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In silico screening of hispolon and its analogs: Pharmacokinetics and molecular docking studies with cyclooxygenase-2 enzyme

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ABSTRACT

Hispolon is a phenolic compound with diverse biological activities. The analgesic action of hispolon is due to the inhibition of prostaglandins biosynthesis. However, the molecular basis of this inhibition has not been explored yet. Therefore, we have carried out theoretical investigations to evaluate the molecular basis of analgesic action. Furthermore, we have conducted high throughput *in silico* screening of a library of compounds to get novel cyclooxygenase 2 (COX-2) inhibitors with better pharmacokinetic and analgesic properties. The docking study was conducted using PyRx and the drug-like properties were calculated by MarvinSketch. Furthermore, the pharmacokinetic properties were computed on the online server PreADMET. In this study, our virtual screening based on structure similarity search using hispolon as reference structure afforded 1,699 compounds. These compounds were subjected to molecular docking with COX-2. These were then filtered based on binding affinity, binding poses, and drug-like properties which yielded seven compounds. The *in silico* pharmacokinetic study revealed that these compounds possess good human intestinal absorption and moderate permeability. Moreover, molecular docking of these compounds revealed that all the ligands possess moderate to good binding affinity (−7.6 to −8.9 Kcal/mol). Our computed properties may assist in developing hispolon derivatives with better pharmacokinetic and COX-2 inhibitory activity.

INTRODUCTION

Medicines from natural origin have been used for centuries to treat human diseases since they contain chemicals of therapeutic value (Beutler, 2009; Nostro *et al*., 2000). The World Health Organization published a report in 2008 stating that over 80% of the world's populace depends on traditional medicine for their primary healthcare needs (Vital and Rivera, 2009). So, traditional medicines serve as important sources of new drugs or drug candidates (Papia *et al*., 2016) and more than 80% of drug candidates are either directly obtained from natural products or developed from natural compounds (Lautié *et al*., 2020; Maridass and De Britto, 2008). In addition, about 50% of pharmaceuticals are derived from substances first identified or isolated from natural sources such as herbs/plants, organisms, animals, and insects (Krief *et al*., 2004).

Pain is manifested as a defensive response resulting from either cellular dysfunction or imbalance in its functions due to harmful stimulus. Pain is accompanied by human and animal diseases and has become the focus of global scientific research (Malarvizhi *et al*., 2020; Sarker *et al*., 2012). Many drugs are used to relieve pain, but none are devoid of side effects (Devaraj and Karpagam, 2011). Generally, compounds obtained from natural sources have little or no side effects, thus searching for new analgesic drugs from traditionally used plant species is still a rational strategy.

6-(3,4-Dihydroxyphenyl)-4-hydroxyhexa-3,5-dien-2-one (Hispolon) (Fig. 1) is a natural phenolic type bioactive

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compound first isolated from *Inonotus hispidus* in 1996 (Ali *et al*., 1996) then from *Phellinus linteus* (Chen *et al*., 2013; Toopmuang *et al*., 2014) and *Phellinus igniarius* (Mo *et al*., 2004). Hispolon possesses diverse biological activities, including antioxidant (Huang *et al*., 2011; Sarfraz *et al*., 2020), hepatoprotective (Huang *et al*., 2012), analgesic and anti-inflammatory (Chang *et al*., 2011; Sarfraz *et al*., 2020), antiproliferative (Huang *et al*., 2011), antimetastatic (Huang *et al*., 2010), antidiabetic (Sarfraz *et al*., 2020) immunomodulatory, and antiviral effects (Ali *et al.*, 1996, 2003). Hispolon also inhibits the chemiluminescent response of human mononuclear cells and downregulates nitrogen-induced proliferation of spleen lymphocytes of mice (Ali *et al*., 1996).

The analgesic action of hispolon is due to the inhibition of prostaglandin biosynthesis by cyclooxygenase (COX) enzyme (Chang *et al*., 2011), as it inhibits the nociception induced by acetic acid and formalin (late phase). Furthermore, hispolon demonstrated a significant inhibition of edema development induced by Carr in the third phase, indicating inhibition of prostaglandin synthesis.

