CHEMISTRY & BIODIVERSITY

Supporting Information

Deoxyribonuclease I Inhibitory Properties, Molecular Docking and Molecular Dynamics Simulations of 1-(Pyrrolidin-2-yl)propan-2-one Derivatives

Budimir S. Ilić, Mihajlo Gajić, Bojan P. Bondžić, Zdravko Džambaski, Gordana Kocić, and Andrija Šmelcerović*

Supporting Information

Deoxyribonuclease I Inhibitory Properties, Molecular Docking and Molecular Dynamics Simulations of 1-(Pyrrolidin-2-yl)propan-2-one Derivatives

Budimir S. Ilić,[a] Mihajlo Gajić,[b] Bojan Bondžić,[c] Zdravko Džambaski,[c] Gordana Kocić,[d] Andrija Šmelcerović*,[c]

[a] University of Niš, Faculty of Medicine, Department of Chemistry, Blvd. Dr. Zorana Đinđića 81, 18000 Niš, Serbia

[b] University of Niš, Faculty of Medicine, Department of Pharmacy, Blvd. Dr. Zorana Đinđića 81, 18000 Niš, Serbia

[c] University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, 11000 Belgrade, Serbia, e-mail: andrija.smelcerovic@medfak.ni.ac.rs (or a.smelcerovic@yahoo.com)

[d] University of Niš, Faculty of Medicine, Department of Biochemistry, Blvd. Dr. Zorana Đinđića 81, 18000 Niš, Serbia

Table of content

1. Experimental Section

1.1. Chemicals

DNase I from bovine pancreas, DNA (sodium salt from calf thymus, type I, fibers), DMSO and perchloric acid were purchased from Sigma-Aldrich. Crystal violet was purchased from Lach-Ner.

1.2. Compounds

The synthesis of 1-(pyrrolidin-2-yl)propan-2-one derivatives was conducted according to the description in our previous study.[1]

1.3. Evaluation of deoxyribonuclease I inhibition

Compounds were assessed for inhibitory properties against DNase I from bovine pancreas. Evaluation of *in vitro* enzyme inhibition was conducted as spectrophotometric measurement of acid-soluble nucleotides formation at 260 nm, according to our previously published procedures[9 - 13] Studied compounds were assayed for DNase I inhibitory activity at concentration of 200 μM. Those exhibiting inhibition greater than 50% at these concentrations were tested in a broader series of concentrations to allow calculation of IC_{50} values. IC_{50} curves were generated using three concentrations of studied compounds (200, 150 and 100 μM). Crystal violet was used as a positive control. All experiments were performed in triplicate and averaged.

1.4. *In silico* molecular and ADMET properties

Physico-chemical and pharmacokinetic properties of proposed compounds were assessed using SwissADME online tool,[2,3] together with ADMETlab,[4,5] while OSIRIS DataWarrior software[6] was utilized for toxicity prediction.

1.5. *In silico* PAINS and promiscuity assessment

Web platform HitDexter 2.0 was employed to evaluate similarity to known PAINS and predict promiscuity of tested compound.[7,8]

1.6. Molecular docking

1.6.1. Ligand preparation

Examined 1-(pyrrolidin-2-yl)propan-2-ones have been generated using the builder panel in the Molecular Operating Environment (MOE) 2019.0101 software.[14] Using the MOE LigX module, partial atomic charges were ascribed and possible ionization states were generated at a pH of 7.0. The MMFF94x force field was used for optimization and the resulting structures were used

for modeling studies. Conformational search was carried out by MOE LowModelMD method which performs molecular dynamic perturbations along with low frequency vibrational modes with energy window of 7 kcal/mol, and conformational limits of 1000.

1.6.2. Receptor preparation

The X-ray crystallographic structure of DNase I (PDB code: 1DNK), retrieved from the Protein Data Bank, was prepared using the Structure Preparation process in MOE. After the correction, hydrogens were added and partial charges (Gasteiger methodology) were calculated. Energy minimization (AMBER14:EHT, RMS gradient: 0.100) was performed.

1.6.3. Binding site selection

The Site Finder module of the MOE was used to identify possible ligand-binding sites within the optimized structure of DNase I. Hydrophobic or hydrophilic alpha spheres served as probes denoting zones of tight atom packing. These alpha spheres were utilized to define and rank potential ligand-binding sites according to their propensity for ligand binding (PLB) score, which was based on the amino acid composition of the pocket.[15]

1.6.4. Docking protocol

The molecular docking study was performed using the MOE to understand the ligand/protein interactions in detail. The default Triangle Matcher placement method was used for the induced fit docking. GBVI/WSA dG scoring function which estimates the free energy of binding of the ligand from a given pose was used to rank the final poses. Each ligand/protein complex with lowest relative binding free energy (ΔG) score was selected for further study.

