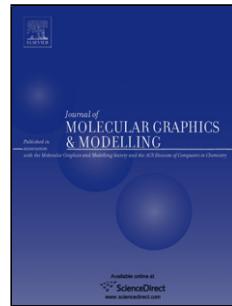


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# The study of dual COX-2/5-LOX inhibitors by using electronic-topological approach based on data on the ligand-receptor interactions

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## Highlights

- Conformational and electronic structure of COX-2/5-LOX inhibitors were investigated.
- Pharmacophores and anti-pharmacophores analyzed using ETM combined with Neural Networks.
- A prediction system developed recognizing correctly 93% of compounds as active ones.

- Peculiarities of the ligand-receptor binding obtained with docking and DFT.
- New active compounds were modelled based on the developed prediction system.

### Graphical abstract

### Abstract

Structural and electronic factors influencing selective inhibition of cyclooxygenase-2 and 5-lipoxygenase (COX-2/5-LOX) were studied by using Electronic-Topological Method combined with Neural Networks (ETM-NN), molecular docking and Density Functional Theory (DFT) in a large set of molecules. The results of the ETM-NN calculations allowed for the selection of pharmacophoric molecular fragments, which could be taken as a basis for a system capable of predicting the COX-2/5-LOX inhibitory activity. For the more effective extraction of the pharmacophoric molecular fragments, docking of molecules into the active sites of the two enzymes was carried out to get data on the ligand-receptor interaction. To make an assessment of these interactions, stabilization energies were calculated by using Natural Bond Orbital (NBO) analysis. Docking and data on the electronic structures of active sites of enzymes helped to reveal effectively the peculiarities of the ligand-receptor binding. The system for the selective COX-2/5-LOX inhibitory activity prediction that has been developed as the result of the ETM-NN study recognized correctly 93% of compounds as highly active ones. Thus, this system can be successfully used for carrying out computer screening and synthesis of potent inhibitors of COX-2/5-LOX with diverse molecular skeletons.

**Keywords:** NSAID; COX-2/5-LOX; Electronic-topological approach; Molecular docking; Density functional theory.

### 1. Introduction

Dual inhibitors are drugs that are able to block both the cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) pathways. A large number of pharmacological studies have been devoted to the development of such compounds. In contrast to single inhibitors of COX and LOX pathways, dual inhibitors possess a wide range of anti-inflammatory activity and are almost exempt from side effects of COX inhibitors [1]. Nevertheless, results of the clinical trials performed with dual inhibitors are currently incomplete and wait for continuation.

The search for dual-acting molecules is based on the rational design that uses a combination of pharmacophores extracted from the libraries of known drugs. The screening may be executed both *in vitro* and

*in silico*. It was shown that the most appropriate way is to discover molecules acting on proteins from the same or close protein families [2]. The study of ligand structures acting on different targets and parallel analysis of protein similarity, size, and active site features can be used to find potential targets for dual-acting.

The first commercialized dual COX-2/5-LOX inhibitor was benoxaprofen. Unfortunately, this drug appeared to be toxic in acute overdosage causing by this fatal self-poisoning. Several compounds belonging to various structural families of dual inhibitors are currently undergoing preclinical and clinical trials as promising new drugs. Recent data strongly suggest the potential use of dual inhibitors in the prevention and therapy of cancer, thrombosis, atherosclerosis and neurodegenerative diseases. As an example, below you can find a few species that are being currently used in the medical practice.

First, they are dual inhibitors derived from indomethacin and flufenamic acid [3-5]. Another important source of dual COX-2/5-LOX inhibitors is the di-tert-butylphenol class [6-9]. The authors have shown that the 2,6-di-tert-butyl-1-hydroxy substitution pattern of the benzene ring is optimal for obtaining dual COX-2/5-LOX inhibitors. A large number of di-tert-butylphenols have been synthesized and widely evaluated pharmacologically. Flavonoids and other phenolic derivatives are also effective COX-2/5-LOX inhibitors capable of binding with the Fe-ion belonging to the active site of the enzyme.

In the series of pyrazole derivatives and pyridinyl pyrrole-imidazoles, several compounds showed a dual COX-2/5-LOX inhibitory profile as well [10]. Potent dual COX-2/5-LOX inhibitors among the derivatives of thiophene and pyrrolizine are such compounds as L-652,343, RWJ-63556, and ML-3000. Several pyrazolinic and hydrazone derivatives also belong to this group [11, 12]. Other examples can be found in the recent publications [13-20].

Previously [21], a few large series of NSAIDs were studied with the help of the Electronic-Topological Method (ETM). These compounds were taken from different structural classes and possessed different levels of the COX-2 inhibitory activity. As the result of this study, a system for the activity prediction was developed, which included a set of the most representative pharmacophores and conditions that are necessary for the activity demonstration by a compound. It should be noted that the system was capable of identifying active compounds with high enough probability (~0.96 in average). The structure-activity relationships (SAR) were also investigated for a few series of cyclooxygenase-2 (COX-2) inhibitors by using advanced ETM combined with Neural Networks (ETM-NN) [22].

The present study is aimed at the search of the most potent dual inhibitors of COX-2/5-LOX by applying the combination of molecular docking, electronic structure calculations, and our own ETM-NN approach. Each of them has been already successfully applied, but separately, to a wide enough variety of tasks on the SAR investigation [23-25]. To find new dual COX-2/5-LOX inhibitors, we carried out the electronic-topological study of the peculiarities of their inhibitory activity, as a continuation of our previous studies.

## 2. Materials and Method

### 2.1. Data set

As initial data for the search of the structure-activity dependency by the ETM, a training set of compounds represented by their structures and activities is to be selected. Here, inhibiting activities of molecules relative to 5-LOX and COX-2 enzymes are investigated in the series of 160 molecules with different enough skeletons, selected from literature (Fig. 1). Because of the large volume of the series of compounds intended for the study, they are presented as supporting information (Table S1). References related to the compounds are also given in the supplementary material (Fig. S1).

**[INSERT FIG. 1 ABOUT HERE]**

All compounds were divided into classes of active molecules (105 compounds) and inactive ones (the rest 55), relative to the COX-2/5-LOX inhibiting activity. Compounds with the IC<sub>50</sub> less than 15 μM (for both COX-2 and 5-LOX enzymes) were considered as active ones.

## 2.2. Description of Methods

Since ETM is widely explained [26-30], we give here only the most distinguished properties of the ETM relative to other approaches to the SAR study. ETM belongs to the so-called structural methods, which use specific languages for the compound structure description. The language of the ETM reflects the discrete nature of compounds as groups of atoms, where some of the atoms are chemically bonded. By this, labeled graphs are used as the most appropriate mathematical counterparts of chemical structures represented by the sets of atoms and bonds together with relationships on them. As known, such graph's representative is a matrix of the order  $n \times n$ , where  $n$  is the number of the graph's vertices. Therefore, the ETM proposes the language of Electronic-Topological Matrices of Contiguity (ETMC) to be its own, a very special language for the chemical skeletons descriptions. These matrices are symmetrical relative to their left diagonal (bonds have no orientation), and it is enough to have only the right upper triangle of any such matrix alone with its diagonal.

Vertices of such graphs are naturally labeled by values representing atomic characteristics (charges, HOMO/LUMO coefficients, etc.), and the values are diagonal elements  $a_{ii}$  of the corresponding matrices. For the off-diagonal elements  $a_{ij}$  there are two possibilities. If they represent chemical bonds, then one of the bond characteristics is to be chosen and fixed for all off-diagonal elements of the matrices. Otherwise, the value of corresponding distance is taken for the non-bonded pair of atoms. Thus, after the training set of compounds has been selected and the activities of the compounds have been estimated (as active/inactive or quantitatively), the main steps of the ETM-study are as follows:

1. Calculate spatial and electronic characteristics for atoms and bonds (for all compounds).
2. Form the corresponding ETMCs by choosing appropriate values from the data calculated (usually, charges are diagonal elements, bonds are the Wiberg's indices, and non-bonded atoms are distances).
3. Set a desirable level for the activity prediction (in %) and precision values to compare all ETMCs with a template selected. The ETMCs are mathematical equivalents of the 3D molecular structures, and their comparison is being done only by elements of the same nature. Atomic and bond characteristics, so as distances, usually have different precisions.

4. Make pair-wise comparison with the ETMC of the most active compound for all ETMCs, and select a set of steric and electronic features  $S_i$ ,  $i \in I$  (known as “activity features” or pharmacophores) that are common for all active compounds, only.
5. Calculate the probability of the fragments realization relative to the probabilistic criteria  $P_a$  and  $\alpha_a$  (see below) and choose those that correspond to the desired prediction level set before. If the fragments found are not informative enough, change some initial settings (or all of them) and repeat steps 3-5.