Prostaglandins are biosynthesized by the COX enzyme (Goodman, 1996; Malarvizhi *et al*., 2020). COX-1 and COX-2 are isoforms of COX enzyme. The COX-1 is constitutively expressed in the stomach, kidneys, and platelets and plays a vital role in protection of the mucosa and functioning of platelets, whereas COX-2 is inducible and causes prostaglandin biosynthesis in inflammatory cells (Griswold and Adams, 1996; Malarvizhi *et al*., 2020). Recently, Wang *et al*. (2017) concluded that hispolon likely inhibits COX-2 and demonstrates analgesic and anti-inflammatory action.

The literature survey revealed no report on the molecular mechanism of COX-2 inhibition by hispolon, which may open a window to develop new COX-2 inhibitors, i.e., analgesic and anti-inflammatory drugs. Therefore, we have carried out a widely accepted virtual screening (molecular docking) of hispolon and a compound library containing hispolon skeleton curated from the PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/search/search.cgi#) to explore the molecular mechanism of COX-2 inhibition and to find novel COX-2 inhibitors. Also, *in silico* pharmacokinetic studies such as absorption [cellular permeability and human intestinal absorption (HIA)] and distribution [plasma protein binding (PPB), brain to blood partitioning, and interaction with P-Glycoprotein] of hispolon and its top hits (H1–H7) were carried out with PreADMET (https://preadmet.bmdrc.kr/).

METHODOLOGY

Molecular docking study

Preparation of target protein

The ligand bound X-ray crystal structure of target protein is essential to carry out a molecular docking study since docking software searches compatible binding site(s) of ligands within the protein of interest (Khan *et al*., 2018). Hence, for a molecular docking study, the availability of ligand-bound structure is a prerequisite. Furthermore, the structure should have acceptable values for Root mean square error (RMS) deviation from ideality, *R*work/*R*free, and validation parameters such as rotamer outliers, Ramachandran outliers, bad bond, or bad angle count. In the current investigation, the crystal structure of mammalian COX-2 complexed with celecoxib (PDB ID: 3LN1) (Wang *et al*., 2010) has been selected since the structure was solved at 2.4 Å resolution with *R*work/*R*free of 0.232/0.264, and the validation parameters suggests good quality of the modeled structure. At first, the protein molecule was prepared by removing water, ligands, heteroatoms, and chains B, C, and D, and adding polar hydrogen to the macromolecule using PyMOL (Version 1.7.4.4, Schrödinger). The energy of the protein structure was minimized with YASARA Energy Minimization Server (http://www.yasara.org/minimizationserver.htm).

Preparation of ligands

The structures of hispolon (CID 53395354) and celecoxib (CID 2662) were downloaded from PubChem. Molecular geometry was optimized with MMFF94 level of theory available in Open Babel (O'Boyle *et al*., 2011). Appropriate charges were then assigned to the optimized structure of ligands.

Preparation of ligand library

The library was prepared by utilizing the structure of hispolon as a substructure. The simplified molecular-input line-entry system string of hispolon was used to search for similar structures in the PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/search/search.cgi#) which yielded 1,699 structures. These structures were then optimized and prepared for docking by the method described above.

Protein–ligand docking

AutoDock Vina (Trott and Olson, 2010) implemented in PyRx 0.8 (Dallakyan and Olson, 2015) was used to conduct docking of target protein with the ligand. Docking was carried out with a box volume of 25.00 × 25.00 × 25.00 Å3 with a center of 30.7423, −22.209, and −15.738. Throughout the docking study, the flexible ligands searched its complementary site(s) within the search space of rigid macromolecule. Ligands with lowest binding affinity and promising binding pose were chosen as the best conformation. The interactions of protein residues with hispolon and other ligands were analyzed by PyMOL (DeLano, 2002).

Initially, celecoxib was re-docked into the binding pocket of COX-2 to validate the docking method. The root mean square deviation (RMSD) between the docked and experimental celecoxib was then calculated. The result revealed that the first pose of celecoxib nearly superimposes (RMSD < 1) with the experimental structure of celecoxib (Fig. 2). Thus, the docking method was reasonably accurate and reproducible.

