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Study of Heparanase Inhibitors (Genus: Felis) Among Bioactive Compounds Produced by Plants in Volgograd Regions

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***Abstract* — The paper describes a search and selection of potential heparanase inhibitors. This enzyme is inhibited to protect an organism against various diseases such as chronic kidney disease and diabetic nephropathy. These diseases are widely distributed and often lead to death. Searching low-cost plant analogs of compounds which are able to inhibit their target protein activity can make the treatment cheaper and more efficient. The study has been carried out by means of Autodock Vina software to perform molecular docking and Protein Data Bank to obtain a crystal structure. Molecular docking has computed Folate, Folate, Riboflavin to be the most potential heparanase ligands. These compounds are produced by widely distributed plants and known to be harmless to different species.**

Keywords: inhibitors, heparanase, HPSE, docking, screening, PhytoChem, Autodock Vina

I. INTRODUCTION

Anthropogenic impact on the environment affects human and animals' organisms causing various diseases such as chronic kidney disease and diabetic nephropathy. These diseases are caused by disturbing biochemical processes that leads to increased heparanase synthesis. Treatment aims to inhibit this enzyme. This way today it is very important to find harmless and low-cost raw materials that can efficiently be used as a source of heparanase inhibitors.

According to the principle of green chemistry, a process of selecting compounds and their forms in the industry should aim its harm degree to be zero. Thus, today it is getting more common to search pharmaceutical effects in chemical compounds produced by plants. These materials are easy to get that drops the production expenditures. Using plant compounds as raw materials allows us to avoid high expenditures for toxicity analysis and drops the total production costs comparing with medicines produced from synthetic compounds. For this reason, we decided to search compounds in widely distributed plants: the ones which are well known for their pharmaceutical effects as well as the ones which are less commonly used in medicine.

Heparanase acts both at the cell surface and within its ECM, regulating different processes such as cellular communication, autophagy and gene transcription. Moreover, this enzyme is the only known mammalian endoglycosidase which is able to degrade polymeric heparan sulfates. Given the functional diversity of heparan sulfate, its degradation by heparanase deeply affects important pathophysiological processes, including tumour development, inflammation and neovascularization as well as progression of kidney disease [1,2]. Increased expression of heparanase can be observed in numerous malignancies, glomerular inflammation and albuminuria. Heparanase inhibition in animals protects an organism against glomerular disease and nephropathy including the type caused by diabetes [1]. Diabetic nephropathy often appears as a diabetes complication and leads to kidney disease which is one of the most serious pathologies of this disease [3]. Diabetes mellitus is a common endocrine disorder in animals [4]. At the end stage nephropathy leads to chronic kidney disease (CKD) which is another common mammalian pathology. However modern medicines are quite expensive and often not very effective (lead to death with a high probability). This way, studying

the new approaches to nephropathy and CKD treatment is a very important problem in modern medicine. Thus, this study is to search plant inhibitors of heparanase for CKD and diabetic nephropathy treatment.

II. Materials and Methods (Model)

23 plants were selected and analyzed: common yarrow (Achillea millefolium); chives (Allium schoenoprasum); tarragon (Artemisia dracunculus); caraway (Carum carvi); coriander (Coriandrum sativum); cumin (Cuminum cyminum); wild carrot (Daucus carota); true lavender (Lavandula angustifolia); fennel (Foeniculum vulgare); lovage (Levisticum); chamomile (Matricaria recutita); lemon balm (Melissa officinalis); peppermint (Mentha piperita); basil (Ocimum basilicum); marjoram (Origanum majorana); oregano (Origanum vulgare); garden parsley (Petroselinum crispum); rosemary (Rosmarinus officinalis); garden sage (Salvia officinalis); summer savory (Satureja hortensis); lemon thyme (Thymus citriodorus); garden thyme (Thymus vulgaris), summer squash (Cucurbita-pepo).

Following software and databases were used in the study: PhytoChem [5] database for the plants' chemical composition analysis; ChEMBL and BindingDB databases to obtain data for the models' training; AutoDock Vina [7] for molecular docking; the Blastn algorithm [8] to analyze the homology of human and genus Felis's heparanase sequences.

III. Results and Discussion

Chemical composition analysis was carried out for each plant. 826 unique compounds were obtained.

