Research Article

Potential Smoothened Inhibitor from Traditional Chinese Medicine against the Disease of Diabetes, Obesity, and Cancer

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Nowadays, obesity becomes a serious global problem, which can induce a series of diseases such as type 2 diabetes mellitus, cancer, cardiovascular disease, metabolic syndrome, and stroke. For the mechanisms of diseases, the hedgehog signaling pathway plays an important role in body patterning during embryogenesis. For this reason, smoothened homologue (Smo) protein had been indicated as the drug target. In addition, the small-molecule Smo inhibitor had also been used in oncology clinical trials. To improve drug development of TCM compounds, we aim to investigate the potent lead compounds from TCM compounds in TCM Database@Taiwan. The top three TCM compounds, precatorine, labiatic acid, and 2,2'-[benzene-1,4-diylbis(methanediolxybenzene-4,1-diyl)]bis(oxoacetic acid), have displayed higher potent binding affinities than the positive control, LY2940680, in the docking simulation. After MD simulations, which can optimize the result of docking simulation and validate the stability of H-bonds between each ligand and Smo protein under dynamic conditions, top three TCM compounds maintain most of interactions with Smo protein, which keep the ligand binding stable in the binding domain. Hence, we propose precatorine, labiatic acid, and 2,2'-[benzene-1,4-diylbis(methanediolxybenzene-4,1-diyl)]bis(oxoacetic acid) as potential lead compounds for further study in drug development process with the Smo protein.

1. Introduction

Nowadays, obesity, which is caused by the body’s inability to handle excessive energy intake, becomes a serious global problem. It can induce a series of diseases such as type 2 diabetes mellitus, cancer, cardiovascular disease, metabolic syndrome, and stroke [1, 2]. In fact, the diseases of diabetes, obesity, and cancer have the dysregulated intracellular signaling and altered metabolic state [3]. Nowadays, increasing numbers of distinct mechanisms of diseases have been determined [4–6]. According to these mechanisms, increasing numbers of potential target proteins for drug design against each disease have been identified [7, 8]. The hedgehog signaling pathway plays an important role in body patterning during embryogenesis [9]. Abnormalities in hedgehog signaling pathway can lead to diabetes, obesity, and cancer [10–14]. As hedgehog pathway genes encoding patched homologue 1 (Ptchl) and smoothened homologue (Smo), Smo protein had been indicated as the drug target, and the small-molecule Smo inhibitor had been used in oncology clinical trials [15–18].

Many in silico researches had indicated that compounds extracted from traditional Chinese medicine (TCM) can be used as potential lead compounds for many different diseases [19], such as cancer [20–23], diabetes [24], inflammation...
To improve drug development of TCM compounds, we aim to investigate the potent lead compounds as Smo inhibitor from the TCM compounds in TCM Database@Taiwan [36]. As structural disordered residues in the protein may lead to the side effect and influence the ligand to bind with target protein [37, 38], the disordered residues of Smo protein were predicted before virtual screening. After virtual screening of the TCM compounds, as the interactions between protein and ligand in the docking simulation may not be stable under dynamic conditions, the molecular dynamics (MD) simulations were performed to validate the stability of those interactions.

2. Materials and Methods

2.1. Data Collection. The X-ray crystallography structure of the human smoothened receptor (Smo) was obtained from RCSB Protein Data Bank with PDB ID: 4JKV [39]. PONDR-Fit [40] protocol was employed to predict the disordered amino acids for the sequence of Smo protein from Swiss-Prot (UniProtKB: Q99835). For the protein preparation, Prepare Protein module in Discovery Studio 2.5 (DS2.5) was employed to protonate the final structure of protein with Chemistry at HARvard Macromolecular Mechanics (CHARMM) force field [41] and remove crystal water. The binding site for virtual screening was defined by the volume of the cocrystallized antitumor agent, LY2940680. Prepare Ligand module in DS2.5 was employed to protonate the final structure of TCM compounds from TCM Database@Taiwan [36], and Lipinski’s Rule of Five [42] was applied to filter the TCM compounds after virtual screening.