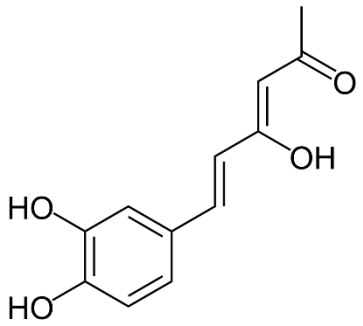

Figure 1. Structure of hispolon.

Calculation of drug-like properties

Since the purpose of docking the ligand database is to find compounds with higher affinity than hispolon, we have selected those with binding affinity greater or equal to the binding affinity of hispolon. This list of compounds was further standardized by applying Lipinski's (2004) rule of five and other drug-like properties in order to achieve compounds with good bioavailability (Veber *et al*., 2002), less adverse effects (Hughes *et al*., 2008), and lower risk of compound attrition (Ritchie and Macdonald, 2009). Lipinski (2004) stated that an orally active drug should have molecular weight below 500 Da, no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, and partition coefficient less than or equal to 5. Good oral bioavailability of compounds was also observed in rats if they contain rotatable bonds (ROTB) and polar surface areas (PSAs) less than 10 and 140 Å2, respectively (Veber *et al*., 2002). Hughes *et al*. (2008) reported that compounds with logP less than 3 and PSA greater than 75 Å2 have 6 times less likely to exhibit adverse events in *in-vivo* tolerance studies. In addition, the number of aromatic rings influences the risk of compound attrition and it was found that aromatic rings greater than 3 significantly increase the risk of compound attrition (Ritchie and Macdonald, 2009). So, we have standardized the list by applying the following drug-like properties:

- molecular weight below 500 Da;
- no more than five hydrogen bond donors;
- no more than 10 hydrogen bond acceptors;
- logP less than 3;
- ROTB less than 10;
- PSA greater than 75 Å2 but less than 140 Å2;
- aromatic rings less than or equal to 3.

Pharmacokinetic study

The pharmacokinetic properties were assessed with PreADMET server (https://preadmet.bmdrc.kr/). Pharmacokinetic parameters such as HIA, Caco-2 cell permeability (PCaco-2), interaction with P-glycoprotein (Pgp), skin permeability (PSkin), penetration of blood–brain barrier, and PPB were calculated and predicted.

RESULTS AND DISCUSSION

Calculation of drug-like properties

Since hispolon (H) demonstrated a binding affinity of −7.6 Kcal/mol (Table 1), we sorted out the list of compounds having binding affinity greater than or equal to −7.6 Kcal/mol, which yielded 696 compounds. Further standardization based on drug-like properties provides a list of seven compounds (H1–H7) (Table 1 and Fig. 3). It is expected that *in vitro* investigation of these ligands would demonstrate good bioavailability, less adverse effects, and a lower risk of attrition.

Molecular docking study

The crystal structure of 3LN1 revealed that COX-2 enzyme appears as a dimer and each monomer consists of an N-terminal Epidermal Growth Factor (EGF)-like domain, a membrane binding domain (MBD), and a large catalytic domain with two active sites, the peroxidase site, and the COX site (Blobaum *et al*., 2015). The critical interactions between the inhibitors and residues at the active site of COX enzymes have been identified by X-ray crystallographic and site-directed mutagenesis studies (Kalgutkar *et al*., 2000; Picot *et al*., 1994; Rowlinson *et al*., 2003). The COX-2 inhibitors are thought to first enter through the four helical (A, B, C, and D) MBDs into an open area called the lobby, consisting of Val74, Leu78, Trp85, Ile98, Tyr101, and Val102 (Blobaum *et al*., 2015; Picot *et al*., 1994). The lobby is separated from the active site by a gate made of three conserved residues Arg106, Tyr341, and Glu510 (Blobaum *et al*., 2015). It has been established that the selectivity of celecoxib and rofecoxib for COX-2 is due to the ability of inserting the sulfonamide and methyl sulfone groups, respectively, into the side pocket of COX-2 constituting of His75, Arg499, Val509, and Glu510 (Blobaum *et al*., 2015). In addition, Val509 at the active site of COX-2 confers the selectivity of selective COX-2 inhibitors since mutation of this residue to Ile abolishes the selectivity (Gierse *et al*., 1996). It has been found that celecoxib also makes hydrophobic interaction with Val509. Therefore, molecules having the ability to insert a functional group into the side pocket of COX-2 and makes hydrophobic interaction with Val509 may act as selective COX-2 inhibitors.

