Lead Screening for HIV-1 Integrase (IN) Inhibited by Traditional Chinese Medicine

Tzu-Chieh Hung, Wen-Yuan Lee, Kuen-Bao Chen, Yueh-Chiu Chan, and Calvin Yu-Chian Chen

1 Department of Biomedical Informatics, Asia University, Taichung 41354, Taiwan
2 School of Medicine, College of Medicine, China Medical University, Taichung 40402, Taiwan
3 Department of Neurosurgery, China Medical University Hospital, No. 2 Yude Road, North District, Taichung 40447, Taiwan
4 Department of Anesthesiology, China Medical University Hospital, Taichung 40447, Taiwan
5 Research Center for Chinese Medicine and Acupuncture, China Medical University, Taichung 40402, Taiwan

Correspondence should be addressed to Calvin Yu-Chian Chen; ycc929@MIT.edu

Received 21 February 2014; Accepted 5 March 2014; Published 11 June 2014

Academic Editor: Chung Y. Hsu

Copyright © 2014 Tzu-Chieh Hung et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Human immunodeficiency virus causes the acquired immunodeficiency syndrome (AIDS) and becomes a serious world-wide problem because of this disease’s rapid propagation and incurability. Integrase strand transfer inhibitors (INSTIs) supports HIV have rapid drug resistance for antitreatment. Screening the traditional Chinese medicine (TCM) database by simulating molecular docking and molecular dynamics may select molecular compounds to inhibit INSTIs against HIV drug resistance. (S)-cathinone and (1S,2S)-norpseudoephedrine are selected based on structure and ligand-based drugs are designed and then get higher bioactivity predicted score from SVM than Raltegravir and other TCM compounds. The molecular dynamics are helpful in the analysis and detection of protein-ligand interactions. According to the docking poses, hydrophobic interactions and hydrogen bond variations define the main regions of important amino acids in integrase. In addition to the detection of TCM compound efficacy, we suggest (1S,2S)-norpseudoephedrine is better than the others based on the analysis of interaction and the effect on the structural variation.

1. Introduction

The acquired immunodeficiency syndrome (AIDS) is caused by a retrovirus, the Human immunodeficiency virus (HIV) [1–4]. In AIDS, the immune system is inhibited by the virus, which makes patients have more opportunities for deadly infections and cancers. The HIV virus is transmitted via unprotected sexual intercourse [5, 6], contaminated medical equipment [7, 8], bodily fluids, and vertical infection (pregnancy, delivery, or breastfeeding) [9, 10].

AIDS has caused nearly thirty-six million deaths since the first case in 1981 and there were approximately seventy-five million carriers as recorded by UNAIDS (http://www.unaids.org/en/resources/campaigns/globalreport2013/factsheet/).

There are still no vaccines or drugs available to kill all the viruses in body; thus, highly active antiretroviral therapy (HAART) had identified the standard of care for patients with advanced infection in these years [11] which decreases the patient’s total burden of HIV success by the complex transcription inhibitors but this treatment is expensive.

HIV-1 integrase (IN) is an essential enzyme which catalyzes the integration of the viral DNA into the host cell genome. According to human without the enzyme integrase, the inhibitor of HIV-1 integrase becomes a promising therapeutic target for AIDS. After the rapid drug resistance of HIV-1 integrase had been found, several drugs approved by the FDA lost their efficacy. There is a reference that indicates the drug target site of integrase and explores the molecular
Table 1: The score of molecular docking and bioactivity of prediction.

| Name                               | DockScore | SVM  | MLR  | Herb                       |
|------------------------------------|-----------|------|------|----------------------------|
| (S)-cathinone                      | 87.568    | 7.513| 8.058| Ephedra sinca stapf        |
| (1S,2S)-norpseudoephedrine         | 80.074    | 7.262| 6.689| Ephedra sinca stapf        |
| Octopamine                         | 81.861    | 7.093| 6.950| FRUCTUS AURANTII           |
| Noradrenaline                      | 95.291    | 6.955| 5.746| Portulaca oleracea          |
| P-synephrine                       | 77.385    | 6.814| 4.015| FRUCTUS AURANTII           |
| 3,4,5-Trimethoxy benzeneethamine   | 83.352    | 6.738| 13.406| Myristica fragrans         |
| D77*                               | 74.525    | 6.667| −11.476| Myristica fragrans         |
| Raltegravir*                       | 43.285    | 6.482| −9.812| Control.                   |

*Control.