After the activity features ( $S_i$ ,  $i \in I$ ) were successfully found for a training set and subsequently proved for a test set, they can be used to predict the activity in view for any new series of compounds with probabilities (Yule’s coefficients [31]) estimated previously for each structural feature separately according to the equations (1) and (2):

$$P_a = (n_1+1)/(n_1+n_3+2) \quad (1)$$

$$\alpha_a = \frac{(n_1 \cdot n_4 - n_2 \cdot n_3)}{\sqrt{N_1 \cdot N_2 \cdot N_3 \cdot N_4}} \quad (2)$$

Here  $N_1$  and  $N_2$  are numbers of molecules in the classes of active and inactive compounds, respectively;  $n_1$  and  $n_2$  are amounts of molecules possessing and not possessing the feature of activity  $S_i$  in the class of active compounds, respectively;  $n_3$  and  $n_4$  have same meaning relative to the class of inactive compounds;  $N_3 = n_1 + n_3$  and  $N_4 = n_2 + n_4$ . Note that the probability value  $P_a$  is related exactly to the compounds that contain an activity feature  $S_i$  found from the ETM calculations, while  $\alpha_a$  takes into account all compounds of the series.

### 2.2.1. ETM–NN approach

For an activity fixed, the ETM approach is aimed in finding pharmacophores (i.e. molecular fragments common for the structures of all active compounds and absent in all inactive compounds with similar structures). Analogously, a set of anti-pharmacophores, or fragments characterizing inactive compounds, exclusively, is found. The combined set of pharmacophores and anti-pharmacophores includes those basic molecular substructures, which constitute a system for the prediction of the activity in view. The system can be used for both testing newly synthesized compounds and modeling new candidates for the purposeful syntheses [22-25].

The procedure of the activity prediction needs the expert’s participation. To automate it, the ETM application was followed by an ANNs (artificial neural networks) application (with unsupervised and supervised learning algorithms), and, for the reason, this approach was named as ETM–NN [22]. The ANNs application uses data of the ETM calculations (they are electronic-topological submatrices of contiguity (ETSC) for pharmacophores and anti-pharmacophores) as input for a new algorithm developed on the base of Volume Learning Algorithm used previously for the CoMFA analysis [32, 33]. This algorithm is implemented as iterative application of the Kohonen self-organizing maps (SOMs) and associative neural networks (ASNNs) [34, 35].

Common block schema of the data analysis is shown in Fig. 2. As seen from the scheme presented, docking is being applied after the series of compounds for study has been determined, and its aim is determining the most profitable conformations for binding with receptor. Earlier, when forming ETMCs for flexible molecules, it was necessary to carry out complicated calculations. A new approach based on the Genetic Algorithms [36, 37] has been applied for the pharmacophoric groups' identification. When ETM and docking are used together, 3D coordinates of a molecule correspond to the most effective position of the molecule within receptor. Molecular docking and electronic structure calculations can be performed with the account of ligand-receptor interaction to allow for the more effective extraction of pharmacophoric molecular fragments in the frameworks of the ETM-NN approach.

**[INSERT FIG. 2 ABOUT HERE]**

The algorithm of the ETM-NN approach, by applying repetitive training, works on clustering the ETMC matrices and minimizing the initial size  $S$  of the Kohonen's maps (SOMs). In parallel, the projection errors are calculated relative to the SOMs for all pharmacophores/anti-pharmacophores represented by ETSC matrices and used for the fragment weights calculation. At the same time, ASNNs application to the ETSC matrices results in the average prediction error ( $E_c$ ) calculation. When  $E_c < E_p$ , that is a value found at the preceding stage of training, the current projection of the ETSC for the fragment is saved. As soon as the best projections of the ETMC fragments have been found (being the result of the ASNN training), the most informative of them are selected by using special pruning methods [38, 39]. The more detailed description of the ETM-NN approach is given in the supporting information (Fig. S2, S3). According to this algorithm, its main steps are as follows:

- 1.** Prepare ETM-data (ETMCs, pharmacophores and anti-pharmacophores) as input set.
- 2.** Initialize the Kohonen's network parameters and, for each molecule, calculate clusters from its ETMC. The initial size of Kohonen's maps is taken as  $S=2*S_{ETMC}$ , where  $S_{ETMC}$  is the size of the largest molecular matrix.
- 3.** For each ETSC, calculate its projection on the units of the Kohonen's SOM. Calculate the projection error ( $E_q$ ). Calculate the weight of each fragment as  $1/E_q$  and create new data set by using the calculated fragment weights as parameters.
- 4.** Analyze ETM-data related to fragments, i.e. ETSC matrices, by using ASNNs; calculate the average prediction error ( $E_c$ ).
- 5.** Compare the  $E_c$  value to that one found at the preceding stage of training,  $E_p$  (initially,  $E_p = 10^{-3}$ ). If  $E_c < E_p$ , the current projection of the ETSC for the fragment is saved.
- 6.** Decrease the size of the Kohonen's map.
- 7.** Repeat steps 2–6 until the map size,  $S$ , decreases to  $S_{min}$  ( $S_{min}$  equals to eight nodes).
- 8.** Select the best projections of the ETMC fragments, relative to the minimal value of  $E_c$ , and predict the activity of new compounds.
- 9.** Select the most informative ETMC fragments (after the ASNN training) by using special pruning methods [38, 39].
- 10.** Predict activities of new compounds.

Optimized ETMC fragments are used to visualize those regions of molecules under study that have been found important for the activity demonstration by the molecules.

### *2.2.2. Molecular docking analysis*

Molecular docking is one of visual approaches to computer-assisted molecular modelling (CAMM). It plays a very important role in the design of potential ligands that are both sterically and chemically compatible with the binding site of a target bio-macromolecule [40, 41]. Docking may reveal a great number of possible conformations and orientations for the inhibitors at the binding site. Knowing the binding site conformations helps to find the most important interactions that stabilize the ligand-receptor complex.

Crystal structures of COX-2 and 5-LOX enzymes were obtained from the RCSB Protein Data Bank (<http://www.rcsb.org/pdb/>), under the accession codes 4COX [42] and 3O8Y [43], respectively. Before the procedure of molecular docking, initial molecular skeletons were built with GaussView 5 software [44]. As far as the structures in view are rigid molecular skeletons of heterocyclic and ring form, and flexibility of their bonds is restricted, different optimizing methods cause very similar results. Structure optimization with docking is useful when compounds are flexible enough and have a lot of conformations. Docking can help to select an appropriate conformer in this case. Molecular mechanics techniques were used to minimize the energy of ligand in the binding pocket, starting from a series of random positions and orientations within the binding site. The ligand structures had been energy-minimized by using the MMFF94s force field and the conjugated gradient method until the default derivative convergence criterion of 0.05 kcal/(mol Å) was reached.

Molecular docking calculations were performed using the MOE (Molecular Operating Environment) software [45] to estimate free energy of binding for ligands in the poses given. The enzyme-ligand complexes were minimized up to a gradient of 0.01 kcal/(mol Å), and hydrogens were added using the force field AMBER99. Charges on the protein were assigned using the force field AMBER99, while the charges on the ligands were assigned by using force field MMF94X. The docked poses were scored using London dG scoring function for finding the best docking pose. The free energy of hydration was calculated using the Generalized Born/Volume Integral (GB/VI) solvation model [46].

## **3. Results and Discussion**

### *3.1. Analysis of pharmacophores and anti-pharmacophores obtained from ETM*

Vectors are discrete representations used for the chemical structure descriptions. But they do not take into account all electron effects and their influence on the compounds activities. The more complete and detailed are 3D structural descriptions of compounds, which are able to reflect conformational and electronic properties of the latter. To solve the problem of finding the proper initial conformation for flexible molecules, the later version of ETM with addition of neural networks for data optimization and features extraction was working with compounds structures optimized through their interaction with receptor.

Conformational analysis and the electronic properties study were carried out for all compounds by means of Gaussian 09 [47] program. Its results were used to form ETMCs for all compounds. Charges on atoms ( $q_i$ )

were selected as diagonal elements; the Wiberg's indices ( $W_{ij}$ ) were taken as off-diagonal elements for chemically bonded atoms; otherwise, optimized distances between non-bonded two atoms ( $R_{ij}$ ) were used.

In the process of the matrices comparison, optimal values of variations allowable for the atoms and bonds matching were found as  $\Delta_1=\pm 0.12$  for diagonal elements ( $q_i$ ) and  $\Delta_2=\pm 0.20$  for off-diagonal values ( $W_{ij}$  and  $R_{ij}$ ). To determine the most informative activity features, the desired values of probabilistic estimations  $\alpha_a$  and  $P_a$  were set as 0.50 and 0.80, correspondingly. For anti-pharmacophores (APh<sub>i</sub>), ( $n_2+1$ ) was used in place of the ( $n_1+1$ ) in the equation (1). After the ETMCs had been processed, a set of pharmacophores (activity features) was found. The features formed a basis for a system capable of both carrying out computer screening and forecasting activities for new drug prototypes.

The results of the ETM-NN application to the series of compounds in view are pharmacophoric and anti-pharmacophoric molecular fragments, which are characteristic of the class of compounds demonstrating dual inhibition activity (or inactivity) relative to the COX-2/5-LOX receptors. The selected pharmacophores formed the basis of a system for the inhibitory activity prediction.