1.7. Molecular dynamics simulation

The molecular dynamics simulation of 1-(pyrrolidin-2-yl)propan-2-ones on DNase I, was carried out using the Desmond Molecular Dynamics System (Desmond) 2018.4 software.[16] The structure of the added water was based on the simple point charge (SPC) solvent model. The system was neutralized with Na^+ ions to balance the net charge of the whole simulation box to neutral. The final system contained approximately 29500 atoms. The system was passed through a 6-step relaxation protocol before molecular dynamics simulations. The relaxed system was simulated for 10 ns, using a normal pressure temperature (NPT) ensemble with a Nosé–Hoover thermostat at 300 K and Martyna–Tobias–Klein barostat at 1.01325 bar pressure. Atomic coordinate data and system energies were recorded every 1 ps. The root mean square deviation (RMSD) and root mean square fluctuation (RMSF) of the inhibitor/enzyme complexes were analyzed with respect to the simulation time.

2. Supplementary tables

Table S1. Summary of the top five inhibitor-binding sites in DNase I.

Site	Size	PLB	Hyd	Side	Residues
1	34	2.63	9	39	Asn 7, Arg 9, Glu 39, Glu 78, Arg 111, His 134, Pro 137, Asp 168, Asn 170, Tyr 175, Thr 203, Thr 205, Thr 207, Tyr 211, Asp 251, His 252
2	10	0.51	13	16	Val 125, Lys 126, Glu 127, His 159, Leu 160, Asn 161, Asp 162, Leu 220
3	17	0.28	3	19	Tyr 76, Glu 78, Ser 110, Arg 111, His 134, Ser 135, Ala 136, Pro 137, Asn 170
4	21	0.23	9	14	Pro 137, Ser 138, Asp 139, Ala 140, Val 141, Ala 142, Ser 174, Tyr 175, Gln 180
5	12	0.18	8	14	Val 66, Val 67, Ser 68, Phe 82, Thr 94, Tyr 95, Gln 96, Ala 114, Val 115, Val 116

3. References

[1] Z. Džambaski, B. P. Bondžić, 'Dehydrogenative C(sp^3)–H bond functionalization of tetrahydroisoquinolines mediated by organic oxidants under mild conditions', *Org. Biomol. Chem.* **2019**, *17*, 6420-6425.

[2] http://www.swissadme.ch; Accessed November 16, 2020.

[3] A. Diana, O. Michielin, V. Zoete, 'SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules', *Sci. Rep.* **2017**, 7, 42717.

[4] http://admet.scbdd.com; Accessed January 27, 2021.

[5] J. Dong, N. N. Wang, Z. J. Yao, L. Zhang, Y. Cheng, D. Ouyang, A. P. Lu, D. S. Cao, 'ADMETlab: a platform for systematic ADMET evaluation based on a comprehensively collected ADMET database', *J. Cheminform.* **2018**, *10*, 29.

[6] T. Sander, J. Freyss, M. von Korff, C. Rufener, 'DataWarrior: an open-source program for chemistry aware data visualization and analysis', *J. Chem. Inf. Model.* **2015**, *55*, 460-473.

[7] https://nerdd.zbh.uni-hamburg.de/hitdexter; Accessed January 27, 2021.

[8] C. Stork, Y. Chen, M. Šícho, J. Kirchmair, 'Hit Dexter 2.0: Machine-learning models for the prediction of frequent hitters', *J. Chem. Inf. Model.* **2019**, *59*, 1030-1043.

[9] A. Smelcerovic, A. Zivkovic, B. S. Ilic, A. Kolarevic, B. Hofmann, D. Steinhilber, H. Stark, '4-(4-Chlorophenyl) thiazol-2-amines as pioneers of potential neurodegenerative therapeutics with anti-inflammatory properties based on dual DNase I and 5-LO inhibition', *Bioorg. Chem.* **2020**, *95*, 103528.

[10] A. Kolarević, B. S. Ilić, N. Atanassova, A. T. Mavrova, D. Yancheva, G. Kocić, A. Šmelcerović, 'Benzimidazoles as novel deoxyribonuclease I inhibitors', *J. Cell. Biochem.* **2018**, *119*, 8937-8948.

[11] A. T. Mavrova, S. Dimov, D. Yancheva, A. Kolarević, B. S. Ilić, G. Kocić, A. Šmelcerović, 'Synthesis and DNase I inhibitory properties of some 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidines', *Bioorg. Chem.* **2018**, *80*, 693-705.

[12] A. Kolarević, B. S. Ilić, G. Kocić, Z. Džambaski, A. Šmelcerović, B. P. Bondžić, 'Synthesis and DNase I inhibitory properties of some 4-thiazolidinone derivatives', *J. Cell. Biochem.* **2019**, *120*, 264-274.

[13] A. Kolarevic, A. Pavlovic, A. Djordjevic, J. Lazarevic, S. Savic, G. Kocic, M. Anderluh, A. Smelcerovic, 'Rutin as deoxyribonuclease I inhibitor', *Chem. Biodiversity* **2019**, *16*, e1900069.

[14] MOE, Molecular Operating Environment 2019.0101, Chemical Computing Group ULC, Montreal, QC, Canada, 2019.

[15] S. Soga, H. Shirai, M. Kobori, N. Hirayama, 'Use of amino acid composition to predict ligand-binding sites', *J. Chem. Inf. Model.* **2007**, *47*, 400-406.

[16] Desmond, Desmond Molecular Dynamics System, version 2018.4, D. E. Shaw Research, New York, NY, 2018.