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LOWERING THE BLOOD BRAIN BARRIER PERMEATION PROPERTY OF 6-GINGEROL THROUGH A STRUCTURAL MONOSUBSTITUTION MODIFICATION APPROACH FOR THE PREVENTION OF PSYCHOTROPIC SIDE EFFECTS: AN IN-SILICO PHARMACOKINETICS STUDY

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Abstract

Different studies have shown that the regular consumption of certain vegetables and fruits can reduce disease risks. The *Zingiber officinale* (ginger) rhizome is consumed worldwide as a spice and herbal medicine. It is made up of pungent phenolic substances collectively regarded as gingerols. 6-Gingerol exhibits a good number of biological activities as the basic active ginger compound. It is chemically a relative of capsaicin and piperine, the compounds which give chilli peppers and black pepper their respective spiciness. 6-gingerol and its analogues have a favourable toxicity profile, but are cytotoxic towards a range of cancer cell lines including blood cancer and lung cancer. The compound has also been investigated in vitro for its effect on cancerous tumors of the bowel, breast tissue, ovaries, and pancreas, with positive results. Each of the 6-gingerol analogues used for the purpose of this study were designed with the aid of the ChemAxon software while the 2D structure of 6-gingerol was view and downloaded from the PubChem repository. Each of the chemical structures were converted into SMILES strings using the OpenBabel software and the pharmacokinetic parameters which includes the blood-brain barrier permeation properties of each experimental compounds was predicted using the SwissADME online server. 7 analogues were obtained through the modification of 6-gingerol (C=O, COOH, $CONH_2$, NH_2, OH, C_2H_5 and CH_3) by the substitution of each functional groups for the OCH_3 group attachment to the carbon-1 (C1) of 6-gingerol. Results obtained from the in silico pharmacokinetics study showed that the COOH, $CONH_2$ and the NH_2 analogues of 6-gingerol lost their blood-brain barrier permeation activity while others including 6-gingerol retained theirs. These three compounds can be applied as therapeutic alternatives to 6-gingerol and non neuroactive agents for the prevention of interference with the Central Nervous System (CNS). The laboratory synthesis of these compounds is therefore recommended.

Keywords: *Zingiber officinale; 6-Gingerol; SMILES; Pharmacokinetics; Blood-Brain Barrier*

Introduction

Ginger (Zingiberofficinale Roscoe, Zingiberaceae) is a medicinal plant that has been widely used in Chinese, Ayurvedic and other global herbal medicinal practices since ancient times for a wide array of ailments including arthritis, rheumatism, sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, dementia, fever, infectious diseases and helminthiasis [1]. 6-Gingerol is the major pharmacologically-active component of ginger [2]. It is known to exhibit a variety of biological activities including anticancer, anti-inflammation, and anti-oxidation [3]. 6-Gingerol has been found to possess anticancer activities via its effect on a variety of biological pathways involved in apoptosis, cell cycle regulation, cytotoxic activity, and inhibition of angiogenesis [4]. Thus, due to its efficacy and regulation of multiple targets, as well as its safety for human use, 6-gingerol has received considerable interest as a potential therapeutic agent for the prevention and/or treatment of various diseases [5]. 6-Gingerol is isolated from ginger root using ethanol and other organic solvents [6].

The blood-brain barrier (BBB) is a highly regulated and complex layer of cells that has evolved to protect the brain from toxic molecules and infectious agents [7]. The Blood Brain Barrier can also be said to be the physical barrier in the circulatory system that stops many substances from traveling into the Central Nervous System (CNS) [8]. The blood-brain barrier (BBB) functions to protect the brain from toxic molecules and infectious agents that can impact brain health and cognitive function [9]. It also serves as the interface between the brain and the other tissues of the body. Under normal conditions, the BBB permits the selective transport of molecules that are essential for brain function and metabolism while preventing the passage of most proteins and small molecules, including the majority of drugs in current use [10].

The drugs which are intended to interact with their molecular targets in the CNS must cross the BBB in order to be used as therapeutic agents [11]. The peripherally acting agents should not cross the BBB so as to avoid the CNS related side effects. In both cases the BBB permeability of the molecules must be known [12]. Common disease states, environmental toxins, certain medications and the aging process itself can compromise the integrity of the BBB [13]. Drug developers and regulatory agencies are increasingly interested in measuring and monitoring cognitive function as part of a drug's safety profile and risk management strategy [14, 15].

The aim of this study is to effect structural modifications on the 2D structure of 6-gingerol thereby affecting its blood brain barrier permeability attribute. The sole objective of performing this experiment is to find alternative 6-gingerol structural analogues that will pose less threat to the functionality of the brain and yet effectively perform biological functions as a peripherally acting therapeutic agent.

