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Extra precision docking studies of novel luteolin analogues for the inhibition of Tankyrase II: A theoretical-based approach toward novel cancer target

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ABSTRACT

Introduction: Luteolin, a natural flavonoid is known to possess variety of pharmacological activity, particularly growth inhibition. Reports are available to substantiate the interaction of luteolin with tankyrase II, one of the key enzymes responsible for sustenance of telomeres and involved in promoting cellular function. Objective:In our study, we have identified synthetic analogues of luteolin from a chemical compound database (E molecule). After suitable modifications of these analogues, we have screened them for their binding affinity for the active site of tankyrase II (PDB ID: 4HKN). **Methods:** 15 analogues were subjected to the molecular docking process, which was executed using Glide™ module in Maestro Molecular Modeling platform (version 10.5) from Schrodinger, LLC, using both the standard precision (SP) as well as extra precision (XP) mode. Further, we also attempted to assess the ADME toxicity profiles of the compounds using QikProp application from Schrodinger, LLC. **Result:** In the present study, we have successfully identified 3 analogues (flav 10, flav 11, flav 12) which exhibited comparable dock scores (-10.02 -10.438 and -10.083 kcal/mol respectively) when compared to the standard luteolin (-11.472). Further, these 3 analogues also demonstrated favourable pharmacokinetic profiles as compared to luteolin. **Conclusion:** Therefore, the present study will provide insight for further structural modifications and aid in generating a suitable scaffold for enhanced binding affinity to tankyrase II. Future perspectives reside in attempting their synthesis, *in vitro* and *in vivo* evaluation to develop a novel anticancer agent.

INTRODUCTION

Flavonoids are naturally occurring group of secondary metabolites with variable phenolic functional groups found in various parts of plants.[1] They are biosynthesized in plants from the aromatic amino acid precursors such as phenylalanine and tyrosine.[2] Luteolin, a tetrahydroxyflavone, is found extensively in plants and has drawn much attention in the recent times owing to its diversified activities. Structure-activity relationships studies on naturally occurring flavonoids have revealed that the hydroxy substitution and 2−3 alkenic double bond contributes to the salient structural features in luteolin, which is responsible for its biological properties.[3] Further, plants containing luteolin have also been used in traditional remedies for the treatment of cancer.[4] The antiproliferative effect of luteolin is associated with its capacity to induce apoptosis, suppressing metastasis, and angiogenesis. It is found to cross blood-brain barrier, rendering it as a promising candidate as central nervous system drug, in particular, brain tumors.[5]

In recent times, Tankyrase I and Tankyrase II have become potential targets for cancer. Much attention has been drawn on the fact that Tankyrase inhibition negatively regulates the wnt/beta-catenin pathway in colon cancer cells resulting in axin stabilization. The Tankyrases get overexpressed in several human cancers, including breast cancer,[6] colon cancer,[7] chronic myeloid leukemia,[8] brain tumors,[9] and gastric[10] and

bladder cancers.[11] The highly homologues human Tankyrase isoforms, Tankyrase I and Tankyrase II, belong to poly (ADP-ribose) polymerase (PARP), a proteins that share a common catalytic domain.[12] Tankyrase I and II, share 85% sequence homology.[13,14] PARP catalyzes the formation of poly (ADP-ribose) on a cellular response to DNA damage.[15] In humans, they play an important role in maintenance of telomeres. Telomeres are important components, residing at the end portion of chromosomes, that consist of associated proteins, that play a crucial role in cellular responses, apoptosis, and tumorigenesis.[16,17] PARP catalyzes the formation of poly (ADP-ribose) on a cellular response to DNA damage.[15]

In a study, to determine the Tankyrase inhibition potential of flavones, Narwal *et al*. have reported luteolin to have an IC50 of 2.4 μM against Tankyrase I, but with an IC50 of 1.1 μM against Tankyrase II.[18] This gives a clear indication that luteolin is more active against Tankyrase II. Therefore, on the basis of literature reports, we have designed novel luteolin analogues and studied their binding affinity at the active site of Tankyrase II. The extra precision (XP) docking approach used in this study is one of the most accurate docking protocols available with Schrödinger molecular design suite. It utilizes longer time compared to other docking protocols such as standard precision and high throughput docking protocols. It gives us an idea about the rewards and penalties. A drug designer can carefully use this information to design potent analogues.