Compounds **44** and **16** possessing good dual activity were taken as templates for comparison. In Fig. **3a**, a sub-matrix of the template ETMC (ETSC) is given, which corresponds to one of the pharmacophores revealed (Ph1).

#### [INSERT FIG. 3 ABOUT HERE]

One of the pharmacophores, Ph1, which has been found in 41 active compounds, consists of 6 atoms adjusted to the indole ring (Fig. 3a). Probability of its realization ( $P_a$ ) is 0.96. The highest negative charges are concentrated on the C<sub>2</sub>, N<sub>7</sub>, C<sub>10</sub> ( $q_i = -0.19 \text{ e}^-$ ) and O<sub>18</sub> atoms ( $q_i = -0.21 \text{ e}^-$ ). The carbon atoms, C<sub>5</sub> and C<sub>12</sub>, have a small negative charges (-0.03 and -0.08 e<sup>-</sup>). The Ph2 pharmacophore found relative to the template compound **16** and realized in 37 molecules inhibiting COX-2/5-LOX enzymes, is shown along with its ETSC in Fig. **3b**. The probability of the Ph2 realization in active compounds is 0.93. As one can see, this pharmacophore is presented by 9 atoms, which include tert-butyl and hydroxyl groups attached to phenyl ring. Negative charges ( $q_i=-0.21 \text{ e}^-$ ) are concentrated on atoms C<sub>9</sub>, C<sub>10</sub>, C<sub>13</sub>, C<sub>14</sub> entering tert-butyl groups and on the O<sub>22</sub> atom of hydroxyl group ( $q_i=-0.28 \text{ e}^-$ ). The remaining pharmacophores, Ph3-Ph5, have been found analogously, and the probabilities of their realization in the class of active compounds are in the range of 0.86-0.90.

To determine anti-pharmacophores ('breaks of activity'), ETMCs of inactive compounds were taken as templates. Five anti-pharmacophores, APh1-APh5, were found, in total. The ETSCs that correspond to APh1 and APh2 are given in Fig. 4 along with structures of the corresponding templates after which the anti-pharmacophores have been found.

#### [INSERT FIG. 4 ABOUT HERE]

As seen from Fig. **4a**, APh1 (based on the inactive template **69**) consists of 7 atoms. APh1 is found in 25 inactive and 1 active molecules, thus, the probability of its realization is 0.93. As seen from the matrix presented, APh1 includes the N<sub>3</sub> atom and carbon atoms of the phenyl rings. On all of them small negative charges are observed ( $q_i=-0.10 - -0.14 \text{ e}^-$ ). APh2 (based on the inactive template **77**) is composed of 7 atoms, and

probability of its realization is 0.88 (Fig. 4b). Distinct from APh1, this anti-pharmacophore contains atoms with alternating charges. Maximum positive charge ( $q_s=0.55 \text{ e}$ ) is on the sulphur atom, while maximum negative one on the oxygen atom of the methylsulfonyl group (-SO<sub>2</sub>CH<sub>3</sub>).

Analysis of submatrices that correspond to APh1 and APh2 has shown their close similarity, although they are calculated relative to different template compounds. The statistical characteristics of five pharmacophores (Ph<sub>i</sub>) and five anti-pharmacophores (APh<sub>i</sub>) entering the forecasting system, are given in Table 1. As seen from the table, probability of realization for the rest of pharmacophores and anti-pharmacophores is a bit lower than for the first two ( $P_a=0.91$  and 0.86, correspondingly).

#### [INSERT TABLE 1 ABOUT HERE]

When comparing the structures of pharmacophores and anti-pharmacophores, one should pay close attention to the differences in their spatial and electronic characteristics. Thus, the complex of pharmacophores and anti-pharmacophores taken as a whole plays an important role in the activity prediction, as well as in the search for new drugs. The set of activity/inactivity fragments found as a result of this study form a basis for the development of a system for the anti-inflammatory activity prediction.

#### *3.2. Results of the ETM-NN approach application*

The first stage of the ETM-NN data analysis was to find a cluster distribution model capable of reflecting realistic internal structure of the data (Table 2).

#### [INSERT TABLE 2 ABOUT HERE]

For the training set, 231 clusters were found. ASNNs recognized correctly 94% (126 from 134 compounds), while for the test set the predictive ability ( $P_a$ ) was a bit lower, 0.86 (22 compounds from 26). For the total set, the result is  $P_a=0.92$  (148 compounds from 160). The results obtained tell in favor of high quality of cluster distribution models and their fitness for the analysis of new data sets, so as to search for pharmacophores.

At the second stage, only 125 fragments were selected for the training and test sets (Table 2). On the base of weights calculated for the molecular fragments represented by ETMSs, ASNNs were capable of recognizing activity with probability  $P_a=0.95$  (127 compounds from 134) in the training set, and  $P_a=0.91$  (24 compounds from 26), in the test set. In total, the network classified correctly  $P_a=0.93$  (149 compounds from 160).

The pruning methods application resulted in the selection of only 23 the most influential ETMC fragments. By this, ASNN classified correctly 129 compounds from 134 in the training set ( $P_a=0.96$ ), and 24 compounds from 26 in the test set ( $P_a=0.93$ ). ASNNs classified correctly 95% (153 compounds from 160).

In our case, comparison of two models (one model based on the cluster distribution found in a straightforward manner, and another based on the ETMC fragments used for the network learning) tells in favor of close correspondence between their results. However, the first model is not stable enough and depends severely on the structures of compounds selected for the training set.

In comparison with other commonly used approaches, the approach presented in this study has shown quite satisfactory results. This fact tells about workability of the both models found, and both can be applied for

the design of new potent COX-2/5-LOX inhibitors. The system of the inhibitory activity prediction recognized 96% of compounds as highly active ones. In this way, the present study resulted in a system that is not only capable of predicting the COX-2/5-LOX inhibitory activity but also can successfully carry out computer screening followed by synthesis of potent dual inhibitors with diverse enough molecular skeletons.

### *3.3. Results of the docking and DFT application*

In the frameworks of the ETM-NN study, docking of dual inhibitors to the COX-2 and 5-LOX active sites has been carried out. By taking selected template compounds **44**, **16**, **69** and **77** as an example, let us consider peculiarities of their binding with the active sites of COX-2 and 5-LOX receptors. Amino acid residues of COX-2 and 5-LOX, which interact with ligands **44**, **16**, **69** and **77**, are presented as supporting information (see Fig. S4).

In Fig. 5, compounds **44** and **69** are given as an example of interaction with COX-2 and 5-LOX receptors. For the compounds **16** and **77** this information is presented in the supporting document (Fig. S5a, b). Three hydrogen bonds formation, two of them between the carboxylic group and Arg120 and Tyr355, and one more between the carboxylic group and Ser530, is characteristic of the molecule **44** (Fig. 5a). Inactive molecule **69** does not form any bonds with COX-2 enzyme (Fig. 5b).

#### [INSERT FIG. 5 ABOUT HERE]

Compound **16** (Fig. S5a) forms hydrogen bonds with Tyr355 through carboxylic group, and also demonstrates weak hydrophobic interaction with COX-2 enzyme. Compound **77** (Fig. S5b) makes a hydrogen bond between the oxygen atom of methylsulfonyl group and Arg513.

The character of interaction of the studied compounds with the active side of the 5-LOX receptor substantially differs, however. Thus, compound **44** forms three hydrogen bonds with Tyr181, Gln363 and Thr364, for the account of carboxylic and methoxy groups (Fig. 5c). Additionally, a weak  $\pi$ - $\pi$  interaction arises for the account of chlorophenyl group and His372. Weak  $\pi$ - $\pi$  interaction between phenyl ring and Phe421 is formed as the result of the compound **69** and 5-LOX receptor interaction (Fig. 5d). A hydrogen bond formation between carbonyl group and Tyr181 is characteristic of highly active compound **16**. For the inactive compound **77**, a hydrogen bond arises between methylsulfonyl group and Thr364, and aromatic interaction occurs between fluoro-phenyl ring and His372 (Fig. S5c, d).

In Table 3, the results of docking are given for all template compounds selected. From the data it is seen that high values of scoring energy (between -21 and -26 kcal mol<sup>-1</sup>) and hydration energy (-27 kcal mol<sup>-1</sup> for MM/GBVI) are observed for dual inhibitor **44**. Lower values of the parameters are characteristic of the molecule **16** that can be related to the hydrophobic interaction between tert-butyl groups and active sites of COX-2/5-LOX receptors. Scoring energy values are -10.7 (COX-2) and -18 kcal mol<sup>-1</sup> (5-LOX) for molecule **16**.