Figure 1: The disorder region prediction and binding site detection. The blue curve is the disorder disposition of each amino acid, and pink regions are the residues of the important amino acids.

Figure 2: Relation of observed activity (pIC50) and predicted activity (pIC50), (a) MLR and (b) SVM.
mechanism of drug resistance [12]. Based on this research, the drug inhibiting integrase and preventing the resistance is feasible.

Computer-aided drug design (CADD) is an *in silico* simulation technique to screen for molecular compounds by the structure and to predict the biological activity of drug character. In comparison with traditional drug design, CADD has the advantages of both greater speed and lower cost. The structure-based drug design and ligand-based drug design are two major application areas of CADD. We used CADD to investigate the molecular simulation in drug design on the basics of structure-based drug design and molecular dynamics [13–18].

In these two decades, the personalized medicine and biomedicine are important knowledge [19, 20] for the mutation [21, 22], the pathway [23, 24], the cause for special disease [25–27], and the clinical diagnosis [28]. The traditional Chinese medicine (TCM) is identified as one of personalized medicines. TCM has an important role in Asia, especially in Chinese culture. The TCM Database@Taiwan (http://tcm.cmu.edu.tw/) [29] is the largest traditional Chinese medicine database established in 2011. There are 2D
chemical structures, 3D chemical structures, bioactivity, and molecular information for over 61,000 compounds of traditional Chinese medicinal herbs in this database. Since 2011, the TCM Database@Taiwan has made successful discoveries of novel compounds for cancer treatment [30–33], stroke prevention [34], EGFR inhibition [35], inflammation inhibition [36], pain relief [14], and antivirals [37–41]. The TCM Database@Taiwan could be valuable for TCM application and drug design with the application of the website [42] and the cloud computing platform [43].

In this research, we screen a possible compound against HIV from the TCM Database@Taiwan. We use the molecular docking screening to select ligands, and then we apply molecular dynamics (MD) simulation to investigate variations from protein ligand interactions. This program may contribute to the evaluation of the effect of integrase inhibition.

2. Materials and Methods

2.1. Data Set. Accelrys Discovery Studio 2.5 (DS 2.5) was used as a docking platform for the molecular simulations. A total of 61,000 TCM compounds had been downloaded from the TCM database (http://tcm.cmu.edu.tw/). The HIV-1 integrase crystal structure was obtained from Protein Data Bank (PDB ID: 2B4J), and Raltegravir, as control drug, helped design the docking site [12].

2.2. Disorder Protein Detection. We take the sequence of protein structure from Uniprot (http://www.uniprot.org/) and docking site to predict the disorder region by the Database of Protein Disorder (DisProt: http://www.disprot.org/) [44]. The result of prediction could analyze the character of the docking site and the efficacy of the drug.

To compare the region of the disorder protein and the docking sites, we could assess the protein-ligand interaction and drug efficacy effect from disorder.

2.3. Molecular Docking. The docking simulation used the LigandFit [45], a receptor-rigid docking algorithm program in Discovery Studio 2.5 (DS 2.5), module in the force field of CHARMM [46] to dock Raltegravir and TCM compounds to HIV integrase. The docking site of HIV integrase was identified by the research [12, 47].

