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ORIGINAL ARTICLE

In Silico Investigation of Surfactants as Potential Permeation Glycoprotein Inhibitors for Formulation Development

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ABSTRACT
Over the past few decades, microbial multidrug resistance (MDR) has posed significant challenges to various industries, including pharmaceuticals, animal husbandry, and agriculture. This has necessitated the exploration of effective strategies to address this issue. Molecular docking studies were conducted using the Schrodinger Maestro 9.1 software program, where specific ligands were examined. The selected receptors were prepared using the Protein Preparation Wizard. The molecular docking simulations highlighted the critical role of various factors in the interaction between ligands and proteins. These factors encompassed hydrogen bonds, lipophilic contacts, metal interactions, pi-pi interactions, and pi-cation interactions. Scoring functions, which are computational algorithms, were employed to estimate the strength of non-covalent contacts between molecules after docking. The findings of this study provide valuable insights into the potential inhibitory activity of these excipients on P-glycoprotein (P-gp) and contribute to understanding their molecular mechanisms. Further validation will involve formulation development using P-gp substrate drug molecules, along with in vitro and in vivo studies, to confirm these findings conclusively.

Keywords:*In silico, Molecular docking, P-gp inhibition, Schrodinger*

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INTRODUCTION

A change in bowel habits that is not typical of the patient is one of the symptoms of colorectal cancer, which is a disease that is characterised by aberrant cell division in the colon and rectal region [1].When it comes to cancer patients, colon cancer is regarded as one of the primary causes of death [2], and this is the case in a number of Arab nations, including Saudi Arabia.People who are between the ages of 35 and 65 have an increased likelihood of developing colorectal cancer. A sedentary lifestyle, as well as exposure to chemical vapours emanating from factories, were identified as risk factors for the aforementioned condition [3]. Cytokines, on the other hand, are thought to be another factor that contributes to the pathophysiological states that patients have. Cytokines are proteins that are secreted by cells and that are responsible for the growth and activity of other cells in the immune system. Cytokines are responsible for the proliferation and activity of other cells in the immune system. Macrophages that have been stimulated create the vast majority of pro-inflammatory cytokines, which are then implicated in the process of upregulating inflammatory responses. There is mounting evidence that points to specific pro-inflammatory cytokines being implicated in the pathogenesis of pathological pain [4, 5, and 6]. [7] Research has shown that interleukins and transforming growth factor B both enhance the proliferation and inversion of cancer cells. The activation of cytokine receptors and the coordination of intercellular signalling both hasten the growth of tumours [8].P-gp is an abbreviation for multidrug resistance protein 1, which also happens to be the name of a permeability glycoprotein. P-gp is a key membrane transporter that plays a vital role in the metabolism of foreign particles and the subsequent efflux of these particles out of the cell. P-gp is a component of the permeability gradient that separates the cytosol from the plasma membrane. Once foreign particles have been metabolised, P-gp is the protein that is responsible for clearing the cell of them [1, 2]. In order for the substrate-dependent efflux activity of P-gp to take place, adenosine triphosphate, more commonly known as ATP, must first be present. It is thought that this protein is involved in the process of protecting the body from substances or organisms that are not naturally there. It has

been found in a broad variety of microorganisms, some of which include bacteria, mammals, and fungi, and the fact that it is present in these creatures gives support to this notion [3]. P-gp is widely dispersed throughout the body and is an essential component of the efflux processes that occur in the gut, bile ducts and liver cells, kidney cells (such as proximal tubules), and capillary endothelium. Capillary endothelium are essentially endothelial cells and include the blood-testis barrier as well as the blood-brain barrier. Not only in the colon, but also in other organs such as the pancreas and the adrenal glands, it has been found [4-6]. P-gp is frequently overexpressed in cancer cells, which prevents a significant variety of anticancer drugs from entering the cell and lowers the effectiveness of treatment for cancer. On the other hand, it is created in the lumen of the digestive system by the bile ducts, which means that it protects tissues from potentially harmful noxious components and also assists in the disposal of metabolites. P-gp expression was discovered to a significant degree in the great majority of cancer patients, regardless of the type of malignancy that was being investigated. The development of drug resistance is a common factor in the failure of chemotherapy therapies for breast cancer [7-8]. The epithelial lining of the ducts (responsible for 85% of breast cancer cases) or the lobules (responsible for 15% of breast cancer cases) is the area where cancer develops in the vast majority of cases of breast cancer. When cancer first starts to form, it is often contained (also known as "in situ") within a duct or lobule, and it is extremely rare for it to produce symptoms or spread to other parts of the body. Cancer typically begins to form in a duct or lobule. These in situ tumours have the potential to metastasize over time, eventually entering the breast tissue of surrounding neighbors and spreading to lymph nodes in the region as well as further afield. This can happen if they are allowed to grow unchecked for a long enough period of time. When a woman dies from breast cancer, the cause of death is almost always the result of the disease having spread to other parts of the body [9, 10]. Using immunohistochemistry, researchers in a variety of laboratories looked into how proteins were expressed in their samples. In contrast to the majority of breast tumours that could be surgically removed, which were reported in the various studies [11, 12], the protein expression levels of Pgp, Ki-67, and p53 were much greater in locally progressed breast cancers. This was found to be the case in breast cancers that had already spread locally. Numerous excipients chemicals have been recorded, some of which include HPMC, Polysorbate 20, Vitamin E- TPGS, Polysorbate 80, Rhamnolipids, Tetrglycerol Monooleate, Glyceryl Mono Stearate, Glyceryl Monoleate, Pentaethylene Glycol, Decussate sodium, and Nanoxynol, amongst others. For both in silico drug design and drug discovery, the technique known as'molecular docking' is the one that is utilised the majority of the time. It is a form of structure-based virtual screening that looks for new compounds that have an affinity for a certain target protein. The goal of this type of screening is to find new drugs. In light of the above information, the objective of the current work is to locate natural compounds produced from a wide variety of sources that contain excipients that have the ability to inhibit P-gp activity [13-15]. This will be accomplished by identifying natural compounds that contain excipients that have the capacity to inhibit P-gp activity.

