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Molecular Docking and Quantitative Structure Activity Relationship for the Identification of Novel Phyto-inhibitors of Matrix Metalloproteinase-2

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Abstract

Cancer is a deadly disease that affects humans of all races, gender, and age. The matrix metalloproteinase-2 (MMP-2) protein is a good target when designing an anticancer drug. The expression of this protein influences cell growth and division. The activation of this protein opens the extracellular matrix and provides entry of the new cells into the body system. Phytochemicals are known to possess the ability to cure human diseases with little or no side effect. In this study, phytochemicals from yellow mombin, turmeric, green chiretta, African basil and ginger were evaluated against the MMP-2 orthosteric sites and three-dimensional quantitative structure activity relationship (3D-QSAR) was used to generate a model for MMP-2 inhibitors. The drug-like properties of the lead compounds and the standard drug were tested by employing the Lipinski rule of five. Azulene from ginger, Andrographidine A from green chiretta and Isovitexin from African basil with the docking scores of -7.3 kcal/mol, -9.3 kcal/mol, and -8.2 kcal/mol, respectively, were found to be the lead compounds as potential MMP-2 inhibitors. A robust regression model for the inhibition of MMP-2 was generated. Andrographidine A with the highest docking score stood out as a potential inhibitor of MMP-2 by sharing selective interactions with his-120 and his-130. The QSAR model proposed herein was thoroughly validated and hence offers a tool for the identification of potential MMP-2 inhibitors in the future.

Introduction

Cancer alters the normal properties of cells via different molecular events [1]. Normal control systems that prevent cell overgrowth and the invasion of other tissues are disabled in cancer cells [2]. Cancer is one of the deadliest diseases in both developed, and developing countries. Lung, breast, prostate and large bowel cancers are the most common types of cancer and are responsible for more than half of all cases [3]. Zinc-dependent proteases like matrix metalloproteinases (MMPs) or matrixins cleave and rebuild connective tissue components like elastin, collagen, gelatin and casein [4]. MMPs also degrade extracellular matrix during growth, morphogenesis and other developmental stages. Due to their physiological functions, MMPs have been reported for their high activity in diseases and pathological processes like inflammation and cancer [4]. High MMPs expression levels contribute to the development of cancer [5]. Due to the importance of MMPs in diseases, efforts have been made in the area of drug development to develop small molecule drugs that can inhibit MMPs. However, all efforts failed in clinical trials due to their low specificity, MMP inhibitors binding to Zn^{2+} and other heavy metals (active sites) in various proteins in the body, hence they are highly toxic [6, 7]. Also, drugs directed at multiple MMP family members evoked surprising effects because MMP activities are numerous. In fact, some MMPs have been reported for the roles they play as anti-tumorigenic agents [8, 9]; hence, there is a need to develop inhibitors that target only one or a narrow range of MMPs. Studies have repeatedly shown that consuming fruits and vegetables regularly helps greatly in reducing risks of developing chronic diseases such as cancer owing to the antioxidant activity of the phytochemicals present and are as well less toxic even after reacting with Zn^{2+}, which is responsible for activating MMP-2 [10]. Some traditional healers in Nigeria claim that they can successfully cure cancer using herbs [11].

In the present study, phytochemicals from *Spondias mombin*, *Curcuma longa*, *Andrographis paniculata*, *Oscimum gratisimum*, and *Zingiber officinale* were screened for their inhibitory properties against MMP-2. The conformity of the lead compounds to Lipinski's rule of five (RO5) was assessed. The accuracy of the docking results was validated by a coefficient correlation with reported MMP-2 inhibitors. In addition, 3-dimensional quantitative structure-activity relationship (3D-QSAR) was used to generate a model for MMP-2 inhibitors, and the residues involved in amino acid interactions of the leads were determined.

