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Research Article

Improving the Drug Bioavailability Property of Myricetin through a Structural Monosubstitution Modification Approach: an *In-Silico* Pharmacokinetics Study

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ABSTRACT

Myricetin belongs to the members of polyphenolic compounds that make up the flavonoid class, which possess antioxidant properties. Myricetin is mostly obtained from vegetables, fruits, nuts, berries, tea, and is also found in red wine. It is also similar structurally to quercetin, fisetin and luteolin and is known to possess similar functions as the other members in the flavonol class of flavonoids. The health benefits of myricetin cuts across being an anticarcinogen compound to its antiviral, antithrombotic, antidiabetic, antiatherosclerotic, neuroprotective and anti-inflammatory properties among others. It also plays a role as a cyclooxygenase 1 inhibitor, an antineoplastic agent, an antioxidant, a plant metabolite, a food component and a hypoglycemic agent. It is a hexahydroxyflavone and a 7-hydroxyflavonol. The 2D structure of myricetin was obtained from the PubChem database while the MarvinSketch software was used to effect the various structural modifications on the compound. The structural modifications entails the substitution of the OH group attached to the C_1 of myricetin with different functional groups such as the C=O, C_2H_5, CH_3, CHO, $CONH_2$, H and OCH_3 which were saved as mrv files. The saved mrv files for each 2D structures were converted into canonical SMILES with the aid of the Open Babel software while the pharmacokinetic parameters for each compound was predicted using the SwissADME server. Results from this study showed that the C_2H_5, CH_3 and H analogues of myricetin showed a higher gastrointestinal absorption rate compared to their C=O, CHO, $CONH_2$ and OCH3 counterparts. This result shows that the C_2H_5, CH_3 and H analogues of myricetin might be more orally bioavailable compared to myricetin and the other modified analogues. Preclinical studies on these compounds are therefore recommended

Keywords: *Myricetin; Flavonoid; Antioxidant; SMILES*

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INTRODUCTION

Bioavailabilityis used in the description of the fraction of anunchanged administered drug dose that gets to the systemic circulation, one of the drugs principal pharmacokinetic properties. Going by this definition, an intravenously administered drug possesses a 100% bioavailability (Griffin, 1978). However, administering a medication through other routes (such as the oral administration) generally reduces the drug bioavailability (due to absorption and first-pass metabolism that remains incomplete) and this may also vary amidst patients. Bioavailability remains one of the basic essentials of pharmacokinetics, as the bioavailability properties of drug-like compounds must be considered in dosage calculationsfor administration routes that are non-intravenous.In the case of herbs, dietary supplements and other nutrients where the route of administration is mostly oral, generally, the bioavailability simply refers to the ingested dose quantity or fraction that is absorbed (Ong, and Khoo, 1997).The bioavailability definition for drugs is slightly different in comparison to the dietary supplements and this difference is due to the administration method coupled with thespecified regulations from the Food and Drug Administration (Heaney, 2001).

Myricetin has a wide variety of effects in the body biologically (Ross and Kasum , 2002). Several studies have shown that it possess anti-cancer and antimutagenic properties and likewise has been implicated in the promotion of mutagenesis by utilizing the *Ames Test (*Mortelmans and Zeiger, 2000).The anti-inflammatory properties of this flavonoid were also discovered (Santhakumar et al., 2013). Myricetinin some other studies have been revealed to inhibit the tumor necrosis factor-alpha expression, a cytokine which aids the inflammatory response and is implicated in inflammatory diseases (Hollman and Katan, 1999).

Furthermore, myricetin consumption is good for the functionality of the heart (Flamini, 2013). Flavonoids like myricetin may improve the heart health through the prevention the oxidation of low density lipoproteins(LDL) and likewise reducing the oxidized LDL macrophages uptake. Myricetin improves the brain health and may offer benefits to people suffering from such brain diseases as the Parkinson and Alzheimer's disease through the suppression of the ROS production caused by glutamate and reduced glutamate-induced activation of caspase-3. Myricetin has also been shown to restore dopamine level in laboratory animals with induced Parkinsonism. It also has been shown to inihibit the formation of beta-amyloid fibril in Alzheimer's disease patients (Gupta, 2014).

