OnLine Journal of Biological Sciences

Original Research Paper

Designing of Disulfide Cyclic Peptide for Inhibiting Polymerase A and B1 (PA_C-$PB1_N$) in H1N1 Virus using Molecular Simulation Approach

[1]Usman Sumo Friend Tambunan, [1]Mochammad Arfin Fardiansyah Nasution, [1]Arli Aditya Parikesit, [1]Harry Noviardi and [2]Djati Kerami

[1]*Bioinformatics Research Group, Department of Chemistry,*
[2]*Mathematics Computation Research Group, Department of Mathematics,*
Faculty of Mathematics and Natural Science, University of Indonesia, Depok 16424, Indonesia

Article history
Received: 30-05-2016
Revised: 23-08-2016
Accepted: 23-08-2016

Corresponding Author:
Usman Sumo Friend Tambunan
Bioinformatics Research Group, Department of Chemistry, Faculty of Mathematics and Natural Science, University of Indonesia, Depok 16424, Indonesia
Tel: +62217270027,
Fax: +62217863432
Email: usman@ui.ac.id

Abstract: The drug resistance A/H1N1 flu virus is emerging rapidly. Therefore, looking for potential therapy is very important. PB2, PB and PA are subunits of viral RNA-dependent RNA polymerase (RdRp). They play an important role in viral replication. The PA and PB1 binding sites can be considered as potential targets for the development of new influenza drugs. The peptide inhibitors can be designed specifically due to their high-preferred activity. In this study, the cyclic peptide ligands were designed based on the crystal structure of PA_C-$PB1_N$ in the surface of the molecule, resulting 1728 cyclopentadienyl compounds. The MOE 2008.10 software was utilized for molecular docking and dynamics simulation approach, while Lipinski's Rules of Five were utilized to evaluate the feasibility of drug candidates. Thus, molecular dynamics simulation was applied, in order to facilitate the interaction between the ligand and enzyme. The simulations have successfully produced two cyclopentyl peptides, namely CKKTC and CKTTC, which results in both ligands providing a potent inhibitor of polymerase PA_C-$PB1_N$ of Influenza A/2009 (H1N1).

Keywords: Five-Ring Peptides, Polymerase PA_C-$PB1_N$, Influenza A, Molecular Docking, Molecular Dynamics

Introduction

Since March 2009, a new strain of Influenza A/H1N1 flu has spread rapidly and become a pandemic. The H1N1 virus infection has been officially reported in many countries (WHO, 2014). This could be a big burden to the health care system in the world due to the high mortality and morbidity associated with H1N1 pandemics (Donaldson *et al*., 2009). The antigenic seasonal flu vaccine can give protection against the new strain A/2009 (H1N1) and render it ineffective. Until now, there are two classes of anti-influenza drugs that exist in the market; neuraminidase inhibitors (e.g., zanamivir and oseltamivir) and M2 inhibitors (e.g., amantadine, rimantadine and adamantine). Neuraminidase (NA) inhibitors prevent offspring distribution and release of viral particles (Ushirogawa and Ohuchi, 2011). The Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) have recommended oseltamivir as the most effective anti-influenza therapy (Rungrotmongkol *et al*., 2009). However, the rapid mutation of influenza virus showed several strains of drug resistance (Regoes and Bonhoeffer, 2006). Therefore, identifying new potential drug candidates are necessary. Hence, pharmaceutical biotechnology has determined the core components of the polymerase complex, namely Polymerase B1 (PB1), Polymerase B2 (PB2) and Polymerase A (PA) (Toyoda *et al*., 1996). Cyclopentyl peptides were chosen due to their durability in utilizing vesicles delivery system (Pfaff *et al*., 1994). The hydrophobic pockets of PA_C-$PB1_N$ crystal structure were located on the molecular surface. Hydrophobic pockets would facilitate an array of hydrogen bonds in the PA_C-$PB1_N$ (Moen *et al*., 2014). Hydrophobic interactions could be a significant contribution to the binding energy (Obayashi *et al*., 2008). The PB1 N-terminal ($PB1_N$) of 48 amino acids is known as the α-binding domain. It is required for the PA that contains residues (Perez and Donis, 2001).

In this study, our approach is was based on bioinformatics computation (Fischer and Serapião, 2010). Bioinformatics tools have been widely applied for drug development and molecular medicine (Bartoli, 2011). Moreover we constructed the cyclopentyl peptide ligands from two cysteine and three amino acids which can be found in the PB1 sequences, such as asparagine, glutamine, methionine, threonine, aspartic acid, alanine, valine, leucine, glycine, lysine, proline and phenylalanine. Thus, we obtained 1728 cyclopentyl peptide ligands by this approach. Those ligands would be piped into the Absorption, Distribution, Metabolism, Excretion and Toxicology (ADMET) screening. Further processing of the screening approach was based on the molecular docking and dynamics methods (Sudi *et al*., 2012). The purpose of this study is to inhibit the activity of PA_C-$PB1_N$ domain. We expected that the lead compounds from this study could be developed further as drug candidates.