#### [INSERT TABLE 3 ABOUT HERE]

On the other hand, tert-butyl groups are shielding hydroxyl group situated between them, and this makes impossible the hydrogen bond formation by –OH group with amino acids of the receptor. All these observations determine low values of the scoring energy for the compound **16**. A bit higher values of the parameters are observed for the compounds **69** and **77**. The energy parameters (S and MM/GBVI) by themselves do not give the equivocal answer on the activity of inhibitors for each enzyme separately. The role of the stabilization energy on activity ( $E(2)$ , see Table 3) will be explained later.

For the deeper understanding of the ligand-receptor interaction, electronic structures of COX-2 and 5-LOX active sites were calculated relative to corresponding active and inactive inhibitors. The electronic structure calculations were carried out with the Gaussian 09 using DFT at the Becke three parameter hybrid exchange functional combined with Lee-Yang-Parr correlation functional (B3LYP)/6-31G (d,p) level of theory [48, 49].

Frontier orbitals (HOMO and LUMO) of the molecular systems are of special interest for the analysis of the specificity of the ligand-receptor interaction. The analysis of the electron density distribution on the frontier orbitals tells in favor of mainly donor-acceptor character of interaction inside ligand-receptor complexes under study. The electron density distribution on the frontier orbitals formed by active sites of COX-2 and 5-LOX with ligands **44** and **69** are given in Fig. 6. Figure of the electron density distribution for the compounds **16** and **77** can be found in the supporting information (Fig. S6).

#### [INSERT FIG. 6 ABOUT HERE]

Analysis of electron density has shown that when dual inhibitors **44** and **16** bind with COX-2, frontier orbitals are formed between atoms of amino acid residues and ligands (Fig. 6a and Fig. S6a). As can be seen from Fig. 6a, for the compound **44** the orbital interaction between ligand and receptor is realized by the atoms of both aromatic ring and oxygen-containing functional groups (-COOH, -OCH<sub>3</sub>) with such amino acid residues as Arg120, Tyr355, Gly526, Leu531, Ser530, and Ser353. For inactive compound **69** (Fig. 6b), this interaction happens for the account of  $\pi$ -conjugated system including ligand and 3 amino acid residues, Gly526, Ser353, and Val349.

In the case of 5-LOX enzyme and templates **44** and **69**, the electron density distribution on the border orbitals is shown in Fig. 6c and 6d. The basic electron interaction goes, as in the case of interaction with COX-2, between  $\pi$ -orbital system of the ligand **44** and such residues as His 372, Thr364, Ile415, and Asn425. For the template compound **69** (Fig. 6d), this interaction is limited by three amino acids, namely, His372, Gln363, and Phe421.

To make an assessment of all these interactions, energies of stabilization were calculated for arising ligand-receptor complexes. The second-order perturbation theory analysis of Fock matrix in Natural Bond Orbital (NBO) basis was applied by using NBO module implemented in Gaussian 09 [50]. This analysis was carried out by examining all possible interactions between "filled in" (donor) Lewis-type NBOs and "empty" (acceptor) non-Lewis NBOs, and by estimating their energetic importance by the second-order perturbation theory. For each donor NBO (*i*) and acceptor NBO (*j*), the stabilization energy  $E(2)$  associated with delocalization (2e-stabilization)  $i \rightarrow j$

$$E(2) = \Delta E_{ij} = q_i \frac{F(i,j)^2}{\varepsilon_j - \varepsilon_i}$$

where  $q_i$  is the donor orbital occupancy,  $\varepsilon_i$ ,  $\varepsilon_j$  are diagonal elements (orbital energies), and  $F(i,j)$  is an off-diagonal NBO Fock matrix element [51].

$E(2)$  stabilization energy values for the ligand-receptor complexes are given in Table 3. As seen from the data, dual inhibitors **44** and **16** have high values of  $E(2)$  for both COX-2 (41.2 for **44** and 41.7 kcal mol<sup>-1</sup> for **16**) and 5-LOX (27.8 for **44** and 21.6 kcal mol<sup>-1</sup> for **16**). Inactive inhibitors have lower values of  $E(2)$  for COX-2 (16.3 for **69** and 0.4 kcal mol<sup>-1</sup> for **77**) and 5-LOX (13.5 for **69** and 6.4 kcal mol<sup>-1</sup> for **77**). In this way, by taking into account details of the electronic structure of the enzyme-ligand complex and combining them with the data obtained from the ETM-NN approach, we can successfully use them for the search and design of new highly active dual inhibitors of the COX-2/5-LOX receptors.

It should be noted that results of docking and results of the ETM-study for the series of COX-2/5-LOX dual inhibitors in view completely agree. The molecular fragments found, which enter the pharmacophores Ph1 and Ph2, play an important role in the interaction with receptor (Fig. 3), but atoms entering anti-pharmacophores APh1 and APh2 (Fig. 4) play minor role in the interaction with the COX-2/5-LOX receptors. Analogous conclusions were obtained as the result of analysis of frontier orbitals in the active sites of COX-2 and 5-LOX receptors (Fig. 6).

The system of the COX-2/5-LOX dual inhibitory activity prediction developed after results of the ETM-NN approach was applied to design of new model compounds possessing good enough selectivity. As an example, Fig. 7 contains a few modelled molecules.

#### [INSERT FIG. 7 ABOUT HERE]

From all compounds that have been modelled after the results of ETM-NN, six of them, **t1 – t6**, contain only characteristic pharmacophores that are realized in the class of dual inhibitors and no anti-pharmacophores (APhi). Docking of the compounds into the active sites of COX-2 and 5-LOX enzymes revealed high values of scoring energy and stabilization energy ( $E(2)$ ). Thus, for example, scoring energy for **t1** is -22.0 kcal mol<sup>-1</sup> and  $E(2)$  is 12.6 kcal mol<sup>-1</sup> (COX-2 enzyme). For the rest of compounds these values vary between -16.2 and -26.5 kcal mol<sup>-1</sup> (COX-2) and between -18.2 and -26.0 kcal mol<sup>-1</sup> (5-LOX) for S. For  $E(2)$  the ranges are 12.6 – 35.9 kcal mol<sup>-1</sup> (COX-2) and 15.0 – 27.1 kcal mol<sup>-1</sup> (5-LOX).

From calculations, compounds **t1-t6** were predicted as dual acting COX-2/5-LOX inhibitors with  $IC_{50}<15\ \mu M$ . As other molecular skeletons, we can model new potent COX-2 inhibitors in the same way. I.e. after the compound geometry is optimized and its electronic structure calculated, ETMC matrix will be formed and checked on the correspondence with necessary parameters of the system for the activity prediction. In this way, the system of prediction developed for dual COX-2/5-LOX inhibitors is capable of fast enough assessment of the compounds' activity and can be an alternative to the voluminous total screening of chemical compounds.

#### 4. Conclusions

A large set of COX-2/5-LOX inhibitors including 160 structurally diverse molecules were studied with the aim of finding peculiarities of their conformational and electronic structures. 105 of them actively inhibit

COX-2/5-LOX receptors, and the rest 55 are inactive. The results of the ETM-NN application to the series of compounds in view are pharmacophoric and anti-pharmacophoric molecular fragments, which are characteristic of the class of compounds demonstrating activity (correspondingly, inactivity) relative to the COX-2/5-LOX receptors. The probabilistic estimations for five pharmacophores ( $\text{Ph}_i$ ) and five anti-pharmacophores ( $\text{APh}_i$ ) entering the forecasting system, are 0.91 and 0.86, correspondingly. After applying pruning methods, the probability of qualifying the compounds under study as dual acting inhibitors is higher and equals to 0.96 for training set and 0.93 for the test set.

The results of docking for template compounds have shown that ligand-enzyme interaction happens for the account of both hydrogen bonds formation and weak  $\pi$ - $\pi$  interactions of aromatic rings. In some cases, hydrophobic interaction may affect the character of bonds formed by ligand with the active site of an enzyme. Based on the analysis of the electron density distribution on the frontier orbitals (HOMO/LUMO) of the ligand-enzyme complex, the conclusion on the donor-acceptor interaction can be done.

The energy of stabilization ( $E(2)$ ) for the ligand-receptor complex was calculated using NBO analysis. A system of prediction for the dual inhibitory activity of COX-2/5-LOX was developed on the base of the pharmacophores found, docking results, and electronic structure calculated. It allows for screening and design of new potent COX-2/5-LOX inhibitors. The percentage of correctly classified compounds that is the result of the ETM-NN application to the test set equals to 93%.

### **Acknowledgement**

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### **Appendix A. Supplementary data**

Supplementary material provided with this article.

