Dhttps://doi.org/10.31925/farmacia.2020.2.24 **ORIGINAL ARTICLE**

HOMOLOGY MODEL, DOCKING ANALYSIS AND MOLECULAR DYNAMICS SIMULATION OF CANNABINOID CB2 RECEPTOR

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Abstract

Using cannabinoid CB1 as structural template, the 3D model of CB2 receptor was established with homology model method, and refined with molecular dynamics method. The docking of well-known reference antagonist compounds with CB2 was studied according to the model. Then the structure and components of active site in CB2 were investigated by aromatic interactions, hydrogen bond interactions and binding free energy analysis.

Rezumat

Folosind receptorul canabinoid CB1 ca model structural, a fost stabilită structura 3D a receptorului CB2 prin metoda modelului de omologie și definită prin intermediul dinamicii moleculare. Amestecul de compuși antagoniști de referință ai CB2 a fost studiat conform modelului de andocare. Ulterior, structura și componentele situsului activ din CB2 au fost cercetate prin intermediul interacțiunilor aromatice, legăturilor de hidrogen și analiza energiei libere de legare.

Keywords: CB2 receptor, homology model, molecular dynamics

Introduction

Cannabinoid receptors are involved in a variety of physiological processes such as appetite, mood, pain-sensation and memory. The potential therapeutic applications targeted cannabinoid receptors include management of glaucoma, attenuation of nausea and vomiting, suppression of muscle spasticity and therapeutic effects of analgesia [1, 2]. Cannabinoid receptors were abbreviated CB and numbered by a subscript in the order of their discovery. Now two cannabinoid receptor types were identified unequivocally, named CB1 and CB2. Although they share a certain degree of structural homology, there are differences in their tissue distribution and their signalling mechanisms. CB1 was located primarily in the central and peripheral nervous system. The antagonists or inverse agonists of CB1 have potential therapeutic application, such as treatment schizophrenia [3] and appetite suppressants [4]. The side effects of this therapeutic application are involved in dysphoria, sedation, euphoria and alterations in cognition and memory [5]. CB2 was located mainly in immune cells including tonsils, thymus, T lymphocytes, B lymphocytes, monocytes, macrophages, polymorphonuclear cells and natural killer (NK) cells. CB2 plays roles in immune regulation and inhibiting the release of cytokines. It was showed that CB2 is down-regulated at protein and mRNA levels during B-cell differentiation [6]. Recent studies showed that CB1 and CB2 were overexpressed on tumour cells in various types of cancers, and therefore could be used as novel targets for cancer [7].

Both cannabinoid receptors belong to the G-protein-coupled receptors (GPCRs) [8]. The characteristics of GPCRs are seven hydrophobic transmembrane helices (TMH) and are integral membrane proteins. Therefore, the structure of cannabinoid receptors is very important for their function and for drug design. The human CB1 receptor, which was performed the point mutation T210A and replaced the third intra-cellular loop (ICL3) with the thermostable *Pyrococcus abyssi* glycogen synthase (PGS) domain, was determined at high resolution by X-ray crystallography [9]. This 3D structure of CB1 has allowed modelling CB2 with greater reliability.

Classical cannabinoids are tricyclic terpenoid derivatives such as D^9-tetrahydrocannabinol bearing a benzopyran moiety. There are some cannabimimetic including tricyclic (such as CP55244) and bicyclic (such as CP55940) analogues which lack the pyran rings. Diarylpyrazole such as SR144528 is another type of cannabinoid analogues that is a selective and potent CB2 antagonist. SR144528 displays sub-nanomolar affinity (Ki = 0.6 nM) for both the rat spleen and cloned human CB2 receptors [10]. Other cannabinergics are the amino-alkylindoles that are potential non-steroidal anti-inflamatory agents. AM-630 as aminoalkylindoles

derivatives is the first selective CB2 receptor antagonist (Figure 1) with a Ki of 32.1 nM at CB2 and 165-fold selectivity over CB1. AM630 behaved as a competitive antagonist of WIN55212-2, CP55940, AM356 and anandamide [11].