MATERIALS AND METHODS

Ligand selection: The construction of a formulation is impossible without the utilisation of several excipients. Investigations on the compounds' potential to inhibit P-gp have ushered in a new era in which such substances are capable of being used as P-gp inhibitors. Polymers, surfactants, lipids, and a variety of other chemicals are all examples of P-gp inhibitors. In the field of excipients, a vast number of research organizations have examined and addressed the function of specific P-gp inhibitors for improved medication administration. We based our selection of the several excipients that would be utilised in the p-gp interaction investigation on the findings of a survey of the relevant literature (Table 1), which can be found here.

Table 1: Primary selection of the excipients on the basis of literature survey [16]

Sr. No.	Surfactants Name	Sr. No	Surfactants Name	Sr. No	Surfactants Name
1	Arginine	12	Chitosan	23	HPMC (Hydroxypropyl methyl cellulose)
2	Oleylamine	13	SLS (sodium lauryl sulphate)	24	Proline
3	Polyethylene glycol Monooleate	14	B-Octylglucoside	25	Tween 80 (polysorbate 80)
4	Benzalkonium Chloride	15	Sodium stearate	26	PVP (Polyvinyl Pyrrolidone)
5	polyethylene lauryl ether	16	Docusate sodium	27	PVA (Polyvinyl Alcohol)
6	Sodium Alginate	17	cyclodextrin	28	TPGS (Tocopherol Polyethylene Glycol Succinate)
7	Ammonium Bromide	18	Dendrimer	29	PEG (Polyethylene Glycol)
8	Nanoxynol	19	Gaur Gum	30	Poloxamers (188, 407)
9	Rhamnolipid	20	Miglyol	31	Triton x-100 (Polyoxyethylene glycol octylphenol ethers)
10	Xanthine	21	Cetostearyl alccohol	32	Tween-20, 80(polysorbate 20, 80)
11	Sorbitan tristearate	22	Polysorbates (20, 80)	33	Cremophor EL (Polyethoxylated Caster oil)

Protein Selection: The construction of a formulation is impossible without the utilisation of several excipients. Investigations on the compounds' potential to inhibit P-gp have ushered in a new era in which such substances are capable of being used as P-gp inhibitors. Polymers, surfactants, lipids, and a variety of other chemicals are all examples of P-gp inhibitors. In the field of excipients, a vast number of research organisations have examined and addressed the function of specific P-gp inhibitors for improved medication administration. We based our selection of the several excipients that would be utilised in the p-gp interaction investigation on the findings of a survey of the relevant literature (Table 1), which can be found here.

Molecular Docking: We used the Schrodinger Maestro 9.1 software programme to run the molecular docking studies on the ligands of our selection. The chosen receptors were prepared using the Protein preparation wizard. Protein Data Bank's crystal structure was retrieved using proteins that include the P-gp domain (PDB ID: 3G60). Protonation was used to remove water molecules from the protein's structure, with the exception of those that were identified close to the active site. The chemical compounds' structural elements were diagrammed using the ChemDraw programme. The ligands were created using an application called the Ligand preparation wizard. The complexity of the chemical and protein structural geometries was reduced by using the OPLS-2005 force field. The process of ligand docking required the creation of the receptor grid. The XP mode of the Glide programme created by Schrodinger Inc. in the United States of America was used for ligand docking tests into the P-gp (PDB ID: 3G60) binding pocket.