Methods

Ligand Preparation

The 2D structure of 6-gingerol and its modified analogues were designed using the MarvinSketch software [16]. All designed structures were downloaded and saved as mrv files in preparation for docking.

File Conversion

Saved mrv files from the ligand preparation process were converted into SMILES strings (Simplified Molecular Input-Line-Entry System) using the Open Babel Open Source Chemistry Toolbox. Open Babel, a chemical toolbox is designed to speak many of the languages of chemical data [17]. It's an open and collaborative project allowing anyone to make searches, conversions, analysis, or storage data from molecular modelling, chemistry, solid-state materials, biochemistry, or related areas [18].

Ligand Minimization

6-Gingerol and its modified analogues were minimized using the UCSF Chimera software [19]. UCSF Chimera is an extensible program for analyzing and interactively visualizing molecular structures and related data which include supramolecular assemblies, density maps, alignment of sequences, results from molecular docking, trajectories and conformational ensembles [20].

Visualization of Atoms

Atoms making up 6-gingerol and its modified analogues were visualized using the Pymol molecular visualizer [21]. PyMOL is an open-source tool for model visualization and it is made available for utilization in structural biology [22]. The Py aspect of the name of the software is a reference pointer that it is extensible and extends by the python programming language [23].

Results and Discussion

Structure of 6-Gingerol

Figure 1 shows the 2D structure of 6-gingerol as designed by the MarvinSketch software. Modifications that resulted into the derivatives of this compound were made through the substitution of the OCH3 group attachment to the carbon-1 (C1) of the compound with other functional groups such as the C=O, C2H5, CH3, CONH2, COOH, NH2 and OH groups.

The 3D Structure of 6-Gingerol

The 3D structure of 6-gingerol was generated though the input of the canonical SMILES obtained from the OpenBabel conversion of the 2D structure of the compound into the Chimera visualizer. The "Build Structure" function of the Chimera visualizer was enacted to generate a 3D structure which was saved as a Mol2 file. The saved Mol2 file was viewed using the Pymol visualize and this same software was used in labeling and viewing each atom making up the compound. The 6-gingerol modifications as reported in figure 1 were made by substituting the OCH3 functional group attachment of the compound with other functional groups. This OCH3 group is clearly depicted in the 6-gingerol 3D structure in figure 2. The oxygen component of the functional group is labeled O4 while the CH3 component is labeled C17, H24, H25, H26

The polar surface area (PSA), also known as the topological polar surface area (TPSA) of a molecule is defined as the sum of all polar atoms (oxygen and nitrogen), with the inclusion of the hydrogen atom attachments. The polar surface area is a metric that is often used in medicinal chemistry to optimize the cell permeation ability of drugs. Molecules with a PSA value higher than 140 angstroms squared are known to be poor in cell membrane penetration [24]. For molecules to penetrate the blood–brain barrier (BBB) (in order to act on the central nervous system receptors), the value assigned to the polar surface area must be less than 90 angstroms squared [25]. 6-Gingerol and five of its modified analogues (C=O, C2H5, CH3, NH2 and OH) might possess the blood-brain barrier permeation attributes as their TPSA values appeared lower than 90 angstroms

The partition coefficient between n-octanol and water (log Po/w) serves as the classical method for the description of lipophilicity. The diversity of the models backing the predictors will increase the accuracy in the prediction using the consensus log Po/w [26]. The lipinski's rule [27] was used as the drug likeness descriptor for the purpose of this study and the optimal lipophilicity range (Log Po/w) allowed should not exceed 5. The observation from the concensus lipophilicity column of table 1 shows that 6-gingerol and all the analogues derived from it are within the optimal lipophilicity range and as such can be regarded as druglike compounds.

Activities regarding drug development can be facilitated and made easier in cases where molecules are soluble. This brings about ease in drug handling and its formulation [28]. Moreover, for discovery projects that target the oral form of administration, one of the major absorption property influencers the solubility of the compound [29]. Also, drugs that are designed for parenteral administration requires a high solubility attribute to aid the delivery of an appreciable amount of the active ingredient in smaller volumes of pharmaceutical dosage [30]. A compound can be considered as soluble if the Log S value is less than 6 [28]. 6-Gingerol and its modified analogues used for the purpose of this study, according to the column projecting the solubility result in table 1 are all water soluble, implying that they might be easily absorbed.