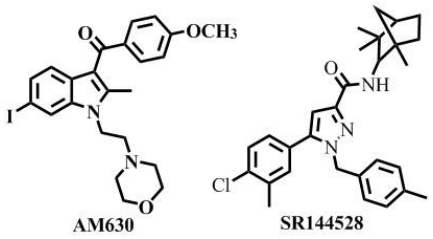

Figure 1.
CB2-selective antagonist

In this article, a homology model of CB2 receptor was established by means of the structural template of X-ray crystal structure CB1. A molecular docking approach with Autodock and molecular dynamics simulation was employed to investigate the active site.

Materials and Methods

Homology model of CB2 receptor

The sequence of human CB2 was extracted from the Uniprot database with accession code P34972 and the RCSB Protein Data Bank was searched with human CB2 sequence [12]. Human CB1 receptor (PDB ID: 5U09), as the template, was selected for homology model due to relatively high sequence identity to CB2 (46%), high-resolution crystal structure (2.6 Å) and completeness of sequence. The sequences of CB1 and CB2 were aligned using the CLUSTALW which is a sequence alignment program [13]. Using the method of MODELLER, 3D models were generated automatically by optimization of a molecular probability density function (pdf) containing all hydrogen atoms [14]. Then the models were minimized automatically by the program with the conjugate gradient method and the Charmm force field. Of the 20 models obtained with MODELLER, the one was selected for further analysis corresponding to the fewest restraints violations and lowest value of the pdf. The final model was assessed with ERRAT [15] and PROCHECK [16] which are the structure assessment programs.

Molecular docking

It is necessary to investigate the ligand binding mechanisms in order to get more potent and selective drugs. Therefor we performed a molecular docking study of CB2-selective antagonists with the CB2 model, and further investigated the critical residues of active site. Molecular docking was made with AutoDock4.2 package that is an automated procedure for predicting the interaction between ligands and biomacromoleculars [17]. When docking, CB2 model was kept rigid, while the torsional angles and torsional bonds in AM630 and SR144528 were set free in order to carry out flexible docking. We added Kollman united atom partial charges and polar hydrogens to CB2 model by means of AutoDock Tools (ADT) [18]. With AutoGrid the grid maps of model were calculated. The dimensions of grids were 60 Å × 60 Å × 60 Å in order to include the active site and important portions of the surrounding active site with a spacing of 0.375 Å. We performed fifty independent docking runs with the Lamarckian genetic algorithm and the empirical free energy function. According to the criterion of 1.0 Å root-mean-square deviation (RMSD) the results were clustered.

Molecular Dynamics Simulation

According to the homology model and molecular docking results, MD simulations were performed with and without ligand using the GROMACS 5.1.4 [19] package and the amber99SB force field [20]. The receptor models were solvated with TIP3P water model and placed in the centre of octahedron box [21], and electro-neutralized by adding 0.15 mol/L NaCl ions. The size of the box was chosen with a margin distance of 10 Å. The dynamics were carried out by means of the leapfrog algorithm with a fixed time step of 2 fs in the NPT ensemble. The pressure and temperature were kept constant at 1.0 bar and 300 K respectively using Parrinello-Rahman method and v-rescale method. First energy minimization of MD systems was performed with the steepest descent method; then 100 ps of position restraining simulation was performed by relaxing the solvent; finally a 10 ns MD simulation without the structural restraints was performed with the particle mesh Ewald (PME) method. A 14 Å cut-off for van der Waals interactions, a 9Å cut-off for coulomb interaction and the LINCS algorithm for bond constraints were used [22]. Periodic boundary conditions (PBC) were used for avoiding edge effects.

Binding free energy calculations

For CB2-AM630 and CB2-SR144528 complex systems, 200 snapshots were extracted to carry out binding free energy calculations from the last 2 ns that was stable MD trajectory by means of G_MMPBSA package [23]. The free energy of each molecular species (protein, ligand and complex) was computed for each snapshot, and then the binding free energy is calculated by equation (1).

$$\Delta G_{bind} = \Delta G_{MM} + \Delta G_{sol} - T\Delta S \qquad (1).$$

The molecular mechanics energy (ΔG_{MM}) is computed from the van der Waals and electrostatic interactions. In case of the solvation free energy (ΔG_{sol}), it involves both the nonpolar and the polar contributions. The nonpolar solvation free energy component was computed from solvent accessible surface area (SASA) method, whereas the polar solvation free energy component was obtained from the Poisson-Boltzmann equation by using the MM/PBSA method [24]. $T\Delta S$ is the entropy term. Usually entropic changes were not considered, but binding free energy could be used to compare against similar systems.