Materials and Methods

Sequence Retrieval

The Database resources of the National Center for Biotechnology Information (NCBI) were employed to retrieve the H1N1 virus polymerase B1 (PB1) (http://www.ncbi.nlm.nih.gov/) (Parikesit, 2009). About 1644 sequences were downloaded. The virus was isolated from human and the data archive was not later than December 2009. ClustalW program was used to align the PB1 sequences (http://www.ebi.ac.uk/Tools/clustalw2/index.html) (Thompson *et al*., 2002). According to the results of ClustalW2 computation, all sequences (716 amino acids) have about 99% similarity.

Protein Structure

Templates were used to identify PB1 by using Swiss model (http://swissmodel.expasy.org) (Biasini *et al*., 2014). Polymerase PA_C-$PB1_N$ complex was found with Protein Data Bank (PDB) entry code of 3CM8. The identification results score based on the template is 844. Thus, PA_C-$PB1_N$ 3D structure was extracted from Research Collaboration Structural Bioinformatics-Protein Data Bank (RCSB-PDB) database at http://www.rcsb.org/pdb/static.do?p=search/index.html (Rose *et al*., 2013). The 3D structure was saved in .pdb format.

Construction of the Ligand Structure

In this study, 1728 cyclopentadienyl peptide inhibitors were developed. At present, the ligand is composed of five disulfide bridges in the amino acid residues (cyclo pentapeptide) (Tambunan *et al*., 2014). The ACD/Phys Chem Suite 12.0 was used for drawing the ligands (Spessard, 1998).

Preparation of the Ligand

The ligands were converted into .mol format using VegaZZ software and then opened on database viewer of Molecular Operating Environment (MOE, version 2008.10). The ligands went through several steps of preparation, including “Wash”, “Partial Charge” and “Energy Minimize” step. The selected force field in these process was Merck Molecular Force Field 1994 (MMFF94).

Molecular Docking

Protein-Ligand Docking

The docking simulation was conducted using MOE 2008.10 software with the default settings (Vilar *et al*., 2008). Docking simulations determine the optimal ligand-protein interaction (Shoichet *et al*., 2002). Thus, our results would be screened by Lipinski’s rules using SCFBio-IT (Shaikh *et al*., 2007). The protein structure of 3CM8 was used for the preparation of the docking. The missing hydrogen atom was added. The protein-ligand complex was obtained for docking constitution. In order to estimate the free energy of binding of the ligands, the score composed is selected as molecular docking score function (London dG) (Ben *et al*., 2013).

Molecular Dynamics

Molecular dynamics simulations were conducted with a fixed Number of particles, Volume and Temperature (NVT) and born solvation stages. Thus, Nose'-Poincaré-Andersen (NPA) method was utilized (Sturgeon and Laird, 2000). It leads to the stability and durability of the simulation of 300 K and 312 K. The heating stage requires 20 picoseconds (ps), while to observe the enzyme-ligand enzyme inhibitor interaction stability needs 5 nanoseconds (ns) simulation calculations for all ligands (Durrant and McCammon, 2011).

ADMET Properties

The ADMET properties of the ligands were computed by using Toxtree2.1.0 and Molinspiration (http://www.molinspiration.com/) (Jarrahpour *et al*., 2010).

Results

Sequence Analysis

In molecular systems, the crystal structure PA-PB1 with 2,3 and 2,9Å resolutions are 2ZNL and 3CM8, respectively. The existence of PA-PB1 3D structure provides a starting point for further investigation and dissemination of the structure of viral polymerase. In this study, the polymerase sequences were aligned to obtain the conserved regions.

Molecular Docking Simulation

In this study, the docking process was performed on 1728 cyclopentadienyl peptide inhibitors into PA-PB1 protein. There are three regions binding site between the Polymerase A and Polymerase B1, which is (1) Trp706 and Phe411, (2) Phe710 and Leu666 and (3) Leu640, Val636, Met595 and Trp619. Unlike the other two binding sites, The Trp706 and Phe411 binding sites have a great contribution to the bonding interactions of polymerase PA-PB1 (Liu and Yao, 2010). Therefore, the docking simulations were carried out at this binding site. Based on docking simulation results on the Trp706 and Phe411 binding sites, we obtained two ligands, namely CKTTC and CKKTC, as the best ligands which have the lowest $\Delta G_{binding}$ value compared to other ligands. The ligand CKTTC itself has a $\Delta G_{binding}$ value of -20.9946 kcal/mol and ligand CKKTC has $\Delta G_{binding}$ value of -18.0293 kcal/mol. The docking results showed that both ligands have better $\Delta G_{binding}$ value than the standard ligand PTLLFL and ribavirin, both of which each have $\Delta G_{binding}$ value of -14.4066 kcal/mol and -9.7573 kcal/mol, respectively.

Molecular Dynamics Simulation

The Conformation of Ligand CKKTC with the Enzyme

The conformational changes in the molecular dynamics are shown in Fig. 1. It illustrates Root Mean Square Deviation (RMSD) as a function of elapsed time of MD simulations for the main chain alpha carbon atom. At 300 K and 312 K, RMSD value at 0 ps is the same with the value of RMSD in complex enzyme without ligand. Increased RMSD values also occur in molecular dynamics simulation. Ligand CKTTC and CKKTC are more stable than ligand PTLLFL. Upon disulfide formation, the cross-linked vesicles remained stable under conditions that disrupted the non-crosslinked peptide ones.