2.2. Docking Simulation. LigandFit protocol [43] in DS 2.5 was employed to virtually screen the TCM compounds by docking ligands into the binding site using a shape filter and Monte-Carlo ligand conformation generation. The result of docking was then optionally minimized with CHARMM force field [41] and evaluated with Dock Score energy function as follows:

\[
\text{Dock Score} = - (\text{ligand receptor interaction energy} + \text{ligand internal energy}).
\]  

The clustering of saved docking pose was performed to reject the similar poses.

2.3. Molecular Dynamics (MD) Simulation. Gromacs [44] was employed to simulate each protein-ligand complex under dynamic conditions using classical molecular dynamics theory. The pdb2gmx protocol of Gromacs and SwissParam program [45] were provided to employ topology and parameters for Smo protein with charmm27 force field and each ligand, respectively. The Gromacs program sets the dimensions of the cubic box based upon setting the box edge approx 12 Å from the molecules periphery and solvated using TIP3P water model. Steepest descent [46] is one of the common algorithms for minimization. For this algorithm, new positions are calculated by the equation as follows:

\[
r_{n+1} = r_n + \frac{F_n}{\max |F_n|} h_n
\]

If \( V_{n+1} < V_n \), \( r_{n+1} \) accepted and \( h_{n+1} = 1.2h_n \)

otherwise, \( r_{n+1} \) rejected and \( h_n = 0.2h_n \),

where \( r \) is the vector of all 3N coordinates, \( h_n \) is the maximum displacement and initial \( h_0 \) is given in unit of 0.01 nm, and \( F_n \) is the force or the negative gradient of the potential \( V \).

The algorithm stops when \( \max |F_n| < \varepsilon \) or complete the maximum number of iterations defined in the protocol. After a steepest descent minimization with a maximum of 5,000 steps was employed to remove bad van der Waals contacts, it created a neutral system using 0.145 M NaCl model. Then another steepest descent minimization with a maximum of 5,000 steps was employed to remove bad van der Waals contacts. For the equilibration, the position-restrained molecular dynamics with the Linear Constraint algorithm for all bonds was performed with NVT equilibration, Berendsen weak thermal coupling method, and Particle Mesh Ewald method. The Berendsen weak thermal coupling method mimics with first-order kinetics an external heat bath with given temperature 300 K and slowly corrected the temperature deviation of the system by the equation as follows:

\[
\frac{dT}{dt} = \frac{T_0 - T}{\tau},
\]

where \( T_0 \) is given temperature 300 K and \( \tau \) is a time constant in unit of 0.1 ps.

The MD program was then employed to simulate a total of 5000 ps production simulation with time step in unit of 2 fs under Particle Mesh Ewald (PME) option and NPT ensembles. A series of protocols in Gromacs was employed to analyze the MD trajectories.

3. Results and Discussion

3.1. Disordered Protein Prediction. The disordered disposition for the sequence of Smo protein from Swiss-Prot (UniProtKB: Q99835) predicted by PONDR-Fit was illustrated in Figure 1. As the residues in the binding domain do not lie in the disordered region, the binding domain of Smo protein has a stable structure in protein folding.

3.2. Docking Simulation. Before virtual screening, the cocrystallized antitumor agent, LY2940680, had been redocked by LigandFit protocol into the binding site defined by the volume of LY2940680 (Figure 2(a)) to validate the accuracy of LigandFit protocol. The Root-mean-square deviation value between crystallized structure and docking pose of LY2940680 is 0.5106 Å (Figure 2(b)). After virtual screening, the chemical scaffold top TCM compounds ranked by Dock Score [43] and LY2940680 are illustrated in Figure 3. The scoring function of Dock Score indicates that the top three TCM compounds have higher binding affinities than LY2940680. The top three TCM compounds, precatorine, labiatic acid, and 2,2’-[ben-zene-1,4-diylbis(methanediylxybenzene-4,1-diyl)] bis(oxoa-ctec
Figure 1: Disordered disposition predicted by PONDR-Fit. Sequence alignment with disordered residues (yellow regions) and residues in the binding domain (magenta regions).
Figure 2: (a) Binding site of Smo protein defined as the volume of LY2940680. (b) Root-mean-square deviation value between crystallized structure (orange) and docking pose (green) of LY2940680.

Figure 3: Chemical scaffold of controls and top three TCM candidates with their scoring function and sources.