The aim of this study was to carry out an *in silico* pharmacokinetic study on myricetin and its monosubstituted analogues which were derived through the structural modification of myricetin with the basic objective of increasing the bioavailability of the drug-like compound..

MATERIALS AND METHODS

Ligand preparation: The 2D structure of myricetin and its modified analogues were designed using the MarvinSketch software (Chen, 2005). 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 (Noel et al., 2011). It's an open and collaborative project allowing anyone to make searches, conversions, analysis, or storage data from molecular modeling, chemistry, solid-state materials, biochemistry, or related areas (Bultinck et al., 2002).

Ligand minimization: Myricetin and its modified analogues were minimized using the UCSF Chimera software (Pettersen et al., 2004). 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 Goddard et al., 2017).

Visualization of atoms: Atoms making up myricetin and its modified analogues were visualized using the Pymol molecular visualizer (Zhu 2014). PyMOL is an open-source tool for model visualization and it is made available for utilization in structural biology (Salam et al., 2014). The *Py* aspect of the name of the software is a reference pointer that it is extensible and extends by the python programming language (Beard et al., 2013).

RESULTS

2D Structure of myricetin and its analogues

Figure 1 shows the 2D structure of myricetin as designed by the MarvinSketch software. Modifications that resulted into the derivatives of this compound were made through the substitution of the OH group attached to the C_1 of myricetin with different functional groups such as the C=O, C_2H_5, CH_3, CHO, $CONH_2$, H and OCH_3

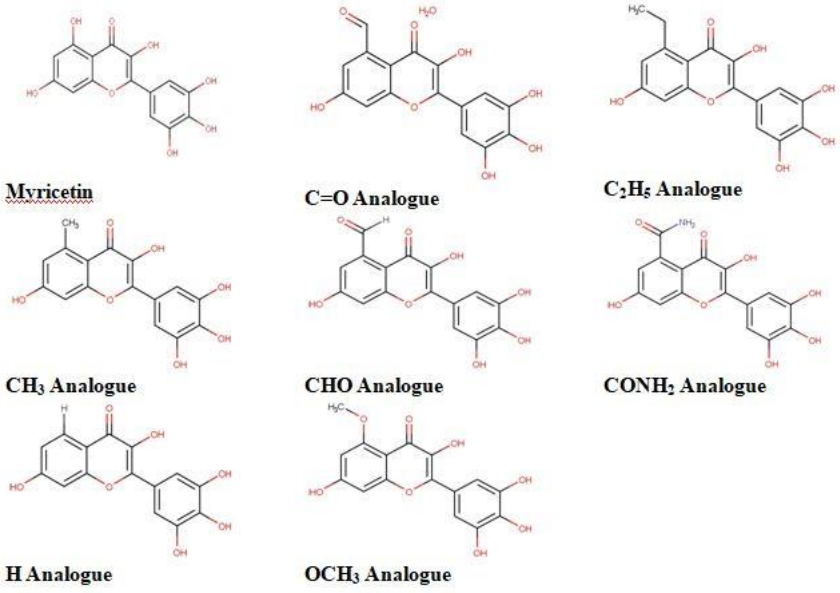

Figure 1.
The two dimensional (2D) structures of myricetin and its modified derivatives as designed using the MarvinSketch software.

