In Silico Molecular Docking Analysis of the Potential role of Reticuline and Coclaurine as Anti-colorectal Cancer Alkaloids

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ABSTRACT

Background: Colorectal cancer (CRC) is a serious global epidemic, being the third most prevalent cancer worldwide, finding novel treatment alternatives for CRC is thus of the greatest importance. The atomic level interaction between a tiny molecule and a protein can be represented using molecular docking. Molecular docking is critical for visualizing ligand-protein interactions at the atomic level highlighting our knowledge of ligands behavior, which aids in the development of structure-based drugs. Methods: We used molecular docking to investigate the anticancer activity for two main ligands (reticuline and coclaurine) and four potential anticancer receptors (TNIK, VEGFR, EGFR and AKT2). Protein Data Bank provided the 3D structures of the receptor proteins, iGEMDOCK and AutoDock vina program were used for molecular docking. Results: Reticuline had the best docked postures and the highest interactive energy with CRC receptors: TNIK, VEGFR, EGFR and AKT2 with the following binding energy; -96.7, -117.8, -120.2, and -108.3 kcal/mol accordingly. Conclusion: According to this study, the investigated ligands were successfully docked onto reticuline and coclaurine ligands for drug interaction studies, the calculated binding energy demonstrate their importance as an anti-carcinogenic target. The current findings lay the groundwork for further research into reticuline and coclaurine as a potential CRC therapeutic option.

Keywords: Alkaloids; Colorectal Cancer; Coclaurine; *In Silico*; Molecular Docking; Reticuline.

1. INTRODUCTION

Colorectal cancer (CRC) is the third most frequent disease diagnosed and the second largest cause of cancer mortality globally, with over 1.9 million new cases and 935,000 fatalities expected in 2020 [1,2]. The prognosis of CRC is relatively dismal, with the patient's fate determined by the degree of local and metastatic tumor dispersion. Combined with advances in the detection and treatment of human CRC during the last decades, this disease remains one of the world's most difficult health issues [3]. Large comprehensive proteome and genomic studies of CRC have also been performed, resulting in the discovery of CRC subtypes, cancer antigens, therapeutic targets, and major signaling pathways linked to CRC development [4].

TNIK (Nck-interacting kinase) belongs to the germinal center kinase family. TNIK was discovered to be a kinase that regulates cytoskeletal structure in several different kinds of cells, and it was latterly suggested as a new curative candidate in many kinds of human malignancies [4]. While earlier research indicates that TNIK has an important furcation in tumor cell survival and predicting treatment, its intervention in hematological tumor cell survival has not been explored [5]. VEGFR-2 is tyrosine kinase receptor expressed in endothelial cells. VEGFR-2 is a main factor in anti-angiogenesis and a potent inhibitor of tumor cell growth and metastasis [6]. Angiogenesis inhibitors block the activities of vascular endothelial growth factor receptors (VEGFR-1, VEGFR-2, and VEGFR-3) in downstream signaling pathways [7]. Preventing VEGFR-2 in tumor cells was discovered to start and expedite apoptosis, which simultaneously enhances the anticancer impact [8]. The Epidermal Growth Factor Receptor (EGFR) is a tyrosine kinase receptor that becomes active following the acquisition of different driver mutations within the kinase domain, causing aberrant cell replication. EGFR is a key indicator for treatment strategies since it is one of the most important goals for kinase inhibition in non-small cell lung tumors [9]. Akt, or protein kinase B, a serine–threonine kinase, has been elucidated in several types of human tumors, involving gastrointestinal, pulmonary, breast, ovarian, head and neck, prostate and thyroid tumors and may be involved in carcinogenesis [10].

Alkaloids have long been identified as vital secondary metabolites belongs to phytoconstituents with a variety of biological

characteristics [11]. The term "alkaloids," is derived from the Arabic name al-qali, which is linked to the plant from which soda was originally extracted [12]. Due to their wide range of physiological and pharmacological properties such as antibiotics and anticancer, as well as their potential exploitation as narcotics, poisons, and stimulants, alkaloids have had a huge effect on human history, with about 12,000 alkaloids are isolated from different genera of the plant kingdom [11,13].

