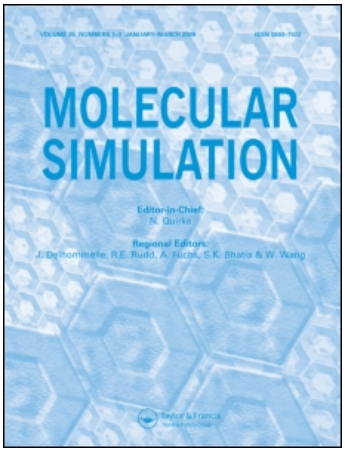

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Screening from the world's largest TCM database for inhibiting DNA repair protein XRCC4

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Screening from the world's largest TCM database for inhibiting DNA repair protein XRCC4

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Human XRCC4 protein is a key component of DNA double-strand break (DSB) repair pathway related to the diseases of stroke and cancer. Cancer cells being treated with the drugs that interfere with DSBs repair mechanism have shown increased radiosensitivity to ionising radiation. Therefore, the development of novel radiosensitiser for radiation therapy becomes important for cancer treatment. We screened from the world's largest traditional Chinese medicine (TCM) database and found potential TCM molecules that can dock at XRCC4 functional site. Among the selected potential TCM compounds, we specifically investigated the top-ranked molecules: glycyrrhizic acid and macedonoside C. The molecular docking and molecular dynamics simulations on these compounds show similar location with high predicted binding affinity. Both compounds form continuous interaction with Lys188 and Arg192 of chain C and Lys187 and Lys190 of chain D. All these protein residues are required to form key hydrophobic interactions with other components participating in DNA repair. We suggested both glycyrrhizic acid and macedonoside C as potential lead compounds for inhibiting XRCC4.

Keywords: traditional Chinese medicine; stroke; XRCC4; molecular dynamics; DNA repair

1. Introduction

XRCC4 is a key player in the DNA repair process that responds to DNA double-strand breaks (DSBs) related to the disease of stroke and cancer. Such DNA damage is hazardous to cells and can cause cells to be susceptible to environmental stress. Increased number of unrepaired or improperly repaired DSB has been reported to increase radiosensitivity to ionising radiation. Interestingly, such increase could favour the application of radiation therapy. Various methods have been developed to target DSB repair mechanism in attempt to enhance the effectiveness in radiosensitising tumour cells [1,2].

XRCC4 facilitates the process in joining DSB ends in the non-homologous end joining (NHEJ) pathway. The NHEJ process is initiated by the binding of Ku heterodimer to the DSB sites, and then the recruitment of DNA-dependent protein kinase catalytic subunit (DNA-PKcs). The resulting complex, DNA-PK, has critical roles in maintaining broken DNA ends in proximity as well as in recruiting XRCC4–DNA ligase IV complex [3,4]. The XRCC4–DNA ligase IV complex then actively joins the broken DNA ends, where XRCC4 enhances the joining activity of DNA ligase IV. Single nucleotide polymorphism (SNP) study in oral cancer patients demonstrated that normal XRCC4 gene does not lead to oral cancer susceptibility in comparison to truncated XRCC4 SNPs [5–7]. Conversely, this implies that although XRCC4 gene facilitates DNA repair, it also reduces the effectiveness in radiation therapy. Intriguingly, using XRCC4 fragments to interfere with the functions of XRCC4–DNA ligase IV complex was demonstrated to radiosensitise tumour cells [8]. This suggests that abolishing XRCC4 functions may enhance the effectiveness in radiation therapy, particularly to oral cancer.

It is possible to develop XRCC4 small-molecule inhibitors which result in increased radiosensitivity in cancer cells for anti-tumour purposes. In this study, we presented a computer-aided drug design (CADD) approach, using both molecular docking and molecular dynamics (MD) simulations to screen for potential XRCC4 inhibitors from the traditional Chinese medicine (TCM) database. More specifically, we focused on identifying potential lead compounds that target XRCC4 and disrupt the formation of XRCC4–DNA ligase IV complex. We employed the TCM Database@Taiwan (http://tcm.cmu.edu.tw) that is the world's largest resource library on TCM

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compounds. Both molecular docking and MD simulations were employed commercially to design novel therapeutic agents targeting proteins such as phosphodiesterase, human epidermal growth receptor 2 and influenza virus neuraminidase [9–24]. In this study, we have combined these two methods to investigate potential lead compound for XRCC4 small-molecule inhibitor.

