Development of Rational Strategy for Selective COX-2 Inhibitors Searching as Potential Anticancer Drugs


Summary
Molecular docking for 145 compounds (4-thiazolidinone derivatives and related heterocyclic systems) has shown in vitro anticancer activity into the active site of enzyme COX-2 using crystallographic models 1CX2 and 6COX (www.rcsb.org). In silico investigations were performed stage-by-stage with OpenEye Scientific Software program package. As a result selected 20 compounds, that are thiopyrano [2,3-d] thiazole-2-ones, 5-arylidene-4-thioxo-2-thiazolidinone and 2-thioxo-(heteryl, imino or oxo)-4-thiazolidinone derivatives selective COX-2 inhibition with blockage of COX-2 pathway in cancerogenesis, which determines a role of selective COX-2 inhibitors in prevention and treatment of cancer.

Key Words: 4-thiazolidinones and related heterosystems, COX-2, anticancer activity, OpenEye, molecular docking.

INTRODUCTION
Cyclooxygenase (COX) is an enzyme which is responsible for biochemical transformations of arachidonic acid and synthesis of its metabolites prostanooids including prostaglandins, prostacyclin and thromboxan. Today three isoforms of the enzyme are known – COX-1, COX-2 and COX-3. COX-3 is defined as the COX-1 analogue bonded with intron, therefore, for the denotation of this isoform abbreviation COX-1b is often used (1-7). COX-3 is identified in the fabric of brain, however, to the present movement the biological role of this isoform is not exactly identified.

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Two isoforms of cyclo-oxygenase (COX), the key enzyme in prostaglandin (PG) and thromboxane (TX) biosynthesis referred to as COX-1 and COX-2 have been identified. Each enzyme is encoded by a separate gene and has a distinct pattern of expression and biological function. COX-1 is expressed constitutively and high levels can be detected in most tissues. In contrast, levels of COX-2 mRNA and protein are usually low or undetectable under basal conditions but are rapidly elevated during inflammation or mitogenic stimulation. Selective inhibition of COX-2 by non-steroidal anti-inflammatory drugs (NSAIDs) has been proposed as an approach to reduce their associated side effects while maintaining efficacy. The improved safety profile of selective COX-2 inhibitors will allow more widespread and sustained use than is currently possible with standard NSAIDs.

The crystalline structures of COX-1 and COX-2 were obtained in 1994 and 1996 respectively that brought in considerable contribution to the understanding of action and consequences of inhibition of this enzyme (8, 9). COX-1 takes part in adjusting vitally necessary functions, including work of stomach and blood clotting system, controls the production of prostaglandins (PG) that regulate the integrity of mucus membrane of gastrointestinal tract, function of thrombocytes, kidney and circulation of blood (10-14). In spite of that this isoform is constitutional; its expression can be stimulated by violations of integrity of the nervous fibers (15-18). Isoenzyme COX-2 takes part in the origin of pain and inflammation, synthesis of PG within the inflammation, the ПГ-гликопrotein activation (transporting protein responsible for the development of the multidrug resistance) (19-25). In addition, in kidney and nervous tissue too there is a constitutional expression of mentioned isoform (26, 27). Constitutional COX-2 has an important role in spinal cord, particularly synthesis of PGE₂ that leads to hyperalgesia (28).

Ghilardi et al29 assumes that blocking of the spinal COX-2 can result in the decline of sensitiveness (centers and periphery) that leads to the certain damages. COX-2 plays an important role in proliferation of cells30-37. The results of numerous researches testify to its overexpression within the non-small linear lung cancer, esophagus and colon adenocarcinomas and beginning from the early stages of cancer in 80-90% of the patients 38-47. An early expression of COX-2 is observed during oncogenesis of major cancer types: lung, breast, prostate, and colon (malignant) tumors.

During oncogenesis of the thyroid gland tumors COX-2 expression is marked that is accompanied by the high level PG, especially PGG2, an increase in the level of matrix metalloproteinase-2 (MMP-2). It has an important value for metastatic potential of these tumors. An increase in the COX-2 and MMP-2 levels in tumors depends on the age of patients that predetermines more aggressive development of malignant tumors of thyroid gland at the patients of senior age comparative with the youths (48-53). Similar situation is characteristic for the Khashimoto illness, follicle cancer and adenoma, medullary carcinoma, and adenoma from Gurtle cells. From the data of literature the special attention is spared to the colon cancer that by the conditioned trained mechanisms of the COX-2 action in oncogenesis, namely by activating of the PPARγ receptors and antiapoptotic gene Bcl-x (increasing of level of antiapoptotic protein Bcl-2) by PGE₂.

Both, COX-1 and COX-2 use arachidonic acid for the synthesis of PGH₂, which is afterwards modified with the formation of bioactive lipids–prostanoids that include prostacyclin, thromboxane A₂ and PGD₂, E₂ and F₂ (Fig. 1) which have an influence on immune, cardiovascular, gastrointestinal, renovascular, respiratory systems, CNS and the reproductive functions.