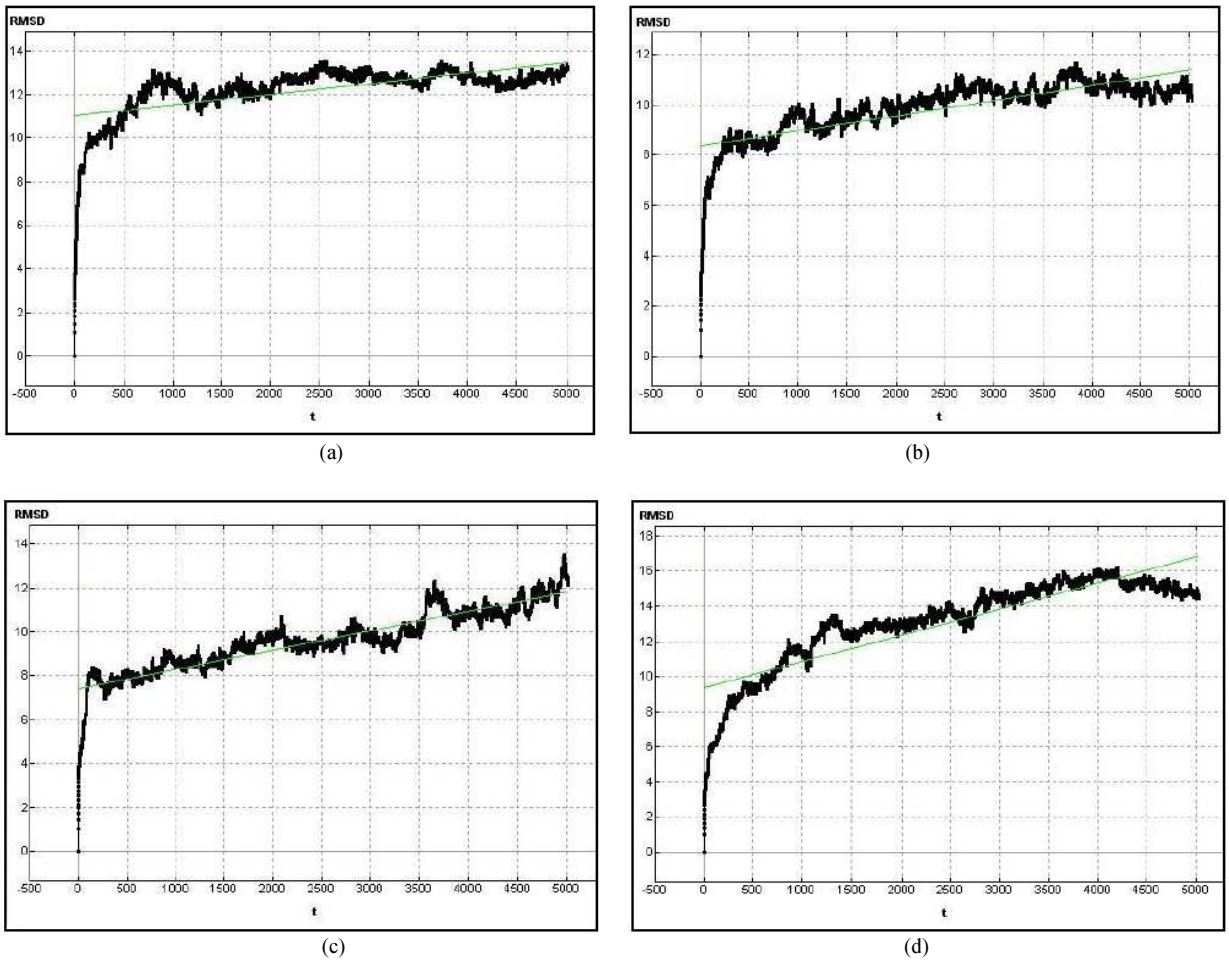

Fig. 1. RMSD values from enzyme-ligand CKTTC and CKKTC complex in molecular dynamics simulation. (a) CKTTC 300 K, (b) CKTTC 312 K, (c) CKKTC 300 K and (d) CKKTC 312 K