Reticuline (Fig 1.A) and coclaurine (Fig 1.B) are alkaloid's chemical compounds that occur naturally and extracted in a wide range of plants [14–19].

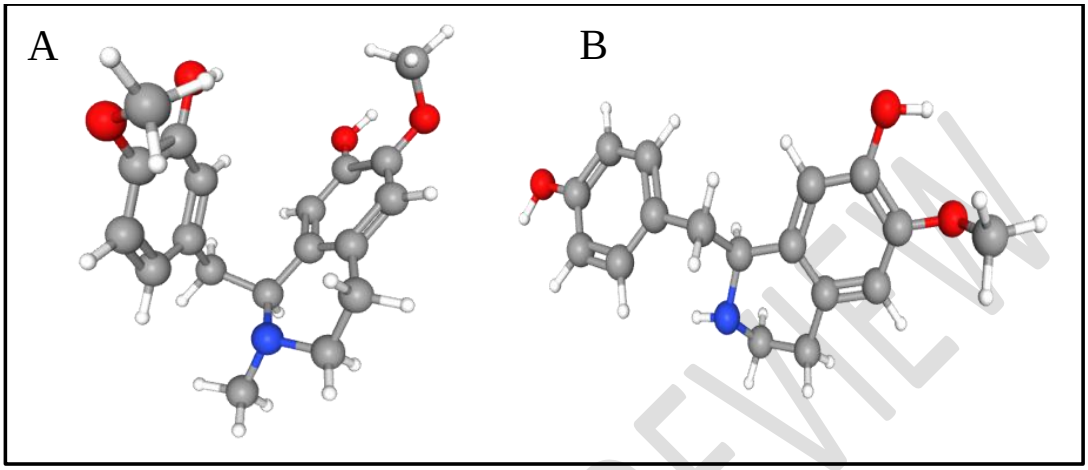

Figure 1. Chemical Structure of (A) Reticuline and (B) Coclaurine [20]

An *in silico* experiment is one that is carried out on a computer or through computer simulation in biology and other experimental sciences [21]. The term refers to silicon in computer chips and is pseudo-Latin meaning 'in silicon' (in Latin, it would be *in silico*). In 1987, it must have been developed as a play on the Latin terms *in vivo, in vitro, and in situ*, which are usually utilized in biology (especially systems biology) [22]. *In silico* medical research has the ability to accelerate the rate of innovation, minimizing the requirements for costly laboratory efforts and new treatments. One method to accomplish this is to increase the efficiency with which drug candidates are produced and screened [23]. Researchers discovered possible inhibitors to an enzyme related with malignancy activity *in silico* and used the protein docking method EADock [24].

This approach varies from the use of delay and cost overrun screening (HTS) robotic labs that physically test hundreds of different compounds per day, with a predicted hit rate of 1% or fewer, and even lower projected to be genuine leads after additional examination (like drug discovery) [25]. It has been attempted to develop computer models of cellular behavior. Researchers, for illustration, constructed an in silico model of tuberculosis to assist in medicinal development in 2007, with the major purpose of being better than meaningful predicted growth rates, providing phenomena of importance to be detected in minutes but instead of months [26].

The molecular docking methodology may be used to represent the atomic level interaction between a small molecule and a protein, allowing us to define novel molecular behavior in target protein binding sites as well as elucidate key biochemical pathways [27]. The lock-and-key theory proposed by Fischer [28], in which the ligand fits into the binding site like a lock and key, was the first explication of the ligand-receptor binding phenomenon. The initial docking approaches [29] were highlighted in this section, and the ligand and receptor were both considered as rigid entities. The "induced-fit" theory [30,31] proposed by Koshland extends the lock-and-key theory by claiming that as ligands engage with the protein, the active region of the protein is constantly altered by interactions with the ligands. Molecular docking has been utilized to identify potential inhibitors for numerous disease's receptors and associated pathways, including but not limited to; inflammation, viral infection, Alzheimer's disease, cardiovascular disease [32–37] and various cancer types [38–42].