Results and Discussion

Homology model

Both cannabinoid receptor types contain seven hydrophobic TMH belonging to the large family of GPCRs [25]. CB2 shared 46% of identity with CB1. The sequential alignment of CB1 and CB2, produced by CLUSTALW program, was showed in Figure 2.

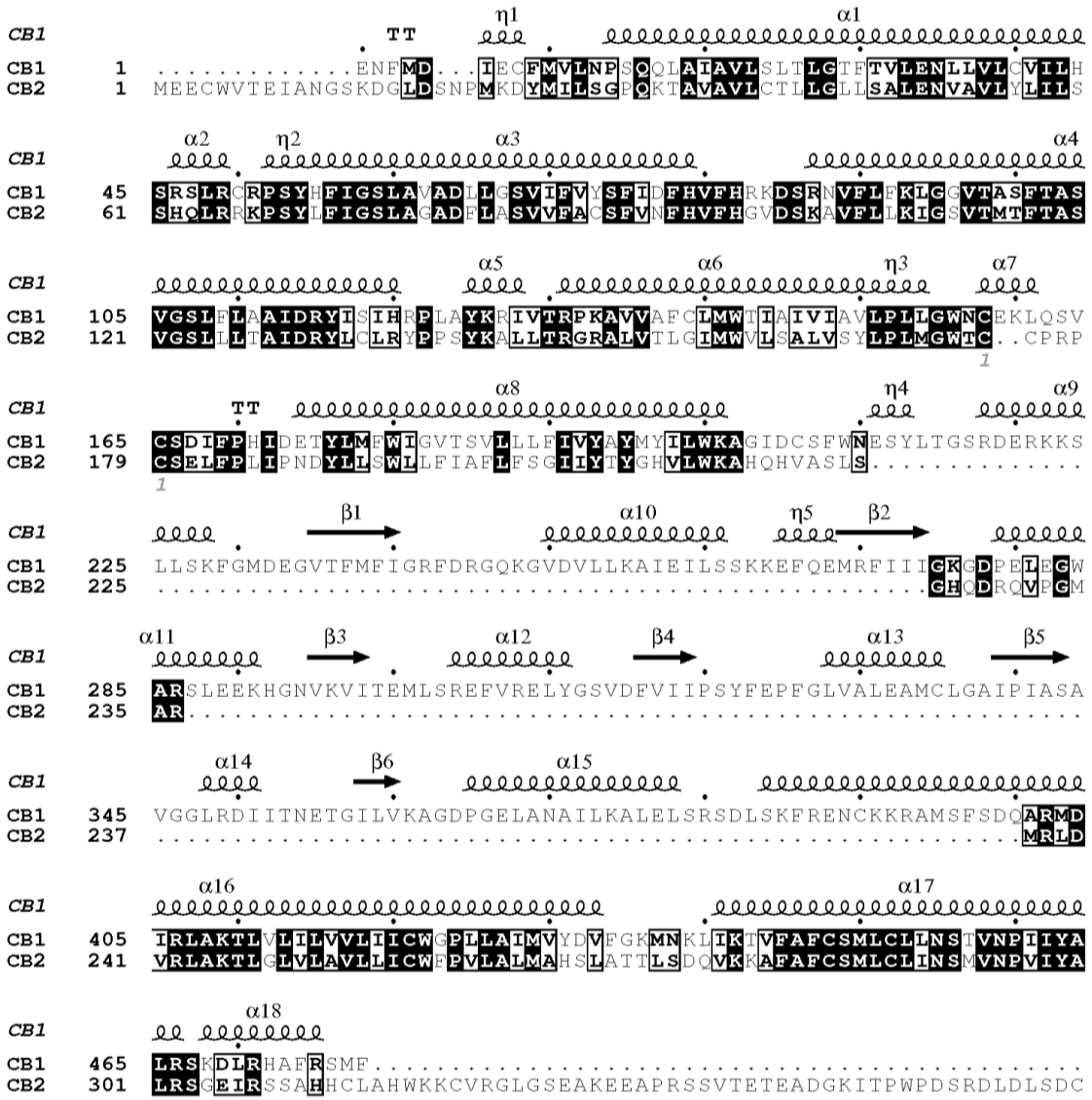

Figure 2.

