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MOLECULAR DOCKING STUDY OF SOME ACTIVE PRINCIPLES FROM *SILYBUM MARIANUM*, *CHELIDONIUM MAJUS*, *GINKGO BILOBA*, *GELSEMIUM SEMPERVIRENS*, *ARTEMISIA ANNUA*, AND *TARAXACUM OFFICINALE*

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Abstract. In this study, it was investigated by molecular docking, the interaction of fourteen natural compounds (artemisinin, bilobalide, bilobetin, chelerythrine, chelidonin, epicatechin, gelsemic acid, ginkgolide A, isosilybin, silicristin, silybin, taraxacin, taraxacoside, and taraxinic acid) from *Silbum marianum*, *Chelidonium majus*, *Ginkgo biloba*, *Gelsemium sempervirens*, *Artemisia annua*, and *Taraxacum officinale* with three cancer-related GPCRs: the apelin receptor, the *β*2-adrenoceptor, and the A2B adenosine receptor. QuickVina2 was used to determine the binding affinities and identify the nature of the strongest interactions. Several compounds (bilobetin, isosilybin, chelidonin, silicristin, and artemisinin) showed high binding affinities and interactions with key residues responsible for the receptor activity. These results highlight the potential of phytochemicals in modulating the activity of GPCRs and may form the basis for further experimental validation.

Keywords: natural compound, molecular docking, apelin receptor, *β*2-adrenoceptor, A2B adenosine receptor.

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Introduction

Cancer continues to be among the major problems facing the international health system. Although significant progress has been made in treatment, drug resistance and adverse effects remain a major challenge. In recent years, a promising approach to tackle these problems has been targeting GPCRs. Data from literature show that the inhibition of these receptors can suppress the growth of various cancer tumours and also prevent resistance [1-5].

Simultaneously with these developments, natural products and their derivatives remain an important source of anticancer agents. It was selected here a number of such plants that can provide natural products as potential agents against cancer. *Silybum marianum* is renowned for its hepatoprotective properties, primarily attributed to a group of flavonolignans collectively known as silymarin. Key constituents include silibinin, isosilibinin, silychristin, and others. Advancements in the genetic pathways of these compounds have enhanced our understanding of their therapeutic potential [6]. *Chelidonium majus* contains alkaloids such as chelidonine and chelerythrine. While these compounds have been studied for their biological activities, it is essential to note that *Chelidonium majus* has been associated with cases of hepatotoxicity. Therefore, caution is advised when considering its medicinal use [7]. *Gingko biloba* contains a variety of pharmacologically active compounds, including ginkgolide A, bilobalide, and flavonoids such as bilobetin. These constituents have been extensively studied for their neuroprotective, antioxidant and vasoregulatory effects. However, Gingko may increase bleeding risk, particularly when combined with anticoagulants, and should therefore be used with appropriate clinical caution [8]. *Gelsemium sempervirens* contains indole alkaloids such as gelsemine, gelseminine and gelsemic acid, known for their action on glycine and GABA receptors. These compounds have been explored for anxiolytic, analgesic, and sedative properties. However, the plant exhibits a narrow therapeutic index and toxic effects. Caution is essential due to its potent neurotoxicity [9]. *Artemisia annua* is a rich source of artemisin, a sesquiterpene lactone with well-established antimalarial activity. In addition to artemisinin, the plant contains

flavonoids, phenolic acid, and essential oils that exhibit anti-inflammatory, antioxidant, and antiviral properties. Recent studies also suggest potential anticancer effects through modulation of apoptosis and oxidative stress pathways. Due to its potent bioactivity, standardised extracts are preferred for therapeutic use [10]. *Taraxacum officinale* is characterised by a rich phytochemical profile, including taraxacin, taraxacoside and taraxinic acid. These compounds underline the plant's extensively studied hepatoprotective, anti-inflammatory, antioxidant, diuretic, and anticancer effects, ultimately supporting its potential role in prevention and adjunctive treatment of hepatic, inflammatory, and neoplasic disorders [11].