2.4. Ligand-Based Prediction. Bioactivity prediction was assessed by the MLR and SVM models. The pIC$_{50}$ of model drugs for integrase was set as the template to assist with model assessment [48]. Before creating the prediction model, the descriptors of these ligands were evaluated by the genetic approximation (GA) algorithm of the Calculate Molecular Properties module in Accelrys Discovery Studio 2.5 [49].

The MLR was established by the five descriptors and the Matlab Statistics Toolbox was used to select the ligand based on activity [50]. After prediction, the result should be detected by the Leave One Out Validation [51].

Setting the SVM model used the same ligand template and the production of descriptors and descriptors should be normalized to transform the range from −1 to 1. Screening
the best training model was based on the Five-fold Cross Validation [52].

The results were ranked by the score of SVM prediction. The top compounds were selected with the protein as complex to analyze the hydrophobic interactions by Ligplus [53, 54] and then be submitted to the molecular dynamics simulation.

2.5. Molecular Dynamics Simulation. Before applying MD simulation, selected ligands must be reprepared based on the reference force field [55] of GROMACS 4.5.5 [56] through SwissParam (http://swissparam.ch/) [57]. The HIV integrase combines with ligands going into the buffer (or solution) simulation box. This cubic box was solvated with the TIP3P water model in which sodium and chloride ion were added to neutralize complex charges within a minimum distance of 1.2 Å from the complex to box. The complex was minimized with the steepest descent method for 5,000 steps, and then the last structure with lowest energy was transferred to MD simulation. The electrostatic interactions were calculated based on the particle-mesh Ewald (PME) method [58], in which situation, each time step was 2 fs and the numbers of steps were 10,000,000 times. According to the Berendsen weak thermal coupling method, the equilibration was under the 100 ps constant temperature (PER ensemble). The total simulation time of MD was 20 ns. The Gromacs 4.5.5 had protocols to analyze MD trajectories, RMSD, and energy variations of the complex.
3. Results and Discussion

3.1. The Detection of Disorder Protein. The disorder protein is an unstructured protein which makes the drug dock to protein hardly and the complex will stabilize with difficulty. But some references [41, 42] indicate that the interaction with the disorder region might cause lower side effect than with the widespread domain; thus, the disorder region cannot be defined as a bad docking site for selection. The disorder regions of HIV integrase are defined as having a disposition of over than 0.5 (Figure 1) which indicates that the docking and functional region do not consist of disorder regions, thus the ligand docking to the selected site has a weaker effect from disorder protein.

3.2. Candidate Compounds Detection. The ligand based prediction should be detected correctly (Figure 2). The correlation coefficient ($r^2$) of both SVM and MLR is higher than 0.8 which means our bioactivity prediction is credible and the selected compounds may have the same efficacy as the template drug with the function of integrase inhibition. The top two TCM compounds can be selected (Table 1). These TCM compounds, (S)-cathinone and (1S,2S)-norpseudoephedrine selected from TCM database, are both extracted from the herb *Ephedra sinica stapf* which was defined as anti-HIV herb [59–61]. Cathinone has reported the function for HIV [62] and immunity [63]. Thus we suggest the selected compounds might be against HIV through the inhibition of integrase.

The structure of the candidate compounds (Figure 3) and the docking poses sign, the docking site, and the amino acid neighbors by ligands is shown (Figure 4). From this result, we observe compounds interact the A and C substructures of whole HIV integrase (A, B, C, and D subunits).
After the hydrophobic interaction is analyzed by Ligplus, Glu170 of A unit and Asn367 of C are also found the interaction with ligand (Figure 5). These results may present that Glu170 of A unit and Asn367 of C have an effect on HIV integrase.

3.3. Molecular Dynamics Simulation. The total energy variation of complex and apo (unbound protein) range between $-2428$ and $2422 \times 10^3$ KJ/mol and have a tendency towards $2424 \times 10^3$ KJ/mol (Figure 6). The RMSD calculate average-ment of residue position variation caused by the protein-ligand interaction (Figure 7). According to lowest energy and RMSD, we find the structure of apo is more stable than the complex before 16 ns. Thus, we suggest the integrase may be inhibited while compounds interact to make the structure unstable.