2. Materials and methods

2.1 Protein and ligand preparation

A total of 20,000 TCM constituents were downloaded from the world's largest TCM Database@Taiwan (http://tcm.cmu.edu.tw). All downloaded TCM molecules were prepared using Prepare Ligand module in Discovery Studio 2.5 to adjust the ionisation state to physiological setting. In addition, the TCM molecules have been screened with ADMET models in Discovery Studio 2.5 to remove the compounds that have unfavourable pharmacokinetic profile. The Chemistry at HARvard Molecular Mechanics (CHARMm, [25]) force field was applied to all TCM molecules.

The X-ray crystallography structure for XRCC4 was downloaded from the Protein Data Bank, PDB ID: 3II6 [26]. All water molecules and non-bounded heteroatoms present in the XRCC4 protein crystal were removed prior to adding missing hydrogen and CHARMm force field. The DNA ligase IV BRCT domain co-crystallised with XRCC4 was also removed. XRCC4 and DNA ligase IV interacting surface was set as the ligand-binding site for the docking of TCM molecules.

2.2 Docking

Docking of TCM molecules was performed using the LigandFit module implanted in Discovery Studio 2.5. LigandFit dock ligands based on a receptor-rigid method. The ligand conformations, generated from Monte Carlo trials, were assessed based on shape complementary with the receptor. The docked TCM molecule was energetically minimised using the steepest descent algorithm. The Dock Score was used to rank the TCM molecules. This function evaluates candidate ligand based on two energy terms, the interaction energy of the ligand with the receptor and the internal energy of the ligand. In addition to ranking and selecting candidate ligands based on Dock Score, we also visually inspected all the top-ranking TCM molecules. The selection criteria include whether the ligand has formed favourable interactions with key residues within the binding site.

2.3 MD simulation

To further investigate receptor–ligand interactions, we performed MD simulation on the selected TCM molecule–XRCC4 complexes. Each complex was solvated in a cubic water box using explicit periodic boundary condition. Counter ions were also added. The solvated system was pre-applied with CHARMm force field and then went through energy minimisation with fixed atom constraint onto the TCM molecule and XRCC4. The minimisation protocol used was 2000 steps of steepest descent and 2000 steps of conjugate gradient. The fixed atom constraint was later removed, and the unconstrained system went through another round of energy minimisation. The energetically minimised system was then heated from 50 to 310 K in 20 ps and undertook 100 ps of equilibration at 310 K. The production stage was conducted in the NVT ensemble in 310 K for 20 ns. The particle mesh Ewald method was used to calculate the long-range electrostatic interaction. The SHAKE algorithm was applied throughout the entire simulation to restraint bonds involving hydrogens. The time step was 0.001 ps. Snapshots for monitoring dynamics trajectory was taken every 5 ps.

3. Results and discussions

The docking result, ranked in descending order of the Dock Score, is shown in Table 1. We used the Dock Score to rank the TCM molecules and select potential poses. A study done by Venkatachalam and colleagues [27] has demonstrated good correlation between the Dock Score and binding affinity. The four top-ranked TCM molecules are glycyrrhizic acid, macedonoside C, lithospermic acid and salvianolic acid B, which all are extracts from common TCM herbs. Glycyrrhizic acid and macedonoside C can be obtained from licorice (*Glycyrrhiza glabra*). Licorice has been used to cure digestive disease, neutralise food poisoning and mitigate drug toxics in TCM [28]. It is regarded as one of the most useful herbal medicines in TCM. Lithospermic acid can be isolated from dry root of *Lithospermum erythrorhizon*; and salvianolic acid B can be obtained from root or rhizome of *Salvia miltiorrhiza*. *Lithospermum erythrorhizon* and *S. miltiorrhiza* are used against sepsis [29] and circulation disorders [30], respectively, in TCM. Furthermore, both medicines were investigated for pharmacological potentials [13–15].

Table 1. TCM docking result.