In this research we used molecular docking approach to analyze and visualize ligand-protein interactions between (reticuline and coclaurine) and CRC receptors: TNIK, VEGFR, EGFR and AKT2.

2. MATERIALS AND METHODS

This study was carried out in the computer laboratories at king Abdulaziz University during 2020-2021.

2.1 Molecular Docking Analysis

The docking procedure consists of two main steps: predicting the ligand structure including its location and orientation within certain sites (known as pose) and determining the binding affinity. Knowing where the binding site would be before starting this same docking process improves docking efficiency dramatically. Throughout many cases, the target protein is identified already when ligands are docked into it. Also, by comparing the target protein to a group of proteins with comparable functions or proteins co-crystallized with other ligands, one can learn more about the locations.

Docking energy is utilized to pick the lowest-energy pose(s) among a huge variety of conformations created for each molecule. While binding energy is released when a drug molecule binds to a target, decreasing the total energy of the complex, the higher the energy released when a ligand binds to a protein, the greater the tendency of the ligand to associate with that protein [43].

2.2 Selection of Receptors

The receptors used in this investigation for performing In-silico studies, the receptors were chosen based on their physiological roles and pathways. The receptors for the current research were chosen based on the published target locations of ligands (Table 1). These receptors three-dimensional structures were obtained from the PDB (Protein Database) [44]. These receptors pathways were investigated utilizing KEGG (Kyoto Encyclopaedia of Genes and Genomes) pathways [45].

Four different types of receptors have been selected to perform this experiment, which mainly includes TNIK protein (Nck interacting kinase), VEGFR (Vascular endothelial growth factor), EGFR (Epidermal growth factor receptor), and AKT2 (Protein kinase B).

Table 1: Potential therapeutic targets from a variety of CRC receptors for structure-based drug screening*.

Gene name	Receptor name	PDB id
TNIK	Nck Interacting kinase	5AX9
VEGFR	Vascular endothelial growth factor	3VHK
EGFR	Epidermal growth factor receptor	1XKK
AKT2	Protein kinase B	1MRV

*Source: PDB (Protein Database)

2.3 Selection of ligands

For this study, ligands belong to alkaloid phytochemicals were screened with the specific anti-cancer receptor. Two alkaloids were selected for this study (reticuline and coclaurine) because of the highest binding energy exhibited by them. Chemspider database and PDB (Protein Database) [46] were used to obtain the structures of reticuline and coclaurine. The pKCSM (Prediction of small-molecule Pharmacokinetics and Toxicity) programme was used to screen the pharmacokinetic features of the various ligands. The absorption, distribution, metabolism, and excretion features of every substance are defined by its pharmacokinetics profile [47]. Many tools are accessible online for forecasting a compound's pharmacokinetics and toxicological qualities depending on the chemical structure or composition, spanning from data-based approaches as QSAR (Quantitative Structure-activity Relationship), identical studies [48,49], and 3-dimensional QSAR [50-54].

2.4 Multi-Receptor Docking

The anticancer (colorectal tumour) capabilities of the selected reticuline and coclaurine were predicted using multi-receptor docking. iGEMDOCK and AutoDock vina software were used to conduct docking investigations [55]. The features of active sites, including as physical and chemical qualities, will allow the ligand to be recognized and bound. The parameters (population size 200, generations 70, and solutions 10) were used to produce different conformations of docked structures, and the best confirmation was chosen based on the lowest binding energy.

3. RESULTS

3.1. *In Silico Prediction of the Anti-Cancer Properties of Reticuline and Coclaurine*

The inhibitory activity of the ligands Reticuline and Coclaurine with various cancer receptors that are thought to be potential therapeutic targets was investigated using multi-receptor docking. The optimum docked pose of structure was chosen based on the lowest binding energy, interacting residues, and hydrogen bond number. Among the trsted alkaloids , reticuline had the best docked postures and the highest interactive energy. TNIK, VEGFR, EGFR and TNIK are the receptors that demonstrated the best binding relationships. -96.7, -117.8, -120.2, and -108.3 kcal/mol (Fig 2 and Fig 3). On the other hand with respect to Coclaurine ligand the receptors showed less binding energy which were -87.8, -102.3, -76.7, and -96.6 kcal/mol were discovered to represent the docked energies of receptor-ligand complex-1, accordingly (Fig 4 and Fig 5).

