Available online at www.sciencedirect.com

ScienceDirect

Mendeleev Commun., 2020, **30**, 430–432

Mendeleev Communications

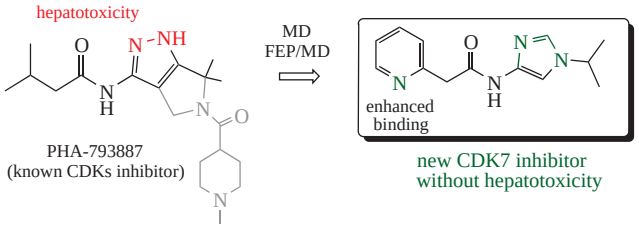
Modeling of novel CDK7 inhibitors activity by molecular dynamics and free energy perturbation methods

Polina V. Rusina,*[a,b] Ilya Yu. Titov,[a,c] Maria V. Panova,[a] Victor S. Stroylov,[a,c] Yana R. Abdyusheva,[a,d] Elizaveta Yu. Murlatova,[a,d] Igor V. Svitanko[a,d] and Fedor N. Novikov[a,c]

[a] *N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russian Federation. Fax: +7 499 135 5328; e-mail: polyanarus@gmail.com*
[b] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation*
[c] *MolTech Ltd., 119992 Moscow, Russian Federation. E-mail: nfn@moltech.ru*
[d] *National Research University Higher School of Economics, 101000 Moscow, Russian Federation*

DOI: 10.1016/j.mencom.2020.07.008

Although CDK7 inhibitors are considered to be potential anticancer drugs, all inhibitors developed so far have significant disadvantages preventing their further use. We have developed a new CDK7 inhibitor scaffold lacking hepatotoxicity using molecular dynamics (MD) and free energy perturbation (FEP/MD) methods, and were able to double its binding affinity after additional research. The combination of MD and FEP/MD methods was shown to be a valuable instrument for the development of novel and potent CDK7 inhibitors for anticancer therapy.

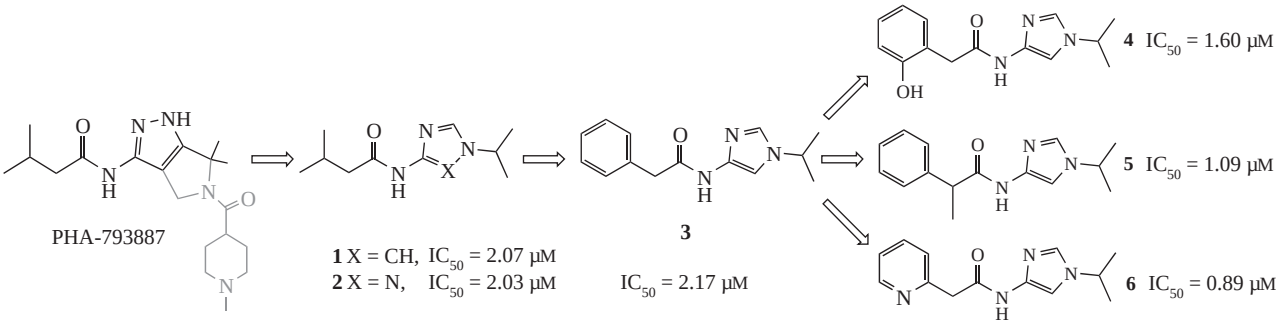


Keywords: cyclin-dependent kinase 7, CDK7 inhibitors, hepatotoxicity, PHA-793887, non-hepatotoxic scaffold, molecular dynamics, free energy perturbation, relative binding free energy.

Cyclin-dependent kinase 7 (CDK7) is involved in regulating mRNA processing and controls cell cycle progression.[1] Given that CDK7 activity is moderately elevated in different types of tumors, targeting this kinase for anticancer therapy appears attractive.[2] Previously developed CDK7 inhibitors were shown to have antitumor activity,[3] but the downsides were supposed toxicity and intravenous delivery. Therefore, the search for new CDK7 inhibitors is an important step in development of anticancer drugs.

In medicinal chemistry, preliminary modeling methods for targeted organic synthesis cover two basic directions depending on reaction kinetics and thermodynamics: (1) static and dynamic modeling of structural and physicochemical parameters of ligand-target complex[4–6] and (2) modeling the mechanism of reaction leading to target products.[7–10] The modern paradigm of directed synthesis includes only those modeling options which lead to the target structure with the desired properties. It is not limited by synthetic preferences or reagent availability, and studies in the field regard modeling not as a way of describing a reaction already carried out but as the first stage of a synthesis chain.

This work was accomplished in accordance with these requirements, using preliminary modeling for directed synthesis of compounds possessing desired properties using methods proven to be the best in various tests.[11] The key stage in development of new drugs is an accurate prediction of protein–ligand binding affinities. Over the past decade, full-atom explicit solvent-based FEP methods using MD methodology have become a useful tool in predicting binding affinity, because they represent the most rigorous physical approach to calculating binding free energies in complex biological systems.[12] In this work, we aimed to evaluate the potential of MD and FEP/MD methods in predicting affinities of new CDK7 inhibitors.