Compound	Dock Score
Glycyrrhizic acid	214.035
Macedonoside C	206.674
Lithospermic acid	199.655
Salvianolic acid B	197.448
2-*O*-Caffeoyl tartaric acid	194.868
Mumefural	188.703
Chicoric acid	187.478
2-*O*-Feruloyl tartaric acid	186.828
Glutinic acid	172.647
Chebulinic acid	149.136

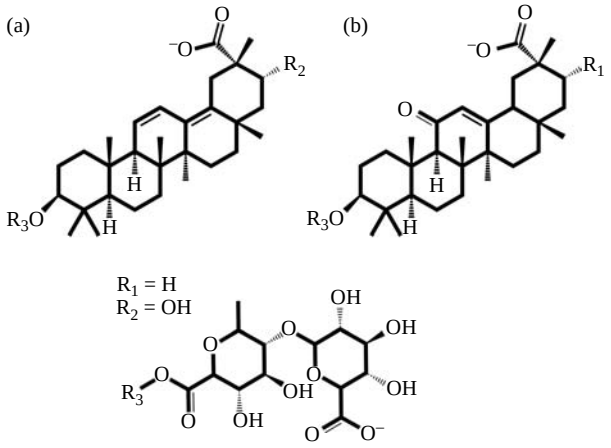

Figure 1. Structure of (a) glycyrrhizic acid and (b) macedonoside C.

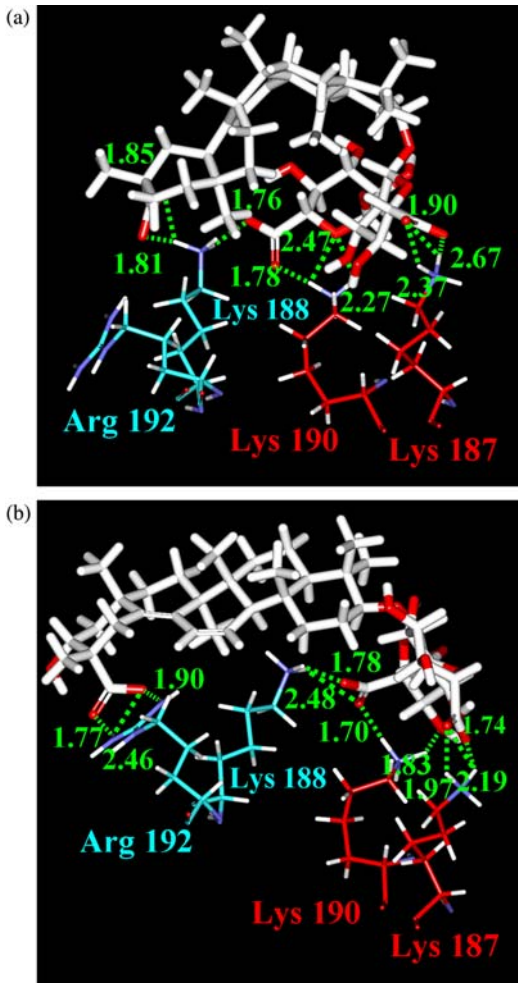

Figure 2. Docking poses of (a) glycyrrhizic acid and (b) macedonoside C in XRCC4 binding site.

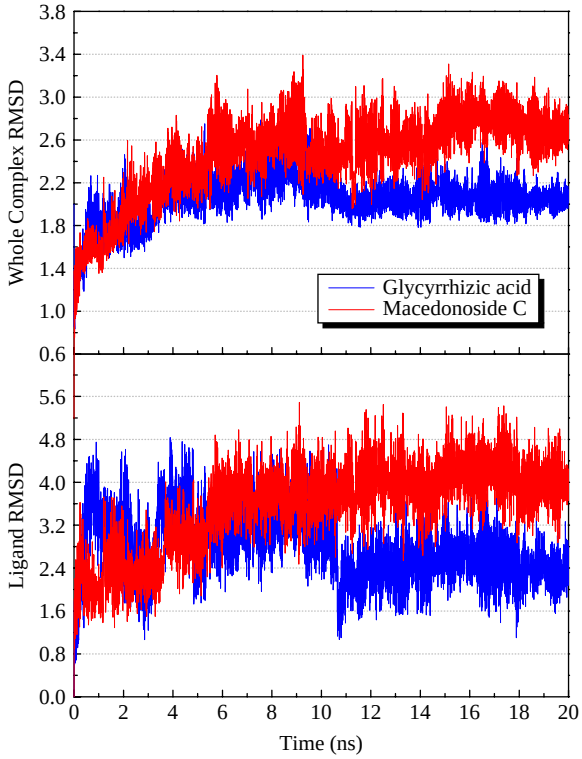

Figure 3. Whole molecule RMSD and ligand RMSD for glycyrrhizic acid and macedonoside C.