Many of these natural compounds or their derivatives have been tested for their anticancer activity, which is mainly due to kinase inhibition, apoptosis induction, or antioxidant pathways. In some cases, the *in vitro* or *in vivo* studies were supported by molecular docking. For example, Kai, K. *et al.* have shown that chelerythrine binds strongly to phosphoinositide 3-kinase (–10.50 kcal/mol) and thus inhibits the proliferation of gastric cancer cell [12]. According to Kumari, M. *et al.*, epicatechin gives a good docking score (–10.85 kcal/mol) when interacting with mitogen-activated protein kinase 2, a protein overexpressed in breast cancer [13]. Silibinin in combination with concanavalin A inhibits the synthesis of Janus kinases, although the binding affinity of silibinin to Janus kinases is only –6.46 kcal/mol [14].

However, a possible interaction between these natural compounds and GPCRs has not yet been extensively investigated by molecular docking. Therefore, this study aims to use molecular docking to investigate the binding affinities and interaction profiles of selected natural compounds with three GPCRs that are associated with cancer proliferation and progression: the apelin receptor, the β2-adrenoceptor, and the A2B adenosine receptor.

Theoretical calculations

Preparation of receptor structures

The three-dimensional structures resulting from X-ray crystallography for the apelin receptor, β2-adrenoceptor, and A2B adenosine receptor were retrieved from the Protein Data Bank (PDB). Their respective PDB identification codes are 7SUS [15], 4LDE [16], and 8HDO [17].

The initial preparation of these structures for molecular docking studies was performed using Python Molecular Viewer(part of MGL Tools, version 1.5.7). The preparation steps included the removal of water molecules, any co-crystallized ligands and ions present in the X-ray structures, as well as the addition of hydrogen atoms, which are typically absent in X-ray data due to their low electron density.

Preparation of ligand structures

The structures of the ligands (artemisin, bilobalide, bilobetin, chelerythrine, chelidonine, epicatechin, gelseminic acid, ginkgolide A, isosilybin, silicristin, silybin, taraxacin, taraxacoside, and taraxinic acid) were retrieved from the PubChem [18] database in 3D format. Prior to docking studies, these ligands were protonated followed by energetic minimization using Open Babel [19] version 3.0. Protonation was performed using the MMFF94 force field in Open Babel with default parameters at physiological pH (7.4).

Molecular docking

Molecular docking studies were conducted using QuickVina2, a widely employed molecular docking software. QuickVina2 utilizes an efficient algorithm for ligand-receptor docking, based on the AutoDock Vina program. The main steps in our procedure are outlined below:

Preparation of receptor grid

Grid boxes were defined around the binding sites of the receptors, encompassing key residues known to be involved in ligand binding based on literature review and structural information of the previous bound ligands [20-22]. Care was taken to allow sufficient box size in order to permit the ligands to correctly explore their degrees of freedom.

Docking procedure

Each ligand was docked into the binding site of the respective receptor using QuickVina2. During docking, ligand flexibility was allowed to explore various conformations, while the receptor was held rigid. The docking procedure was performed in triplicate in order to reduce the stochastic component of the scoring function and the average predicted affinities were calculated. Each docking run was repeated three times independently for every ligand–receptor pair to account for stochastic variation in the scoring function

Scoring and analysis

Docking poses generated by QuickVina2 were scored based on their binding affinity (expressed in kcal/mol), which represents the predicted strength of interaction between the ligand and receptor [23]. The docked complexes were then visually inspected to assess the orientation and interactions of the ligands within the binding sites.

Results and discussion

The two-dimensional molecular structures of the 14 ligands used in this study are given in Figure S1 (see supplementary information). Their interaction with GPCRs was characterised by molecular docking. Molecular docking is a computational tool designed to find the most stable complex between a small molecule and its receptor. It is based on a search algorithm that generates systematic changes within the position and conformation of the small molecule in the receptor pocket. The resulting complexes are evaluated based on so-called scoring functions: the more negative the scoring function, the more stable the complex will be.

The binding affinities of each compound with the three receptors are presented in Table 1. Bilobetin showed the highest affinity for both A2B adenosine (–10.1 kcal/mol) and apelin receptors (–10.4 kcal/mol), while isosilybin had the strongest interaction with the *β*2-adrenoceptor (–10.2 kcal/mol). Generally, values of the binding affinity around –10 kcal/mol indicate strong interactions between the ligands and their respective receptors.