ADME properties: An important factor in the transformation of a potent lead molecule into excipients is its ADME properties. It is crucial to have a thorough understanding of the lead molecule's absorption, distribution, metabolism, and excretion properties in order to use it effectively in people. As a result, numerous compounds' ADME properties were predicted using the QikProp application of Schrodinger Maestro 9.1. Predictions could be made for characteristics including overall CNS activity, MDCK and Caco-2 cell permeability, log BB, and log Khsa for human serum albumin binding. The accompanying table 2 contains the ADME profile of the selected excipients for your reference.

Table 2: ADME Properties of selected excipients [16]

Sr. No.	Compound Name	Mass	Log P	H-Bond Donor	H-Bond Acceptor
1.	HPMC	1261.4369	-2.3208	30	8
2.	Polysorbate 20	522.6684	1.9984	10	3
3.	Vitamin E- TPGS	574.8300	8.3557	6	1
4.	Polysorbate 80	604.8117	4.4626	10	3
5.	Rhamnolipids	650.7940	2.5485	13	6
6.	Tetraglycerol Monooleate	504.6961	3.6753	8	4
7.	Glyceryl Mono Stearate	358.5551	5.1443	4	2
8.	Glyceryl Monooleate	356.5392	4.9203	4	2
9.	Pentaethylene Glycol	238.2779	-0.9626	6	2
10.	Decussate sodium	444.5601	4.8903	7	0
11.	Nanoxynol	264.4023	4.3508	2	1

RESULTS AND DISCUSSION

The specific effects that various factors have on the protein ligand interaction profile were revealed by the docking simulations. Hydrogen bonds, lipophilic contacts, metal interactions, pi-pi interactions, and pi-cation interactions are a few of these elements. In the realm of computational chemistry and molecular modelling, scoring functions are quick approximation mathematical techniques that are used to predict the strength of a non-covalent bond between two molecules once the molecules have been docked. The energy that the posture uses up while it is trapped within the binding site is estimated by the docking score. It is supported by molecular dynamics and physics. It accounts for free energy resulting from changes in vibrational mode, the action of the solvent, conformational changes in proteins and ligands, internal rotations, the association energy of ligand and receptor to form a single complex, and internal rotations. Based on the protein's docking score, it has been identified which molecules were chosen. Excipients with a docking score and their interaction docking scores with different amino acids discovered on the receptor were taken into consideration for further discussion (Table 3) [16–18].

The constraint preventing further lead optimisation is the inability to create new drug candidates with reasonable chemical and biological properties of the substances under study. Because of this, having a precise forecast of the pharmacokinetic profile of medications is crucial [19–22]. Only 11 of the 33 compounds were selected for the following phase based on molecular docking (Table 3).

Table 3: Compilation of selected excipients docking result [16]

Sr. No.	Excipients	Docking score
1.	HPMC	-14.16
2.	Polysorbate 20	-11.0
3.	Vitamin E- TPGS	-11.0
4.	Polysorbate 80	-9.0
5.	Rhamnolipids	-11.0
6.	Tetraglycerol Monooleate	-9.0
7.	Glyceryl Mono Stearate	-8.29
8.	Glyceryl Monooleate	-7.19
9.	Pentaethylene Glycol	-7.0
10.	Decussate sodium	-6.0
11.	Nanoxynol	-6.0

In addition, research was carried out in silico on the pharmacokinetic properties of the selected drugs in conjunction with the receptors that they correspond to. When conducting therapeutic development, having a solid understanding of the pharmacokinetic properties of a molecule is essential for developing a more powerful lead compound. The acceptable levels of variation for these characteristics have been figured out. The molecular weight can range anywhere from 130 to 725 kDa, and volume is defined as the anticipated number of hydrogen bonds that the solute will receive from water. It is expected that there will be a total of five hydrogen bond acceptors and ten hydrogen bond donors. The symbol for the octanol/gas projected partition coefficient is Q P log Poct (8.0-35.0). This represents the octanol/gas projected partition coefficient. instances of expected partition coefficients for water and gas include Q P log Pw, the projected octanol/water partition coefficient log p (acceptable range: -2.0 to 6.5), P log Po/w, and Q log S, the predicted aqueous solubility and S in mol/L (acceptable range: -6.5 to 0.5). [23-25] P log Po/w and Q log S are instances of predicted aqueous solubility.

All of the excipients showed pharmacokinetics that were within acceptable criteria, and all of the characteristics that were discussed before fell within the boundaries of what is considered acceptable. The pharmacokinetic profiles of compounds that displayed hydrogen bond donar and hydrogen bond acceptor values that were outside of the permitted range should be altered to reflect this new information. The molecular weight, the projected octanol/water partition coefficient, the Q log S, the predicted aqueous solubility, and the S in mol/L values are all within a range that is considered to be acceptable. Additionally, the octanol/gas partition coefficient that was predicted falls well below levels that are considered acceptable [26-29].