The torsion of compounds could help us understand the interaction site while the compound affects protein (Figure 8). In this result, the main structure is less variant and the side is larger which might affect the interaction of different amino acids and then the direction of torsion turn.

The clustering is calculating the RMSD variation and divides the similarity to the same group thus the structure may be the same in a group (Figure 9). For this reason, the largest group at the end means this complex might be more stable or the simulation might be balanced. In this result, we find (S)-cathinone is more stable than the others and the (1S,2S)-norpseudoephedrine has the largest group.

Figure 9: Clustering the ligand-protein interaction, (a) (S)-cathinone, (b) (1S,2S)-norpseudoephedrine, and (c) apo.
in this clustering. We suggest that the different kinds of two compounds’ interaction may indicate two kinds of influence that the (S)-cathinone will target as complex to interact the protein, and the (1S,2S)-norpseudoephedrine might make the structure unstable to inhibit integrase.

To compare the H bond and structure variation in MD 0 ns and 20 ns could help the definition of interaction (Figures 10 and 11). In H bond variation, we find the distance is longer which means the compounds move away from the docking site. From this situation, we suggest the integrase is important for virus and this enzyme wants to prevent the function inhibited; thus, this kind of protein will make weak interaction between ligands. Even in the short time interaction, we also could find the structure variation (we only signed around docking site focus on our discussion, but the variation of other sites might have more function effect).

The pathway could help understanding the path for ligand interaction with protein could be defined based on the calculation of caver 3.0 to determine the interpath protein path during MD simulation (Figure 12) [64]. Most of the pathways...
are not inside the protein, then the present interaction is located on the surface and the ligand could not target the protein closely; thus, the efficacy will decrease.

4. Conclusion

Based on the above discussion, we find the top two TCM compounds, (S)-cathinone and (1S,2S)-norpseudoephedrine, can have an effect on HIV integrase against HIV infection. The ligand impacts integrase through hydrophobic interactions and H-bonds but the protein tries to prevent these influences which make the structure vary and affect the function. With these analyses of interaction and discussion the character of integrase prevents ligand targeting. We suggest both the herb *Ephedra sinica stapf* and (1S,2S)-norpseudoephedrine may have a better effect on the inhibition of integrase based on the larger variation than other compounds and the fact that the result for protein is more unstable.
Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors’ Contribution

Tzu-Chieh Hung, Wen-Yuan Lee, and Kuen-Bao Chen contributed equally to this paper.

Acknowledgments

The research was supported by Grants from the National Science Council of Taiwan (NSC102-2325-B039-001 and NSC102-2221-E-468-027-), Asia University (ASIA100-CMU-2, ASIA101-CMU-2, and 102-ASIA-07), and China Medical University Hospital (DMR-103-058, DMR-103-001, and DMR-103-096). This study is also supported in part by Taiwan Department of Health Clinical Trial and Research Center of Excellence (DOH102-TD-B-111-004) and Taiwan Department of Health Cancer Research Center of Excellence (MOHW103-TD-B-111-03) and CMU under the Aim for Top University Plan of the Ministry of Education, Taiwan.

References

[1] F. Barre-Sinoussi, J. C. Chermann, F. Rey et al., “Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS),” Science, vol. 220, no. 4599, pp. 868–871, 1983.

[2] M. W. Cloyd, J. J. Y. Chen, and L. Q. Wang, “How does HIV cause AIDS? The homing theory,” Molecular Medicine Today, vol. 6, no. 3, pp. 108–111, 2000.

[3] R. A. Weiss, “How does HIV cause AIDS?” Science, vol. 260, no. 5112, pp. 1273–1279, 1993.