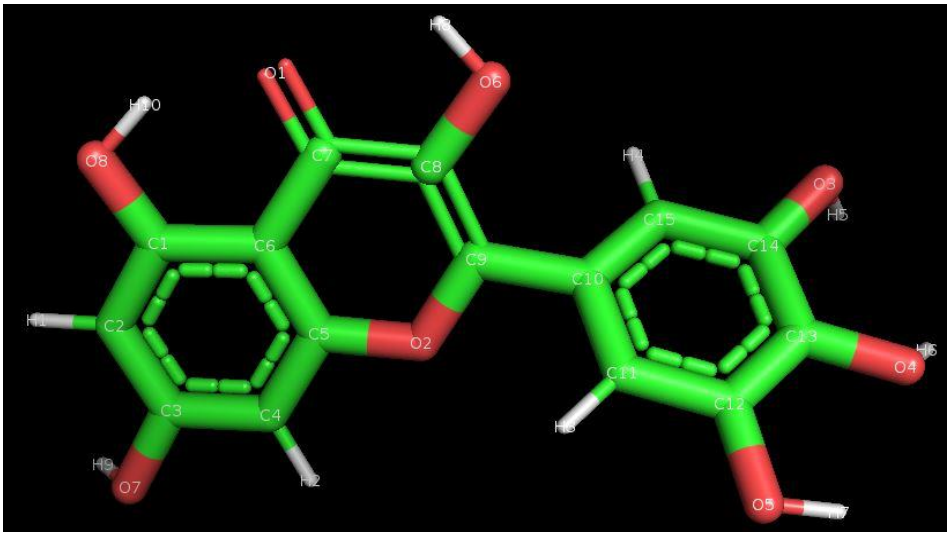

Figure 2:
3D structure of Myricetin showing all labeled atoms

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

Parameters	**Myricetin**	**C=O Analogue**	**C_2H_5 Analogue**	**CH_3 Analogue**	**CHO Analogue**	**$CONH_2$ Analogue**	**H Analogue**	**OCH_3 Analogue**
Formula	$C_{15}H_{10}O_8$	$C_{16}H_{12}O_9$	$C_{17}H_{14}O_7$	$C_{16}H_{12}0_7$	$C_{16}H_{10}O_8$	$C_{16}H_{11}NO_8$	$C_{15}H_{10}O_7$	$C_{16}H_{12}O_8$
Molecular weight g/mol	318.24	348.26	330.29	316.26	330.25	345.26	302.24	332.26
Num. H-Bond acceptors	8	9	7	7	8	8	7	8
Num. H-Bond donors	6	6	5	5	5	6	5	5
TPSA Å²	151.59	157.66	131.36	131.36	148.43	174.45	131.36	140.59
Lipophilicity Consensus Log $P_{o/w}$	0.79	0.44	1.86	1.46	0.73	0.28	1.12	0.98
Water Solubility Log S	Soluble	Soluble	Moderately Soluble	Soluble	Soluble	Soluble	Soluble	Soluble
GI absorption	Low	Low	High	High	Low	Low	High	Low
BBB permeant	No	No	No	No	No	No	No	No
P-gp substrate	No	No	No	No	No	No	No	No
CYP1A2 inhibitor	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
CYP2C19 inhibitor	No	No	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No	No	No
CYP2D6 inhibitor	No	No	Yes	Yes	No	No	Yes	No
CYP3A4 inhibitor	Yes	No	Yes	Yes	No	No	Yes	Yes
Lipinski Druglikeness	Yes; 1 Violation	Yes; 1 Violation	Yes; 0 Violation	Yes; 0 Violation	Yes; 1 Violation	Yes; 1 Violation	Yes; 0 Violation	Yes; 0 Violation
Synthetic accessibility	3.27	3.39	3.42	3.36	3.29	3.26	3.21	3.36

Table 2
Name of compounds, their chemical formulas and the canonical SMILES strings for each compound.

Compounds	**Chemical Formula**	**Canonical SMILES**
Myricetin	$C_{15}H_{10}O_8$	c1(cc(cc2c1c(=O)c(c(o2)c1cc(c(c(c1)O)O)O)O)O)O
C=O Analogue	$C_{16}H_{12}O_9$	c1(cc(cc2c1c(=O)c(c(o2)c1cc(c(c(c1)O)O)O)O)O)C=O.O
C_2H_5 Analogue	$C_{17}H_{14}O_7$	c1(cc(cc2c1c(=O)c(c(o2)c1cc(c(c(c1)O)O)O)O)O)CC
CH_3 Analogue	$C_{16}H_{12}O_7$	c1(cc(cc2c1c(=O)c(c(o2)c1cc(c(c(c1)O)O)O)O)O)C
CHO Analogue	$C_{16}H_{10}O_8$	c1(cc(cc2c1c(=O)c(c(o2)c1cc(c(c(c1)O)O)O)O)O)C=O
$CONH_2$ Analogue	$C_{16}H_{11}NO_8$	c1(cc(cc2c1c(=O)c(c(o2)c1cc(c(c(c1)O)O)O)O)O)C(=O)N
H Analogue	$C_{15}H_{10}O_7$	c1cc(cc2c1c(=O)c(c(o2)c1cc(c(c(c1)O)O)O)O)O
OCH_3 Analogue	$C_{16}H_{12}O_8$	c1(cc(cc2c1c(=O)c(c(o2)c1cc(c(c(c1)O)O)O)O)O)OC

The 3D structure of myricetin

The 3D structure of myricetin 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.