MATERIALS AND METHODS

All the docking simulations were performed with maestro molecular modeling platform (version 10.5) from Schrödinger, LLC.[19]

Protein Preparation

The luteolin bound X-ray crystal structure (protein data bank [PDB]ID: 4HKN) of Tankyrase II was retrieved from PDB with a resolution of 2A°. The enzyme is further refined by protein preparation wizard.[20] The protein preparation process has two major steps including preparation and refinement. The preparation component adds missing hydrogen adds missing residues and caps terminals. It assigns proper bond orders. Optimized potential for liquid simulations (OPLS) - all atom force field was used for this purpose and then active site of protein was defined. In the next step, the water molecules were removed (except near the active site) and hydrogen atoms were added. Then, the disulfide bridges were created. Missing chains and missing loops were filled using prime. Water molecules were deleted beyond 5å units.

Ligand Preparation

The designed ligands were prepared using ligprep tool.[21] OPLS 2005 was used as force field. The ionization states were generated at the pH between 7 and 23 using EPIK application. The structures were desalted and the possible tautomers were generated. Specified chiralities were fixed and the low-energy ring conformations were generated per ligand. In this study, a series of 15 luteolin derivatives were employed in the docking process [Figure 1].

Ligand Alignment

Ligand alignment is one of the prerequisites for generating better docking poses. The generated ligands were aligned using flexible ligand alignment. The aligned ligands were presented in Figure 8.

Glide™ Docking

Docking is a two-step process; in the first step, the receptor grid was defined. In the next step, the actual docking of analogues to the active site of receptor was carried out. The ligand docking process utilizes Glide™ program from Schrödinger (grid-based ligand docking with energetics).[22] Glide™ identifies most significant interactions between one or more chemical entities and a receptor, usually a protein. Glide™ was operated in rigid as well as flexible modes. The grid generated was represented by different fields around and inside the ligand binding cavity. Receptor grid was generated from grid generation panel available under glide utility. The electrostatic and Vander Waals potentials of the binding pocket were calculated using grid-based method. In the second step, the actual docking of analogues at the binding site took place. For this purpose, the option XP docking method was used. The XP Glide method semi-quantitatively ranks the ability of ligands to bind to a bioactive conformation of the protein receptor.

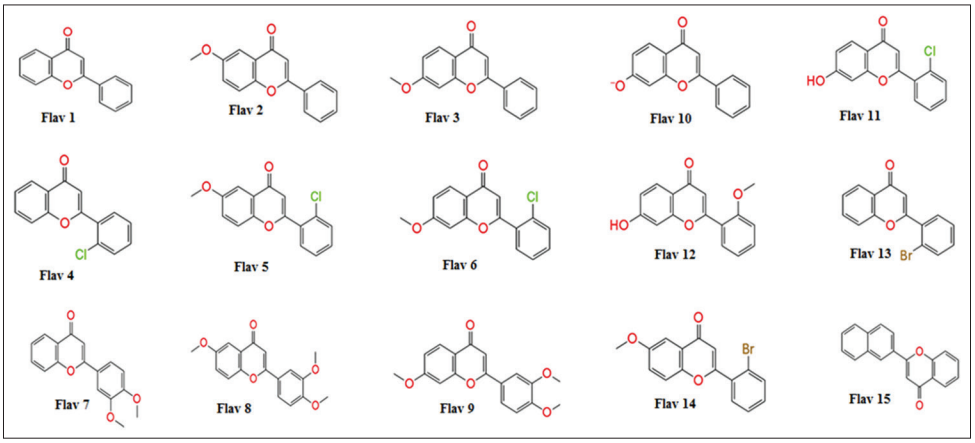

Figure 1: Structures of the designed luteolin analogues

Validation of Docking Process

The most suitable accurate method of evaluating the accuracy of a docking protocol is to determine how closely the lowest energy pose predicted by the scoring algorithm resembles an experimental binding mode as evidenced by X-ray crystallography. After the receptor grid generation,[23] the luteolin moiety was extracted from 4HKN and it was redocked into the receptor. Using superposition option, both the ligands were aligned and root mean square deviation (RMSD) was measured. The RMSD was found to be 0.3, which was well below the limit of 2.