The binding affinity and interactions of H and top hits (H1, H2, H3, H4, H5, H6, and H7) are presented in Tables 1 and 2 and Figures 4 and 5. H displays binding affinity of −7.6 Kcal/mol and forms hydrogen bonding with the side chains of TYR371, SER516, and with the backbone of PHE504. Furthermore, H associates via hydrophobic interaction with VAL509, the crucial residue involved in COX-2 selectivity. The aromatic ring of H is accommodated in the side pocket of COX-2 (Fig. 4); thereby, it is expected that H may inhibit the biosynthesis of prostaglandins by selectively inhibiting COX-2.

H1 interacts with a binding affinity of −8.9 Kcal/mol to the side chain of TYR341 and the backbone of LEU338 through hydrogen bond formation and the complex is further stabilized by the hydrophobic interactions with VAL335, LEU338, SER339, and VAL509. H2 shows a binding affinity of −8.6 Kcal/mol and forms only hydrophobic interactions with VAL335, VAL509,

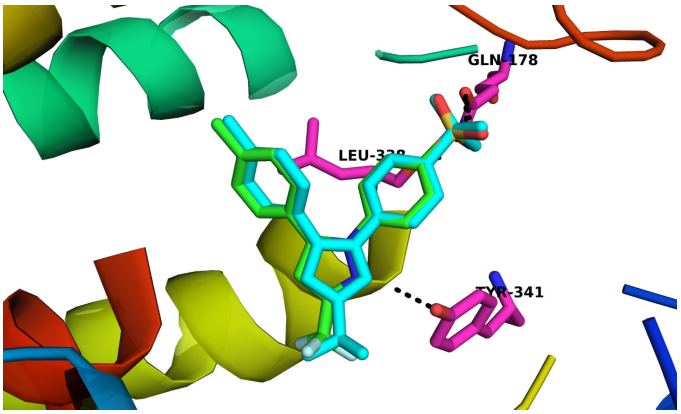

Figure 2. The superposition of the best docking pose of celecoxib (PDB ID: 3LN1) with the X-ray structure. (■) Experimental celecoxib; (■) Docked celecoxib.

and ALA513. H3 makes hydrophobic interactions with SER339, VAL509, and ALA513 with a binding energy of −8.4 Kcal/mol. The compound H4 demonstrates a binding energy of −8 Kcal/mol and forms hydrogen bonding with GLN178, ILE503, and PHE504 which is further stabilized by the hydrophobic interactions with VAL335, LEU338, VAL509, GLY512, and ALA513. H5 forms hydrogen bond with the –OH of TYR341 and the complex is stabilized by hydrophobic interactions with VAL509 and ALA513 with a binding affinity of −8 Kcal/mol. H6 and H7 interact through hydrogen bond formations with TYR341 and ARG106, and TYR371 and PHE504 (with the backbone), and SER516 and GLN178 with the binding energy of −7.8 and −7.7, respectively. Both of them form hydrophobic interactions with VAL509.

The analysis of binding mode reveals that ligands H1, H2, H3, H4, and H7 form hydrophobic interaction with VAL509 and insert their aromatic ring in the site pocket of COX-2; hence, it may be assumed that these ligands exert their inhibitory action on the biosynthesis of prostaglandins by selectively inhibiting COX-2.

Pharmacokinetic study

Pharmacokinetic studies, including absorption (HIA and cellular permeability) and distribution (PPB, brain to blood partitioning, and interaction with P-Glycoprotein) of H and its selected top hits (H1–H7) were performed with PreADMET server (https://preadmet.bmdrc.kr/). The predicted absorption and distribution parameters are presented in Table 3.

Table 1. Binding affinity (Kcal/mol) and other drug-like properties of hispolon and its analogs.