The nature of the gastrointestinal mucosal membrane surface area plays an important role in the process of drug absorption and it has a varying and differential effect from the stomach to the rectum. The physiochemical properties of the luminal content are also implicated to have an influence in drug absorption process [31]. The absorption process itself is continually described in

terms of hypothesis of simple partition of pH, where absorption is controlled by the equilibrium position between the ionized and non-ionized forms of the drug at varying physiological pH values encountered in the gastrointestinal tract [32]. All the experimental possess a high gastrointestinal absorption rate, indicating their ability to aid drug bioavailability.

Overcoming the ability of a non neuroactive drug to cross the blood brain barrier is a major challenge to be solved in the processes of designing drugs. Only neuroactive drugs are required to possess the blood brain permeation attribute for functionality On the contrary, non neuroactive drugs should not cross the blood brain barrier for the avoidance of psychotropic side effects [33]. Blood-brain barrier permeation results from table 1 showed that three of the modified analogues of 6-gingerol ($CONH_2$, COOH and NH_2) cannot penetrate the blood brain barrier. It was also interesting to note that the NH_2 analogue of 6-gingerol did not possess the blood-brain barrier permeation attribute even though the TPSA value is below 90 angstroms.

The P-glycoprotein (P-gp) is involved physiologically in the reduction of the harmful effects of toxic compounds, xenobiotics and drugs which the body is exposed to by constantly pumping them out of cells. The need for the role played by the P-glycoprotein has led to the recognition of the modulation it confers on many important and clinical therapeutic agents and this pharmacokinetic importance has led to the incorporation of its screening in any process involving drug discovery [33]. Drug pharmacokinetic parameters can also be affected through various drug induced induction or inhibition directed at modulating drug transporters and this can lead to a significant drug-drug interaction [34]. Excluding the C_2H_5 and NH_2 analogues of 6-gingerol, other experimental ligands were no substrates to the P-glycoprotein hence their oral bioavailability remains intact.

The bioavailability of drugs designed for oral administration can be determined by the biotransformation process mediated by the intestinal CYP3A4 and the constant pumping of absorbed drugs out of the cell which is a process mediated by the P-glycoprotein. It has been hypothesized that the action of the CYP3A4 and P-glycoprotein may be in concert to reduce oral drug bioavailability and viewing this hypothesis from a theoretical point of view makes it more attractive [34]. The recent test on the hypothesis of the possibility of the enhancement of substrate disappearance mediated by the CYP3A4 being stimulated by drugs interacting with the apical efflux pump suggests that the P-gp/CYP3A4 are cosubstrates and that P-glycoprotein increases the potentials of CYP3A4-mediated disappearance of drugs during secretary detoxification in the intestine [35]. It is also possible for the P-glycoprotein to have an influence on first-pass metabolism in a manner describing co-operativity [35]. Table 1 showed that the C=O analogue of 6-gingerol unlike other experimental ligands might exhibit a higher bioavailability, being an inhibitor of the CYP3A4. Other experimental ligands might undergo CYP3A4-mediated intestinal biotransformation which in turn lowers their bioavailability. The results from this column of table 1 also showed that the C_2H_5 analogue of 6-gingerol can inhibit the CYP3A4 and this suggests the drug might be orally bioavailable even though it appeared to be a P-glycoprotein substrate.

Many areas in the process of drug discovery are in need of estimation models and methods for the determination of the ease of synthesizing drug-like molecules (synthetic accessibility).. The assessment of the synthetic accessibility (SA) of a lead candidate is a task which takes part in the discovery of lead, disregarding methods the lead candidate has been known with. The synthetic accessibility score ranges from 1 (very easy) to 10 (very difficult) after normalization process [36]. This confirms the ease of the laboratory synthesis 6-gingerol and all its modified analogues as their synthetic accessibility score do not exceed 3.0.

Conclusion

Results from the above analysis have shown that structural modifications can be a potent tool in altering the pharmacokinetic properties of selected compounds. All the experimental ligands have shown drug-likeness attributes while the COOH, $CONH_2$ and the NH_2 analogues of 6-gingerol have stood out to be the only ones that can act as non

neuroactive agents with regards to their inability to permeate the blood brain barrier. The laboratory synthesis of these compounds is therefore recommended for further studies.

