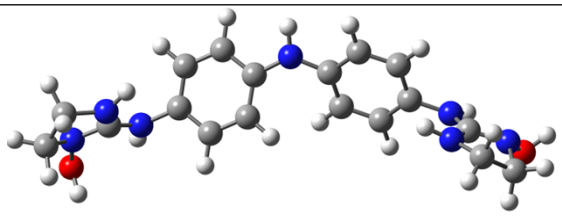
	
4	**4a**
	
5	**5a**

2) Table S1: predicted binding affinities of the compounds with dsDNA containing CGAATTCG and CATATATAT

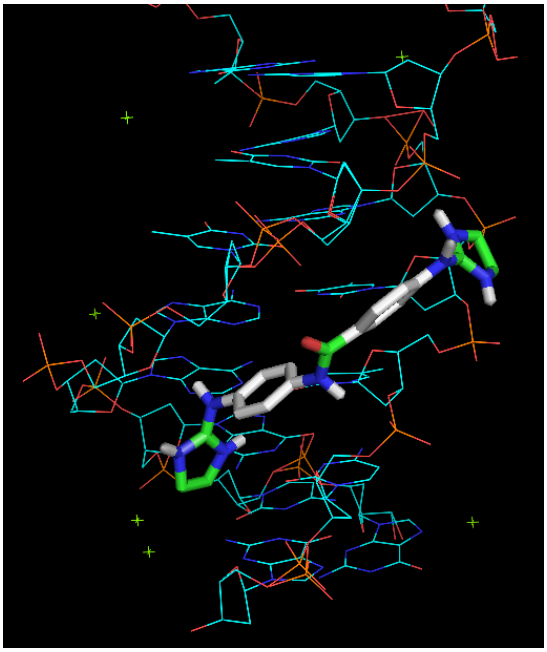
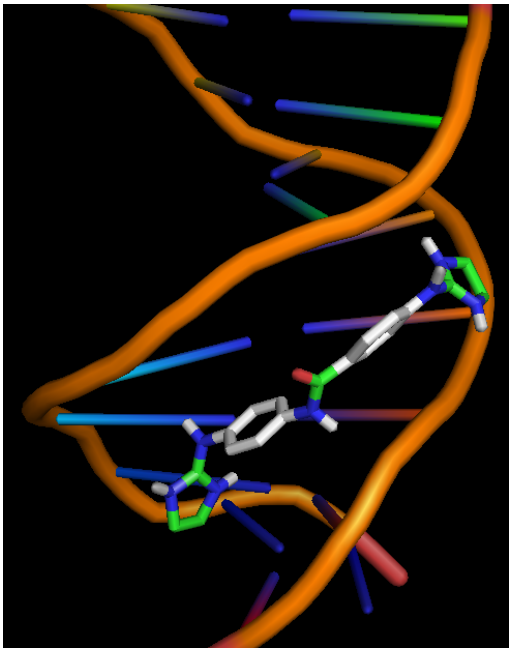
Docking calculations were performed with the AutoDock Vina 1.1.2 modelling software[1] using the crystallographic structures of d(GCGAATTCG) [pdb: 1ENN] and d(CCATATATATGC) [pdb: 3TED].

Table S1. Predicted binding affinities (kcal/mol)