Materials and Methods

Protein preparation for docking

The MMP-2 protein with protein data bank (PDB) ID; 1hov and crystallographic resolution of 2.50Å (Fig. 1) [12] was downloaded from the protein data bank (http://www.rcsb.org). Employing PyMOL Autodock/Vina Plugin, the co-crystallized ligand from the MMP-2 protein was extracted. Tanomastat, an anticancer drug that targets the MMP family was downloaded from the PubChem repository (https://pubchem.ncbi.nlm.nih.gov) and used as the standard drug (Fig. S1).

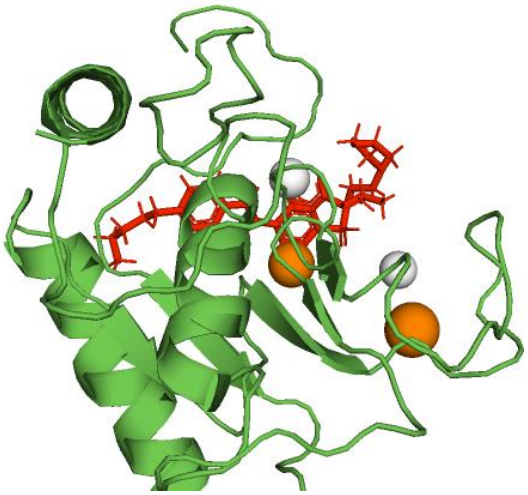

Fig. 1 Matrix metalloproteinase-2 (green) and a co-crystallized compound (red). The grey colored balls represent Ca while orange colored balls represent Zn. PDB ID: 1ho

Ligand preparation for molecular docking

A total of fifty-eight, fifty-five, thirty, fifty-three and fifty-five phytochemicals characterized from yellow mombin (*Spondias mombin*), turmeric (*Curcuma longa*), green chireta (*Andrographis paniculate*), African basil (*Oscimum ratisimum*) and ginger (*Zingiber officinal*), respectively, were downloaded in the structure-data file format (sdf) from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The phytochemicals were catenated and converted to pdb format using Open Babel and later to pdbqt with the lig prep command lines in AutoDock Vina.

The phytochemicals (206) from the plants were docked into the active sites of MMP-2.

Molecular properties and Lipinski's rule of five

The Mavin Viewer software was employed (www.chemaxon.com) in the present study to access the conformity of the lead compounds to the Lipinski's rule of five (RO5). The RO5 helps to determine the drug-ability and check the absorption, distribution, metabolism, and excretion (ADME) properties of the leads. The number of rotatable bonds and polar surface area, which help in differentiating orally active compounds from those that are not was also obtained for the leads [13].

Validation of the docking results

Validation of the docking result was performed with the multiple alignments of the MMP-receptor sequences obtained from PubMed. Using the online available ChemBL Database, the MMP-2 sequences were blasted on www.ebiac.uk/chembl/. The result produced with the identity of 100%, IC_{50} (half maximal inhibitory concentration) value of 5000 and KI value of 721, this was downloaded in text format and converted to sdf format with the Data Warrior version 2 (www.openmolecules.org). This sdf file was then converted to pdb and pdbqt. A total of two hundred and ten compounds were obtained from the chemBl file and were docked into the MMP-2 catalytic site, as it was with the phytochemicals. A correlation graph was plotted with the pIC_{50} and docking scores obtained from docking the chemBl's compounds into the MMP-2 catalytic site. The significance of correlation between the negative log of the IC_{50} (pIC_{50}) and the docking scores was determined at $P<0.05$.

Three-dimensional quantitative structure-activity relationship (3D-QSAR)

Data collection and descriptor calculation

The MMP-2 bioassay IC_{50} was downloaded from the PubChem-ChemBl database, and saved in the excel format. The bioassay was converted to sdf form with DataWarrior, and the sdf structures were catenated and converted to their 3-dimensional structures using command lines. The chemistry development kit (CDK) 1.4.6 was used for the calculation of the molecular descriptors [14].