PubChem ID	Compound name/code	Binding affinity (Kcal/mol)	Molecular weight	H acceptor (≤10)	H donor (≤5)	logP (<3)	Rotatable bond (<10)	PSA (<75) Å²	Number of aromatic ring (≤3)
53395354	Hispolon (H)	−7.6	220.22	4	3	1.71	3.00	77.76	1
11459516	Echinotinctone (H1)	−8.9	256.25	4	2	2.38	0	69.59	3
57328490	CID 57328490 (H2)	−8.6	269.23	5	0	1.26	0	71.06	2
54018219	CID 54018219 (H3)	−8.4	349.40	5	1	2.88	3	70.78	3
58422829	SCHEMBL12305066 (H4)	−8	244.24	4	2	2.37	2	66.76	2
70491116	SCHEMBL11011120 (H5)	−8	268.26	4	1	2.86	2	55.76	3
10589108	CID 10589108 (H6)	−7.8	284.31	4	2	2.81	5	66.76	1
44413103	CHEMBL377858 (H7)	−7.7	234.25	4	2	1.86	4	66.76	1

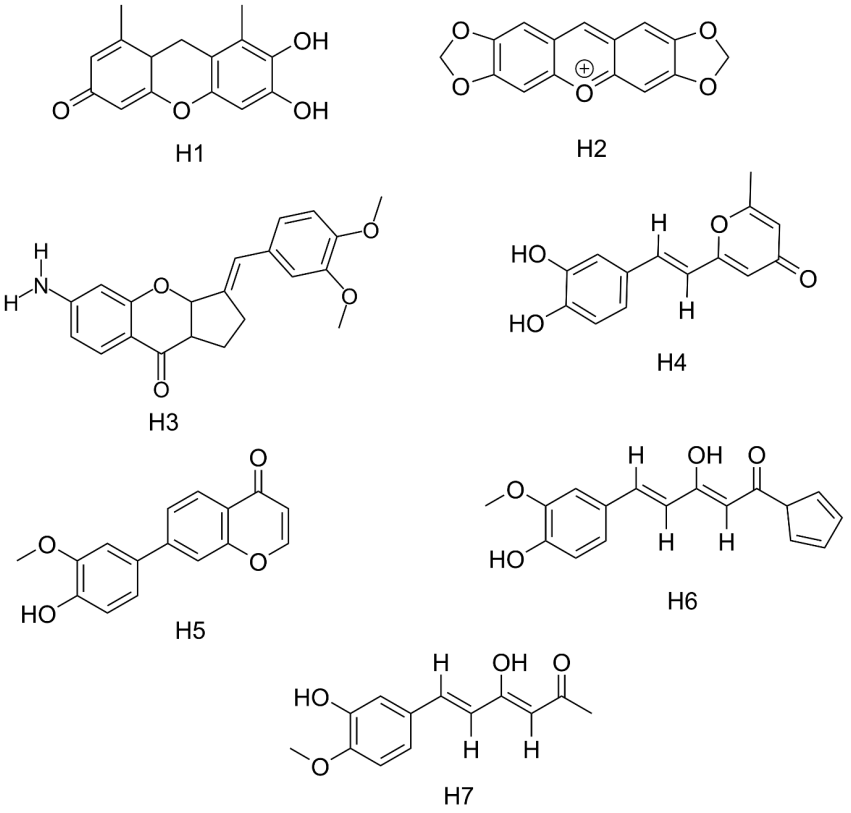

Figure 3. Structure of hispolon derivatives.

The computed HIA of H and top hits (Table 3) ranged from 84.38%–98.34%, suggesting all the compounds are well absorbed through the intestinal cell (Yee, 1997). H and top hits were moderately permeable since values for absorption through Caco-2 cells (PCaco-2) were 9.76–56.89 nm/s (Yazdanian *et al*., 1998). PSkin is a crucial parameter for assessing drugs and chemicals requiring transdermal administration (Singh and Singh, 1993). All compounds were found to be impermeable through the skin since the calculated PSkin were −2.65 to −4.49.