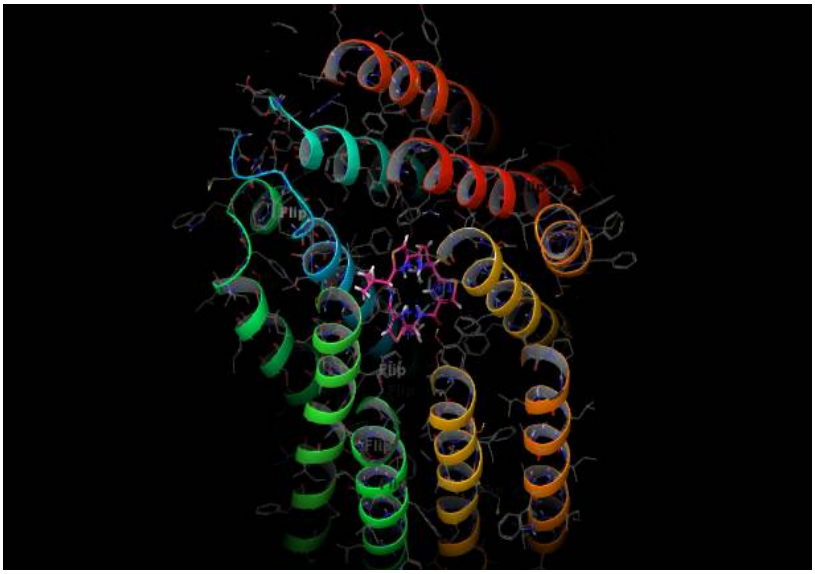

Figure 2: Excipients the hydrogen bond is shown as a yellow dotted line in the 3D ligand interaction, and the polar interaction is shown as a blue dotted line

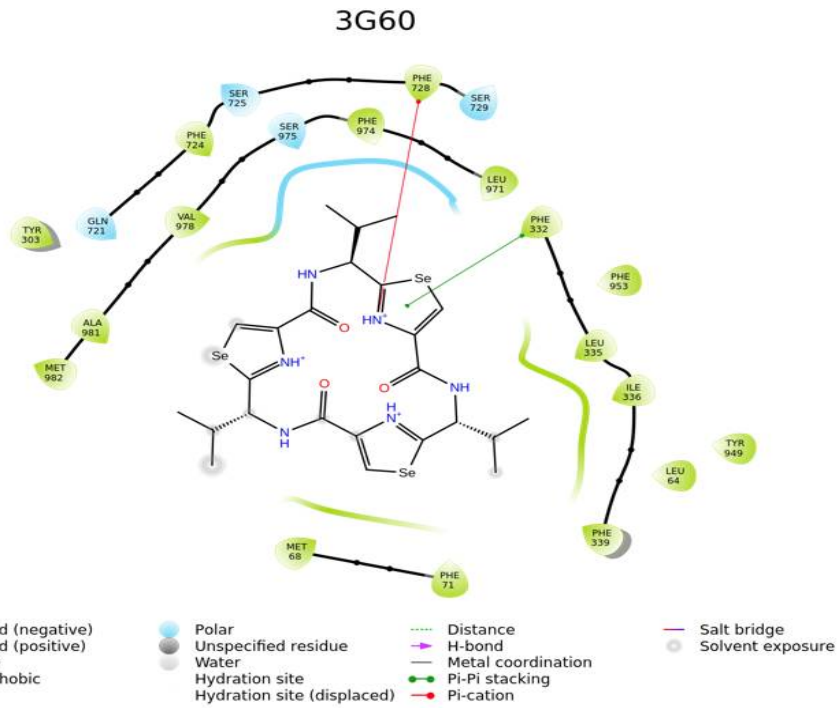

Figure 3: The Schrodinger Glide SP programme was used to get a 2D view of the interaction between the ligand and P-gp (PDB ID: 3G60). The essential amino acid residues at the binding site are marked. Electrostatic amino acids are shown by the purple (+ve) and brown (-ve) circles. Hydrophobic amino acids are shown by the green circle. Polar interactions are shown by the blue circle, and the purple arrow shows the hydrogen bond.

CONCLUSIONS

If it turns out that these excipients are efficient at inhibiting P-gp, then the findings of this study could be helpful in understanding the molecular mechanism behind how they do so. The present findings will be further validated by the development of a formulation using any P-gp substrate pharmaceutical molecule, as well as by research that will be carried out in both in vitro and in vivo settings.

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AUTHOR'S CONTRIBUTIONS

Both authors contributed equally to the study's conception, data collection, analysis, and interpretation, as well as to the writing and composing of the manuscript. The final draught was read and authorized by both authors.

ETHICAL APPROVAL

This study does not involve any animals or human subjects.

CONFLICT OF INTEREST

The authors declare no conflict of interests for this manuscript.

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