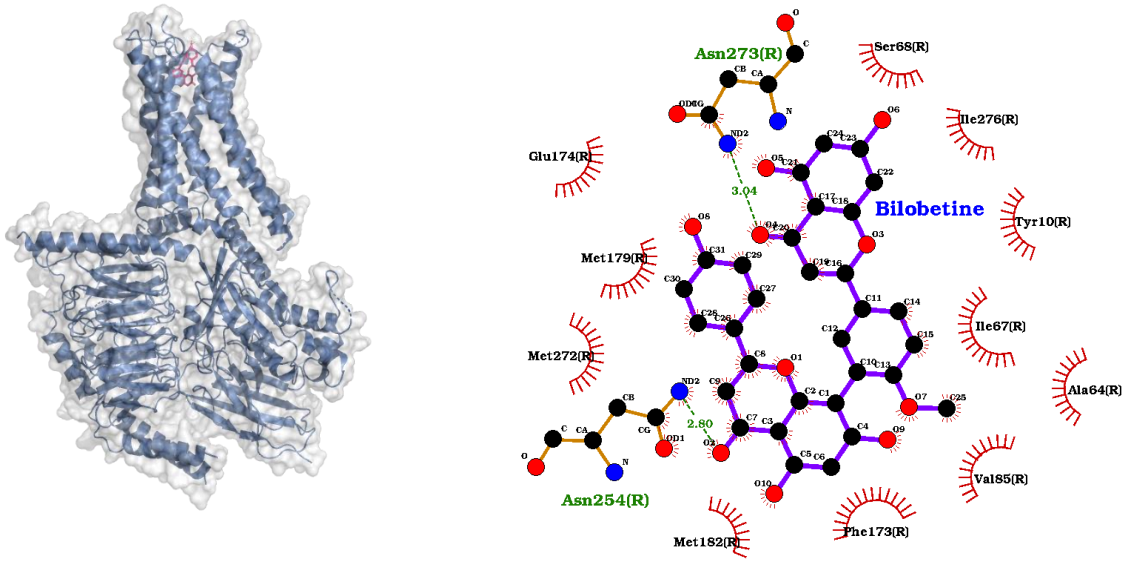
The structures of the most stable ligand-receptor complexes and their key interactions are illustrated in Figures 1, 2 and S2. From Figure 2, it can be observed that the interaction between the A2B adenosine receptor and bilobetin is mediated by two hydrogen bonds with asparagine residues 273 and 254, as well as by numerous hydrophobic interactions involving other amino acids (Glu174, Tyr10, Ile67, Ser68, among others). Among the types of interactions that can occur between two molecules, hydrogen bonds are the strongest. Hydrophobic interactions occur between nonpolar fragments of both molecules, which tend to remain as close as possible to each other while avoiding contact with water molecules. The stability of a ligand-receptor complex is largely attributed to this combination of hydrogen bonding and hydrophobic interactions. Temirak, A. *et al.* reported similar interactions for *p*-(1-propylxanthin-8-yl)benzene sulphonyl fluoride, an irreversible antagonist of the A2B adenosine receptor: hydrogen bonds with Asn254, Asn273, Ser68 and hydrophobic contacts with the Ile67 residue [24].

In the case of the *β*2–adrenoceptor, the stability of the complex with isosilybin is due to hydrogen bonds with three amino acids: Tyr1316, Asp1300, and Asp1192, as well as numerous hydrophobic interactions with His1093, Asp1113, Trp1109, Phe1193, Phe1194, His1178, Glu1180, Lys881, His1296, Lys1305, Ile1309, Asn1312, and Tyr1308 (Figure 2). By combining molecular docking and machine learning, Jimenez-Roses, M. *et al*. identified the key molecular interactions to distinguish what determines the agonist and antagonist function of ligands that bind to the *β*2-adrenoceptor [25]. Thus, they found that to be a *β*2-adrenoceptor agonist, the ligand must interact with residues Lys1097, Phe1194, Ser1203, Ser1204, Ser1207, Trp1286, and His1296, whereas for a *β*2-adrenoceptor antagonist, interactions with residues Lys1305 and Tyr1316 are important. A comparison with the results obtained for isosilybin shows that, among the residues mentioned, isosilybin interacts with Tyr1316, His1296 and Phe1194. The strongest interaction is through hydrogen bonding with Tyr1316, suggesting that isosilybin may be a *β*2-adrenoceptor antagonist.