### **References**

- [1] C.L. Nickerson-Nutter, E.D. Medvedeff, The effect of leukotriene synthesis inhibitors in models of acute and chronic inflammation, *Arthritis & Rheumatism*, 39 (1996) 515-521.
- [2] R. Morphy, C. Kay, Z. Rankovic, From magic bullets to designed multiple ligands, *Drug discovery today*, 9 (2004) 641-651.
- [3] D.H. Boschelli, D.T. Connor, M. Hoefle, D.A. Bornemeier, R.D. Dyer, Conversion of NSAIDS into balanced dual inhibitors of cyclooxygenase and 5-lipoxygenase, *Bioorganic & medicinal chemistry letters*, 2 (1992) 69-72.
- [4] T. Kolasa, C.D. Brooks, K.E. Rodrigues, J.B. Summers, J.F. Dellarria, K.I. Hulkower, J. Bouska, R.L. Bell, G.W. Carter, Nonsteroidal anti-inflammatory drugs as scaffolds for the design of 5-lipoxygenase inhibitors, *Journal of medicinal chemistry*, 40 (1997) 819-824.
- [5] D.H. Boschelli, D.T. Connor, D.A. Bornemeier, R.D. Dyer, J.A. Kennedy, P.J. Kuipers, G.C. Okonkwo, D.J. Schrier, C.D. Wright, 1, 3, 4-Oxadiazole, 1, 3, 4-thiadiazole, and 1, 2, 4-triazole analogs of the fenamates: in vitro inhibition of cyclooxygenase and 5-lipoxygenase activities, *Journal of medicinal chemistry*, 36 (1993) 1802-1810.

- [6] M. Inagaki, T. Tsuri, H. Jyoyama, T. Ono, K. Yamada, M. Kobayashi, Y. Hori, A. Arimura, K. Yasui, K. Ohno, Novel antiarthritic agents with 1, 2-isothiazolidine-1, 1-dioxide ( $\gamma$ -sultam) skeleton: cytokine suppressive dual inhibitors of cyclooxygenase-2 and 5-lipoxygenase, *Journal of medicinal chemistry*, 43 (2000) 2040-2048.
- [7] J.M. Janusz, P.A. Young, J.M. Ridgeway, M.W. Scherz, K. Enzweiler, L.I. Wu, L. Gan, R. Darolia, R.S. Matthews, D. Hennes, New cyclooxygenase-2/5-lipoxygenase inhibitors. 1. 7-tert-butyl-2, 3-dihydro-3, 3-dimethylbenzofuran derivatives as gastrointestinal safe antiinflammatory and analgesic agents: discovery and variation of the 5-keto substituent, *Journal of medicinal chemistry*, 41 (1998) 1112-1123.
- [8] Y. Song, D.T. Connor, A.D. Sercel, R.J. Sorenson, R. Doubleday, P.C. Unangst, B.D. Roth, V.G. Beylin, R.B. Gilbertsen, K. Chan, Synthesis, structure-activity relationships, and in vivo evaluations of substituted di-tert-butylphenols as a novel class of potent, selective, and orally active cyclooxygenase-2 inhibitors. 2. 1, 3, 4-and 1, 2, 4-thiadiazole series 1, *Journal of medicinal chemistry*, 42 (1999) 1161-1169.
- [9] K. Swingle, R. Bell, G. Moore, Anti-inflammatory activity of antioxidants, *Anti-inflammatory and anti-rheumatic drugs*, 3 (1985) 105-126.
- [10] S. Laufer, H.G. Striegel, K. Neher, P. Zechmeister, C. Donat, K. Stolingwa, S. Baur, S. Tries, T. Kammermeier, G. Dannhardt, Synthesis and Evaluation of a Novel Series of Pyrrolizine Derivatives as

Dual Cyclooxygenase-1 and 5-Lipoxygenase Inhibitors, *Archiv der Pharmazie*, 330 (1997) 307-312.

- [11] C. Ghiglieri-Bertez, C. Coquelet, A. Alazet, C. Bonne, Inhibiteurs mixtes des voies de la cyclooxygénase et des lipoxygénases: synthèse et activité de dérivés hydrazoniques, *European Journal of Medicinal Chemistry*, 22 (1987) 147-152.
- [12] D.P. Wallach, V.R. Brown, A novel preparation of human platelet lipoxygenase: Characteristics and inhibition by a variety of phenyl hydrazones and comparisons with other lipoxygenases, *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism*, 663 (1981) 361-372.
- [13] S. Barbey, L. Goossens, T. Taverne, J. Cornet, V. Choesmel, C. Rouaud, G. Gimeno, S. Yannic-Arnoult, C. Michaux, C. Charlier, Synthesis and activity of a new methoxytetrahydropyran derivative as dual cyclooxygenase-2/5-lipoxygenase inhibitor, *Bioorganic & medicinal chemistry letters*, 12 (2002) 779-782.
- [14] A. Bertolini, A. Ottani, M. Sandrini, Selective COX-2 inhibitors and dual acting anti-inflammatory drugs: critical remarks, *Frontiers in Medicinal Chemistry-Online*, 1 (2004) 85-95.
- [15] A.A. Geronikaki, A.A. Lagunin, D.I. Hadjipavlou-Litina, P.T. Eleftheriou, D.A. Filimonov, V.V. Poroikov, I. Alam, A.K. Saxena, Computer-aided discovery of anti-inflammatory thiazolidinones with dual cyclooxygenase/lipoxygenase inhibition, *Journal of medicinal chemistry*, 51 (2008) 1601-1609.
- [16] M.G. Chini, R. De Simone, I. Bruno, R. Riccio, F. Dehm, C. Weinigel, D. Barz, O. Werz, G. Bifulco, Design and synthesis of a second series of triazole-based compounds as potent dual mPGES-1 and 5-lipoxygenase inhibitors, *European journal of medicinal chemistry*, 54 (2012) 311-323.
- [17] F.K. Hansen, M. Khankischpur, I. Tolaymat, R. Mesaros, G. Dannhardt, D. Geffken, Efficient synthesis and 5-LOX/COX-inhibitory activity of some 3-hydroxybenzo [b] thiophene-2-carboxylic acid derivatives, *Bioorganic & medicinal chemistry letters*, 22 (2012) 5031-5034.
- [18] I. Apostolidis, K. Liaras, A. Geronikaki, D. Hadjipavlou-Litina, A. Gavalas, M. Soković, J. Glamočlija, A. Čirić, Synthesis and biological evaluation of some 5-arylidene-2-(1, 3-thiazol-2-ylimino)-1, 3-thiazolidin-4-ones as dual anti-inflammatory/antimicrobial agents, *Bioorganic & medicinal chemistry*, 21 (2013) 532-539.
- [19] S. Misra, S. Ghatak, N. Patil, P. Dandawate, V. Ambike, S. Adsule, D. Unni, K. Venkateswara Swamy, S. Padhye, Novel dual cyclooxygenase and lipoxygenase inhibitors targeting hyaluronan-CD44v6 pathway and inducing cytotoxicity in colon cancer cells, *Bioorganic & Medicinal Chemistry*, 21 (2013) 2551-2559.
- [20] S. Ghatak, A. Vyas, S. Misra, P. O'Brien, A. Zambre, V.M. Fresco, R.R. Markwald, K.V. Swamy, Z. Afrasiabi, A. Choudhury, Novel di-tertiary-butyl phenylhydrazones as dual cyclooxygenase-2/5-lipoxygenase inhibitors: Synthesis, COX/LOX inhibition, molecular modeling, and insights into their cytotoxicities, *Bioorganic & medicinal chemistry letters*, 24 (2014) 317-324.
- [21] A. Dimoglo, E. Sim, N. Shvets, V. Ahsen, Electronic-Topological Study of Structurally Diverse Cyclooxygenase-2 Inhibitors, *Mini Reviews in Medicinal Chemistry*, 3 (2003) 281-294.