This is the pilot study in investigating the binding affinity of these compounds on XRCC4 protein.

We visually inspected all the top-ranked TCM molecules. Glycyrrhizic acid and macedonoside C interact with similar XRCC4 binding site residues. The structures of the top two TCM molecules are shown in Figure 1. The docking pose of glycyrrhizic acid and macedonoside C are shown in Figure 2. As illustrated, glycyrrhizic acid and macedonoside C have interaction with Lys188 of chain C and Lys187 and Lys190 of chain D. Macedonoside C has

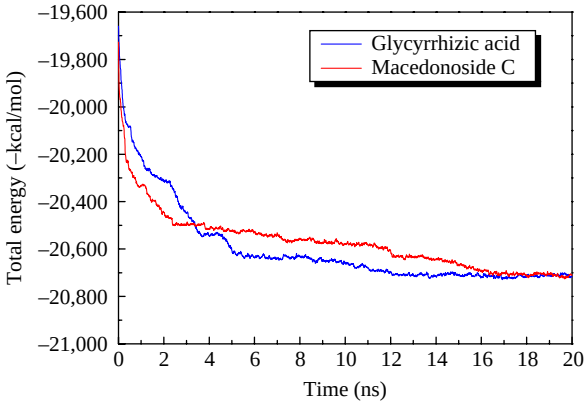

Figure 4. Energy trajectories of glycyrrhizic acid and macedonoside C.

Table 2. Hydrogen bond statistics for glycyrrhizic acid.

Hydrogen bond	Ligand atom	Amino acid	Max. distance	Min. distance	Ave. distance	Hydrogen bond occupancy (%)
H-bond_1	O47	C:Lys188HZ1	3.907	1.556	2.497	49.15
H-bond_2	O47	C:Lys188HZ2	3.972	1.603	2.798	25.63
H-bond_3	O47	C:Lys188HZ3	3.929	1.568	2.796	33.55
H-bond_4	O48	C:Arg192H21	4.538	2.054	2.182	15.93
H-bond_5	O47	C:Arg192H22	4.776	1.953	2.673	25.73
H-bond_6	O48	C:Arg192H22	2.915	1.676	2.136	98.35
H-bond_7	O100	D:Lys187HZ1	4.084	1.558	2.429	58.35
H-bond_8	O100	D:Lys187HZ2	4.196	1.58	3.027	20.48
H-bond_ 9	O100	D:Lys187HZ3	4.319	1.579	3.174	16.00
H-bond_10	O104	D:Lys187HZ1	3.403	1.637	2.340	69.88
H-bond_11	O104	D:Lys187HZ2	3.533	1.558	2.120	78.53
H-bond_12	O104	D:Lys187HZ3	3.614	1.628	2.780	35.85
H-bond_13	O85	D:Lys190HZ1	3.71	1.572	2.616	38.73
H-bond_14	O85	D:Lys190HZ2	3.699	1.56	2.846	27.80
H-bond_15	O85	D:Lys190HZ3	3.738	1.533	2.482	48.48
H-bond_16	O86	D:Lys190HZ1	4.898	1.784	3.241	19.80
H-bond_17	O86	D:Lys190HZ2	4.714	1.75	3.195	14.63
H-bond_18	O86	D:Lys190HZ3	4.749	1.665	2.754	47.88

Table 3. Hydrogen bond statistics for macedonoside C.