ADME Toxicity Analysis

The ADME toxicity and kinetic parameters were determined using QikProp application from Schrödinger.[24] The important substitution patterns required for effective binding was determined using site map application from Schrödinger.[25]

RESULTS AND DISCUSSIONS

XP Docking Results

The XP docking results were promising with the emergence of the analogue flav 11 (dock score of -10.438 Kcal/mole) with the dock scores comparable to the standard luteolin (dock score of -11.472 Kcal/mole) [Table 1]. The results showed that luteolin had the ability to form additional hydrogen bonding interactions at the active site of Tankyrase II. Luteolin appeared to have dominant interactions with the amino acids glutamine 1135, glycine 1032, and serine 1028 [Figures 2 and 3]. The incorporation of hydrogen bond donor or acceptor groups might increase the binding affinity at the active site of Tankyrase II. The extra precision rewards and penalties were provided in Tables 2 and 3.

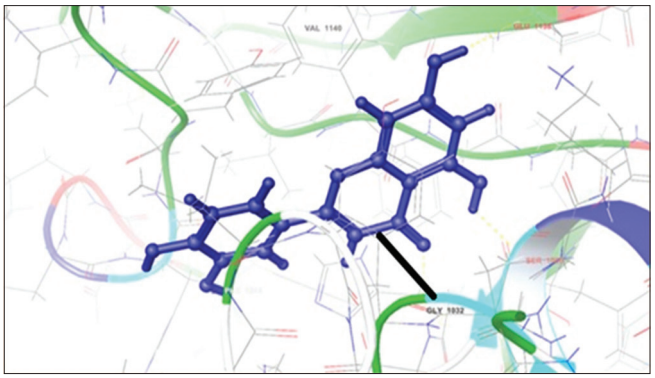

Figure 2: Luteolin hydrogen bonding interactions at the active site of Tankyrase II (protein data bank ID: 4HKN)

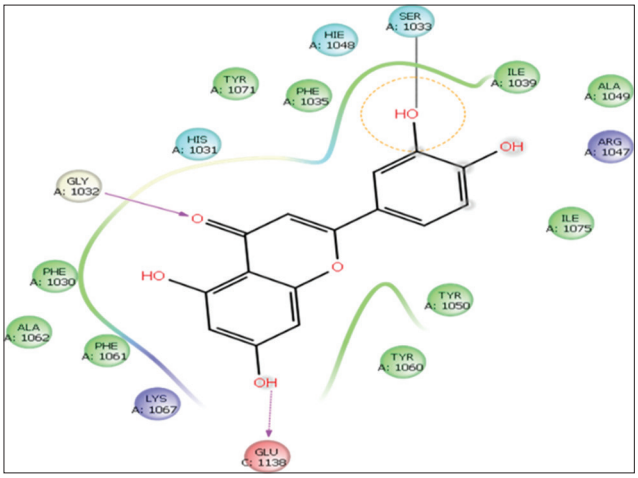

Figure 3: 2D interaction of luteolin at the active site of Tankyrase II (protein data bank: 4HKN)

Table 1: XP docking studies of various luteolin analogues

Title	Docking score (SP) (kcal/mol)	Glide G score (kcal/mol)	Glide E model (kcal/mol)	XP G score (kcal/mol)
Luteolin	−11.472	−11.472	−78.376	−11.472
Flav 11	−10.438	−10.444	−80.528	−10.444
Flav 12	−10.083	−10.089	−56.896	−10.089
Flav 10	−10.02	−10.026	−66.189	−10.026
Flav 15	−9.988	−9.988	−63.832	−9.988
Flav 7	−9.97	−9.97	−61.491	−9.97
Flav 13	−9.856	−9.856	−69.032	−9.856
Flav 4	−9.802	−9.802	−68.256	−9.802
Flav 9	−9.754	−9.754	−74.438	−9.754
Flav 6	−9.549	−9.549	−68.889	−9.549
Flav 1	−9.408	−9.408	−61.566	−9.408
Flav 3	−9.372	−9.372	−56.631	−9.372
Flav 2	−9.318	−9.318	−51.492	−9.318
Flav 5	−7.903	−7.903	−55.001	−7.903
Flav 14	−7.851	−7.851	−51.999	−7.851
Flav 11	−5.811	−8.524	−63.204	−8.524
Flav 8	−5.419	−5.419	−55.582	−5.419