The distribution properties of the compounds were evaluated based on the brain to blood partitioning (Cbrain/Cblood) and PPB values. Strongly bound compounds have a PPB value greater than 90%, whereas lower than 90% PPB indicates weakly bound chemicals (https://preadmet.bmdrc.kr/adme-prediction/). H1, H3, and H5 were considered strongly bound chemicals since they had PPB values of 99.5%, 91.4%, and 93.3%, respectively, whereas H, H2, H4, H6, and H7 were weakly bound since they had PPB values lower than 90%. Due to its strong binding nature,

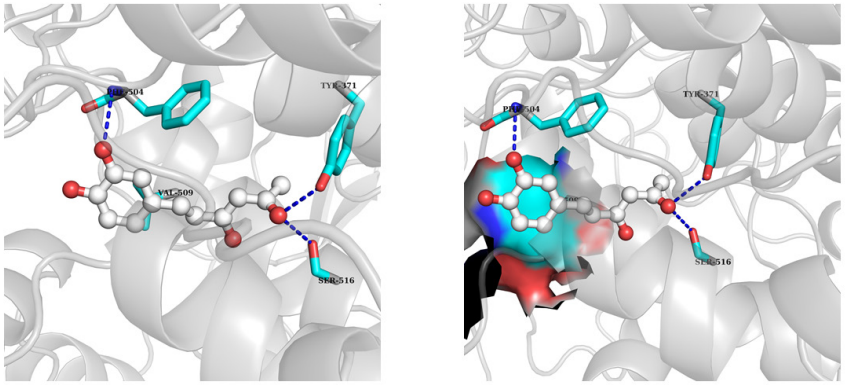

Figure 4. Interactions of hispolon at the binding site of COX-2. A. Interactions of different residues with H. B. Aromatic side chain is at the side pocket of COX-2. Interacting residues are presented in stick model and ligands are in ball and stick model. Blue dash indicates hydrogen bonding.

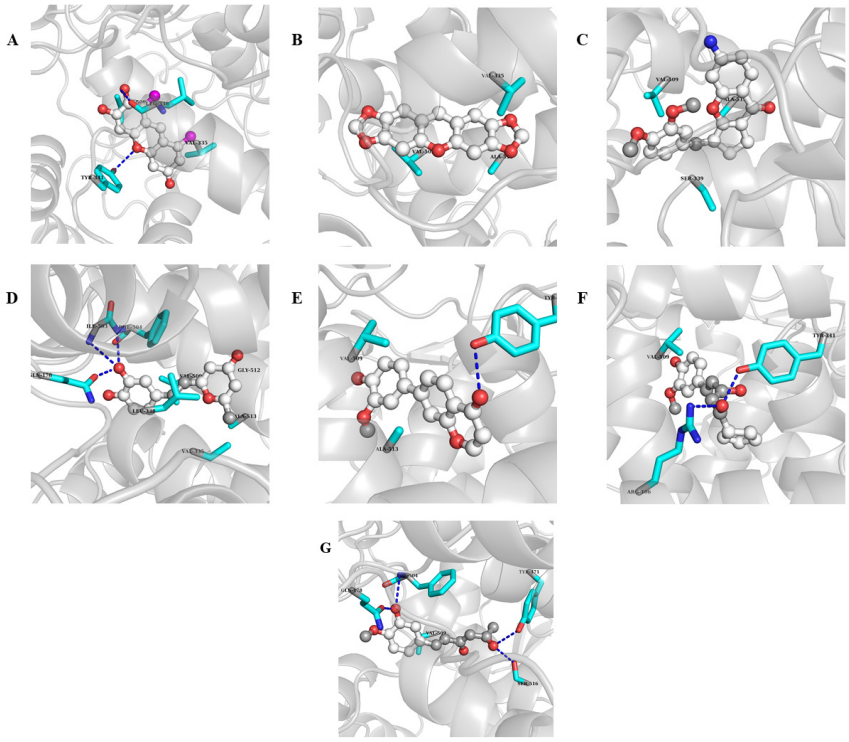

Figure 5. Interactions between COX-2 and A) H1, B) H2, C) H3, D) H4, E) H5, F) H6, and G) H7. Interacting residues and ligands are presented in stick and ball and stick models. Blue dash indicates hydrogen bonding.

Table 2. Interactions of hispolon (H) and its selected analogs (H1–H7) with COX-2 enzyme.