Data pre-treatment

Pretreatment with the aim of removing the co-linearity of descriptors was carried out with the V-WSP algorithm [15]. The variance cut-off was set at 0.0001, while the correlation coefficient cut-off was set at 0.5.

Data set division: training and test sets

A dataset of 100 MMP-2 inhibitors was obtained from the chEMBL database (http://ebi.ac.uk). The data were split into training (70%) and test set (30%) using the Kennard Stone algorithm technique built into the Dataset Division GUI 1.2 [16].

Genetic algorithm and multiple linear regression analysis

A genetic algorithm, a search heuristic system that mimics the natural selection process, was used to perform the selection of significant variables (descriptors). The individual descriptor was picked through a fitness function, which assesses each descriptor, and as a result of this fitness function, the best descriptors were picked. A training set of 70 MMP-2 molecular structures was used as the training set and an equation length of ten descriptors (variables) was adopted. Multiple linear regression (MLR) and generation of the unbiased model equation was carried out with the R software for statistic computing.

Results and Discussion

Molecular docking

In the present study, phytochemicals from yellow mombin, turmeric, green chireta, African basil and ginger screened against the MMP-2 protein catalytic site revealed azulene from ginger, andrographidine A from green chireta and isovitexin from African basin with the docking scores of -7.3 kcal/mol, -9.3 kcal/mol, and -8.2 kcal/mol, respectively, as the lead compounds (Table 1; Table S1-S5; Fig. S1). The docking score, (-9.2 kcal/mol) of tanomastat against the catalytic site of MMP-2 was used as the cut-off for the selection of the leads. Tanomastat possesses both antiangiogenic and antimetastatic properties [12]. It is worthy of note that the leads identified herein through molecular docking screening have all been reported elsewhere to have anticancer properties

Table 1 The docking scores of lead phytocompounds from ginger, green chireta, and African basil and the standard drug.

Plant	Phytochemical	Docking score (kcal/mol)
Ginger	Azulene	-7.3
Green chireta	Andrographidine A	-9.3
African basil	Isovitexin	-8.2
Standard drug	Tanomastat	-9.2

Table 2 Lipinski's physiochemical properties of the lead compounds.

Plants	Hit compounds.	Docking scores kcal/mol	HBA ≤10	HBD ≤ 5	RB ≤10	XLOGP3 ≤5	MW <500	PSA 140)
Ginger	Azulene	-9.5	0	0	0	3.2	128.17	0
Green chireta	Andrographine A*	-9.2	10	4	6	0.7	426.45	144.14
African basil	Isovitexin**	-10.2	10	7	3	3	432.38	177.14
Standard drug	Tanomastat*	-9.2	4	1	8	5.5	410.91	54.37

HBA = hydrogen bond acceptor; HBD = hydrogen bond donor; RB = rotatable bond; XlogP = octanol-water partition coefficient; MW = molecular weight; PSA = polar surface area

* Those that disobeyed one of the Lipinski rules; ** Those that disobeyed two of the Lipinski rules.

[17-19]. This clearly shows that this technique (molecular docking screening) can be applied for the identification of novel anti-cancer compounds.

Lipinski rule of five (RO5)

The RO5 helps to evaluate drug-likeness or determine whether a chemical compound with a certain pharmacological or biological activity possesses properties that qualify it, as a likely orally active drug in humans [20]. According to Lipinski, an orally active drug must not violate more than one of these rules: (1) not more than 5 hydrogen bond donors, (2) not more than 10 hydrogen bond acceptors, (3) a molecular mass less than 500 Daltons and (4) an octanol-water partition coefficient log-*P* not greater than 5. This also includes an additional rule proposed by Veber and coworkers [13], (5) not less than 10 rotatable bonds and polar surface area (PSA) less than 140. Andrographidine A disobeyed one of the Lipinski rules while isovitexin disobeyed two of the Lipinski rules as those have values that deviate a little from the Lipinski prescribed values (Table 2). Azulene and andrographidine A with just one violation of the rules are likely orally active phytocompounds. Isovitexin with two violations of the rules may not be an orally active drug (Table 2). However, there are reports of exceptions to Lipinski's rule of five, mostly among natural products [21].