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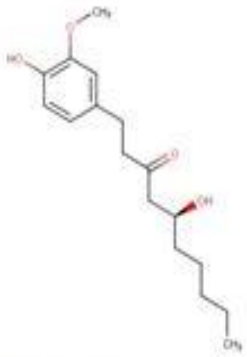
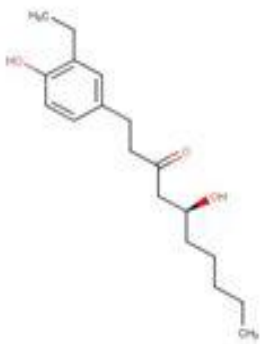
Table 1: Physicochemical properties, lipophilicity, solubility, pharmacokinetics and lipinski druglikeness of antifolate drugs, gedunin and its modified derivatives

Parameters	**6-Gingerol**	**C=O analogue**	**C_2H_5 analogue**	**CH_3 analogue**	**$CONH_2$ analogue**	**COOH analogue**	**NH_2 analogue**	**OH analogue**
Formula	$C_{17}H_{26}O_4$	$C_{17}H_{24}O_4$	$C_{18}H_{28}O_3$	$C_{17}H_{26}O_3$	$C_{17}H_{25}NO_4$	$C_{17}H_{24}O_5$	$C_{16}H_{25}NO_3$	$C_{16}H_{24}O_4$
Molecular weight g/mol	294.39	292.37	292.41	278.39	307.38	308.37	279.37	280.36
Num. H-Bond acceptors	4	4	3	3	4	5	3	4
Num. H-Bond donors	2	2	2	2	3	3	3	3
TPSA Å^2	66.76	74.60	57.53	57.53	100.62	94.83	83.55	77.76
Lipophilicity Consensus Log $P_{o/w}$	3.13	2.96	3.81	3.49	2.47	2.80	2.55	2.75
Water Solubility Log S	Soluble	Moderately Soluble	Moderately Soluble	Moderately Soluble	Moderately Soluble	Soluble	Soluble	Soluble
GI absorption	High	High	High	High	High	High	High	High
BBB permeant	Yes	Yes	Yes	Yes	No	No	No	Yes
P-gp substrate	No	No	Yes	No	No	No	Yes	No
CYP3A4 inhibitor	No	Yes	Yes	No	No	No	No	No
Lipinski Druglikeness	Yes; 0 Violation	Yes; 0 Violation	Yes; 0 Violation	Yes; 0 Violation	Yes; 0 Violation	Yes; 0 Violation	Yes; 0 Violation	Yes; 0 Violation
Synthetic accessibility	2.81	2.67	2.77	2.58	2.71	2.84	2.57	2.61

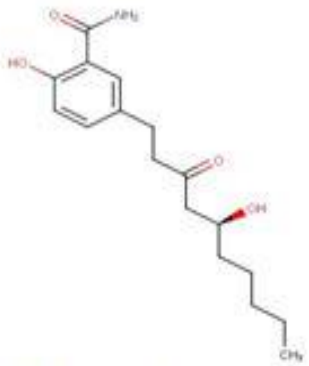
Figure 1: The two dimensional (2D) structure of 6-gingerol and its modified derivatives as designed using the MarvinSketch software.



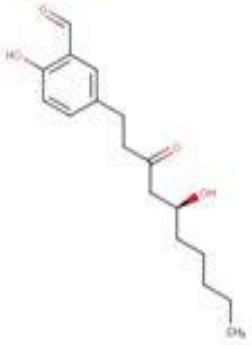
6-Gingerol



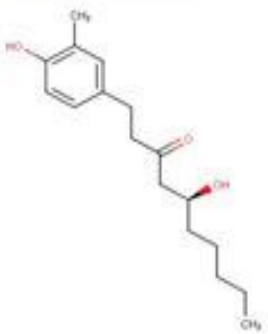
C_2H_5 Analogue



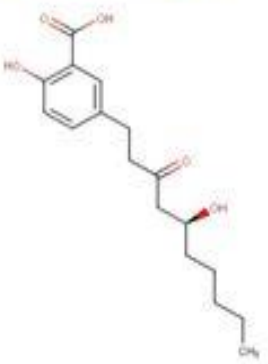
$CONH_2$ Analogue



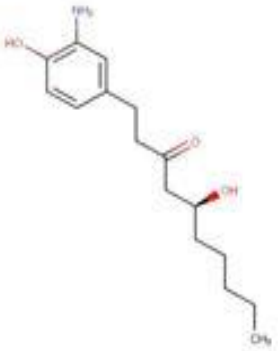
C=O Analogue



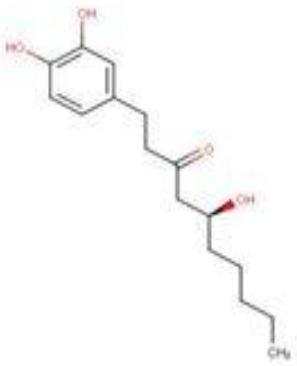
CH_3 Analogue



COOH Analogue



NH_2 Analogue



OH Analogue

Figure 2: 3D structure of 6-Gingerol showing all labeled atoms