Scheme 1 Development of novel CDK7 inhibitors from PHA-793887 prototype.

PHA-793887 is a potent inhibitor of multiple cyclin dependent kinases, *i.e.* CDK2 (IC_{50} = 8 nM), CDK7 (IC_{50} = 10 nM), CDK1 (IC_{50} = 60 nM).[13] It was speculated to overcome potential compensatory activities and resistance-based mechanisms and hence represent a valuable option in cancer treatment. However, it was previously established that PHA-793887 induces dose-related hepatic toxicity, which precludes its further clinical development.[14] We suggested that PHA-793887 hepatotoxicity is associated with a N–N bond in a pyrazole cycle since the latter is known to induce oxidative liver injury.[15] Needless to say, hepatotoxicity poses a significant problem for any drug, and CDK7 inhibitors are intended as drugs for different cancer types, particularly severe liver cancer.[16]

In this work we proposed a compound based on PHA-793887 as a scaffold for new inhibitors, in which a pyrazole core was changed to an imidazole one. Isopropyl substitution in the ring was retained in novel scaffold **1** (Scheme 1).

To study binding of minimal inhibitors based on the scaffold and evaluate the effect of additional nitrogen we performed molecular docking and molecular dynamic simulations of imidazole and 1,2,4-triazole derivatives **1** and **2** (see Scheme 1). Analysis of MD trajectories of inhibitors bound to kinase active site uncovered Met94 backbone O and NH as key interaction sites has shown that the additional nitrogen atom is not critical for binding. FEP/MD simulations and *in vitro* experiments have substantiated these results with negligible differences in relative dG energies and IC_{50} for these compounds (Scheme 1). Thus, imidazole derivatives were selected for further improvements.

Upon analysis of compound **1** bound to CDK7 active site after MD simulations, it was decided to modify isovaleryl substituent into phenylacetyl (**3**, see Scheme 1) to improve binding since it also facilitates further modifications. Compound **3** has demonstrated similar location in the active site and nearly the same experimental activity as **1** (ΔIC_{50} = 0.10 μM).

The influence of hydrogen bond donors on binding affinity was tested using compound **4** with an OH-group as an example, however the latter failed to form any notable H-bonds with kinase active site. Inhibitor structure was stabilized *via* intramolecular H-bond of OH-group with carbonyl oxygen (Figure 1). FEP and experimental relative free energies suggest that this stabilization enhances binding (Table 1).

Binding site surface analysis has revealed a small hydrophobic region which could be filled by a lipophilic group (see Figure 1). A methyl group added to the α position relative to carbonyl group resulted in a so-called 'magic methyl effect'[17]: FEP dG and experimental IC_{50} values for the synthesized racemate demonstrated a twofold increase in binding affinity (see Table 1).

We have also examined the effect of H-bond acceptors by introducing nitrogen into the phenyl substituent changing it into pyridyl (**6**, see Scheme 1). Pyridine derivative **6** was found to have enhanced binding due to the interaction between pyridine nitrogen and Asp97 residue *via* bridging water molecule (see Figure 1). This hydrogen bond had significant impact on binding: compound **6** had the highest binding affinity to CDK7 according to both calculated and experimental data (see Table 1).

Table 1 Correlation between activities predicted by FEP and measured experimentally.

FEP-transformation	ΣddG/kcal mol^{-1}	
	Calculated	Experimental
2 → 1	0.00	0.00
4 → 3	–0.31	–0.41
5 → 3	–0.23	–0.18
6 → 3	–0.59	–0.53

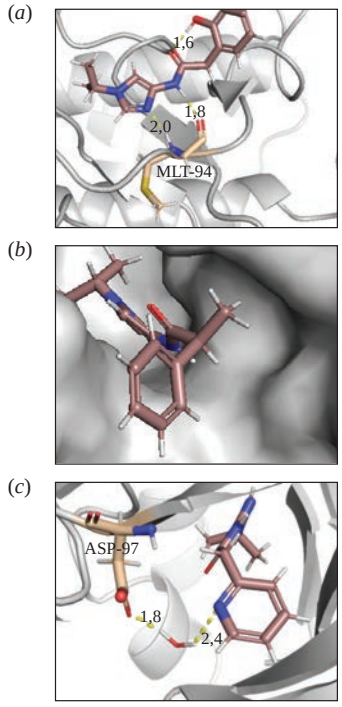


Figure 1 Binding of compounds (*a*) **4**, (*b*) **5** and (*c*) **6** to the active site of CDK7 predicted with molecular modeling.

To verify whether our new inhibitors are hepatotoxic, we tested compounds **1** and **2** on the Vienna LiverTox Workspace[18] unbiased and easily accessed information on the diagnosis, cause, frequency, clinical patterns and management of liver injury attributable to prescription and nonprescription medications and selected herbal and dietary supplements. The LiverTox site is meant as a resource for both physicians and patients as well as for clinical academicians and researchers who specialize in idiosyncratic drug induced hepatotoxicity. Information on specific medications or supplements can be found by entering its name in the 'Search this book' box shown above or by browsing the list of agents by its first letter using the alphabetic list shown below. LiverTox is produced by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). While 1,2,4-triazole derivative **2** had a high probability of causing cholestasis and drug-induced liver injury, imidazole one **1** was free of these disadvantages.