Sequence alignment of 5U09 with CB2 sequence. The conserved patterns are boxed.

Most of the characteristic residues of CB2 are conserved in CB1. Compared to other transmembrane region, TM5 shows a rich aromatic region in CB1 and CB2, more remarkable in CB2. The most important differences are located in extracellular loop II (EL2), the N-terminal, C-terminal of TM7 and the C-terminal between CB1 and CB2 receptors. CB1 and CB2 possess a long N-terminal, and did not obtain any significant hit by searching in PSI-BLAST [26], and did not present in template structure. A region of rich proline was located in EL2 of CB2, while a region (RxAFRS) was located in C-terminal of CB1 which is well conserved in cation channel receptors (e.g. vanilloid receptor VR1) [27]. In CB1 crystal structure, the conformation of EL2 is constrained by the presence of an intra-loop disulphide bond (Cys158-Cys165), also in CB2 there are two cysteines conserved (Cys174-Cys179) that could form a disulphide bridge. The sequence identity percentage of the TMH is 48%, 68%, 62%, 36%, 36%, 59% and 77% for TM1, TM2, TM3, TM4, TM5, TM6 and TM7 respectively. Except TM1, TM4 and TM5, the identity percentage between CB1 TMH and CB2 TMH is around 60%. This is consistent with the substrates selectivity in these regions.

A homology model of human CB2 receptor was established with human CB1 (PDB ID: 5U09) as template (Figure 3). The 3D-model was confirmed by ERRAT and PROCHECK. Ramachandran plot of the model

exhibited that 99.1% of residues are in "most favoured regions" and "additional allowed regions", and only two residues are in "disallowed regions", which means a good stereochemistry for over 99% of the residues. An overall quality factor of 94.9% was produced by ERRAT which is a program for verifying protein structures and compares to reliable high-resolution structures. It means that the calculated error values of 94.9% of all residues are below the 95% rejection limit (Figure 4). The transmembrane helices of model were superposed with the CB1 template in order to identify the orientation of the CB2 helixes. The root-mean-square (RMS) value is 0.35 Å. So it is highly consistent between the transmembrane helices. The 3D model of CB2 constructed here is agreed with the studies of Martinelli *et al*. [28].

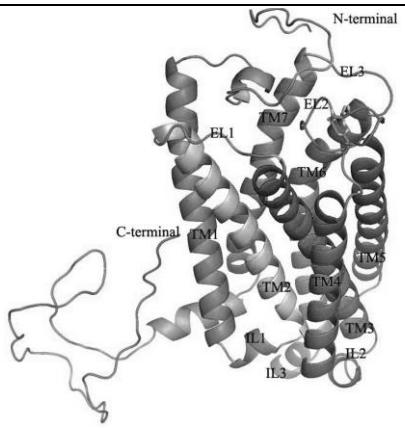

Figure 3.
The model of CB2 receptor drawn as cartoon diagram. The disulphide bond is shown as sticks models

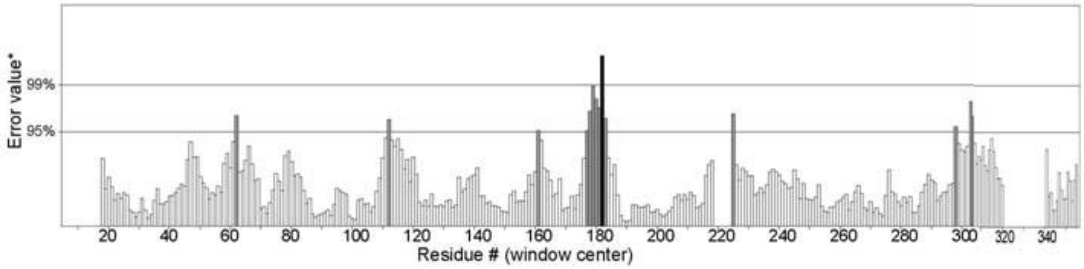

Figure 4.
The error values of each residue in CB2 model produced by ERRAT

The CB2 model conformation did not change drastically during the MD simulation of 10 ns. The RMSD values for CB2 backbone stayed within 1 nm range (Figure 5). In the root mean square fluctuation (RMSF) plot, residues with the largest fluctuations are highlighted during the simulation (Figure 6). We can conclude that the increase of the average RMSD was involved in fluctuations of flexible loops, N-terminal and C-terminal. By contrast, TMHs exhibited the lowest fluctuations and appeared stable during the MD simulation.