Validation of docking results

The veracity of the docking results in the present study was validated by the coefficient correlation analysis of the docking results generated from docking 271 reported MMP-2 inhibitors from the Chembl database against their corresponding pIC_{50}. There was a significant positive correlation (R^2 = 0.459) between the docking score of MMP-2 inhibitors and their corresponding experimentally derived pIC_{50} at P<0.001 (Fig. 2; Table 3). This is a revelation that computers can accurately predict experimental values and hence the docking scores obtained herein are correct and reliable.

Quantitative structure-activity relationship (QSAR) and regression Analysis

QSAR predicts the relationship that exists between the structure and activity of a compound. Seventy (70) MMP-2 inhibitors were used as the training set. The linear regression analyses of the training set were carried out with R software for statistical computing. The Pearson correlation (R) when all the ten (10) descriptors were used was 0.976 (Table S6). This represents a very strong correlation. The R^2 value of 0.952 shows that the model in the present study could predict a wanton 95% of the variation in the predicted pIC_{50} (The IC_{50} values were converted to pIC_{50} with the formula (pIC_{50} = -log IC_{50}) that is accounted for by the ten (10) descriptors. The adjusted-R^2 is concerned with how the model generalizes, which is the external validation of the model. The adjusted-R^2 was close to the R^2 value (the difference between the R^2 value and the adjusted-R^2 value was 0.044) (Table S6), this signifies that our model had experienced just a paltry of 4.4% shrinkage in predicting external pIC_{50}. The closeness of the adjusted-R^2 value to the R^2 value shows that the cross validity of the model is very good.

Durbin-Watson statistics, 1.311976 (Table S6) informs that the assumption of independent error is tenable and the model is valid [22]. The Durbin-Watson statistics, as a conservative rule of thumb, predicts values less than 1 or greater than 3 causes

Table 3 Coefficient correlation analysis of the docking score of matrix metalloproteinase-2 inhibitors and their corresponding experimentally derived pIC_{50}.

Correlation coefficient	0.459**
Sig. (2-tailed)	<0.0001
N	271
Bootstrap	
Bias	-0.002
Std. error	0.048
BCa 95% confidence interval	
Lower	0.368
Upper	0.541

**Significant correlation at P<0.001

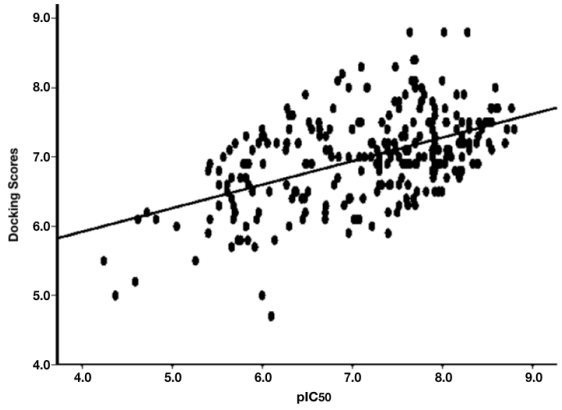

Fig. 2 Coefficient correlation of docking scores of matrix metalloproteinase-2 inhibitors and their corresponding experimentally derived pIC_{50}.