Compounds	Interacting residues and atoms	Bond distance (Å)	Interaction types
Hispolon (H)	[TYR371]OH–O[H]	3.035	Hydrogen bond
	[SER516]OG−O[H]	3.071	Hydrogen bond
	[H]H−O[PHE504]	2.715	Hydrogen bond
	[VAL509]CG2−Pi[H]	3.328	Hydrophobic
Echinotinctone (H1)	[TYR341]OH−O[H1]	2.975	Hydrogen bond
	[H1]H−O[LEU338]	2.552	Hydrogen bond
	[SER339]CA−Pi[H1]	3.493	Hydrophobic
	[VAL509]CG1−Pi[H1]	3.700	Hydrophobic
	[VAL509]CG2−Pi[H]	3.731	Hydrophobic
	[H1]C−C[VAL335]	3.793	Hydrophobic
	[H1]C−C[LEU338]	3.657	Hydrophobic
CID 57328490 (H2)	[VAL335]CG1−Pi[H2]	3.550	Hydrophobic
	[VAL509]CG1−Pi[H2]	3.975	Hydrophobic
	[VAL509]CG2−Pi[H2]	3.329	Hydrophobic
	[ALA513]CB−Pi[H2]	3.793	Hydrophobic
CID 54018219 (H3)	[SER339]CA–Pi[H3]	3.490	Hydrophobic
	[VAL509]CG1–Pi[H3]	3.545	Hydrophobic
	[VAL509]CG2–Pi[H3]	3.645	Hydrophobic
	[ALA513]CB−Pi[H3]	3.729	Hydrophobic
	[GLY512]C,O;[ALA513]N−Pi[H3]	3.821	Hydrophobic
	[ALA513]C−C[H3]	3.498	Hydrophobic
SCHEMBL12305066 (H4)	[PHE504]N−O[H4]	2.995	Hydrogen bond
	ILE503−O[H4]	3.3	Hydrogen bond
	[H4]H−OE1[GLN178]	1.918	Hydrogen bond
	[LEU338]CD1−Pi[H4]	3.956	Hydrophobic
	[VAL509]CG2−Pi[H4]	3.384	Hydrophobic
	[GLY512]C,O;[ALA513]N−Pi[H4]	4.078	Hydrophobic
	[H4]C−C[VAL335]	3.830	Hydrophobic
SCHEMBL11011120 (H5)	[TYR341]OH−O[H5]	2.925	Hydrogen bond
	[VAL509]CA−Pi[H5]	3.777	Hydrophobic
	[ALA513]CB−Pi[H5]	3.370	Hydrophobic
	[ALA513]CB−Pi[H5]	3.849	Hydrophobic
CID 10589108 (H6)	[TYR341]OH−O[H6]	2.750	Hydrogen bond
	[ARG106]NH−O[H6]	3.2	Hydrogen bond
	[VAL509]CA−Pi[H6]	3.694	Hydrophobic
CHEMBL377858 (H7)	[TYR371]OH−O[H7]	3.151	Hydrogen bond
	[PHE504]N−O[H7]	3.090	Hydrogen bond
	[SER516]OG−O[H7]	3.118	Hydrogen bond
	[H7]H−OE1[GLN178]	1.883	Hydrogen bond
	[VAL509]CG2−Pi[H7]	3.371	Hydrophobic

Table 3. Absorption and distribution of hispolon and its analogs.

Compounds	Absorption			Distribution			
	HIA (%)	P_{Caco-2} (nm/s)	P_{skin}	PPB (%)	C_{brain}/C_{blood}	P-glycoprotein (Inhibition)	P-glycoprotein (Substrate)
Hispolon (H)	84.38a	15.59 a	−3.35 a	84.8 a	0.1 a	No	No
Echinotinctone (H1)	92.35	11.60	−3.66	99.5	0.8	No	No
CID 57328490 (H2)	98.34	56.89	−4.49	47.9	0.7	No	No
CID 54018219 (H3)	96.65	32.02	−3.54	91.4	0.03	Inhibitor	Substrate
SCHEMBL12305066 (H4)	92.07	9.76	−3.39	88.6	0.2	No	No
SCHEMBL11011120 (H5)	95.55	25.60	−3.24	93.3	0.05	No	No
CID 10589108 (H6)	93.04	21.63	−2.65	86.0	0.4	No	No
CHEMBL377858 (H7)	91.27	17.36	−2.77	75.9	0.1	No	No

a Khan *et al.*, 2017.

it could be inferred that H1, H3, and H5 would be less likely to be encountered in the systemic circulation.