SP: Standard precision, XP: Extra precision

QikProp Results

QikProp is a quick, accurate application used to predict kinetics, and toxicity parameters. QikProp predicts important physical descriptors and pharmacologically necessary properties of organic structures. Along with providing molecular properties molecular, QikProp also provides range for comparing properties of a molecule with those of known drugs. Analysis of QikProp results suggested that the novel luteolin analogues had superior pharmacokinetic profile compared to standard luteolin. The theoretical results should be authenticated by finding experimental data.

Analysis of Site Map Results

The site map gave possible substitution patterns to enhance binding through several interactions including hydrogen bond donor [Figure 4], acceptor [Figure 5], hydrophobic interactions [Figure 6], hydrophilic interactions [Figure 7], and metal binding interactions. Different color schemes were chosen to differentiate the interactions around the ligand binding site.

CONCLUSION

The present study provided us the most active flavone analogue for effective inhibition of Tankyrase II. The substitution required to elicit strong binding affinity was recorded using site map application. The data obtained from ADME toxicity were promising. All the analogues appeared to satisfy the conditions required to have good absorption and lower toxicity. In the future studies, *in vitro* and *in vivo* experiments will be conducted for most active analogues having well dock scores. The most active analogues will be taken for preclinical studies. Overall the study gave us insight about the exact binding modes of luteolin analogues at the active site of Tankyrase II.