2,2′-[Benzene-1,4-diylbis(methanediolyoxybenzene-4,1-diyl)] bis(oxoacetic acid)
Dock score = 126.221
_Ardisia japonica_

Precatorine
Dock score = 152.053
_Abrus precatorius L._

Labiatic acid
Dock score = 146.216
_Rosmarinus officinalis L._

LY2940680
Dock score = 125.401

acid), are extracted from _Abrus precatorius_ L., _Rosmarinus officinalis_ L., and _Ardisia japonica_, respectively. According to the docking poses in Figure 4, for positive control, LY2940680, there exists a π interaction with residue Phe484 and hydrogen bonds (H-bonds) with residues Asn219 and Arg400. Precatorine has π interactions with residues Tyr394, Arg400, Phe484, and H-bonds with residue Lys395. Labiatic acid has a π interaction with residue Phe484 and H-bonds with residues Tyr207, Lys395, and Arg400. The top 3 compounds have π interactions with residues Tyr394, Arg400, Phe484, and H-bonds with residues Tyr394, Lys395, His470, and Asn521. The docking poses displayed in Figure 4 indicate that each compound has a π interaction with residue Phe484 and interaction with common residues Lys395 and Arg400. Those interactions stabilize each compound in the binding domain of Smo protein.
3.3. Molecular Dynamics Simulation. The docking simulation performed by LigandFit protocol docks compounds into binding site using a shape-based docking. Although the Monte-Carlo techniques had been employed to simulate the flexible compound by generating sets of compound conformations, the structure of target protein is a rigid body of Smo protein from the crystal structure. As the interactions between protein and ligand in the docking simulation may not be stable under dynamic conditions, the molecular dynamics (MD) simulations were performed to validate the stability of those interactions. The root-mean-square deviations (RMSDs) for each protein and ligand were displayed in Figure 5. They indicate the atomic fluctuations during MD simulation for each protein and ligand. Figure 5
shows that the atomic fluctuations of each complex tend to be stable after 4700 ps of MD simulation. The variations of total energy for each complex during 5000 ps of MD simulation were illustrated in Figure 6, which indicate that Smo protein docking with the top three TCM compounds has similar variation of total energy, and there is no significant change of total energy for each complex during 5000 ps of MD simulation. The variation of radius of gyration and mean square displacement (MSD) for proteins in each complex during 5000 ps of MD simulation was illustrated in Figures 7 and 8, respectively. They indicate that Smo protein docking with the top three TCM compounds has similar compactness and diffusion constant under dynamic conditions as LY2940680. The variation of solvent accessible surface area in Figure 9 can also be used to discuss the effect of each ligand for
the Smo protein after docking. In Figure 9, it can be seen clearly that the Smo protein in each complex has similar solvent accessible surface area when the RMSDs tend to be stable after 4700 ps of MD simulation. The smallest distance between residue pairs for Smo protein in each complex illustrated in Figure 10 also has similar distance matrices. They indicate that the top three TCM compounds may cause similar influence for Smo protein as LY2940680.

For the MD simulation, the representative structures of each complex under dynamic conditions were identified by the cluster analysis with a RMSD cutoff of 0.105 nm. According to the RMSD values and graphical depiction of the clusters for Smo protein complexes with LY2940680, precatorine, labiatic acid, and 2,2'-[benzene-1,4-diylbis(methanediyoxybenzene-4,1-diyl)]bis(oxoacetic acid) illustrated in Figure 11, the docking poses of the representative
For LY2940680, there exist the stable H-bonds with residues Asn219 and Arg400 under dynamic conditions. In addition, it forms an H-bond with Tyr394 after MD simulation. Precatorine has stable π interactions with residue Phe484 and H-bonds with Lys395. After MD simulation, it forms an H-bond with residue Asn219. Labiatic acid has stable H-bonds with residues Tyr207, Lys395 and forms the H-bonds with residues Asp384, Gln477, and Glu518. The top 3 TCM compounds have stable π interactions with residue Phe484 and H-bonds with residue Lys395. Moreover, the interaction
with residue Arg400 was changed from $\pi$ interaction to H-bond and forms the H-bonds with residues Tyr207 and Arg285 after MD simulation.