Chemicals can be classified into three groups based on their absorption to Central Nervous System (CNS). Compounds with Cbrain/Cblood values greater than 2.0 have high absorption to CNS, whereas compounds with Cbrain/Cblood values from 0.1 to 2.0 and less than 0.1 are considered to have moderate and low absorption to CNS, respectively. Based on these values, it can be inferred that all compounds except H3 and H5 have moderate absorptions to CNS. Both H3 and H5 have low absorption, and hence are likely to be present in the systemic circulation.

P-glycoprotein (PGP) is an adenosine triphosphate-dependent efflux transport protein encoded by the multidrug resistance gene. It has been shown to influence the absorption, distribution, and excretion of drugs due to its efflux action (Khan *et al*., 2018; Schinkel, 1999). Ineffective cancer and antibiotic chemotherapy is an outcome of increased expression of this gene (Cabrera *et al*., 2006; Kim *et al*., 1998). Compounds that act as substrates for PGP will have low efficacy; hence, substrates for PGP need to be identified and eliminated (de Cerqueira Lima *et al*., 2006; Wang *et al*., 2005). However, this is done through laborious *in vitro* and *in vivo* analyses (Joung *et al*., 2012). The likelihood of compounds to act as substrates for PGP can be predicted by *in silico* tools (Joung *et al*., 2012). The computer-aided screening demonstrated that only H3 is a dual inhibitor and substrate for PGP analogous to quinidine (Zhou, 2008). This phenomenon is due to the complex modulatory interaction of H3 with PGP, and hence H3 acts as both a substrate and an inhibitor (Zhou, 2008). Therefore, coadministration of H3 with a chemotherapeutic agent may demonstrate a beneficial effect in cancer chemotherapy.

CONCLUSION

In the current investigation, our virtual screening based on structure similarity search afforded 1,699 compounds which were further standardized based on binding affinity, binding poses, and drug-like properties. The screening and filtering parameters yielded a list of seven compounds (H1, H2, H3, H4, H5, H6, and H7). Therefore, these compounds likely demonstrate good bioavailability, less adverse effects, and lower risk of attrition.

The *in silico* pharmacokinetic study revealed that these compounds possess good human intestinal absorption and moderate permeability through Caco-2 cells. Furthermore, the Cbrain/Cblood ratio of these drugs indicates moderate penetrability of CNS except H3 and H5, which are low-absorbing agents for CNS and will predominately reside in the systemic circulation. Since H3 acts as both inhibitor and substrate for P glycoprotein due to complex modulatory interactions with the PGP (Zhou, 2008), it is unlikely that H3 would be pumped out of the cells completely and hence reduce the possibility of resistance mediated by the efflux pump. Therefore, the coadministration of H3 with a chemotherapeutic agent may demonstrate a beneficial effect in cancer chemotherapy.

Moreover, molecular docking of H and its selected top hits revealed that all the ligands cause an hydrophobic interaction with Val509, and the H, H1, H2, H3, H4, and H7 ligands can accommodate their aromatic ring inside the side pocket of COX-2. Therefore, these ligands likely exert their analgesic action by selectively blocking the biosynthesis of prostaglandins mediated by COX-2.

These COX-2 inhibitor candidates may be subjected to *in vitro* experiments, followed by *in vivo* experiments to assess the COX-2 inhibitory activity. The investigated pharmacokinetic properties and molecular docking will guide the synthesis of hispolon derivatives with better pharmacokinetic properties and COX-2 inhibitory activity.

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CONFLICTS OF INTERESTS

The authors declare that there is no conflicts of interests.

FUNDING

Not applicable.

CONSENT TO PARTICIPATE

Not applicable.

ETHICS APPROVAL

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Data and material are included in the article.

AUTHORS' CONTRIBUTIONS

Conceived and designed the experiments: MFK and AR. Performed theoretical investigations: MM, MFK, and SMN. Analyzed the data: MM, MFK, RBR, and SMN. Wrote the manuscript: MM, MFK, RBR, and MAR. All authors read and approved the final manuscript for publication.

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