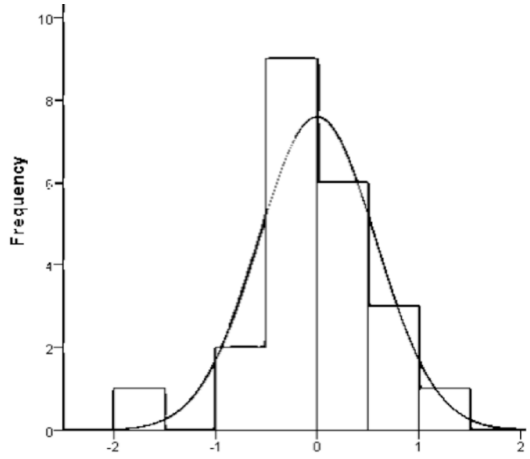

Fig. 3 Histogram plot of the differences between the observed and the predicted pIC50 (residuals).

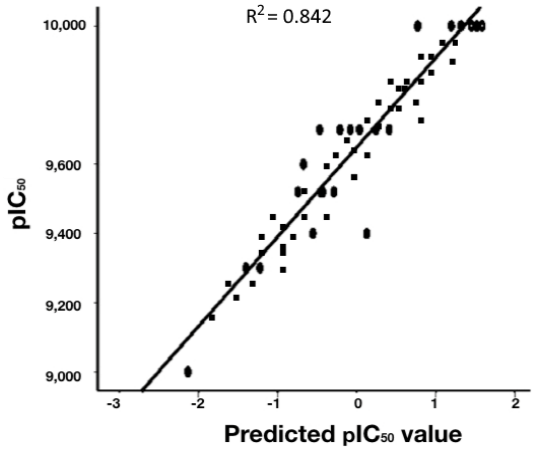

Fig. 4 Scattered plot of the observed pIC_{50} values against the predicted pIC_{50} values of the training set, the R^2 value of 0.872 depicts our model accurately predicts over 87% of the observed pIC_{50} values, hence, the model is unbiased and valid (Table S8).

for concern [22]. The ANOVA table (Table S7) with an *F*-ratio (the ratio of the improvement in the prediction from the model in relation to the inaccuracy in the model) of 21.77 and significant at $P<0.001$, reveals that the model is significantly better at predicting the pIC_{50} from the training set when compared to when the model is not applied. The histogram plot in Fig. 3 shows the normality of the residuals (difference between the observed and the predicted pIC_{50}). The histogram is symmetrical and bell-shaped, this shows the residuals follow a normal distribution and hence the use of multiple linear regression.

Generation of regression model equation

$$Y = MX + C \quad (1)$$

$$Y = B + B1X1 + B2X2 + B3X3 + B4X4 \ldots BnXn \quad (2)$$

$$pIC_{50} = B + B1X1 + B2X2 + B3X3 + B4X4 \quad (3)$$

pIC_{50}= (61.979) + (-0.001*MOMI-Z) + (0.366*MOMI-YZ) + (0.073*nSmallRings) + (0.991*MOMI-XY) + (0.333*khs.sCH3) + (0.012*DPSA-3) + (2.118*ATSc2) + (0.8*Wlambda2.unity) + (0.064*NAtomLAC) + (-4.579*BCUTw-1l) (4)

The Eq. 4 is the regression model equation. Where *Y* or pIC_{50} is the dependent variable, *X* the independent variable, B_0 and B_1 are regression coefficients.

MOMI-Z: moment of inertia along Z-axis, MOMI-YZ: moment of inertia along Y and Z-axis, nSmallRings: an enumeration of all the small rings (sizes 3 to 9) in a molecule, MOMI-XY: moment of inertia along X and Y axis, Khs.sCH3: descriptors that calculates Kier and Hall molecular indices, DPSA-3: difference of PPSA-1 and PNSA-1, ATSc2 (PaDEL;2D): ATS autocorrelation descriptor, weighted by charges, Wlambda2.unity: directional WHIM weighted by unit weights, NAtomLAC: returns the number of atoms in the longest aliphatic chain, BCUTw-1l: Eigen value-based descriptor.