Hydrogen bond	Ligand atom	Amino acid	Max. distance	Min. distance	Ave. distance	Hydrogen bond occupancy (%)
H-bond_1	O85	C:Lys188HZ1	3.918	1.568	2.532	64.78
H-bond_2	O85	C:Lys188HZ3	3.882	1.625	2.476	64.10
H-bond_3	O86	C:Lys188HZ3	4.214	1.617	2.561	49.18
H-bond_4	O48	C:Arg192HH12	3.777	1.687	2.340	89.93
H-bond_5	O49	C:Arg192HH12	4.271	1.699	2.231	96.48
H-bond_6	O49	C:Arg192HH22	2.713	1.597	1.898	100
H-bond_7	O86	D:Lys187HZ1	4.096	1.615	2.670	41.68
H-bond_8	O89	D:Lys187HZ1	4.346	1.584	2.734	37.35
H-bond_ 9	O100	D:Lys190HZ1	4.926	1.641	2.743	39.18
H-bond_10	O100	D:Lys190HZ3	4.202	1.585	2.731	34.45
H-bond_11	O105	D:Lys190HZ1	4.587	1.627	2.733	34.50

an additional hydrogen bond interaction with Arg192 of chain C. In a similar XRCC4 crystal structure, PDB ID: 1IK9 [31], Arg192 (chain C), Lys187 (chain D) and Lys190 (chain D) have been shown to involve in the key hydrophobic contacts with Tyr765 and Phe766 on DNA ligase IV. We specially chose glycyrrhizic acid and macedonoside C for further analyses. We hypothesised that the interactions between the selected TCM molecules and with their target residues on XRCC4 could hinder the binding of DNA ligase IV.

We further conducted MD simulation of the glycyrrhizic acid-bound and macedonoside C-bound XRCC4 complexes for 20 ns to monitor the interactions in a non-static condition. The whole molecule RMSD and ligand RMSD and total energy trajectories of the protein complexes are shown in Figures 3 and 4, respectively. Both compounds reach equilibrium after 11 ns of simulation time. The total energy trajectories show that both complexes reach similar energy status at the end of the simulation (Figure 4).

As illustrated in Tables 2 and 3, both glycyrrhizic acid and macedonoside C maintained strong hydrogen bond when interacting with Arg192 of chain C in the simulation runs. Glycyrrhizic acid, which did not interact with Arg192 initially, shows a continuous interaction with Arg192 at a later stage of MD simulation, with 98.35% of hydrogen bond occupancy (Table 2). Figures 5 and 6 are time-dependent hydrogen bond distances between the TCM molecules and XRCC4. The top 1 has stable interactions with all of the residues observed from docking (Figure 5). The most significant change in the interacting distance for glycyrrhizic acid is observed at 11 ns, which corresponds to a change in ligand conformation (Figure 7(a),(b) and supplementary video 1). This change is in favour of the interaction with Lys190 of chain D. Macedonoside C, however, does not have very stable interaction with Lys187 and Lys190 of chain D, as shown

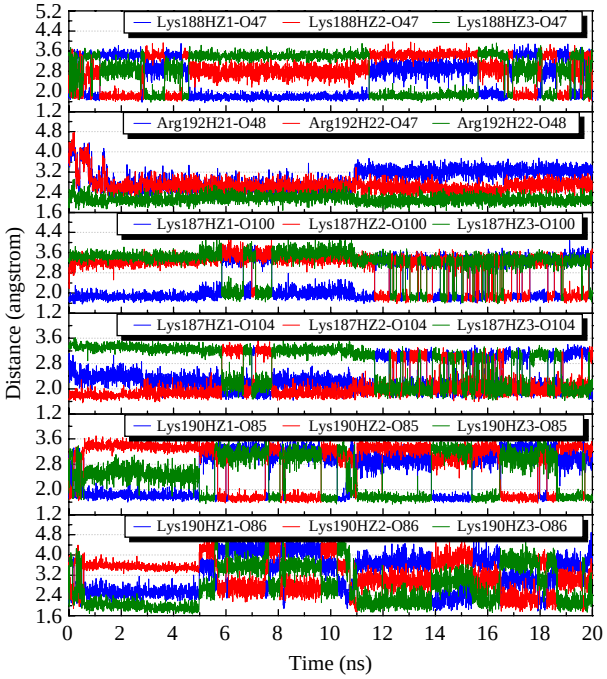

Figure 5. Distances between glycyrrhizic acid and XRCC4 residues during 20 ns of MD simulation.