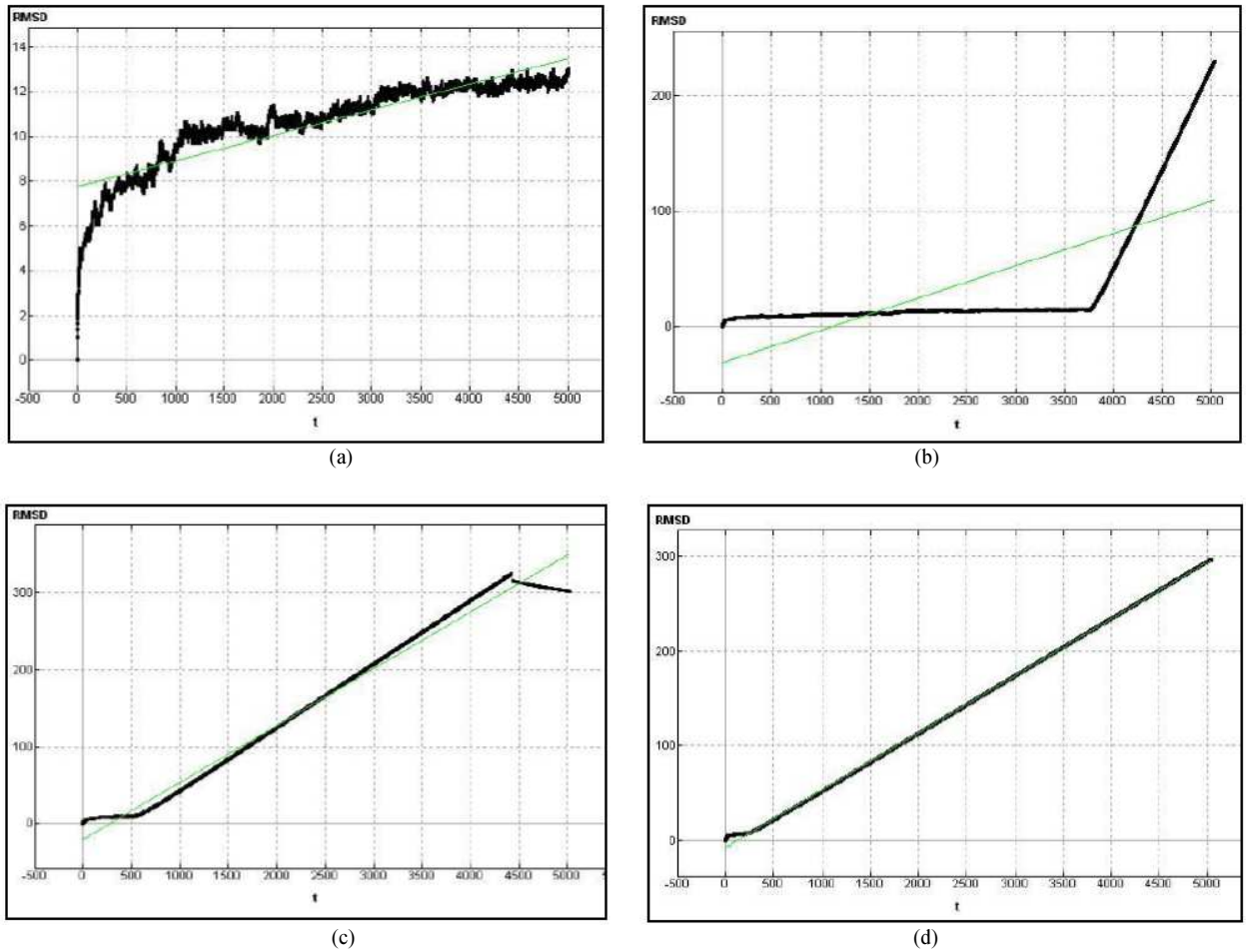

Fig. 2. RMSD values from enzyme-ligand PTLLFL and ribavirin complex in molecular dynamics simulation. (a) PTLLFL 300 K, (b) PTLLFL 312 K, (c) ribavirin 300 K and (d) ribavirin 312 K

Enzyme-Ligand PTLLFL and Ribavirin Three-Dimensional Structure

The RMSD as functions of elapsed time of MD simulations for the main chain alpha carbon atom can be seen in Fig. 2. In molecular dynamics simulations at 300 K, RMSD at 0 ps has the same value with the RMSD in enzyme complex without ligand. The increase in RMSD values also occurs at this temperature. The increase occurred as a result of increasing RMSD values from the influence of solvent molecules.

The Bonding between Enzyme and Ligand

PA_C interacts with $PB1_N$ through an array of hydrogen bonds and hydrophobic contact. Most intersubunit hydrogen bonds were formed through main-chain atoms of $PB1_N$. Residues Asp 2 to Asn 4 formed antiparallel β-sheet-like interaction with Ile621 to Glu623 of PA_C. Based on the consistent information of interaction pair analysis and virtual alanine scanning, several residues of PA_C, such as Trp706, Phe710, Asn412, Gln408, Phe411, Leu666, Trp619, Glu623, Gln670, Leu667, Met595, Thr639 and Leu640, were found to contribute largely to the binding process. If we compare to all of the ligands, based on the $\Delta G_{binding}$ value, CKTTC and CKKTC ligands are the best. Hopefully, these ligands can block the interaction between PA_C and $PB1_N$. We presented the three-dimensional ligand-receptor interaction diagrams (Fig. 3 and 4). In Fig. 3, CKTTC ligand interacted with several residues of PA_C, such as Glu623, Glu630, Lys635, Ala448, Glu449, His452, Ile621, Gly631 and Ser632. CKKTC ligand interacted with the residue of PA_C Glu410, Glu449, His452, Glu410, Phe446, His452, Cys453 and Val636. In Fig. 4, CKTTC ligand interacted with the residue of PA_C such as Asp272, Gly273, Gly622, Glu623, Lys716, Pro271, Pro620, Ile621 and Phe710. CKKTC ligand interacted with Pro267, Leu270, Asp272, Gly273, Glu327, Glu691, Arg266, Leu268, Lys539, Asp682, Tyr687, Trp706 and Phe710. This depiction is relatively strong connections or hydrogen bonds as well as the electrostatic or charge-transfer interaction between a ligand and the residue protein.