To analyze the variation of H-bonds for key residues in each protein-ligand complex, the H-bond occupancy for key residues of Smo protein with top three candidates and LY2940680 overall 5000 ps of MD simulation was listed in Table I, and the distance variations of each H-bond were displayed in Figure 13. They indicate that the H-bonds between LY2940680 and residues Asn219, Tyr394, Arg400 were stabilized over 5000 ps of MD simulation. In addition, the H-bonds between top three TCM compounds and residues mentioned above were also stabilized. Comparing to docking poses between docking simulation (Figure 4) and MD simulation (Figure 12), LY2940680 and the top three TCM compounds maintain most of interactions with Smo protein, which keep the ligand binding stable in the binding domain.

4. Conclusion

This study aims to investigate the potent TCM candidates for Smo protein. The top three TCM compounds, precatorine, labiatic acid, and $2,2'$-[benzene-1,4-diylbis(methanediyoxybenzene-4,1-diyl)]bis(oxoacetic acid), have displayed higher potent binding affinities than the positive control, LY2940680, in the docking simulation. The docking poses of top three TCM compounds have similar $\pi$ interaction with residue Phe484 and interaction with common residues Lys395 and Arg400. The MD simulations are employed to optimize the result of docking simulation and validate the stability of H-bonds between each ligand and Smo protein under dynamic conditions. For the MD simulation, the top three TCM compounds maintain most of interactions with Smo protein, which keep the ligand binding stable in the binding domain. Hence, we propose precatorine, labiatic acid,
Figure 13: Distances of hydrogen bonds with common residues during 5000 ps of MD simulation.

and 2,2’-[benzene-1,4-diylbis(methanediyl)oxybenzene-4,1-diyl]bis(oxoacetic acid) as potential lead compounds for further study in drug development process with the Smo protein.

Conflict of Interests
The authors declared that there is no conflict of interests.

Authors’ Contribution
Kuan-Chung Chen, Mao-Feng Sun, and Hsin-Yi Chen contributed equally to this work.

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Table 1: H-bond occupancy for key residues of Smo protein with top three candidates and LY2940680 overall 5000 ps of molecular dynamics simulation.

| Name          | H-bond interaction | Occupancy |
|---------------|--------------------|-----------|
| LY2940680     | Asn219:HD22/O3     | 96%       |
|               | Tyr394:HH/N27     | 80%       |
|               | Tyr394:HH/N30     | 80%       |
|               | Arg400:HH22/N27   | 61%       |
|               | Arg400:HH22/N30   | 33%       |
| Precatorine   | Asn219:HD22/O8    | 52%       |
|               | Asn219:HD22/O16   | 29%       |
|               | Asn219:HD22/O20   | 18%       |
|               | Asp384:OD1/H29    | 46%       |
|               | Lys395:HZ3/O18    | 55%       |
|               | Lys395:HZ3/O19    | 57%       |
| Labiatic acid | Tyr207:HH/O21     | 7%        |
|               | Tyr207:HH/O23     | 23%       |
|               | Tyr207:HH/O26     | 80%       |
|               | Asn219:HD22/O24   | 17%       |
|               | Asp384:OD1/H40    | 65%       |
|               | Lys395:O/H30      | 9%        |
|               | Lys395:O/H39      | 57%       |
|               | Lys395:HZ3/O23    | 9%        |
|               | Lys395:HZ3/O26    | 24%       |
|               | Glu518:OE1/H41    | 5%        |
|               | Glu518:OE2/H41    | 40%       |
| Top 3         | Tyr207:HH/O18     | 34%       |
|               | Tyr207:HH/O10     | 43%       |
|               | Tyr207:HH/O11     | 8%        |
|               | Tyr394:HH/O29     | 2%        |
|               | Tyr394:HH/O31     | 73%       |
|               | Lys395:HZ3/O8     | 10%       |
|               | Lys395:HZ3/O10    | 23%       |
|               | Lys395:HZ3/O11    | 27%       |
|               | Arg400:HH12/O32   | 14%       |
|               | Arg400:HH22/O31   | 14%       |
|               | Arg400:HH22/O32   | 18%       |
|               | Arg485:HH22/O11   | 2%        |
|               | Arg485:HE/O8      | 48%       |
|               | Arg485:HE/O10     | 10%       |
|               | Arg485:HE/O11     | 25%       |

H-bond occupancy cutoff: 0.3 nm.