in Figure 6 and supplementary video 2. Nevertheless, the strong interactions with Lys188 and Arg192 appear to be sufficient to keep macedonoside C in the binding site. As

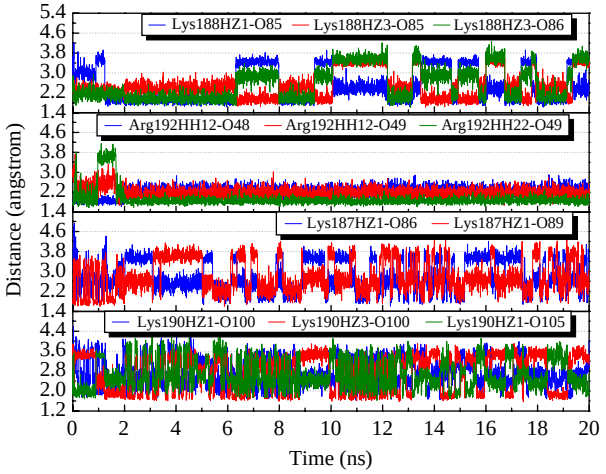

Figure 6. Distances between macedonoside C and XRCC4 residues during 20 ns of MD simulation.

illustrated in Figure 7(c),(d), the interactions with Lys188 and Arg192 of chain C are relatively unchanged whereas interactions with Lys187 and Lys190 appear to be more unstable.

4. Conclusion

The molecular simulation technique is successfully utilised in drug design and discovery [32–41]. We

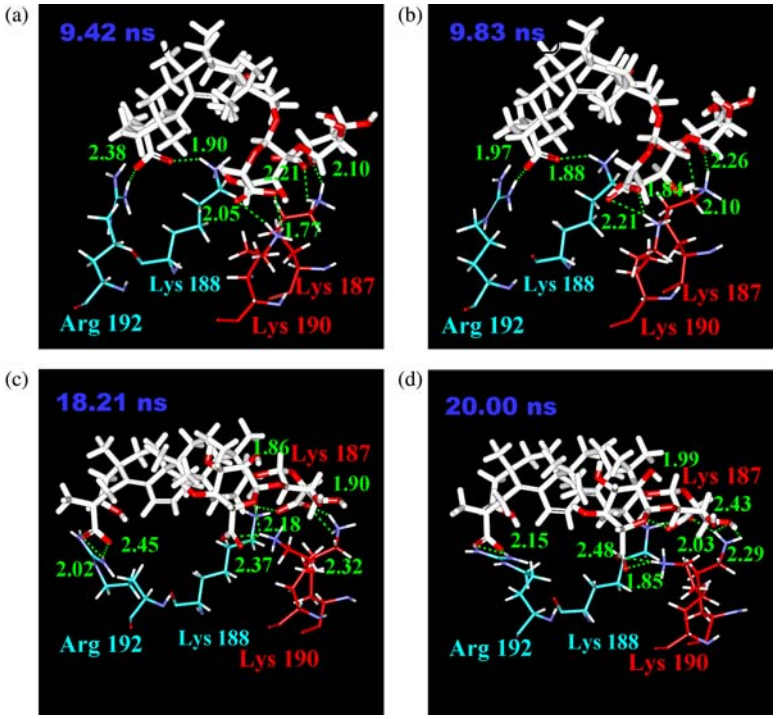

Figure 7. Snapshots taken for glycyrrhizic acid at (a) 9.42 ns and (b) 9.83 ns, and snapshots taken for macedonoside C at (c) 18.21 ns and (d) 20.00 ns.

employed screening, docking and MD for investigating XRCC4 inhibitors. Both TCM constituents, glycyrrhizic acid and macedonoside C, docked to XRCC4 well and established hydrogen bond interactions with the key residues at Lys188 (chain C), Arg192 (chain C), Lys187 (chain D) and Lys 190 (chain D). The extensive hydrogen bond networks formed between XRCC4 and the top-ranked TCM molecules suggest that both compounds can bind to XRCC4 well. The high dock score values also suggest that both compounds have high binding affinity towards XRCC4. We conclude, based on the proposed CADD methods, that both glycyrrhizic acid and macedonoside C are potential anti-tumour lead compounds which interfere with the NHEJ pathway by inhibiting XRCC4 functions.

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