- [22] A. Dimoglo, V. Kovalishyn, N. Shvets, V. Ahsen, The Structure - Inhibitory Activity Relationships Study in a Series of Cyclooxygenase-2 Inhibitors: A Combined Electronic-Topological and Neural Networks Approach, *Mini Reviews in Medicinal Chemistry*, 5 (2005) 879-892.
- [23] E.E. Oruç, S. Rollas, F. Kandemirli, N. Shvets, A.S. Dimoglo, 1,3,4-Thiadiazole Derivatives. Synthesis, Structure Elucidation, and Structure–Antituberculosis Activity Relationship Investigation, *Journal of Medicinal Chemistry*, 47 (2004) 6760-6767.
- [24] N. Karali, A. Gürsoy, F. Kandemirli, N. Shvets, F.B. Kaynak, S. Özbeş, V. Kovalishyn, A. Dimoglo, Synthesis and structure–antituberculosis activity relationship of 1H-indole-2,3-dione derivatives, *Bioorganic & Medicinal Chemistry*, 15 (2007) 5888-5904.
- [25] F. Macaev, Z. Ribkovskaia, S. Pogrebnoi, V. Boldescu, G. Rusu, N. Shvets, A. Dimoglo, A. Geronikaki, R. Reynolds, The structure–antituberculosis activity relationships study in a series of 5-aryl-2-thio-1,3,4-oxadiazole derivatives, *Bioorganic & Medicinal Chemistry*, 19 (2011) 6792-6807.
- [26] A.S. Dimoglo, Compositional approach to electronic structure description of chemical compounds, oriented on computer analysis of structure-activity relationships, *Khimiko-Pharmazevticheskii Zhurnal*, 4 (1985) 438-444.
- [27] A.S. Dimoglo, in: L. Saley (Ed.) *Coordinational and organic biologically active compounds*, Stiinta, Kishinev, 1986, pp. 7-11.
- [28] N. Shvets, Applied program system for the prognosis of biological activity of chemical compounds: development and use, *Computer Journal of Moldova*, 3 (1993) 101-110.
- [29] N.M. Shvets, A. Dimoglo, Structure-odor relationships: results of an applied electron-topological approach, *Nahrung*, 42 (1998) 364-370.
- [30] I.B. Bersuker, A.S. Dimoglo, The Electron-Topological Approach to the QSAR Problem, in: K.B. Lipkowitz, D.B. Boyd (Eds.) *Reviews in Computational Chemistry*, John Wiley & Sons, Inc.2007, pp. 423-460.
- [31] G.U. Yule, On the time-correlation problem, with especial reference to the variate-difference correlation method, *Journal of the Royal Statistical Society*, (1921) 497-537.
- [32] H. Kubinyi, 3D QSAR in drug design: volume 1: theory methods and applications, Springer Science & Business Media1993.
- [33] I.V. Tetko, Neural Network Studies. 4. Introduction to Associative Neural Networks, *Journal of Chemical Information and Computer Sciences*, 42 (2002) 717-728.
- [34] I.V. Tetko, D.J. Livingstone, A.I. Luik, Neural network studies. 1. Comparison of overfitting and overtraining, *Journal of Chemical Information and Computer Sciences*, 35 (1995) 826-833.
- [35] I.V. Tetko, V.V. Kovalishyn, D.J. Livingstone, Volume Learning Algorithm Artificial Neural Networks for 3D QSAR Studies, *Journal of Medicinal Chemistry*, 44 (2001) 2411-2420.
- [36] L. Akyüz, E. Sarıpinar, E. Kaya, E. Yanmaz, 4D-QSAR study of HEPT derivatives by electron conformational–genetic algorithm method, *SAR and QSAR in Environmental Research*, 23 (2012) 409-433.
- [37] L. Akyüz, E. Sarıpinar, Conformation depends on 4D-QSAR analysis using EC-GA method: pharmacophore identification and bioactivity prediction of TIBOs as non-nucleoside reverse transcriptase inhibitors, *Journal of enzyme inhibition and medicinal chemistry*, 28 (2013) 776-791.
- [38] I.V. Tetko, A.E.P. Villa, D.J. Livingstone, Neural Network Studies. 2. Variable Selection, *Journal of Chemical Information and Computer Sciences*, 36 (1996) 794-803.
- [39] V.V. Kovalishyn, I.V. Tetko, A.I. Luik, V.V. Kholodovych, A.E.P. Villa, D.J. Livingstone, Neural Network Studies. 3. Variable Selection in the Cascade-Correlation Learning Architecture, *Journal of Chemical Information and Computer Sciences*, 38 (1998) 651-659.
- [40] C.G. Wermuth, *The practice of medicinal chemistry*, Academic Press, San Diego, 1996.
- [41] N.C. Cohen, *Guidebook on molecular modeling in drug design*, Academic Press1996.
- [42] R.G. Kurumbail, A.M. Stevens, J.K. Gierse, J.J. McDonald, R.A. Stegeman, J.Y. Pak, D. Gildehaus, J.M. Miyashiro, T.D. Penning, K. Seibert, Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents, *Nature*, 384 (1996) 644-648.
- [43] N.C. Gilbert, S.G. Bartlett, M.T. Waight, D.B. Neau, W.E. Boeglin, A.R. Brash, M.E. Newcomer, The structure of human 5-lipoxygenase, *Science*, 331 (2011) 217-219.
- [44] R. Dennington, T. Keith, J. Millam, *GaussView*, version 5, Semichem Inc., Shawnee Mission, KS, (2009).
- [45] Molecular Operating Environment (MOE), 2013.08; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2015.
- [46] P. Labute, The generalized Born/volume integral implicit solvent model: Estimation of the free energy of hydration using London dispersion instead of atomic surface area, *Journal of Computational Chemistry*, 29 (2008) 1693-1698.

- [47] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery Jr., J.E. Peralta, F. Ogliaro, M.J. Bearpark, J. Heyd, E.N. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A.P. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, N.J. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, Ö. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian 09, Gaussian, Inc., Wallingford, CT, USA, 2009.
- [48] C.T. Lee, W.T. Yang, R.G. Parr, Development of the Colle-Salvetti correlation-energy formula into a functional of the electron-density, *Physical Review B*, 37 (1988) 785-789.
- [49] A.D. Becke, Density-functional thermochemistry. III. The role of exact exchange, *The Journal of Chemical Physics*, 98 (1993) 5648-5652.
- [50] E. Glendening, A. Reed, J. Carpenter, F. Weinhold, Gaussian NBO, version 3.1, Gaussian Inc., Pittsburgh PA, (2001).
- [51] A.E. Reed, L.A. Curtiss, F. Weinhold, Intermolecular interactions from a natural bond orbital, donor-acceptor viewpoint, *Chemical Reviews*, 88 (1988) 899-926.

## Figure Captions

**Fig. 1.** Common molecular skeletons of the COX-2/5-LOX inhibitors under study.

**Fig. 2.** Block scheme of data analysis by means of ETM-NN, docking, and DFT.

**Fig. 3.** Two pharmacophores, Ph1 and Ph2, found relative to corresponding active templates **44** (a) and **16** (b).

**Fig. 4.** Two anti-pharmacophores, APh1 and APh2, found relative to corresponding inactive templates **69** (a) and **77** (b).

**Fig. 5.** 3D representations for the docking poses of compounds **44** (a) and **69** (b) in the active site of COX-2, and **44** (c) and **69** (d) in the active site of 5-LOX.

**Fig. 6.** The electron density distribution on the frontier orbitals (HOMO/LUMO) for the active sites of COX-2 (a, b) and 5-LOX (c, d) with ligands **44** (a, c) and **69** (b, d).

**Fig. 7.** 2D structures and corresponding energy values of new compounds as potent COX-2/5-LOX dual inhibitors.

**Table 1**

Statistical characteristics for some of pharmacophores ( $\text{Ph}_i$ ) and anti-pharmacophores ( $\text{APh}_i$ ) calculated by ETM.

Type of pharmacophore (template compound)	$P_a$	$P_{ia}$	Type of anti-pharmacophore (template compound)	$P_a$	$P_{ia}$
Ph1 (44)	0.9 6	0.04	APh1 (69)	0.0 7	0.9 3
Ph2 (16)	0.9 3	0.07	APh2 (77)	0.1 3	0.8 7
Ph3 (2)	0.9 0	0.10	APh3 (82)	0.1 4	0.8 6
Ph4 (54)	0.8 9	0.11	APh4 (140)	0.1 6	0.8 4
Ph5 (137)	0.8 6	0.14	APh5 (152)	0.1 8	0.8 2
Average	0.9 1	0.09	Average	0.1 4	0.8 6