Determination of the Lead's pIC_{50} using the derived QSAR model

The pIC_{50} of the lead phytocompounds (Table S9), azulene, andrographidine A and isovitexin were predicted using the QSAR model generated in the present study (Eq. 4). Isovitexin possessed the highest pIC_{50} value of 10.52, followed by andrographidine A with pIC_{50} of 9.59, while azulene possessed the lowest pIC_{50} value of 8.70. It

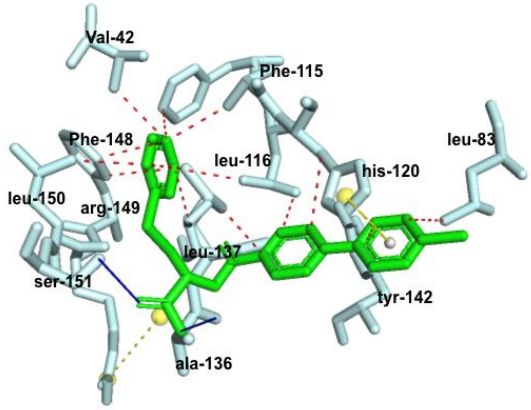

Fig. 5 Molecular interactions of amino acid residues within the active site of matrix metalloproteinase-2 with the standard drug, tanomastat (green stick). The red dotted lines represent hydrophobic interactions, blue dotted lines represent hydrogen bond interactions, yellow dotted lines represent salt bridge and green dotted lines represent pi cation.

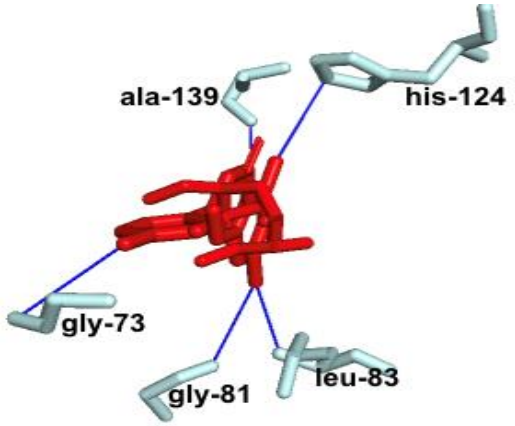

Fig. 6 Molecular interactions of amino acid residues within the active site of matrix metalloproteinase-2 with isovetin (red stick). The blue dotted lines represent hydrogen bond interactions.

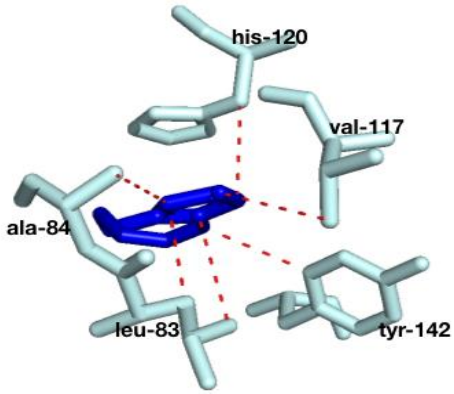

Fig. 7 Molecular interactions of amino acid residues within the active site of matrix metalloproteinase-2 with azulene (blue stick). The red dotted lines represent hydrophobic interactions.

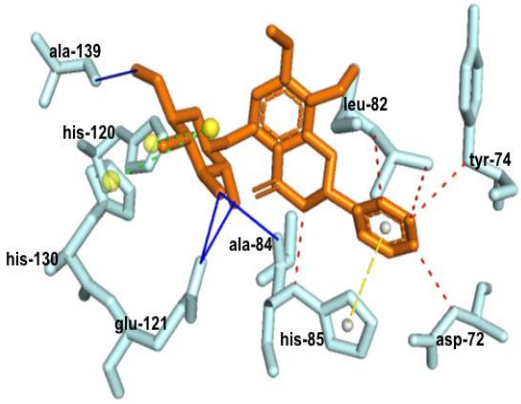

Fig. 8 Molecular interactions of amino acid residues within the active site of matrix metalloproteinase-2 with andrographidine A (orange stick). The red dotted lines represent hydrophobic interactions, blue dotted lines represent hydrogen bond interactions, yellow dotted lines represent salt bridge and green dotted lines represent pi cation.

is worthy of note that the three lead compounds herein have been documented to possess anti-cancer properties [17-19].