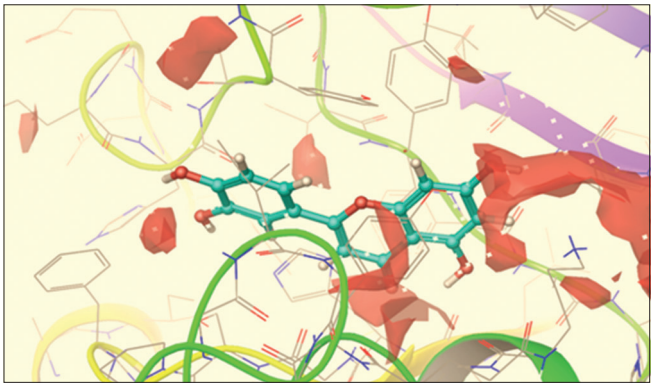

Figure 4: Site map for hydrogen bond donor interactions

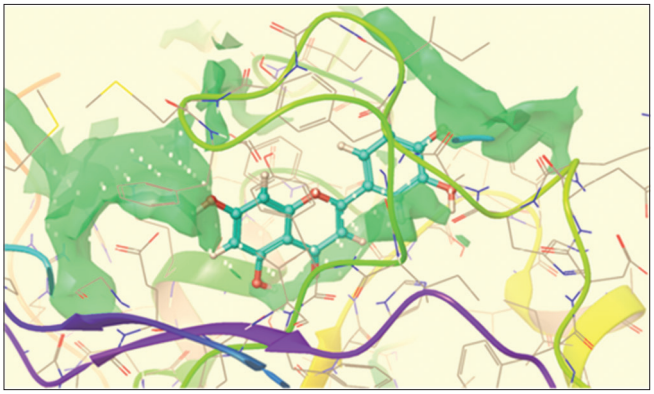

Figure 5: Site map for hydrogen bond acceptor interactions

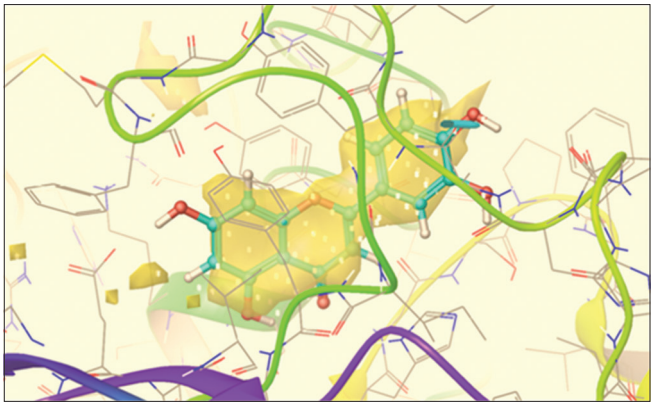

Figure 6: Site map for hydrophobic interactions

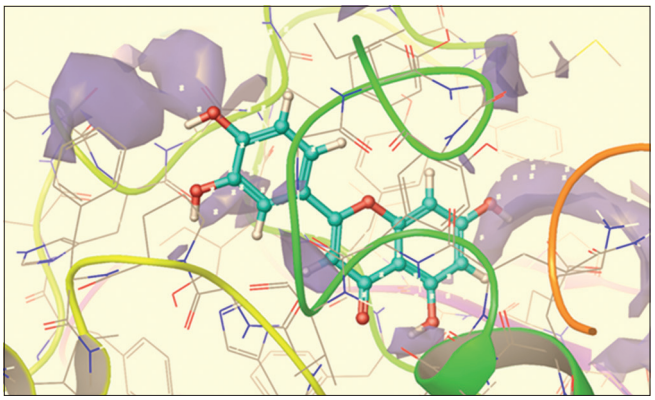

Figure 7: Site map for hydrophilic interactions

Figure 8: Alignment of active ligands

Table 2: Details of extra precision docking rewards of various luteolin analogues

Ligand	Lipophilic EvdW	PhobEn	PhobEnHB	PhobEnPairHB	HBond	Electro	PiCat	ClBr	LowMW
Luteolin	−6.15	−1.34	−1	0	−2.37	−0.86	0	0	−0.5
Flav 10	−5.93	−1.63	−1	0	−1	−0.67	0	0	−0.5
Flav 7	−6.4	−1.5	−1	0	−0.7	−0.31	0	0	−0.5
Flav 11	−6.08	−1.19	−1	0	−1	−0.65	0	0	−0.5
Flav 4	−6.16	−1.67	−1	0	−0.7	−0.27	0	0	−0.5
Flav 9	−6.49	−1.38	−1	0	−0.7	−0.23	0	0	−0.46
Flav 12	−5.91	−1.37	−1	0	−0.99	−0.35	0	0	−0.5
Flav 3	−6.28	−1.33	−1	0	−0.7	−0.15	0	0	−0.5
Flav 1	−5.78	−1.74	−1	0	−0.7	−0.24	0	0	−0.5
Flav 2	−5.9	−1.48	−1	0	−0.7	−0.26	0	0	−0.5
Flav 8	−6.28	−0.73	−1	0	−0.7	−0.32	0	0	−0.46
Flav 6	−6.58	−1.33	−1	0	−0.7	−0.26	0	0	−0.5
Flav 5	−5.61	−1.69	0	0	−0.61	−0.2	0	0	−0.5
Flav 14	−5.23	−0.38	−1	0	−0.7	−0.31	0	0	−0.4
Flav 15	−6.87	−1.47	−1	0	−0.7	−0.28	0	0	−0.5
Flav 13	−6.28	−1.65	−1	0	−0.7	−0.29	0	0	−0.5

Table 3: Details of extra precision docking penalties of various luteolin analogues

Ligand	Penalties	HBPenal	ExposPenal	RotPenal	EpikStatePenalty
Luteolin	0	0	0	0.09	0
Flav 10	0	0	0	0.13	0.01
Flav 7	0	0	0	0.1	0
Flav 11	0	0	0	0.1	0.01
Flav 4	0	0	0	0.11	0
Flav 9	0	0	0	0.08	0
Flav 12	0	0	0	0.11	0.01
Flav 3	0	0	0	0.12	0
Flav 1	0	0	0	0.15	0
Flav 2	0	0	0	0.12	0
Flav 8	0	0	0	0.08	0
Flav 6	1	0	0	0.09	0
Flav 5	0	0	0	0.09	0
Flav 14	1	0	0	0.07	0
Flav 15	4	0	0	0.1	0
Flav 13	4	0	0	0.09	0

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