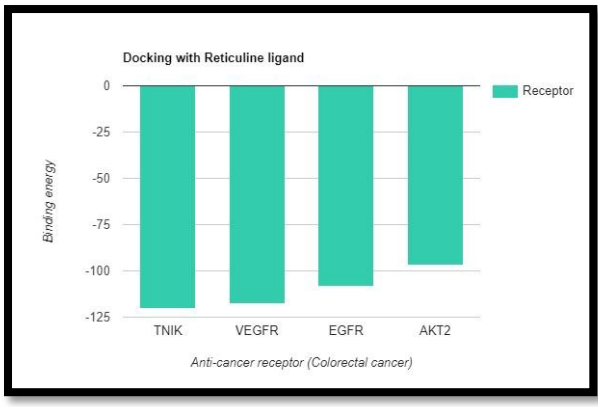

Figure 2. Molecular docking studies of Reticulum ligand with CRC drug targets showing negative binding energy

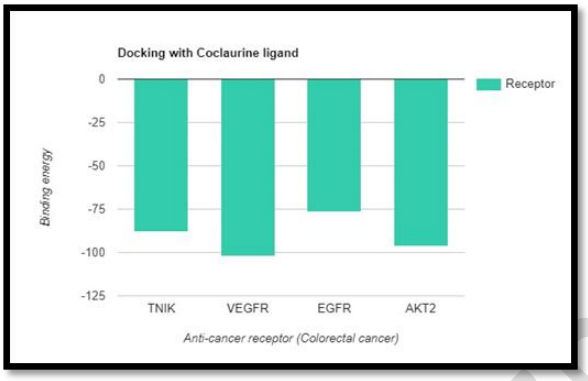

Figure 3. Molecular docking studies of coclaurine ligand with CRC drug targets showing negative binding energy

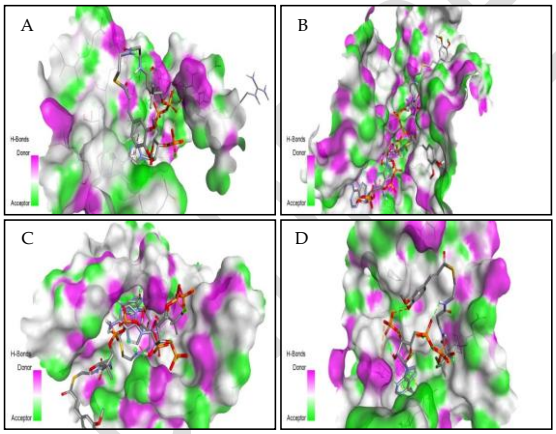

Figure 4. Binding interaction of specific receptor target with Reticuline (A): TNIK, (B): VEGFR, (C): EGFR), (D): AKT2.

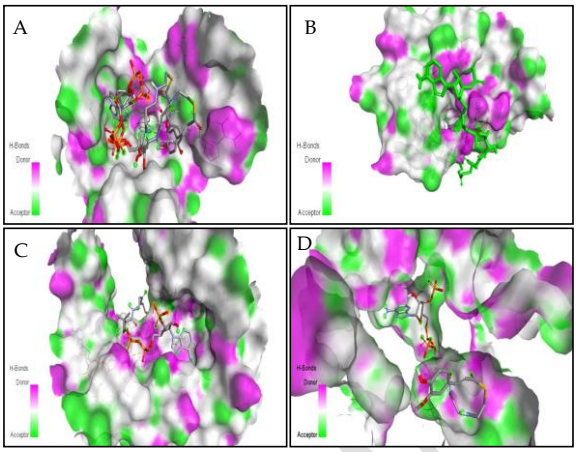

Figure 5. Binding interaction of specific receptor target with Coclaurine (A): TNIK, (B): VEGFR, (C): EGFR), (D): AKT2.