[4] D. C. Douek, M. Roederer, and R. A. Koup, “Emerging concepts in the immunopathogenesis of AIDS,” Annual Review of Medicine, vol. 60, pp. 471–484, 2009.

[5] S. Weller and K. Davis, “Condom effectiveness in reducing heterosexual HIV transmission,” The Cochrane Database of Systematic Reviews, no. 1, Article ID CD003255, 2002.

[6] B. Leynaert, A. M. Downs, and I. de Vincenzi, “Heterosexual transmission of human immunodeficiency virus: variability of infectivity throughout the course of infection,” American Journal of Epidemiology, vol. 148, no. 1, pp. 88–96, 1998.

[7] D. M. Bell, “Occupational risk of human immunodeficiency virus infection in healthcare workers: an overview,” The American Journal of Medicine, vol. 102, no. 5, supplement 2, pp. 9–15, 1997.

[8] J. L. Cleveland, L. Barker, B. F. Gooch, E. M. Beltrami, and D. Cardo, “Use of HIV postexposure prophylaxis by dental health care personnel: an overview and updated recommendations,” Journal of the American Dental Association, vol. 133, no. 12, pp. 1619–1626, 2002.

[9] J. C. Forbes, A. M. Alimenti, J. Singer et al., “A national review of vertical HIV transmission,” AIDS, vol. 26, no. 6, pp. 757–763, 2012.

[10] K. M. Little, P. H. Kilmarn, A. W. Taylor, C. E. Rose, E. D. Rivadeneira, and S. R. Nesheim, “A review of evidence for transmission of HIV from children to breastfeeding women and implications for prevention,” The Pediatric Infectious Disease Journal, vol. 31, no. 9, pp. 938–942, 2012.

[11] D. Finzi, M. Herrmankova, T. Pierson et al., “Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy,” Science, vol. 278, no. 5341, pp. 1295–1300, 1997.

[12] W. Xue, X. Jin, L. Ning, M. Wang, H. Liu, and X. Yao, “Exploring the molecular mechanism of cross-resistance to HIV-1 integrase strand transfer inhibitors by molecular dynamics simulation and residue interaction network analysis,” Journal of Chemical Information and Modeling, vol. 53, no. 1, pp. 210–222, 2013.

[13] H. J. Huang, H. W. Yu, C. Y. Chen et al., “Current developments of computer-aided drug design,” Journal of the Taiwan Institute of Chemical Engineers, vol. 41, no. 6, pp. 623–635, 2010.

[14] W. I. Tou, S. S. Chang, C. C. Lee, and C. Y. Chen, “Drug design for neuropathic pain regulation from traditional Chinese medicine,” Scientific Reports, vol. 3, article 844, 2013.

[15] C. Y. Chen, “A novel integrated framework and improved methodology of computer-aided drug design,” Current Topics in Medicinal Chemistry, vol. 13, no. 9, pp. 965–988, 2013.

[16] C. Y. Chen and W. I. Tou, “How to design a drug for the disordered proteins?” Drug Discovery Today, vol. 18, no. 19-20, pp. 910–915, 2013.

[17] S. C. Basak, “Recent developments and future directions at current computer aided drug design,” Current Computer-Aided Drug Design, vol. 9, no. 1, article 1, 2013.

[18] C. Y. Chen, “Weighted equation and rules—a novel concept for evaluating protein-ligand interaction,” Journal of Biomolecular Structure & Dynamics, vol. 27, no. 3, pp. 271–282, 2009.

[19] W.-L. Liao and F.-J. Tsai, “Personalized medicine: a paradigm shift in healthcare,” BioMedicine, vol. 3, no. 2, pp. 66–72, 2013.

[20] E.-J. Tsai, “Biomedicine brings the future nearer,” BioMedicine, vol. 1, no. 1, article 1, 2011.