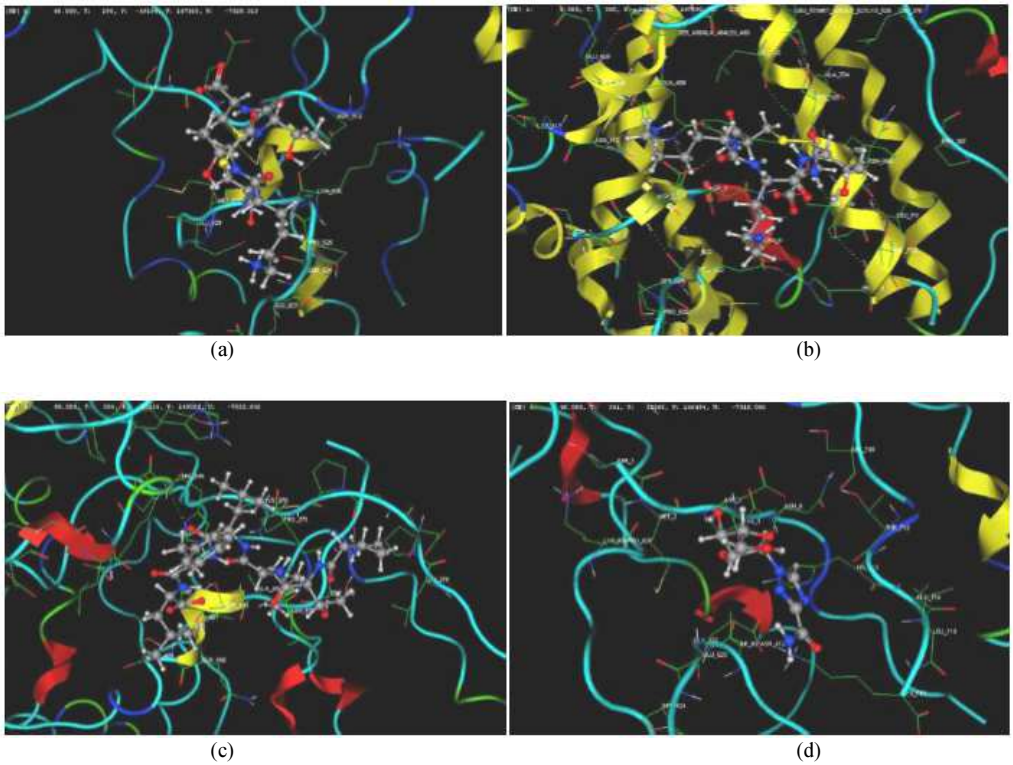

Fig. 3. Interaction plots illustrating between ligand and the respective amino acid residues in molecular dynamics simulation at 300 K. (a) CKTTC-enzyme complex, (b) CKKTC-enzyme complex, (c) PTLLFL-enzyme complex, and (d) ribavirin-enzyme complex

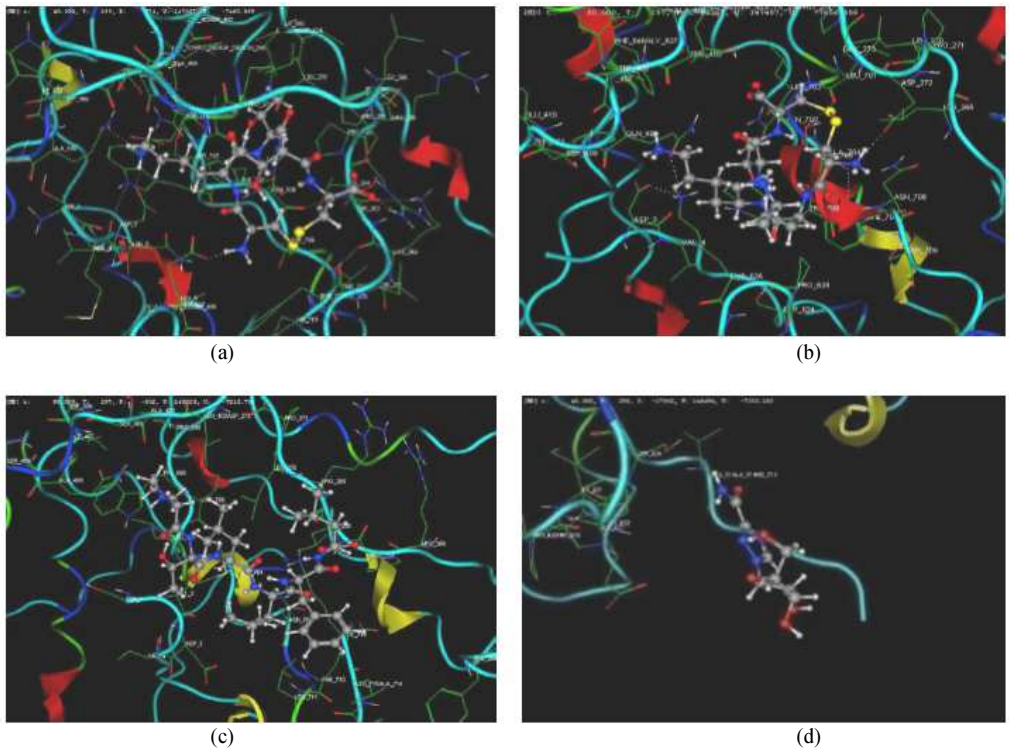

Fig. 4. Interaction plots illustrating between ligand and the respective amino acid residues in molecular dynamics simulation at 312 K. (a) CKTTC-enzyme complex, (b) CKKTC-enzyme complex, (c) PTLLFL-enzyme complex, and (d) ribavirin-enzyme complex