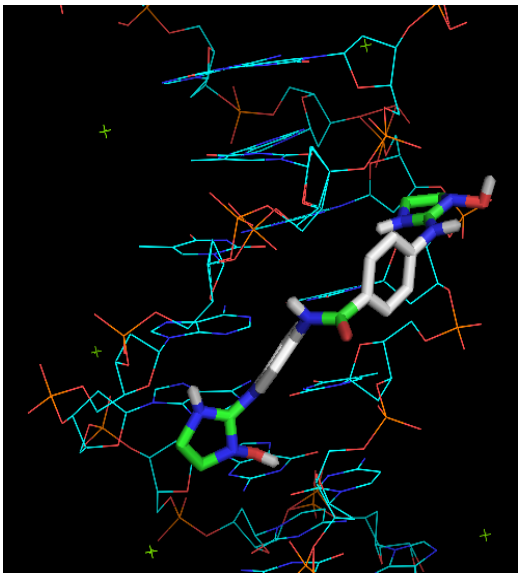
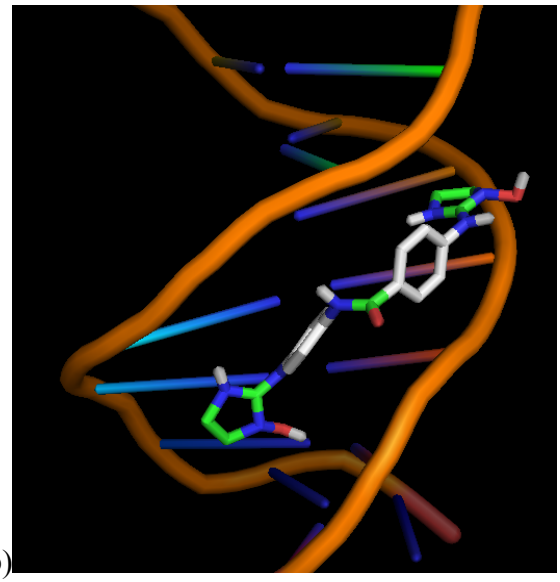
	1ENN	3TED
1	-8.7	-8.4
1a	-9.1	-9.1
3	-7.7	nd[a]
3d	-6.7	nd
4	-8.6	-8.4
4a	-8.6	-8.7
4c	-7.2	nd
5	-8.7	-8.4
5a	-8.8	-8.8

[a] Not determined

3) Figure S3: **1** docked with the d(GCGAATTCG) oligonucleotide (pdb: 1ENN)

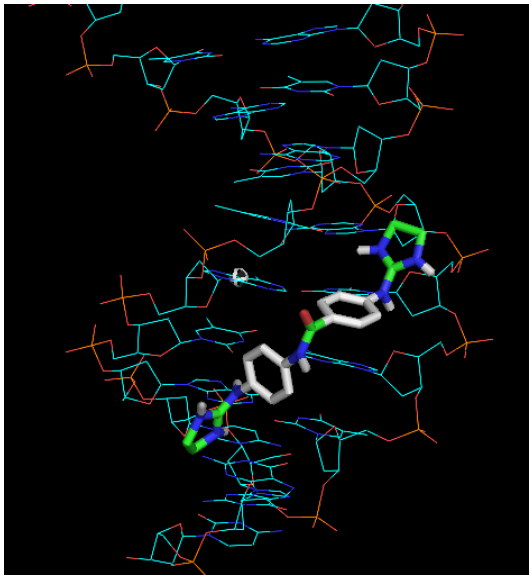
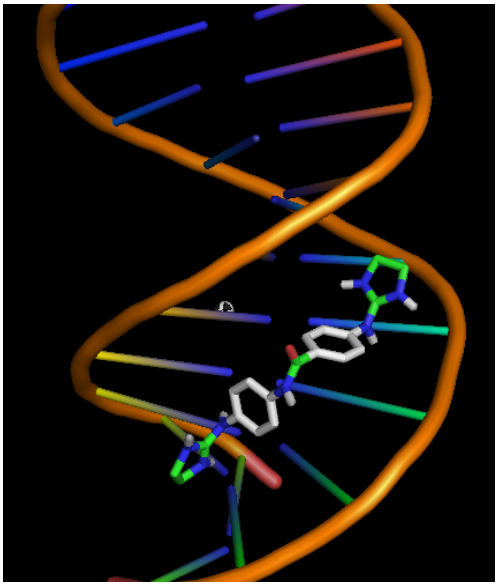
a)  b) 

4) Figure S4: **1a** docked with the d(GCGAATTCG) oligonucleotide (pdb: 1ENN)