4. DISCUSSION

Colorectal cancer (CRC) is among the most lethal and diagnosed malignancies in the world. Targeted treatment is a novel optional strategy that has effectively prolonged overall survival in CRC patients. The current work provides comprehensive information on the binding ability of certain alkaloids, such as reticuline and coclaurine ligands, to prospective cancer treatment targets. In this study anti CRC activity was investigated with two main ligands and four receptors. The ability of reticuline and coclaurine to bind to specific targets was investigated using molecular docking and In-silico studies. TNIK, VEGFR, EGFR and AKT2 were found to have high affinity for reticuline and coclaurine as an anticancer agent according to this research, indicating a promising approach to medication discovery for CRC receptors. TNIK antagonists have recently been demonstrated to decrease cancer cell proliferation *in vitro* and *in vivo*, as well as to diminish CRC cell survival [56]. As a result, identifying TNIK inhibitors might be beneficial in understanding the process of TNIK-mediated cell cycle control and may have promising utility in cancer therapy. Breast cancer, colorectal cancer, and lung cancer all have TNIK protein [57]. Inhibiting VEGFR-2 in cancer cells was discovered to start and expedite apoptosis, which simultaneously enhances the anticancer impact [8]. Another study found that the expression levels of TIPE and VEGFR2 are regulated in CRC angiogenesis [58]. In the treatment of metastatic CRC, EGFR inhibitors are promising therapeutics target [58]. The EGFR signal transduction pathway is often generally thought to have a significant role in tumor genesis and progression, and it is one of the most key targets for a variety of malignancies [59]. The AKT family, which consists of three highly associated isoforms, AKT1, AKT2, and

AKT3, has been linked to cell proliferation, survival, and apoptosis [60]. Overexpressed AKT2 promoted metastasis in CRC [61]. AKT2 is the driving force behind a variety of cellular activities such as DNA replication and DNA repair, and its overexpression has been linked to oncogenesis [62,63]. AKT2 knockdown was reported to inhibit tumor cells proliferation [64].

The inhibitory activity of the ligands reticuline and coclaurine with these anti-cancer receptors are thought to be potential therapeutic targets was investigated before using multi-receptor docking and for In-silico studies [65]. The optimum docked pose of structure was chosen based on the lowest binding energy interacting residues, and hydrogen bond number [66]. Among the alkaloids, reticuline had the best docked postures and the highest interactive energy [67]. Alkaloids have a wide range of inhibitory actions against a variety of cancer receptors making them ideal therapeutic medication for a number of cancers due to their high inhibitory efficacy and superior pharmacokinetic properties [68].

As a result of this research, it documented those alkaloids have an optimal binding feature with the selected cancer receptors.

To the best of our knowledge, this is one of the studies showing alkaloids' therapeutic potential against cancer's key targets. The present research improves our knowledge of how to select and test lead compounds as possible chemotherapeutic drugs in the future. Because of their effectiveness as an inhibitor, reticuline and coclaurine should be considered in *in vitro* and *in vivo* studies for a variety of cancer cell lines.

5. CONCLUSIONS

Alkaloids are vital chemical substances that may be exploited to find new drugs. Various alkaloids isolated from medicinal plants and herbs were shown to have antiproliferative and anticancer effects on a broad range of malignancies *in vitro* and *in vivo*. By using computer-assisted virtual screening, the inhibitory effects of alkaloids against numerous cancer treatment targets were discovered.

According to this study, reticuline and coclaurine inhibited CRC pathogenic gene products of TNIK, VEGFR, EGFR and AKT2 better than their native ligands. These receptors were successfully docked onto reticuline and coclaurine ligands for drug interaction studies, with the best binding energy. Demonstrating its importance as an anti-carcinogenic target by alkaloids. The current findings lay the groundwork for further research into alkaloids as a potential CRC therapeutic option.

Ethical Approval:

This study was carried out in the computer laboratories at king Abdulaziz University during 2020-2021. The study was approved by research ethics committee (29-CEGMR-Bioeth-2021) at the center of excellence in genomic medicine research (CEGMR). All of the data in this investigation was analyzed and presented in accordance with CEGMR ethical requirements.

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