[21] C. C. Lee, C.-H. Tsai, L. Wan et al., “Increased incidence of Parkinsonism among Chinese with β-glucosidase mutation in central Taiwan,” BioMedicine, vol. 3, no. 2, pp. 92–94, 2013.

[22] W.-Y. Lin, H.-P. Liu, J.-S. Chou et al., “Genetic variations within the PSORS1 region affect Kawasaki disease development and coronary artery aneurysm formation,” BioMedicine, vol. 3, no. 2, pp. 73–81, 2013.

[23] C.-H. Wang, W.-D. Lin, D.-T. Bau, J. Chou, C. Tsai, and F. Tsai, “Appearance of acanthosis nigricans may precede obesity: an involvement of the insulin/IGF receptor signaling pathway,” BioMedicine, vol. 3, no. 2, pp. 82–87, 2013.

[24] Y.-M. Chang, B. K. Velmurugan, W.-W. Kuo et al., “Inhibitory effect of alpinate Oxyphyllae fructus extracts on Ang II-induced cardiac pathological remodeling-related pathways in H9c2 cardiomyoblast cells,” BioMedicine, vol. 3, no. 4, pp. 148–152, 2013.

[25] E.-J. Tsai, “Rare diseases: a mysterious puzzle,” BioMedicine, vol. 3, no. 2, article 65, 2013.

[26] I. C. Chou, W.-D. Lin, C.-H. Wang et al., “Mobius syndrome in a male with XX/XY mosaicism,” BioMedicine, vol. 3, no. 2, pp. 102–104, 2013.

[27] Y.-T. Chang, W.-D. Lin, Z.-N. Chin et al., “Nonketotic hyperglycinemia: a case report and brief review,” BioMedicine, vol. 2, no. 2, pp. 80–82, 2012.

[28] C.-H. Wang, W.-D. Lin, and F.-J. Tsai, “Craniofacial dysmorphism, what is your diagnosis?” BioMedicine, vol. 2, no. 2, pp. 49–50, 2012.

[29] C. Y. Chen, “TCM Database@Taiwan: the world’s largest traditional Chinese medicine database for drug screening in silico,” PLoS ONE, vol. 6, no. 1, Article ID e15939, 2011.
[30] H. J. Huang, K. J. Lee, H. W. Yu et al., “Structure-based and ligand-based drug design for HER2 receptor,” Journal of Biomolecular Structure & Dynamics, vol. 28, no. 1, pp. 23–37, 2010.

[31] W. I. Tou and C. Y. Chen, “Traditional Chinese medicine as dual guardians against hypertension and cancer?” Journal of Biomolecular Structure & Dynamics, vol. 30, no. 3, pp. 299–317, 2012.

[32] S. C. Yang, S. S. Chang, and C. Y. Chen, “Identifying HER2 inhibitors from natural products database,” PLoS ONE, vol. 6, no. 12, Article ID e28793, 2011.

[33] C. Y. Chen, “Insights into designing the dual-targeted HER2/HSP90 inhibitors,” Journal of Molecular Graphics & Modelling, vol. 29, no. 1, pp. 21–31, 2010.

[34] K. C. Chen and C. Y. C. Chen, “Stroke prevention by traditional Chinese medicine? A genetic algorithm, support vector machine and molecular dynamics approach,” Soft Matter, vol. 7, no. 8, pp. 4001–4008, 2011.

[35] S. C. Yang, S. S. Chang, H. Y. Chen, and C. Y. Chen, “Identification of potent EGFR inhibitors from TCM Database@Taiwan,” PLoS Computational Biology, vol. 7, no. 10, Article ID e1002189, 2011.

[36] K. C. Chen, M. F. Sun, S. C. Yang et al., “Investigation into potent inflammation inhibitors from traditional Chinese medicine,” Chemical Biology & Drug Design, vol. 78, no. 4, pp. 679–688, 2011.