In order to reduce a set of plants for experiments, we chose the QSAR (Quantitative structure-activity relationship) commonly used methods of virtual screening as the first step of selection and molecular docking as the second one. The QSAR principle is based on the trained model's ability to relate a structure and properties of a chemical compound [6]. Thus, structural features of known heparanase inhibitors would allow us to indicate new compounds having the same features and therefore the same properties. Due to train the model, we selected compounds with experimental values of affinity ki, kd, IC50, EC50 for a human. The compounds were obtained from ChEMBL (206 units) and BindingDB (231 units) databases. Salts had been removed from the set before selection. The first set was divided into two groups: active and inactive. Compounds which ki, kd, IC50 and EC50 values were less or equal to 1000 nmol were considered as active. Compounds which affinity values were over or equal to 100 000 nmol were considered as inactive. Middle as well as duplicate values were removed. Thus, the final set involved 188 active and 31 inactive compounds. These compounds were selected to train the machine learning models which are often used in virtual screening. For each of the models we computed an AUC statistic metric which evaluates a quality of machine learning (5-fold cross validation) (Table 1).

TABLE I. Results of the models' learning

Model	AUC
Decision Tree	0.912
Random Forest	0.98
Naïve Bayes	0.93
Support Vector	0.98

Then these models were used in the process of ensemble forecasting to predict the activity of plant compounds (Figure 1).

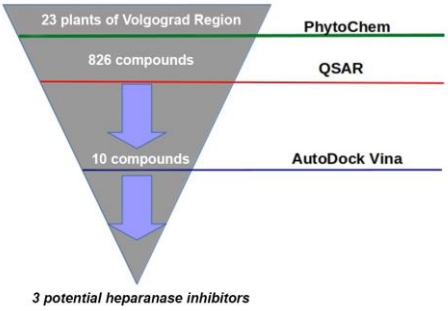

Fig. 1. Models of plant compounds activity in Volgograd Region

At the first step the models selected 10 compounds out of 826 whose properties were similar to known active target inhibitors (Table 2).

TABLE II. Results of natural products forecasting (forecasted by all the models).

Chemical compound	Organ	Molar mass, g/mol	Plant species
PECTIN	Root	194.139	Daucus-carota
PECTIN	Fruit	194.139	Foeniculum-vulgare
PECTIN	Seed	194.139	Cucurbita-pepo
PECTIN	Fruit	194.139	Coriandrum-sativum
PECTIN	Shoot	194.139	Salvia-officinalis
PECTIN	Shoot	194.139	Rosmarinus-officinalis
PECTIN	Shoot	194.139	Mentha-x piperita
RIBOFLAVIN	Plant	376.369	Thymus-vulgaris
RIBOFLAVIN	Seed	376.369	Carum-carvi

Chemical compound	Organ	Molar mass, g/mol	Plant species
RIBOFLAVIN	Root	376.369	Daucus-carota
RIBOFLAVIN	Leaf	376.369	Salvia-officinalis
RIBOFLAVIN	Plant	376.369	Origanum-majorana
RIBOFLAVIN	Leaf	376.369	Mentha-x piperita
RIBOFLAVIN	Plant	376.369	Achillea-millefolium
RIBOFLAVIN	Seed	376.369	Cucurbita-pepo
RIBOFLAVIN	Plant	376.369	Artemisia-dracunculus
RIBOFLAVIN	Leaf	376.369	Coriandrum-sativum
RIBOFLAVIN	Seed	376.369	Cuminum-cyminum
RIBOFLAVIN	Plant	376.369	Rosmarinus-officinalis
RIBOFLAVIN	Plant	376.369	Petroselinum-crispum
RIBOFLAVIN	Seed	376.369	Foeniculum-vulgare
RIBOFLAVIN	Leaf	376.369	Ocimum-basilicum
RIBOFLAVIN	Leaf	376.369	Allium-schoenoprasum
FOLACIN	Plant	441.404	Achillea-millefolium
FOLACIN	Plant	441.404	Petroselinum-crispum
FOLATE	Leaf	441.404	Allium-schoenoprasum
FOLATE	Leaf	441.404	Rosmarinus-officinalis
FOLATE	Leaf	441.404	Mentha-x piperita
PHEOPHYTIN-A	Plant	871.22	Cucurbita-pepo
CODECARBOXYLASE	Plant	247.143	Cucurbita-pepo
GAMMA-GLUTAMYL-S-ALLYLCYSTEINE	Plant	290.334	Allium-schoenoprasum
MALEIC-ACID	Root	116.072	Levisticum-officinale
ASPARAGINE	Plant	132.119	Achillea-millefolium
ASPARAGINE	Plant	132.119	Salvia-officinalis
ASPARAGINE	Root	132.119	Artemisia-dracunculus
HISTIDINE	Leaf	155.157	Ocimum-basilicum
HISTIDINE	Seed	155.157	Cucurbita-pepo
HISTIDINE	Fruit	155.157	Foeniculum-vulgare
HISTIDINE	Plant	155.157	Achillea-millefolium
HISTIDINE	Leaf	155.157	Rosmarinus-officinalis
HISTIDINE	Leaf	155.157	Mentha-x piperita