Table 1. Properties of ligand compound based on Lipinski rules

Ligand	CKTTC	CKKTC	PTLLFL	Ribavirin
Mr (g/mol)	553.682	581.760	702.894	244.207
log P	-6.145	-6.144	-1.352	-2.916
H-donor (free state)	8	8	7	4
H-acceptor (free state)	8	7	8	7
H-donor (binding state)	3	5	1	2
H-acceptor (binding state)	1	0	1	0
molar refractivity	133.81	145.15	188.18	37.88

Table 2. Ligand descriptors value

Descriptors	CKTTC	CKKTC	PTLLFL	Ribavirin
Log P	-5.242	-5.207	0.237	-2.774
TPSA	246.193	251.988	215.044	143.73
Molecular Weight	552.676	579.746	702.894	244.207
n Atoms	36	38	50	17
n ON	14	14	14	9
n OHNH	11	12	8	5
n Violations	3	3	3	0
n Rotatable bonds	7	10	20	3
Volume	480.935	518.024	679.369	197.679
Drug-likeness				
GPCR ligand	0.03	0,00	-0.77	-0.32
Ion channel modulator	-0.38	-0.54	-2.19	-1.35
Kinase inhibitor	-0.43	-0.48	-1.75	-0.33
Nuclear receptor ligand	-0.60	-0.65	-2.03	-1.96
Mutagenicity	Non-mutagen	Non-mutagen	Non-mutagen	Non-mutagen
Carcinogenicity	negative	negative	negative	negative

Ligand's Pharmacological Properties

The pharmacological properties of the ligands were computed by using the Lipinski's rule of five (RO5) (Lipinski *et al*., 1997). The classification of these rules was derived from the physical and chemical properties of the drug compounds. In Table 1, we presented properties of our ligand compound. Lipinski's RO5 helps in distinguishing between a drug like and not a drug like molecules. It computes whether the ligand was breaching with at least 2 rules to determine its feasibility as a drug candidate. The further descriptor analysis helped in the identification of the better inhibitor (Table 2). Based on the RO5 and descriptor analysis, the whole ligands could become candidate drugs for blocking of interaction between polymerase A and B1.

Discussion

On one hand, the current biomedical research has greatly utilized cyclic peptides. The bioengineering methods have been applied to generate robust cyclic peptides (Thapa *et al*., 2014). Thus, structure-based docking method was useful to generate cyclic peptide inhibitor to cope with dengue virus (Idrees and Ashfaq, 2014). Assessment of small cyclic peptides was conducted for generating a library of VEGF inhibitors (Feng *et al*., 2014). Potential application in generating peptidomimetic compounds was in place as well (Razavi *et al*., 2014). On the other hand, the new generation of cyclic peptides databases was already in place, such as antiparasitic and antimicrobial peptides libraries (Gogoladze *et al*., 2014). These strong developments in cyclic peptide design were in line with the interest of our group to work in this field. These are shown in the previous works within the subjects of H1N1 virus (Tambunan *et al*., 2012). The resulted best cyclic peptides, namely the threonine-dominated CKTTC and lysine-dominated CKKTC could be affected by the posttranslational modification, if no cyclization is applied (Tsiboli *et al*., 1997). Threonine can undergo glycosylation while lysine can be hampered with methylation (Zhang *et al*., 2012). Thus, it is already proven that cyclization could prevent the modification of peptides by digestive or biotransforming enzymes (Oliyai, 1996). Hence, the next feasible step is to evaluate the synthetic accessibility of these lead compounds (Ertl and Schuffenhauer, 2009). The synthesized works, bioassay and drug delivery construction will also follow suit (Jois *et al*., 1996).

Conclusion

There are 1728 cyclo-pentapeptide inhibitors already designed in this study. The docking of these inhibitors to determine their interaction activities with polymerase A and B1 was carried out. The small

value of free energy binding and estimated Ki values for all the inhibitors indicate that the molecules bound considerably well with the binding site. We also analyzed our ligand properties based on Lipinski's rules. To ensure the absorption, distribution, metabolism, excretion and toxicity, ADMET calculations on the ligands are recommended. Thus, from this study, cyclo-pentapeptide CKTTC and CKKTC could be proposed as potential inhibitors of the interaction of PA_C-$PB1_N$ in H1N1 virus.

Acknowledgement

This work was supported by the Hibah Penelitian Unggulan Perguruan Tinggi (PUPT) DIKTI 2016 No:1121/UN2.R12/HKP.05.00/2016. We also would like to thank Niken Widayanti, Erwin Prasetya Toepak and Ratih Dyah Puspitasari for proof-read the manuscript.

Author's Contributions

Usman Sumo Friend Tambunan: Supervised this study and designed the research pipeline.

Mochammad Arfin Fardiansyah Nasution: Wrote the manuscript and added more relevant data to the manuscript.

Arli Aditya Parikesit: Wrote the manuscript and worked on the technical details.

Harry Noviardi: Worked on the technical details and analyzed the data.

Djati Kerami: Gave a critical suggestion to improve our research pipeline.