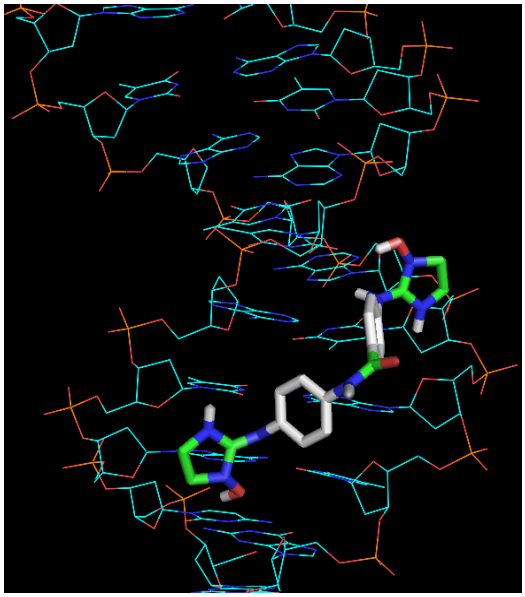
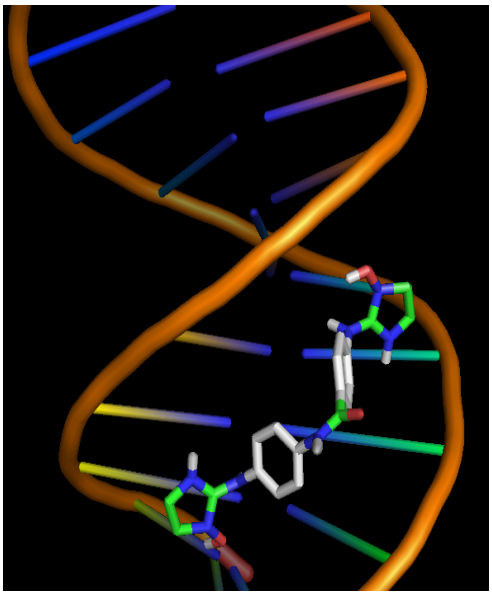
a)  b) 

a) and b) are two images of the same complex drawn with Pymol [The PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC., 2010].

5) Figure S5: **1** docked with the d(CCATATATATGC) oligonucleotide (pdb: 3TED)

a)  b) 

6) Figure S6: **1a** docked with the d(CCATATATATGC) oligonucleotide (pdb: 3TED)

a)  b) 