[37] S. S. Chang, H. J. Huang, and C. Y. Chen, “High performance screening, structural and molecular dynamics analysis to identify 4H inhibitors from TCM Database@Taiwan,” Molecular BioSystems, vol. 7, no. 12, pp. 3366–3374, 2011.

[38] C. H. Lin, T. T. Chang, M. F. Sun et al., “Potent inhibitor design against H1N1 swine influenza: structure-based and molecular dynamics analysis for M2 inhibitors from traditional Chinese medicine database,” Journal of Biomolecular Structure & Dynamics, vol. 28, no. 4, pp. 471–482, 2011.

[39] S. C. Yang, H. J. Huang, and C. Y. Chen, “Two birds with one stone? Possible dual-targeting H1N1 inhibitors from traditional Chinese medicine,” PLoS Computational Biology, vol. 7, no. 12, Article ID e1002315, 2011.

[40] C. Y. Chen, Y. H. Chang, D. T. Bau et al., “Ligand-based dual target drug design for H1N1: swine flu—a preliminary first study,” Journal of Biomolecular Structure & Dynamics, vol. 27, no. 2, pp. 171–178, 2009.

[41] H. J. Huang, Y. R. Jian, and C. Y. Chen, “Traditional Chinese medicine application in HIV: an in silico study,” Journal of Biomolecular Structure & Dynamics, vol. 32, no. 1, pp. 1–12, 2014.

[42] T. Y. Tsai, K. W. Chang, and C. Y. Chen, “iScreen: world’s first cloud-computing web server for virtual screening and de novo drug design based on TCM database@Taiwan,” Journal of Computer-Aided Molecular Design, vol. 25, no. 6, pp. 525–531, 2011.

[43] K. W. Chang, T. Y. Tsai, K. C. Chen et al., “iSMART: an integrated cloud computing web server for traditional Chinese medicine for online virtual screening, de novo evolution and drug design,” Journal of Biomolecular Structure & Dynamics, vol. 29, no. 1, pp. 243–250, 2011.

[44] B. Xue, R. L. Dunbrack, R. W. Williams, A. K. Dunker, and V. N. Uversky, “PONDR-FIT: a meta-predictor of intrinsically disordered amino acids,” Biochimica et Biophysica Acta, vol. 1804, no. 4, pp. 996–1010, 2010.

[45] C. M. Venkatachalam, X. Jiang, T. Oldfield, and M. Waldman, “LigandFit: a novel method for the shape-directed rapid docking of ligands to protein active sites,” Journal of Molecular Graphics & Modelling, vol. 21, no. 4, pp. 289–307, 2003.

[46] B. R. Brooks, C. L. Brooks III, A. D. Mackerell Jr. et al., “CHARMM: the biomolecular simulation program,” Journal of Computational Chemistry, vol. 30, no. 10, pp. 1545–1614, 2009.

[47] P. Cherezov, A. L. Ambroso, S. Rahman, T. Ellenberger, and A. Engelman, “Structural basis for the recognition between HIV-1 integrase and transcriptional coactivator p75,” Proceedings of the National Academy of Sciences of the United States of America, vol. 102, no. 48, pp. 17308–17313, 2005.

[48] U. Velaparthi, P. Y. Liu, B. Balasubramanian et al., “Imidazole moiety replacements in the 3-(1H-benzo[d]imidazo[2,3-yl])pyridin-2(1H)-one inhibitors of insulin-like growth factor receptor-1 (IGF-1R) to improve cytochrome P450 profile,” Biorganic & Medicinal Chemistry Letters, vol. 17, no. 11, pp. 3072–3076, 2007.

[49] D. Rogers and A. J. Hopfinger, “Application of genetic function approximation to quantitative structure-activity relationships and quantitative structure-property relationships,” Journal of Chemical Information and Computer Sciences, vol. 34, no. 4, pp. 854–866, 1994.