Thus, a new scaffold for CDK7 inhibitors with targeted properties was developed using the MD and MD/FEP methods. Taking compound **1** as an example, it was demonstrated that inhibitors obtained by these methods showed no potential hepatotoxicity. Activity ranks predicted by MD and FEP/MD methods were concordant to the experimental IC_{50} values, which suggests their advantageous use for further inhibitor modifications. Investigations of CDK7 inhibitors with improved activity and selectivity are already underway in our laboratory.

This work was supported by the Russian Science Foundation (grant no. 17-13-01526).

Online Supplementary Materials

Supplementary data associated with this paper can be found in the online version at doi: 10.1016/j.mencom.2020.07.008.

References

1 R. P. Fisher, *Transcription*, 2019, **10**, 47.
2 B. Sun, S. Mason, R. C. Wilson, S. E. Hazard, Y. Wang, R. Fang, Q. Wang, E. S. Yeh, M. Yang, T. M. Roberts, J. J. Zhao and Q. Wang, *Oncogene*, 2020, **39**, 50.

3 C. Sánchez-Martínez, M. J. Lallena, S. G. Sanfeliciano and A. de Dios, *Bioorg. Med. Chem. Lett.*, 2019, **29**, 126637.
4 V. I. Polshakov, E. A. Batuev and A. B. Mantsyzov, *Russ. Chem. Rev*, 2019, **88**, 59.
5 B. L. Grigorenko, E. D. Kots, A. I. Krylov and A. V. Nemukhin, *Mendeleev Commun.*, 2019, **29**, 187.
6 M. I. Lavrov, D. S. Karlov, V. A. Palyulin, V. V. Grigoriev, V. L. Zamoyski, G. E. Brkich, N. V. Pyatigorskaya and M. E. Zapolskiy, *Mendeleev Commun.*, 2018, **28**, 311.
7 T. V. Magdesieva and O. A. Levitskiy, *Russ. Chem. Rev.*, 2018, **87**, 707.
8 N. Yu. Kuznetsov, V. I. Malishev, M. G. Medvedev and Yu. N. Bubnov, *Mendeleev Commun.*, 2019, **29**, 190.
9 L. Y. Ustynyuk and A. N. Tikhonov, *J. Organomet. Chem.*, 2018, **867**, 290.
10 A. A. Marjewski, M. G. Medvedev, I. S. Gerasimov, M. V. Panova, J. P. Perdew, K. A. Lyssenko and A. O. Dmitrienko, *Mendeleev Commun.*, 2018, **28**, 225.
11 O. V. Stroganov, F. N. Novikov, V. S. Stroylov, V. Kulkov and G. G. Chilov, *J. Chem. Inf. Model.*, 2008, **48**, 2371.
12 L. Wang, Y. Wu, Y. Deng, B. Kim, L. Pierce, G. Krilov, D. Lupyan, S. Robinson, M. K. Dahlgren, J. Greenwood, D. L. Romero, C. Masse, J. L. Knight, T. Steinbrecher, T. Beuming, W. Damm, E. Harder, W. Sherman, M. Brewer, R. Wester, M. Murcko, L. Frye, R. Farid, T. Lin, D. L. Mobley, W. L. Jorgensen, B. J. Berne, R. A. Friesner and R. Abel, *J. Am. Chem. Soc.*, 2015, **137**, 2695.
13 M. G. Brasca, C. Albanese, R. Alzani, R. Amici, N. Avanzi, D. Ballinari, J. Bischoff, D. Borghi, E. Casale, V. Croci, F. Fiorentini, A. Isacchi, C. Mercurio, M. Nesi, P. Orsini, W. Pastori, E. Pesenti, P. Pevarello, P. Roussel, M. Varasi, D. Volpi, A. Vulpetti and M. Ciomei, *Bioorg. Med. Chem.*, 2010, **18**, 1844.
14 C. Massard, J.-C. Soria, D. A. Anthoney, A. Proctor, A. Scaburri, M. A. Pacciarini, B. Laffranchi, C. Pellizzoni, G. Kroemer, J.-P. Armand, R. Balheda and C. J. Twelves, *Cell Cycle*, 2011, **10**, 963.
15 Y. Lu, P. Gong and A. I. Cederbaum, *Toxicology*, 2008, **252**, 9.
16 C. Wang, H. Jin, D. Gao, L. Wang, B. Evers, Z. Xue, G. Jin, C. Lieftink, R. L. Beijersbergen, W. Qin and R. Bernards, *Cell Res.*, 2018, **28**, 690.
17 H. Schönherr and T. Cernak, *Angew. Chem., Int. Ed.*, 2013, **52**, 12256.
18 *LiverTox: Clinical and Research Information on Drug-Induced Liver Injury*, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2012.

Received: 1st June 2020; Com. 20/6234