***In Silico* Pharmacokinetics**

Table 1 illustrates the specific pharmacokinetics and drug-likeness parameters of each experimental compound while table 2 shows the result of the structural formula conversion into canonical SMILES strings.

DISCUSSION

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(Pajouhesh and Lenz, 2005). For molecules to penetrate the blood–brain barrier (BBB) (in order to act on the central nervous systemreceptors), the value assigned to the polar surface area must be less than 90 angstroms squared(Hitchcock and Pennington, 2006).Myricetin and all its modified analogues (C=O, C_2H_5, CH_3, CHO, $CONH_2$, H and OCH_3) might lack the blood-brain barrier permeation attributes as their TPSA values appeared lower than 130 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 usingthe consensus log Po/w (Mannhold et al., 2009). The lipinski's rule (Lipinski et al., 2001) was used as the drug likeness descriptor for the purpose of this study and the optimal lipophilicity range (Log $P_{o/w}$) allowed should not exceed 5. The observation from the concensus lipophilicity column of table 1 shows that myricetin and all the analogues derived from it fall within the optimal lipophilicity range and as such can be regarded as drug-like 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 (Ritchie et al., 2013). Moreover, for discovery projects that target the oral form of administration, one of the major absorption property influencers is the solubility of the compound (Ottaviani, 2010). 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 (Savjani, 2012). A compound can be considered as soluble if the Log S value is less than 6 (Ritchie et al., 2013). Myricetin 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 lipophilic compounds are absorbed more easily.

The nature of the gastrointestinal mucosal membrane surface area plays an important role in the process of drug absorption and 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 (Bogentoft et al., 1978). 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 (Borgstroem et al., 1957).As displayed in table 1, only the C_2H5, CH_3 and H analogues of myricetin showed attributes of high gastrointestinal absorption which is a pointer to their oral bioavailability properties.

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 (Wolak and Thorne, 2013). Blood-brain barrier permeation results from table 1 showed that none of the experimental ligands can penetrate the blood brain barrier.

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 (Wolak and Thorne, 2013). 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 (Williams and Sinko, 1999).Myricetin and all its analogues appeared to be 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 CYP3A4and 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 (Williams and Sinko, 1999). 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 (Chan et al., 2004). It is also possible for the P-glycoprotein to have an influence on first-pass metabolism in a manner describing co-operativity (Chan et al., 2004). Table 1 showed that the

C_2H_5, CH_3, H, OCH_3 analogues with the inclusion of myricetin are inhibitors of CYP3A4 which implies that they are likely to be orally bioavailable compared to the other experimental ligands which might undergo CYP3A4-mediated intestinal biotransformation and in turn lowers their bioavailability.

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 (Ertl, and Schuffenhauer, 2009). This confirms the ease of the laboratory synthesis myricetin and all its modified analogues as their synthetic accessibility score do not exceed 4.0.

In conclusion, results from the *in silico* pharmacokinetics study on each of the experimental compounds have shown that the monosubstitution approach geared towards the improvement of the oral bioavailability of myricetin through structural modification is a reliable procedure. The C_2H_5, CH_3 and H analogues of myricetin have been shown to exhibit higher gastrointestinal absorption characteristics compared to other experimental ligands and as such might exhibit higher oral bioavailability tendencies.

We have also been able to observe that the monosubstituted groups that displayed the desired gastrointestinal absorption properties according to the *in silico* pharmacokinetics study were without oxygen. It can as such be inferred that the oxygen atom of the myricetin OH group might be implicated for its low gastrointestinal absorption attribute, likewise other modified functional groups with the oxygen moiety.The laboratory synthesis of the 3 compounds that has shown the desired GI absorption traits is therefore suggested for further studies to confirm their oral bioavailability

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