Tabel 1

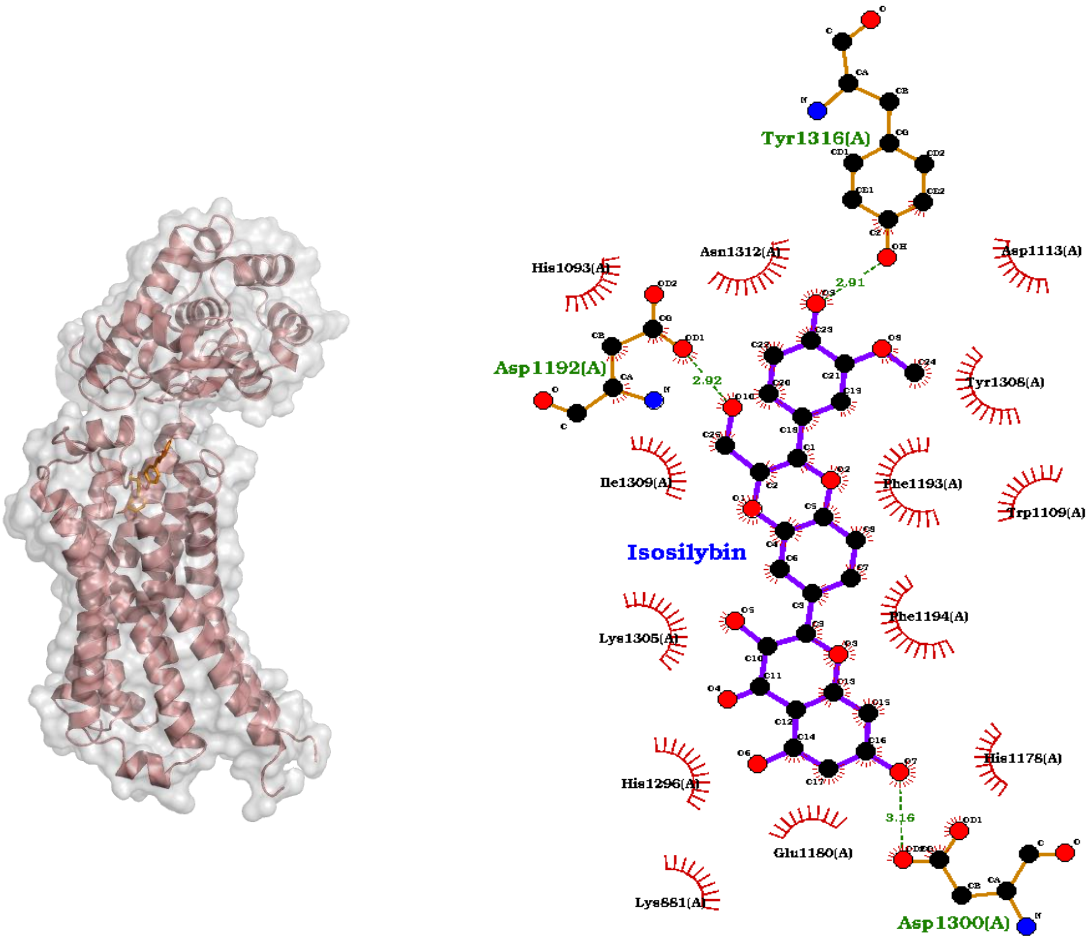
Affinities for some of the compounds studied for each of the receptors.