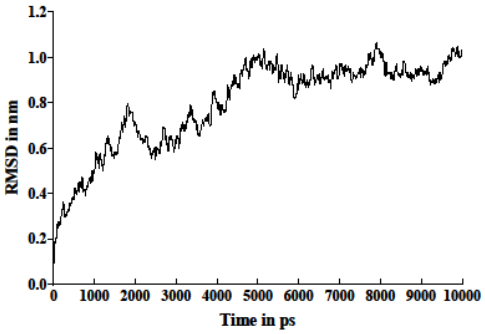

Figure 5.
CB2 backbone root mean squared deviation (RMSD) graph

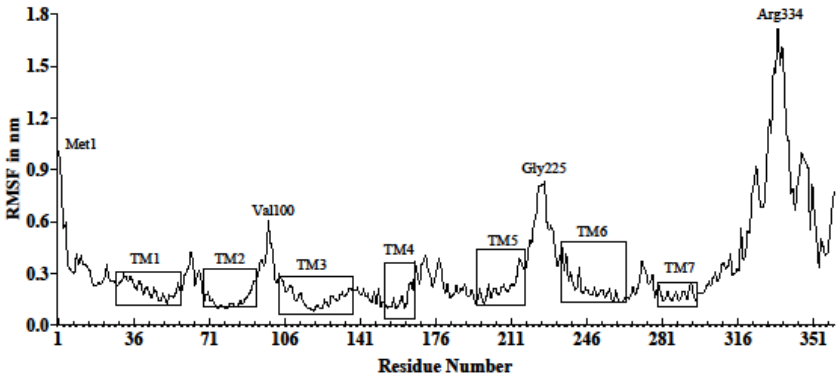

Figure 6.
Residue-specific RMSF during the MD simulation

Cannabinoid ligands docking

The best CB2 model was used with the fewest restraints violations and lowest value of the pdf from 20 models obtained with MODELLER. Docking was carried out on two selective CB2 antagonists to investigate the characteristics of CB2 binding site (Figure 7). Generally, the interactions between ligand and protein are mediated by specific aromatic interactions and hydrogen bond interactions [29]. So, aromatic interactions and hydrogen bond interactions were used as the major criterion for analysis. This binding site was computed by ligand protein contact (LPC) server [30]. Table I illustrated the key LPC data predicted for ligands as well as the *ki* values and the binding free energy. Docking results exhibited that the CB2 active site composed of the TM2-TM3-E2-TM5-TM6-TM7 region. The hydrogen bond interactions and aromatic interactions were predicted by LPC for the docking. The docking studies are consistent with the following binding free energy results.

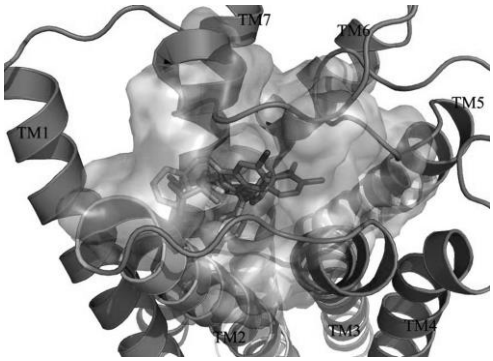

Figure 7.
Binding mode of AM630 and SR144528 with CB2. The compounds are represented as sticks.