Ethics

We hereby declare there are no conflict of interest regarding this manuscript. Furthermore, the whole manuscript has been checked and approved by all authors.

References

Bartoli, L., 2011. Bioinformatics: A machine-learning approach to the prediction of protein, structure, function and interactions. Altran Italia Technol. Rev.

Ben, D.D., M. Buccioni, C. Lambertucci, A. Thomas and R. Volpini, 2013. Simulation and comparative analysis of binding modes of nucleoside and non-nucleoside agonists at the A2B adenosine receptor. Silico Pharmacol., 1: 1-24. DOI: 10.1186/2193-9616-1-24

Biasini, M., S. Bienert, A. Waterhouse, K. Arnold and G. Studer *et al*., 2014. SWISS-MODEL: Modelling protein tertiary and quaternary structure using evolutionary information. Nucleic Acids Res., 42: W252-W258. DOI: 10.1093/nar/gku340

Donaldson, L.J., P.D. Rutter, B.M. Ellis, F.E.C. Greaves and O.T. Mytton *et al*., 2009. Mortality from pandemic A/H1N1 2009 influenza in England: Public health surveillance study. BMJ, 339: b5213-b5213. DOI: 10.1136/bmj.b5213

Durrant, J.D. and J.A. McCammon, 2011. Molecular dynamics simulations and drug discovery. BMC Biol., 9: 1-71. DOI: 10.1186/1741-7007-9-71

Ertl, P. and A. Schuffenhauer, 2009. Estimation of synthetic accessibility score of drug-like molecules based on molecular complexity and fragment contributions. J. Cheminform., 1: 1-8. DOI: 10.1186/1758-2946-1-8

Feng, S., L. Zou, Q. Ni, X. Zhang and Q. Li *et al*., 2014. Modulation, bioinformatic screening and assessment of small molecular peptides targeting the vascular endothelial growth factor receptor. Cell Biochem. Biophys., 70: 1913-1921. PMID: 25069724

Fischer, C.N. and A.B.DS. Serapião, 2010. Bioinformatics: Strategies, Trends and Perspectives. In: New Advanced Technologies. Lazinica, A. (Ed.), InTech, ISBN: 978-953-307-067-4, pp: 126-138.

Gogoladze, G., M. Grigolava, B. Vishnepolsky, M. Chubinidze and P. Duroux *et al*., 2014. DBAASP: Database of antimicrobial activity and structure of peptides. FEMS Microbiol. Lett., 357: 63-68. DOI: 10.1111/1574-6968.12489

Idrees, S. and U.A. Ashfaq, 2014. Discovery and design of cyclic peptides as dengue virus inhibitors through structure-based molecular docking. Asian Pac. J. Trop. Med., 7: 513-516. DOI: 10.1016/S1995-7645(14)60085-7

Jarrahpour, A., M. Motamedifar, M. Zarei, M.H. Youssoufi and M. Mimouni *et al*., 2010. Petra, Osiris and molinspiration together as a guide in drug design: Predictions and correlation structure/antibacterial activity relationships of new n-sulfonyl monocyclic β-lactams. Phosphorus. Sulfur. Silicon Relat. Elem., 185: 491-497. DOI: 10.1080/10426500902953953

Jois, S.D., U.S. Tambunan, S. Chakrabarti and T.J. Siahaan, 1996. Solution structure of a cyclic RGD peptide that inhibits platelet aggregation. J. Biomol. Struct. Dyn., 14: 1-11. DOI: 10.1080/07391102.1996.10508923

Lipinski, C.A., F. Lombardo, B.W. Dominy and P.J. Feeney, 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev., 23: 3-25. DOI: 10.1016/S0169-409X(96)00423-1

Liu, H. and X. Yao, 2010. Molecular basis of the interaction for an essential subunit PA-PB1 in influenza virus RNA polymerase: Insights from molecular dynamics simulation and free energy calculation. Mol. Pharm., 7: 75-85. DOI: 10.1021/mp900131p

Moen, S.O., J. Abendroth, J.W. Fairman, R.O. Baydo and J. Bullen *et al*., 2014. Structural analysis of H1N1 and H7N9 influenza A virus PA in the absence of PB1. Sci. Rep., 4: 5944-5951. DOI: 10.1038/srep05944

Obayashi, E., H. Yoshida and S.Y. Park, 2008. The structural basis for an essential subunit interaction in influenza virus RNA polymerase. Nihon Kessho Gakkaishi, 50: 329-334. DOI: 10.5940/jcrsj.50.329

Oliyai, R., 1996. Prodrugs of peptides and peptidomimetics for improved formulation and delivery. Adv. Drug Deliv. Rev., 19: 275-286. DOI: 10.1016/0169-409X(95)00110-S

Parikesit, A.A., 2009. The role of bioinformatics as auxilliary tools for molecular biology. Proceeding of World-Wide Indonesian Student Association Scientific Writing Olympic, (SWO' 09), Indonesian Student Association of France, Paris, pp: 23-29.