Adenosine receptor A2B		*β2-adrenoceptor*		*Apelin receptor*	
Ligand	*Affinity (kcal/mol)*	*Ligand*	*Affinity (kcal/mol)*	*Ligand*	*Affinity (kcal/mol)*
Bilobetin	–10.1	Isosilybin	–10.2	Bilobetin	–10.4
Chelidonine	–9.7	Silicristin	–10.2	Chelidonine	–9.4
Silybin	–9.4	Artemisinin	–9.4	Epicatechin	–8.7
Chelerythrine	–9.3	Taraxacoside	–9.4	Isosilybin	–8.3
Artemisinin	–9.1	Bilobetin	–9.1	Silicristin	–8.3
Isosilybin	–9.1	Epicatechin	–8.6	Silybin	–8.2
Silicristin	–9.1	Silybin	–8.6	Taraxacoside	–8.1
Taraxacin	–8.7	Chelidonine	–8.4	Chelerythrine	–8.1
Taraxacoside	–8.6	Taraxacin	–8.3	Artemisinin	–8.0
Taraxinic acid	–8.6	Chelerythrine	–8.0	Taraxacin	–8.0
Bilobalide	–8.5	Bilobalide	–7.9	Ginkgolide A	–7.3
Ginkgolide A	–8.3	Taraxinic acid	–7.8	Taraxinic acid	–7.1
Epicatechin	–8.0	Gelseminic acid	–7.3	Gelseminic acid	–6.7
Gelseminic acid	–6.6	Ginkgolide A	–7.2	Bilobalide	–6.6



(a) *(b)*

Figure 1. Structure of the most stable bilobetin–A2B adenosine receptor complex *(a)*. Interactions between bilobetin and the amino acid residues within the binding site *(b)*.
(green indicates residues involved in hydrogen bonds with bilobetin, with distances shown in angstroms; red highlights hydrophobic interactions).



(a) *(b)*

Figure 2. Structure of the most stable isosilybin–*β*2–adrenoceptor complex *(a)*. Interactions between bilobetin and the amino acid residues within the binding site *(b)*.
(green indicates residues involved in hydrogen bonds with bilobetin, with distances shown in angstroms; red highlights hydrophobic interactions).

The interaction between the apelin receptor and bilobetin is facilitated by a hydrogen bond with Tyr264, as well as several hydrophobic interactions: Trp85, Tyr93, Tyr271, Ile109, Phe110, Val267, Lys268, Met288, Thr295, Phe291, and Tyr299 (Figure S2, supplementary information). Recently, a study by Fadhillah, M.R. *et al.* on the interaction of some natural compounds from Indonesian medicinal plants with the apelin receptor indicated binding to the same site [20]. Although none of the top five compounds in their study showed hydrogen bonds with Tyr264, they mentioned interactions with many amino acids with which bilobetin also interacts. For example, gambogic acid interacts with Trp85, Ile109, and Phe 110, procyanidin B2 interacts with Ile109 and Phe291, azelaprag interacts with Trp85 and Ile109, asiaticoside interacts with Ty264, Lys268, Met288, and Thr295, procyanidin B1 interacts with Tyr264, while dihydrocurcumin interacts with Tyr264, Met288, Phe291, and Tyr299.

All these interactions suggest that bilobetin and isosilybin may be valuable molecular scaffolds for future drug development targeting these GPCRs. For the other compounds, besides bilobetin and isosilybin, it was found that they bind at the same sites, but the interaction is weaker, which is reflected in a lower affinity.

Conclusions

This study demonstrates, through computational evaluation, that fourteen natural compounds interact with three G protein-coupled receptors (GPCRs)-the apelin receptor, β2-adrenoceptor, and A2B adenosine receptor-all of which play central roles in cancer biology. Molecular docking highlights bilobetin, isosilybin, chelidonin, silicristin and artemisinin as promising compounds. They have a high binding affinity and interact with the key residues responsible for the activity of these receptors. By comparing our results with the molecular docking results that exist in the literature for other compounds, it was possible to deduce that isosilybin could be a β2-adrenoceptor antagonist. Antagonism of a ligand-receptor pair is a desirable property in cancer therapy when the receptor is involved in immunosuppression and tumour growth. Further *in vitro* and *in vivo* studies are essential to validate the potential of these compounds for anticancer drug development.

Supplementary information

Supplementary data are available free of charge at http://cjm.ichem.md as PDF file.

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