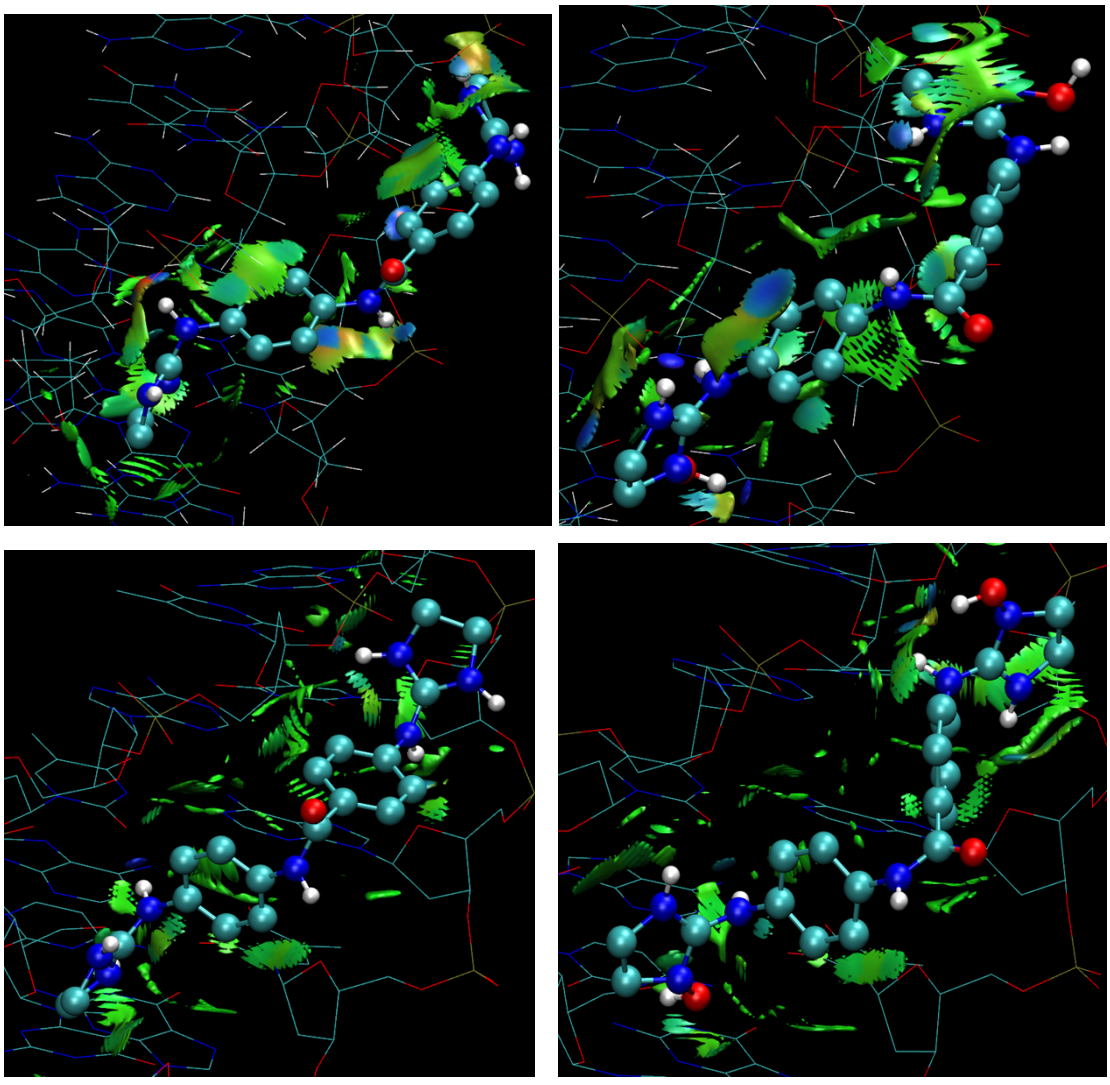
7) NCI Analysis

The Non-covalent Interaction (NCI) program[2] allows visualising the non covalent interactions resulting from complexation using the electron density Hessian. While NCI does not provide quantitative results is a very powerful qualitative tool.

Green areas denote weak ($\lambda_2 \approx 0$) but attractive interactions, while blue ones indicate strong interactions (normally associated with hydrogen bonds). Larger areas correspond to more interaction regions.

Figure S7. NCI plot of non-covalent interaction of compound **1** (left) and **1a** (right) docked with 1ENN (upper pannels) and 3TED (bottom). Green areas correspond to $\lambda_2 \approx 0$ (weak). λ_2 is one of the three eigenvalues of the electron density Hessian with $\lambda_1 \leq \lambda_2 \leq \lambda_3$.

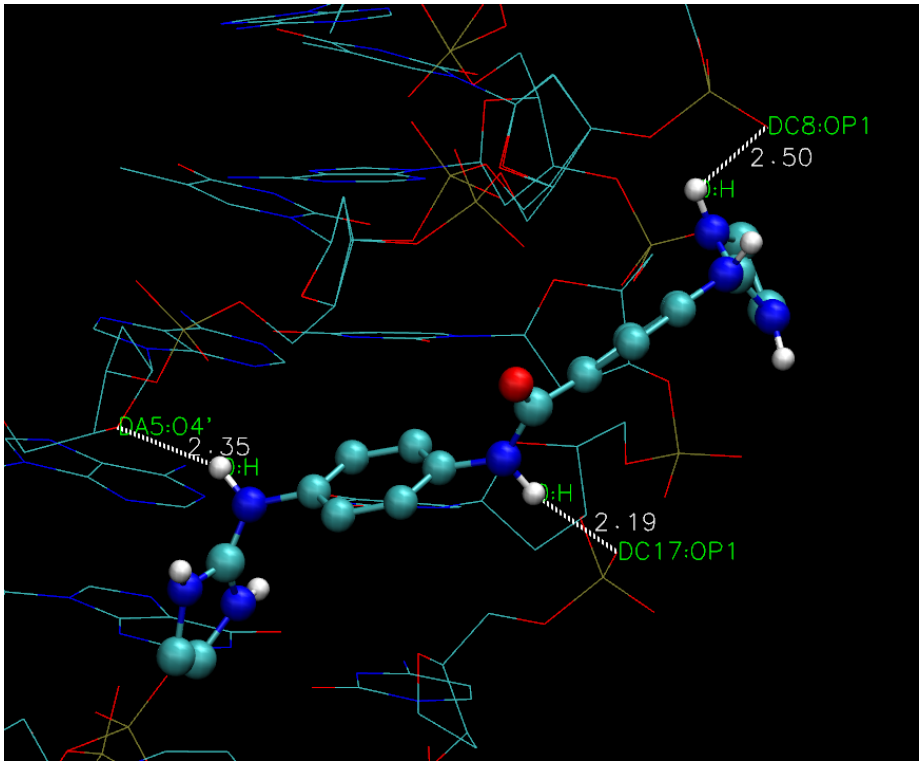
Compound **1** Compound **1a**



8) **Figure S8**: plot of hydrogen bonds (HB) interactions for **1** and **1a** docked with d(GCGAATTCG) (pdb: 1ENN) and d(CCATATATATGC) (pdb: 3TED)