[50] Y. Fujikoshi, T. Noguchi, M. Ohtaki, and H. Yanagihara, “Corrected versions of cross-validation criteria for selecting multivariate regression and growth curve models,” Annals of the Institute of Statistical Mathematics, vol. 55, no. 3, pp. 537–553, 2003.

[51] C. Y. C. Chen, “Computational screening and design of traditional Chinese medicine (TCM) to block phosphodiesterase-5,” Journal of Molecular Graphics & Modelling, vol. 28, no. 3, pp. 261–269, 2009.

[52] R. E. Fan, P. H. Chen, and C. J. Lin, “Working set selection using second order information for training support vector machines,” Journal of Machine Learning Research, vol. 6, pp. 1889–1918, 2005.

[53] R. A. Laskowski and M. B. Swindells, “LigPlot+: multiple ligand-protein interaction diagrams for drug discovery,” Journal of Chemical Information and Modeling, vol. 51, no. 10, pp. 2778–2786, 2011.

[54] A. C. Wallace, R. A. Laskowski, and J. M. Thornton, “LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions,” Protein Engineering, vol. 8, no. 2, pp. 127–134, 1995.

[55] U. D. Priyakumar and A. D. MacKerell, “Comparison of the CHARMM27, AMBER4.1 and BMS nucleic acid force fields via free energy calculations of base flipping,” Abstracts of Papers of the American Chemical Society, vol. 230, pp. U1391–U1392, 2005.

[56] B. Hess, C. Kutzner, D. van der Spoel, and E. Lindahl, “GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation,” Journal of Chemical Theory and Computation, vol. 4, no. 3, pp. 435–447, 2008.

[57] V. Zoete, M. A. Cuendet, A. Grosdidier, and O. Michielin, “GRGMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation,” Journal of Chemical Theory and Computation, vol. 4, no. 3, pp. 435–447, 2008.

[58] A. C. Wallace, R. A. Laskowski, and J. M. Thornton, “LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions,” Protein Engineering, vol. 8, no. 2, pp. 127–134, 1995.

[59] U. D. Priyakumar and A. D. MacKerell, “Comparison of the CHARMM27, AMBER4.1 and BMS nucleic acid force fields via free energy calculations of base flipping,” Abstracts of Papers of the American Chemical Society, vol. 230, pp. U1391–U1392, 2005.

[60] B. Hess, C. Kutzner, D. van der Spoel, and E. Lindahl, “GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation,” Journal of Chemical Theory and Computation, vol. 4, no. 3, pp. 435–447, 2008.

[61] V. Zoete, M. A. Cuendet, A. Grosdidier, and O. Michielin, “GRGMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation,” Journal of Chemical Theory and Computation, vol. 4, no. 3, pp. 435–447, 2008.
activates the replication of latent human immunodeficiency virus type 1 (HIV-1) in a monocytic cell line,” *Biological & Pharmaceutical Bulletin*, vol. 31, no. 12, pp. 2334–2337, 2008.

[61] S. C. Morton, “Statistical collaboration to impact policy decisions,” *Statistics in Medicine*, vol. 24, no. 4, pp. 493–501, 2005.

[62] R. Chintalova-Dallas, P. Case, N. Kitsenko, and Z. Lazzarini, “Boltushka: a homemade amphetamine-type stimulant and HIV risk in Odessa, Ukraine,” *The International Journal of Drug Policy*, vol. 20, no. 4, pp. 347–351, 2009.

[63] R. V. House, P. T. Thomas, and H. N. Bhargava, “Comparison of immune functional parameters following in vitro exposure to natural and synthetic amphetamines,” *Immunopharmacology and Immunotoxicology*, vol. 16, no. 1, pp. 1–21, 1994.

[64] E. Chovancova, A. Pavelka, P. Benes et al., “CAVER 3.0: a tool for the analysis of transport pathways in dynamic protein structures,” *PLoS Computational Biology*, vol. 8, no. 10, Article ID e1002708, 2012.