Analysis of molecular interactions of the standard and the lead compounds

The interactions with key amino acid residues in the catalytic site are of great importance while influencing the inhibitory activity of MMP inhibitors [23]. The molecular interactions of amino acid residues with the active site of MMP-2 are shown in Fig. 5, 6, 7, and 8 for the standard drug, isovitexin, azulene and andrographidine A, respectively. Tanomastat, forms two hydrogen bond interactions with leu-150 and ala-136, it forms hydrophobic interactions with val-42, leu-83, phe-115, leu-116, ala-119, his-120, leu-137, tyr-142, thr-145, phe-148, leu-150, pi cations with his-120 and salt bridge with arg-149 (Fig. 5). Isovitexin on the other hand forms only six hydrogen bond interactions, gly-73, gly-81, leu-83, his-124, and ala-139 within the MMP-2 catalytic site (Fig. 6). Azulene forms five hydrophobic interactions, leu-83, his-120, ala-84, tyr-142, val-117 (Fig. 7) while andrographidine A forms three hydrogen bond interactions with, ala-84, glu-121, ala-139, four hydrophobic interactions with asp-72, tyr-74, leu-82, his-85, one pi stacking with, his-85 and two salt bridge with his-120 and his-130 ((Fig. 8; Table 4). Tanomastat and Azulene share common hydrophobic interactions with tyr-142. Tanomasta, Azulene and Andrographidine A all share his-120. Leu-83 is common to tanomasta, isovitexin and

Table 4 Amino acid residues involved in molecular interactions of the standard drug (tanomastat) and the lead compounds within the active site of matrix metalloproteinase-2.

Lead compounds	Hydrogen bonds	Hydrophobic interactions	Pi stacking	Pi cations	Salt bridge
Tanomastat (Standard drug)	leu 150, ala-136	val-42, leu-83, phe-115, leu-116, ala-119, his-120, eu-137, tyr-142, thr-145, phe-148, leu-150		his-120	arg-149
Isovitexin	gly-73, gly-81, leu-83, his-124, ala-139				
Azulene		leu-83, his-120, ala-84, tyr-142, val-117			
Andrographidine A	ala-84, glu-121, ala-139	asp-72, tyr-74, leu-82, his-85	his-85		his-120, his-130

azulene (Table 4). The active site of MMPs contains histidine residues (his-120 and his-130), which are bound to the Zn atom and are highly conserved in MMPs [24]. According to Agrawal and co-workers [25], the selectivity of MMP-2 inhibitor depends on the nature of the zinc-binding group (ZBG). As evident in the present study, andrographidine A shares selective interactions with his-120 and his-130 and hence a potential MMP-2 inhibitor. The docking score of andrographidine A, azulene and isovitexin is -9.3 kcal/mol, -7.3 kcal/mol and -8.2 kcal/mol while that of the standard is -9.2 kcal/mol. The high docking score of andrographidine A is probably due to its extensive molecular interactions with key residues within the active site of MMP-2 when compared to the other leads (isovitexin and azulene). The higher docking score of isovitexin when compare to azulene is probably due to the absolute hydrogen bond interactions of the former.

Conclusion

Phytochemicals have been shown to possess and demonstrate anti-tumor effects. With the advent of computer-aided drug design, it is now possible to harness the diverse phytocompounds and explore their anti-cancer properties in the designing of novel anti-cancer drugs. The present study reveals azulene, andrographidine A and isovitexin as potential MMP-2 inhibitors. The QSAR model proposed herein is thoroughly validated and hence a tool for the identification of potential MMP-2 leads.

Conflict of Interest

The author declares no conflict of interest in this study.

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