Perez, D.R. and R.O. Donis, 2001. Functional analysis of PA binding by influenza a virus PB1: Effects on polymerase activity and viral infectivity. J. Virol., 75: 8127-8136. DOI: 10.1128/JVI.75.17.8127-8136.2001

Pfaff, M., K. Tangemann, B. Müller, M. Gurrath and G. Müller *et al*., 1994. Selective recognition of cyclic RGD peptides of NMR defined conformation by alpha IIb beta 3, alpha V beta 3 and alpha 5 beta 1 integrins. J. Biol. Chem., 269: 20233-20238. PMID: 8051114

Razavi, A.M., W.M. Wuest and V.A. Voelz, 2014. Computational screening and selection of cyclic peptide hairpin mimetics by molecular simulation and kinetic network models. J. Chem. Inf. Model., 54: 1425-1432. DOI: 10.1021/ci500102y

Regoes, R.R. and S. Bonhoeffer, 2006. Emergence of drug-resistant influenza virus: Population dynamical considerations. Science, 312: 389-391. DOI: 10.1126/science.1122947

Rose, P.W., C. Bi, W.F. Bluhm, C.H. Christie and D. Dimitropoulos *et al*., 2013. The RCSB protein data bank: New resources for research and education. Nucleic Acids Res., 41: D475-D482. DOI: 10.1093/nar/gks1200

Rungrotmongkol, T., P. Intharathep, M. Malaisree, N. Nunthaboot and N. Kaiyawet *et al*., 2009. Susceptibility of antiviral drugs against 2009 influenza A (H1N1) virus. Biochem. Biophys. Res. Commun., 385: 390-394. DOI: 10.1016/j.bbrc.2009.05.066

Shaikh, S.A., T. Jain, G. Sandhu, N. Latha and B. Jayaram, 2007. From drug target to leads--sketching a physicochemical pathway for lead molecule design in silico. Curr. Pharm. Des., 13: 3454-3470. PMID: 18220783

Shoichet, B.K., S.L. McGovern, B. Wei and J.J. Irwin, 2002. Lead discovery using molecular docking. Curr. Opin. Chem. Biol., 6: 439-446. DOI: 10.1016/S1367-5931(02)00339-3

Spessard, G.O., 1998. ACD Labs/LogP dB 3.5 and ChemSketch 3.5. J. Chem. Inf. Model., 38: 1250-1253. DOI: 10.1021/ci980264t

Sturgeon, J.B. and B.B. Laird, 2000. Symplectic algorithm for constant-pressure molecular dynamics using a Nosé–Poincaré thermostat. J. Chem. Phys., 112: 3474-3474. DOI: 10.1063/1.480502

Sudi, I.Y., E.L. Wong, K.H. Joyce-Tan, M.S. Shamsir and H. Jamaluddin *et al*., 2012. Structure prediction, molecular dynamics simulation and docking studies of d-specific dehalogenase from rhizobium sp. RC1. Int. J. Mol. Sci., 13: 15724-15754. DOI: 10.3390/ijms131215724

Tambunan, U.S.F., H. Zahroh, B.B. Utomo and A.A. Parikesit, 2014. Screening of commercial cyclic peptide as inhibitor NS5 methyltransferase of dengue virus through molecular docking and molecular dynamics simulation. Bioinformation, 10: 23-27. DOI: 10.6026/97320630010023

Tambunan, U.S.F., N. Amri and A.A. Parikesit, 2012. In silico design of cyclic peptides as influenza virus, a subtype H1N1 neuraminidase inhibitor. African J. Biotechnol., 11: 11474-11491. DOI: 10.5897/AJB11.4094

Thapa, P., M.J. Espiritu, C. Cabalteja and J.P. Bingham, 2014. The emergence of cyclic peptides: The potential of bioengineered peptide drugs. Int. J. Pept. Res. Ther., 20: 545-551. DOI: 10.1007/s10989-014-9421-0

Thompson, J.D., T.J. Gibson and D.G. Higgins, 2002. UNIT 2.3 Multiple sequence alignment using ClustalW and ClustalX. Curr. Protoc. Bioinformat. DOI: 10.1002/0471250953.bi0203s00

Toyoda, T., D.M. Adyshev, M. Kobayashi, A. Iwata and A. Ishihama, 1996. Molecular assembly of the influenza virus RNA polymerase: Determination of the subunit-subunit contact sites. J. Gen. Virol., 77: 2149-2157. DOI: 10.1099/0022-1317-77-9-2149

Tsiboli, P., G. Konstantinidis, Y. Skendros, A. Katsani and T. Choli-Papadopoulou, 1997. Identification of post-translational modified amino acids. Amino Acids, 13: 13-23. DOI: 10.1007/BF01373045

Ushirogawa, H. and M. Ohuchi, 2011. Novel antiviral activity of neuraminidase inhibitors against an avian influenza a virus. Virol. J., 8: 411-411. DOI: 10.1186/1743-422X-8-411

Vilar, S., G. Cozza and S. Moro, 2008. Medicinal chemistry and the Molecular Operating Environment (MOE): application of QSAR and molecular docking to drug discovery. Curr. Top. Med. Chem., 8: 1555-1572. DOI: 10.2174/156802608786786624

WHO, 2014. Global Alert and Response (GAR): Pandemic (H1N1) 2009 [WWW Document]. World Heal. Organ.

Zhang, X., H. Wen and X. Shi, 2012. Lysine methylation: Beyond histones. Acta Biochim. Biophys. Sin., 44: 14-27. DOI: 10.1093/abbs/gmr100