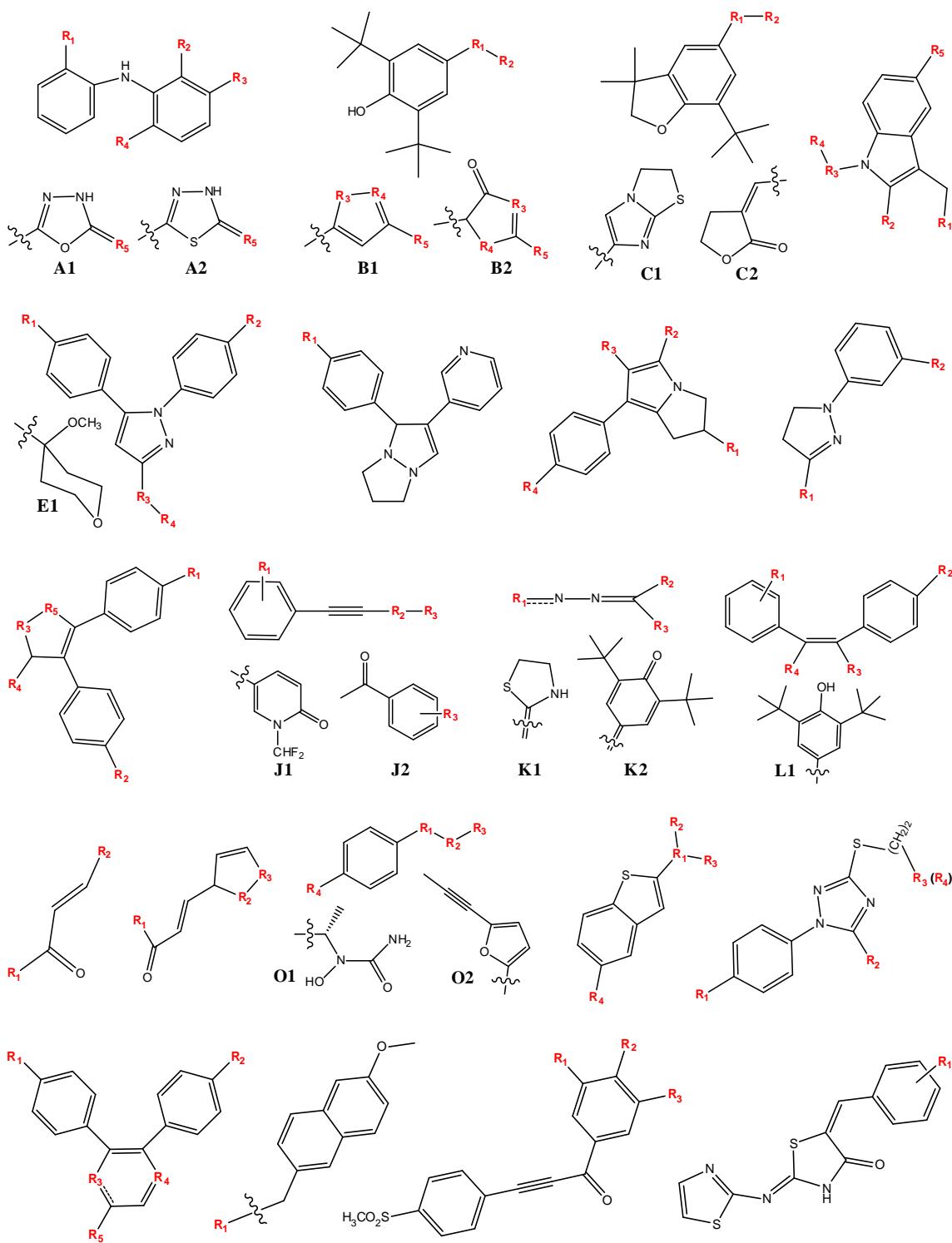
**Table 2**

Cross-validated  $q^2$  coefficients calculated for COX-2/5-LOX inhibitors on the base of fragment data set.

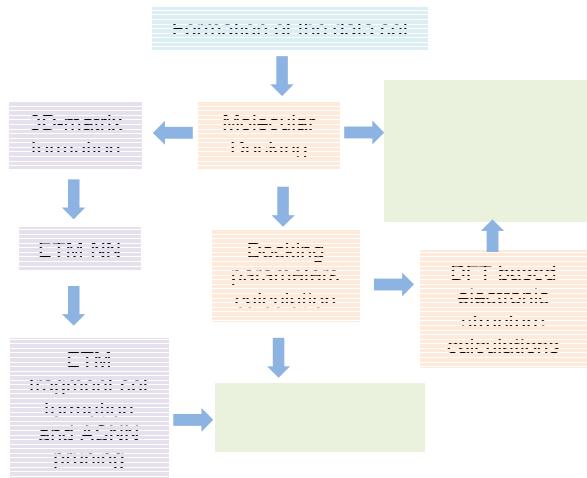
N/N	Data Set	All pharmacophores				Pharmacophores selected by pruning methods				* WD <sub>s</sub> : weig ht desc ripto rs
		S	WD <sub>s</sub> * 5-LOX	MM/GBVI		Para	E(2)	Molecule		
Compound	COX-2	COX-2	5-LOX	COX-2	5-LOX	COX-2	5-LOX	Count	Predicted (P <sub>a</sub> )	
<b>44</b>	-26.1	-21.1	Amc	26.9	-26.8 <sub>d</sub> (P <sub>a</sub> )	41.2	27.8	3	0.95	
<b>16</b>	-10.7	-18.0		-11.1	-20.3	41.7	21.6			
<b>69</b>	-19.8	-19.5		-20.2	-25.3	16.3	0.4			
<b>1 77</b>	Training-17.2	-19.2	13-18.2		-15.6(0.95)	13.5	6.434	129	(0.96)	
<b>2</b>	Test set	125	26	24	(0.91)	23	26	24	(0.93)	desc
<b>3</b>	Total	125	160	149	(0.93)	23	160	153	(0.95)	ripto

**Table 4**

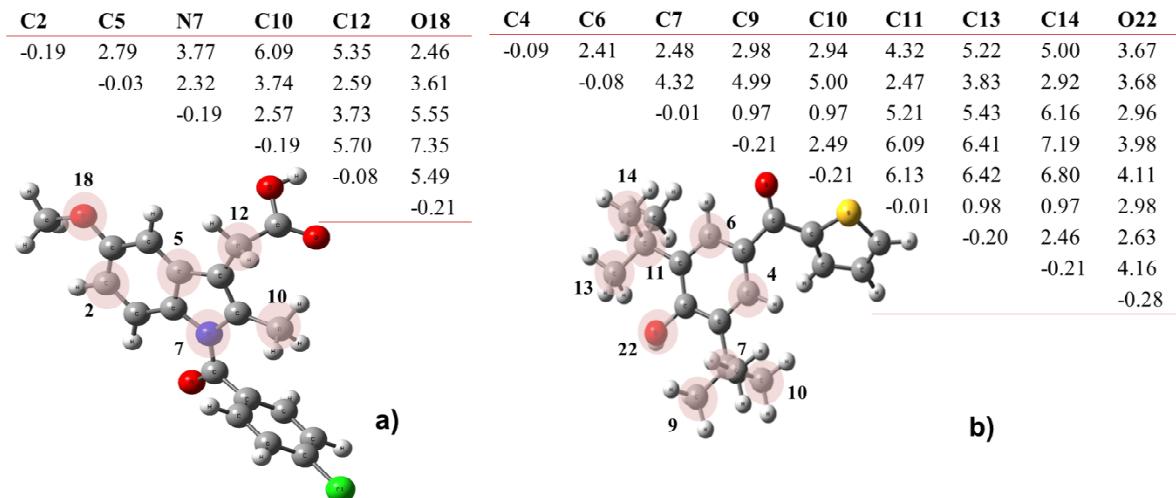
Scoring energy (**S**), the free energy of hydration (**MM/GBVI**) and **E(2)** stabilization energies (in kcal mol<sup>-1</sup>) of template compounds.



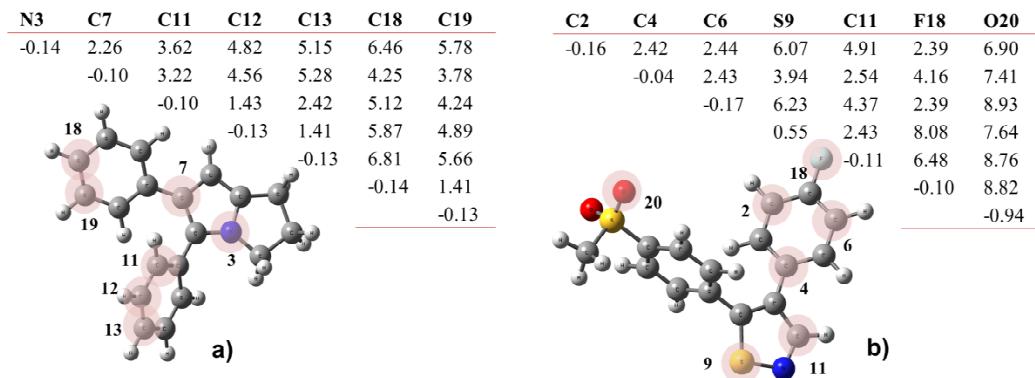
**Fig. 1.** Common molecular skeletons of the COX-2/5-LOX inhibitors under study.