The next step was molecular docking which computed the energy of binding a compound to its target. It was performed by means of the Autodock Vina software. A crystal structure was obtained from Protein Data Bank. In the process of molecular modeling, we bounded selected compounds with a human heparanase 5E8M (a non-mutated, native form of a protein not bounded with heparin). The active site of the protein consists of five amino acids [9]. After QSAR models selected some compounds, their binding energy was computed by the molecular docking method (Table 3). Docking was carried out in two methods: under the complete fixation of target's atoms (DS_rig) and limited flexibility of the active center's atoms (DS_flex) [10]. Results of modeling binding SAR hits to the heparanase active center are presented in Table 3.

TABLE III. RESULTS OF MODELING BINDING SAR HITS TO THE HEPARANASE ACTIVE CENTER

DS_flex, kcal/mol	DS_rig, kcal/mol	Kd_ camputed	Chemical compound	Plant species
-5.7	-5.0		ASPARAGINE	Achillea-millefolium, Salvia-officinalis,
-6.9	-5.9		CODECARBOXYLASE	Cucurbita-pepo
-9.4	-8.9		FOLACIN	Achillea-millefolium, Petroselinum-crispum,
-9.6	-9.0		FOLATE	Allium-schoenoprasum, Rosmarinus-officinalis, Mentha-x piperita
-6.8	-6.1		GAMMA-GLUTAMYL-S-ALLYLCYSTEINE	Allium-schoenoprasum
-5.9	-5.3		HISTIDINE	Ocimum-basilicum, Cucurbita-pepo,

DS_flex, kcal/mol	DS_rig, kcal/mol	Kd_ camputed	Chemical compound	Plant species
				Foeniculum-vulgare, Achillea-millefolium, Zingiber-officinale, Rosmarinus-officinalis, Mentha-x piperita, Carum-carvi, Allium-schoenoprasum, Coriandrum-sativum
-5.1	-4.5		MALEIC-ACID	Levisticum-officinale
-6.8	-6.1		PECTIN	Foeniculum-vulgare, Cucurbita-pepo, Coriandrum-sativum, Salvia-officinalis, Rosmarinus-officinalis, Myristica-fragrans, Mentha-x piperita
-7.9	-5.9		PHEOPHYTIN-A	Cucurbita-pepo
-8.3	-8.2		RIBOFLAVIN	Thymus-vulgaris, Carum-carvi, Salvia-officinalis, Origanum-majorana, Mentha-x piperita, Achillea-millefolium, Cucurbita-pepo, Artemisia-dracunculus, Coriandrum-sativum, Cuminum-cyminum, Rosmarinus-officinalis, Petroselinum-crispum, Foeniculum-vulgare, Ocimum-basilicum, Allium-schoenoprasum

Molecular docking has selected Folate, Folacine, Riboflavin to be the most potential ligands for heparanase (binding energy is less -8 kcal/mol).

IV. Conclusion

Heparanase enzyme plays an important role in various living processes, including cellular communication, gene transcription and autophagy. At the same time, its increased expression is observed in albuminuria, diabetic nephropathy and chronic kidney disease. However, there is no effective and low-cost medicine to treat them. This problem can be solved by searching plant inhibitors of heparanase which is a target of CKD and nephropathy. It can make treatment cheaper and more efficient.

3 plants out of 23 have been selected to have potential heparanase inhibitors: Folate (DS -9,6), Folacine (DS -9,4), Riboflavin (DS -8,3). These compounds are produced by widely distributed plants and known to be harmless to different species.

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