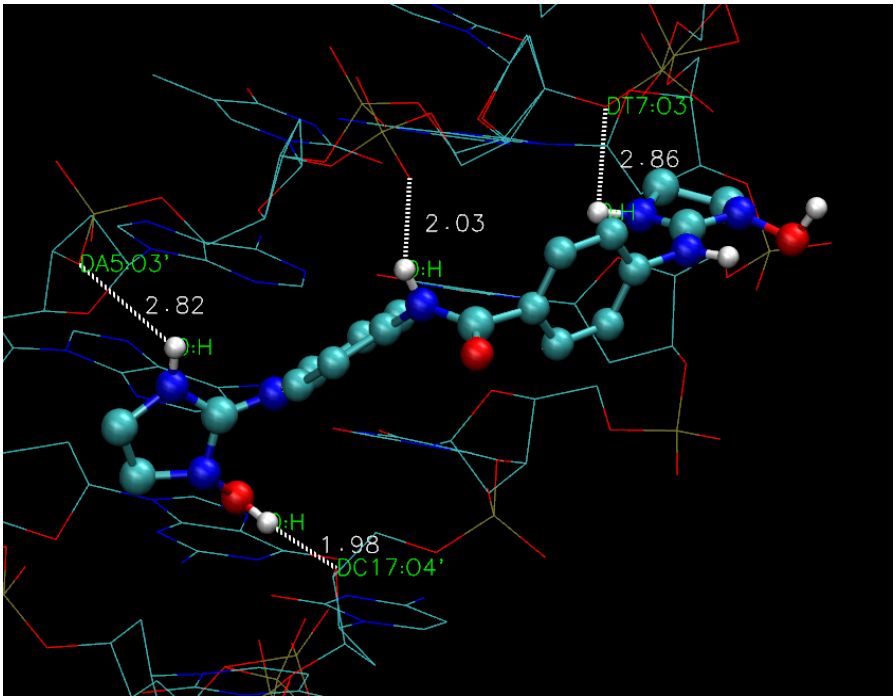
A) HB interactions for compound **1** bound to 1ENN:

- 1HB (2.19 Å) between phosphate (DC17) and amide NH of the ligand
- 1HB (2.35 Å) between the imidazoline exocyclic N(2)H and O4' atom of the adenine (DA5) deoxiribose
- 1HB (2.50 Å) between phosphate (DC8) and the imidazoline N(1)H



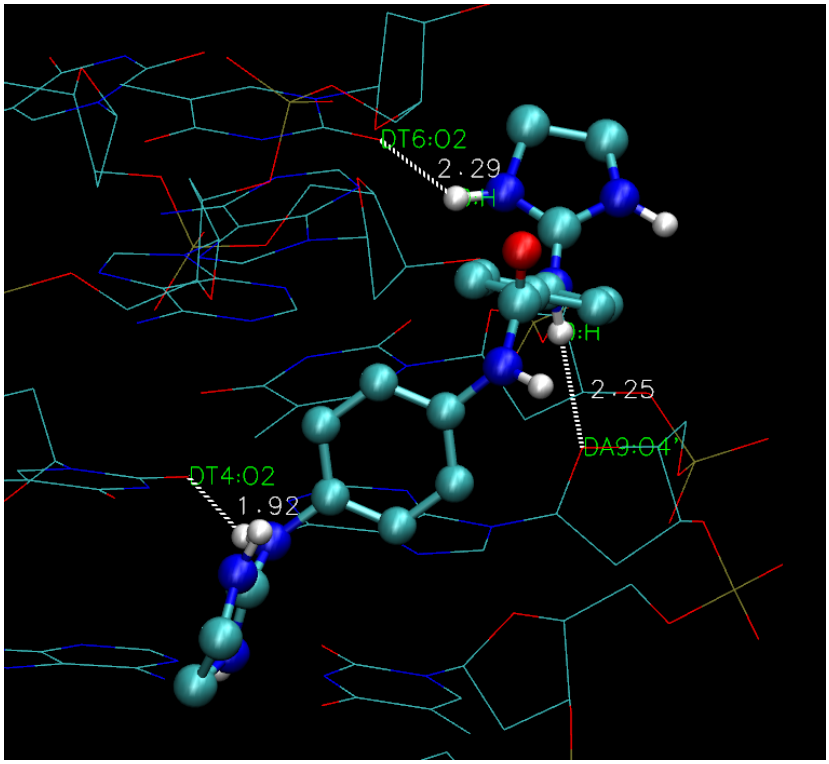
B) HB interactions for compound **1a** bound to 1ENN:

- 1HB (1.98 Å) between the imidazoline N(1)OH substituent and O4’ atom of the cytosine (DC17) deoxiribose
- 1HB (2.03 Å) between phosphate and amide NH of the ligand
- 1HB (2.82 Å) between phosphate (DA5) and the imidazoline endocyclic N(3)H
- 1HB (2.86 Å) between phosphate (DT7) and the imidazoline endocyclic N(3)H of the second imidazoline group



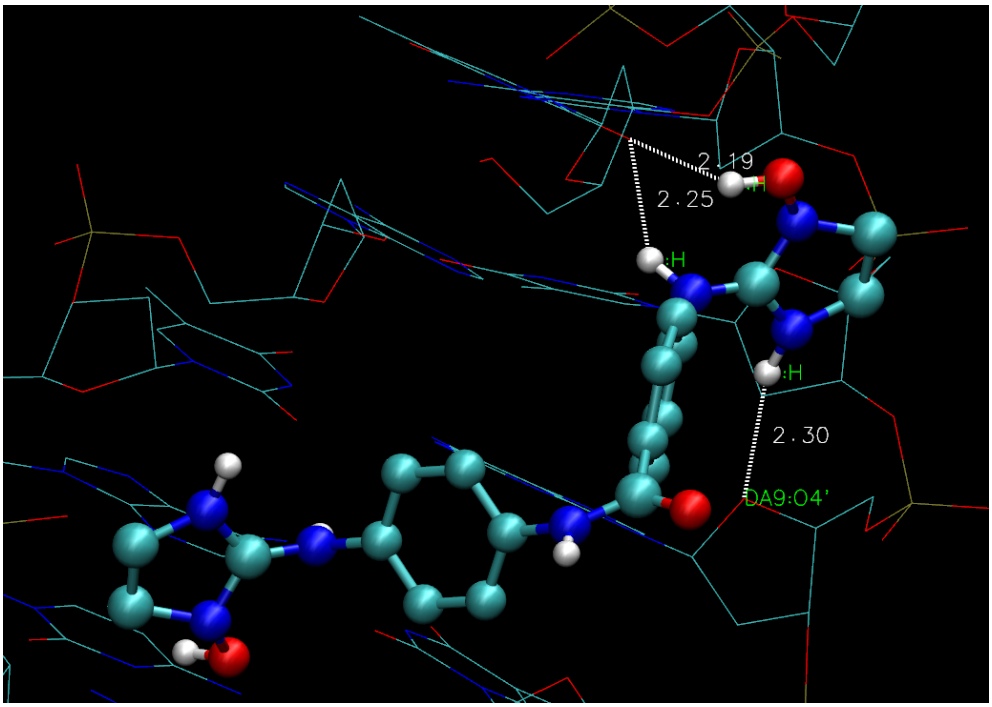
C) HB interactions for compound **1** bound to 3TED:

- 1 HB (1.92 Å) between thymine (DT4) and imidazoline N(2)H
- 1 HB (2.29 Å) between thymine (DT6) and imidazoline N(1)H
- 1 HB (2.25 Å) between the imidazoline exocyclic N(2)H and O4' atom of the adenine (DA9) deoxiribose



D) HB interactions for compound **1a** bound to 3TED:

- 1 bifurcated HB (2.19 and 2.25 Å) between thymine (DT6) and imidazoline N(2)H and N(1)OH.
- 1 HB (2.30 Å) between the imidazoline N(3)H and O4' atom of the adenine (DA9)



References

1. Trott, O.; Olson, A. J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **2010,** 31, 455-461.

2. Johnson, E. R.; Keinan, S.; Mori-Sanchez, P.; Contreras-Garcia, J.; Cohen, A. J.; Yang, W. Revealing Noncovalent Interactions. *Journal of the American Chemical Society* **2010,** 132, 6498-6506.