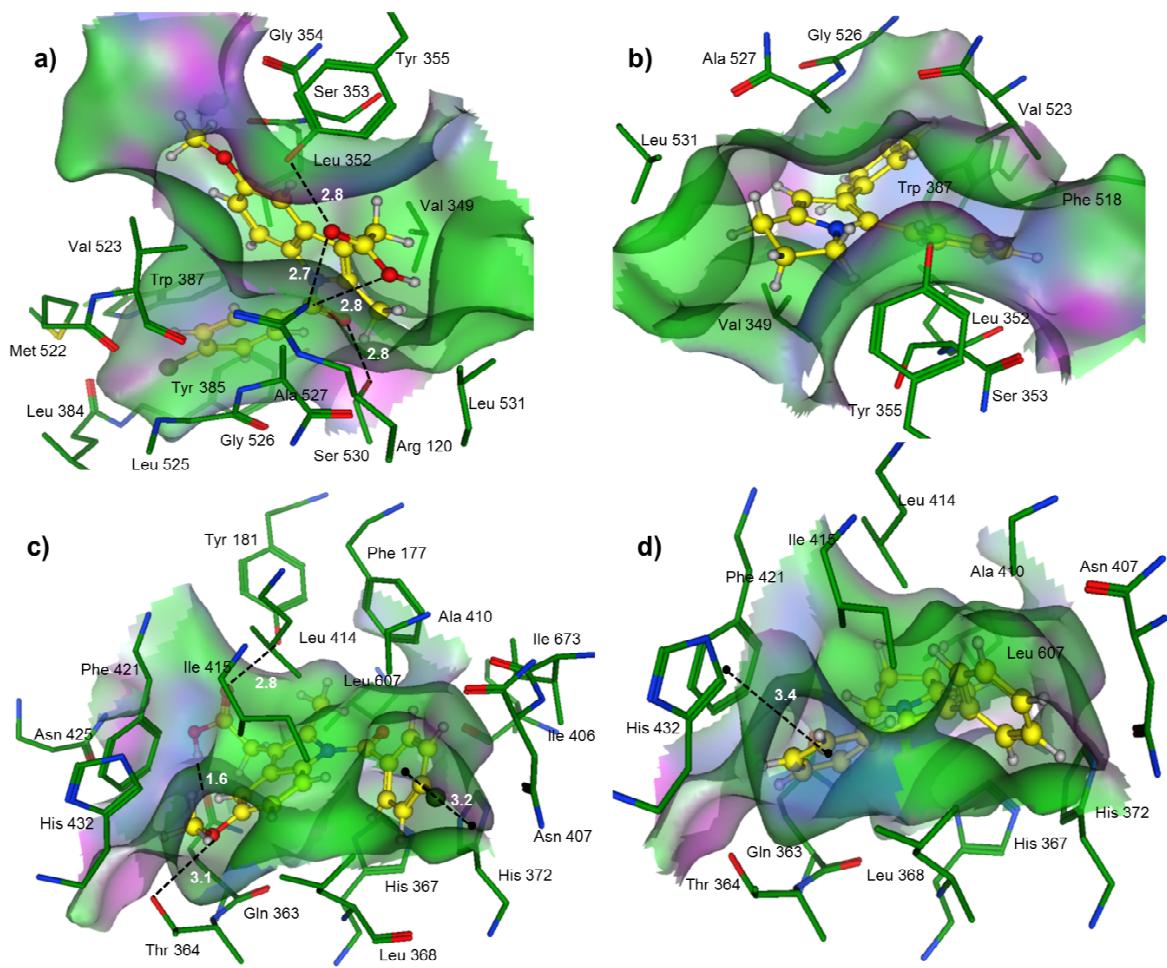
**Fig. 2.** Block scheme of data analysis by means of ETM-NN, docking, and DFT.



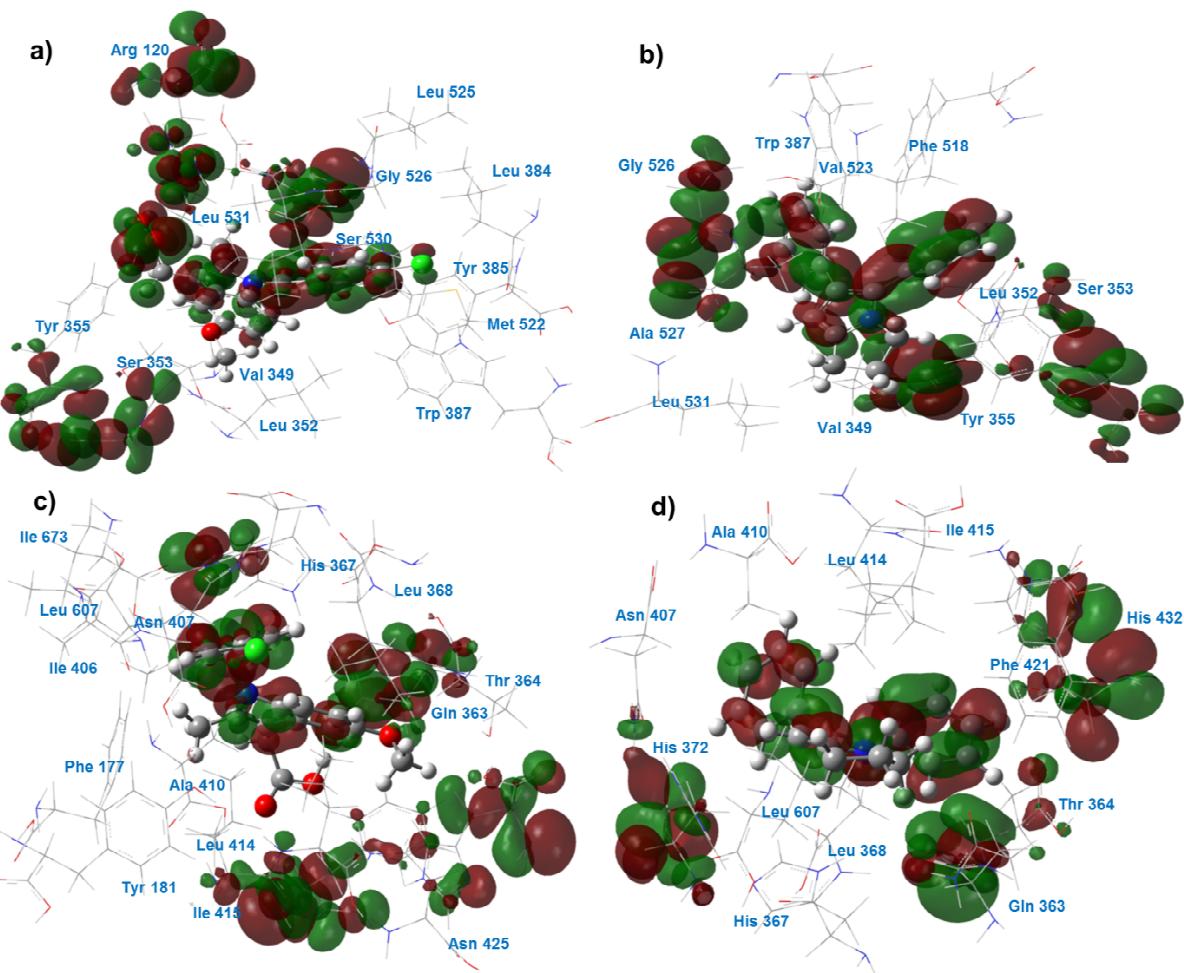
**Fig. 3.** Two pharmacophores, Ph1 and Ph2, found relative to corresponding active templates **44** (a) and **16** (b).



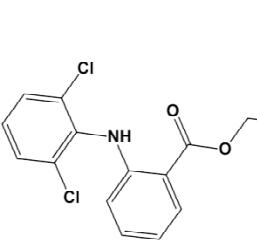
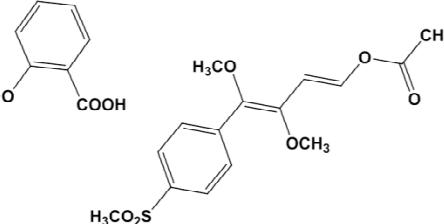
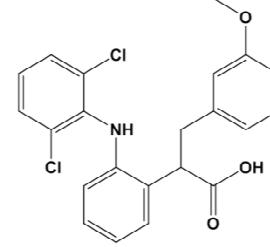
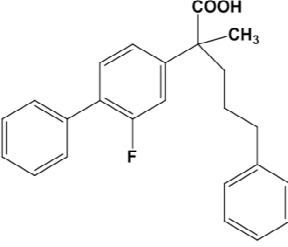
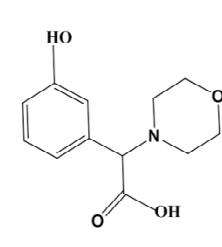
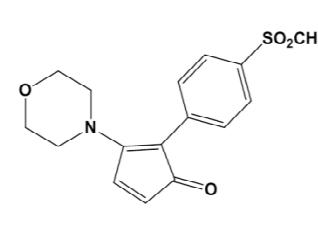
**Fig. 4.** Two anti-pharmacophores, APh1 and APh2, found relative to corresponding inactive templates **69** (a) and **77** (b).



**Fig. 5.** 3D representations for the docking poses of compounds **44** (a) and **69** (b) in the active site of COX-2, and **44** (c) and **69** (d) in the active site of 5-LOX.



**Fig. 6.** The electron density distribution on the frontier orbitals (HOMO/LUMO) for the active sites of COX-2 (a, b) and 5-LOX (c, d) with ligands **44** (a, c) and **69** (b, d).

		
<b>t1</b>	<b>t2</b>	<b>t3</b>
<b>COX-2/5-LOX</b>		
<b>S, kcal mol<sup>-1</sup></b> -22.0/-23.7	<b>-17.6/-20.4</b>	<b>-24.7/-24.1</b>
<b>E(2), kcal mol<sup>-1</sup></b> 12.6/22.2	<b>13.3/18.5</b>	<b>17.2/15.5</b>
		
<b>t4</b>	<b>t5</b>	<b>t6</b>
<b>COX-2/5-LOX</b>		
<b>S, kcal mol<sup>-1</sup></b> -26.5/-26.0	<b>-18.0/-18.2</b>	<b>-16.2/-19.5</b>
<b>E(2), kcal mol<sup>-1</sup></b> 13.5/20.6	<b>24.8/15.0</b>	<b>35.9/27.1</b>

**Fig.7.** 2D structures and corresponding energy values of new compounds as potent COX-2/5-LOX dual inhibitors.