Table I
The docking results of AM630 and SR144528 with CB2

Ligand	Hydrogen bond	Aromatic–aromatic contact	binding free energy (kJ/mol)	*Ki* (nM)
AM630	O(CO)-Phe87	Tyr25, Phe87, Phe106, Phe183, Tyr258, Phe281	-174.8	32.1 [11]
SR144528	N(1)-Ser285 N(2)-Ser285	Phe87, Phe183, Tyr258, Phe281	-185.7	0.6 [10]

Binding free energy analysis

The binding free energy of AM630 is -174.8 kJ/mol predicted by G_MMPBSA, composed of electrostatic energy -20.8 kJ/mol, van der Waals energy -242.8 kJ/mol, SASA energy -24.1 kJ/mol and polar solvation energy 112.8 kJ/mol, while the predicted value of SR144528 is -185.7 kJ/mol, composed of electrostatic energy -19.2 kJ/mol, van der Waals energy -259.6 kJ/mol, SASA energy -27.1 kJ/mol and polar solvation energy 120.2 kJ/mol. Generally the more potent ligands show, the lower binding free energy protein-ligand complexes display. But there is not the linear relationship between biological activity and binding free energy. So, the most significance is the intermolecular van der Waals contribution. The result is in agreement with the fact that the large hydrophobic binding surface was produced between compounds AM630 and SR144528 with CB2. Following the work of Gohlke *et al.* [31], the decomposition of binding free energy, called per-residue decomposition, was performed using g_mmpbsa tool in order to further study the ligand-protein interactions (Figure 8).

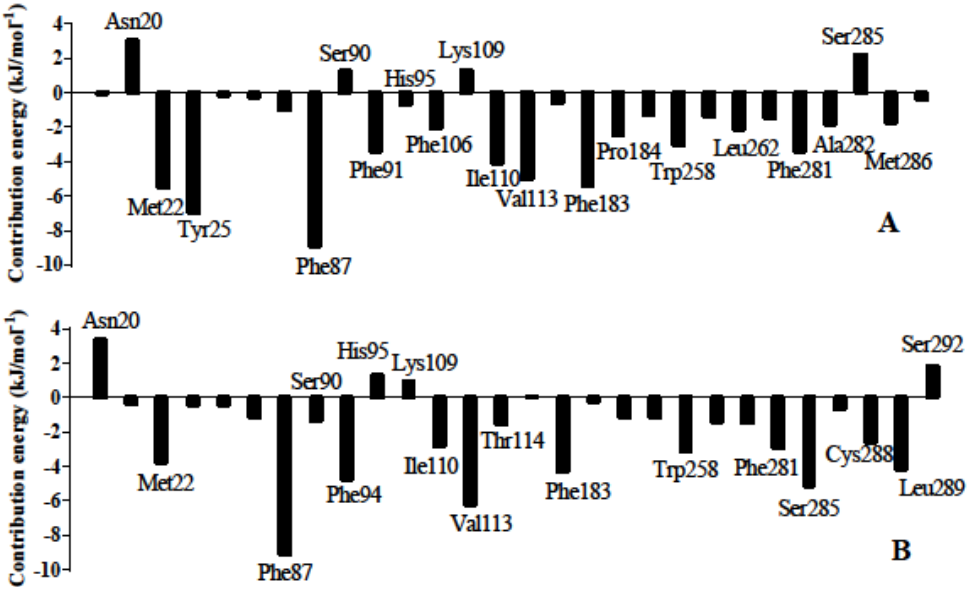

Figure 8.
Energy contribution of each residue to the binding of AM630 (A) and SR144258 (B) with CB2 active site

The binding free energy decomposition analysis exhibits that the common contributions are residues Met22, Phe87, Ile110, Val113, Phe183, Trp258, Phe281 with the binding for compounds AM630 and SR144528. The docking and binding free energy analysis is similar to what Xie and co-workers found that residues, val113, phe183 and phe281, are important for ligand binding to CB2 [32]. Residues Ser90 and Ser285 are unfavourable for binding AM630, while are favourable for binding SR144528. On the contrary, residue His95 is in disfavour for binding SR144528 and in favour for binding AM630.

Conclusions

The 3D model of the CB2 was built on the basis of the highest resolution structural template of CB1. The interactions of the protein–ligand complexes are investigated by molecular docking and MD simulations, then analysed based on aromatic interactions, hydrogen bond interactions and binding free energy analysis. The studies showed that the binding process between ligand and CB2 is governed by hydrogen bond interactions and aromatic interactions. Because the cannabinoid receptors are important targets, the results reported here are very important for the searching of new selective and potent cannabinoid ligands. The design of novel cannabinoid ligands is in progress.

Acknowledgement

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Conflict of interest

The authors declare no conflict of interest.

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