Top 3: 2,2′-[benzene-1,4-diylbis(methanediolxyloxybenzene-4,1-diyl)]bis(oxo-acetic acid).

References

[1] K. Jauch-Chara and K. M. Oltmanns, “Obesity—a neuropsychological disease? Systematic review and neuropsychological model,” Progress in Neurobiology, vol. 114, pp. 84–101, 2014.
[2] A. J. Guri, R. Hontecillas, and J. Bassaganya-Riera, “ Peroxisome proliferator-activated receptors: bridging metabolic syndrome with molecular nutrition,” Clinical Nutrition, vol. 25, no. 6, pp. 871–885, 2006.
[3] R. Teperino, S. Amann, M. Bayer et al., “Hedgehog partial agonism drives warburg-like metabolism in muscle and brown fat,” Cell, vol. 151, no. 2, pp. 414–426, 2012.
[4] Y. Jiang, X. Li, W. Yang et al., “PKM2 regulates chromosome segregation and mitosis progression of tumor cells,” Molecular Cell, vol. 53, no. 1, pp. 75–87, 2014.
[5] Y.-M. Chang, B. K. Velmurugan, W.-W. Kuo et al., “Inhibitory effect of alpineate Oxyphyllae fructus extracts on Ang II-induced cardiac remodeling-related pathways in H9c2 cardiomyoblast cells,” BioMedicine, vol. 3, no. 4, pp. 148–152, 2013.
[6] Y. M. Leung, K. L. Wong, S. W. Chen et al., “Down-regulation of voltage-gated Ca2+ channels in Ca2+-store-depleted rat insulinoma RINm5F cells,” BioMedicine, vol. 3, no. 3, pp. 130–139, 2013.
[7] M. A. Leissring, E. Malito, S. Hedouin et al., “Designed inhibitors of insulin-degrading enzyme regulate the catabolism and activity of insulin,” PLoS ONE, vol. 5, no. 5, Article ID e10504, 2010.
[8] C.-L. Jao, S.-L. Huang, and K.-C. Hsu, “Angiotensin I-converting enzyme inhibitory peptides: inhibition mode, bioavailability, and antihypertensive effects,” BioMedicine, vol. 2, no. 4, pp. 130–136, 2012.
[9] A. P. McMahon, P. W. Ingham, and C. J. Tabin, “Developmental roles and clinical significance of Hedgehog signaling,” Current Topics in Developmental Biology, vol. 53, pp. 1–114, 2003.
[10] S. J. Scales and F. J. de Sauvage, “Mechanisms of Hedgehog pathway activation in cancer and implications for therapy,” Trends in Pharmacological Sciences, vol. 30, no. 6, pp. 303–312, 2009.
[11] A. Sekulic, A. R. Mangold, D. W. Northfelt, and P. M. Lorusso, “Advanced basal cell carcinoma of the skin: targeting the Hedgehog pathway,” Current Opinion in Oncology, vol. 25, no. 3, pp. 218–223, 2013.
[12] R. McMillan and W. Matsui, “Molecular pathways: the hedgehog signaling pathway in cancer,” Nature Reviews Cancer, vol. 18, no. 18, pp. 4883–4888, 2012.
[13] V. Chenna, C. Hu, and S. R. Khan, “Synthesis and cytotoxicity studies of Hedgehog enzyme inhibitors SANT-1 and GANT-61 as anticaner agents,” Journal of Environmental Science and Health A: Toxic/Hazardous Substances and Environmental Engineering, vol. 49, no. 6, pp. 641–647, 2014.
[14] D. Amakye, Z. Jagani, and M. Dorsch, “Unraveling the therapeutic potential of the Hedgehog pathway in cancer,” Nature Medicine, vol. 19, no. 11, pp. 1410–1422, 2013.
[15] D. D. Von Hoff, P. M. LoRusso, C. M. Rudin et al., “Inhibition of the hedgehog pathway in advanced basal-cell carcinoma,” The New England Journal of Medicine, vol. 361, no. 12, pp. 1164–1172, 2009.
[16] A. E. Proctor, L. A. Thompson, and C. L. O’Bryant, “Vismodegib: an inhibitor of the hedgehog signaling pathway in the treatment of basal cell carcinoma,” The Annals of Pharmacotherapy, vol. 48, no. 1, pp. 99–106, 2014.