Non-selective inhibition of COX with drugs such as aspirin, ibuprofen, indomethacin and naproxene that suppress both the COX-1 and COX-2 enzymes which provides effective diminishment of pain syndrome at inflammation, however, at the same time it carries the risk of origin of erosive gastritis and gastrointestinal bleeding.

Selective inhibitors of COX-2 (Celecoxib, Ethoricoxib, Rofecoxib, Valdecoxib, Meloxicam) (Fig. 2) and other derivatives which are in the process of development were created with the purpose of minimization of toxic influence on a gastrointestinal tract that is conditioned by the relatively small expression of COX-2 in a gastrointestinal tract and considerable
Figure 1. COX-pathway of biochemical transformations of arachidonic acid.

Figure 2. Modern selective COX-2 inhibitors.
Figure 3. Celecoxib and rofecoxib in the prophylaxis of certain types of cancer.

in the inflamed fabrics and at all afore-mentioned pathological processes (cancer and multidrug resistance) (54-55).

Harris et al. (56) conducted the epidemiological research of influencing of non-steroidal anti-inflammatory drugs on the risk of origin of colon cancer, breast cancer, lung cancer and prostate cancer. It was found that in a result of in taking 200 mg of Celecoxib or 25 mg of Rofecoxib daily during two years and more is observed high level of prophylaxis of these types of cancer (Fig. 3). 4-thiazolidinone derivatives are one of the perspective classes of heterocyclic compounds as potential selective COX-2 inhibitors (Fig. 4). The aim of the research is to perform a molecular docking of some determined 4-thiazolidinone derivatives into COX-2 active site for the purposeful searching of COX-2 inhibitors as potential anticancer agents.

MATERIALS AND METHODS
Research is performed by the method of molecular docking as computer approach to the search of molecules with affinity to the certain biotargets. Docking studies (57) were conducted with OpenEye Scientific Software program package that include Fred Receptor, Vida, Flipper, Babel3, Omega2 and Fred2. Crystallographic models of COX-2 were obtained from Protein Data Bank (www.rcsb.org) particularly models 6COX (COX-2 in complex with selective inhibitor SC-558 at a resolution of 2,8 Å) and 1CX2 (COX-2 in complex with selective inhibitor SC-558 at a resolution of 3,0 Å). Binding space groups are the differential parts of these crystallographic models (different conformations) (Fig. 5). In Figure 5 one can see images of A chains of COX-2 crystallographic models 1CX2 (chains A, B, C, D) and 6COX (and A, B) with ligands such as selective COX-2 inhibitor SC-558, protoporphyrin IX (contain Fe) and N-acetyl-D-glucosamine. According to the literature data crystallographic models 1CX2 and 6COX are the space groups P 2_1 2_1 2 and I 2 2 2, respectively (58).

The resulted information testifies the difference of conformations of enzymes and consequently about the structural difference of binding sites that exert an influence on ligand-receptor interactions (59). It is, therefore, expedient to use both crystallographic models of COX-2 in docking researches.

As research objects were the selected 145 compounds, 4-thiazolidinone derivatives synthesized in the

Figure 4. Selective COX-2 inhibitors from group of 4-thiazolidinonines.
Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University that has shown in vitro anticancer activity and known selective COX-2 inhibitors such as Celecoxib, Etoricoxib, Rofecoxib, Valdecoxib, Meloxicam and non-selective inhibitor Aspirinum.

RESULTS AND DISCUSSION

Obtained seven scoring function values (Chemgauss2, Chemscore, PLP, Screenscore, Shapegauss, Zapbind and Consenses) were used for in silico estimation of COX-2 compound binding. Consensus (cumulative) scoring function ranking allowed us to select 20 compounds, which can prospectively be selective COX-2 inhibitors at the level of celecoxib for future (in-depth) pharmacological studies as well as could be used as templates for the synthesis of various related analogues.

With the help of Fred receptor, the active site (biotarget) of COX-2 was obtained from each crystallographic model for performing molecular docking. Molecular docking studies include stages such as:

1. Generating R-, S- and cys-trans isomers of ligands using program Flipper and Obtained 461 isomers of studied compounds.
2. 3D optimization of isomers using program Hyper Chem 7.5 (www.hyper.com) (molecular mechanics method MM+ and semi-empirical

Figure 5. Fig. 5. Graphic images of A chains of COX-2 crystallographic models 1CX2 and 6COX with ligands such as selective COX-2 inhibitor SC-558, protoporphyrin IX (contain Fe), N-acetyl-D-glucosamine.
quantum-mechanical method PM3).

3. Conformers generating (Omega2). Further program Fred2 will choose minimum energy conformation for each molecule.

3D molecular docking (Fred2). In a result obtained meaning of seven scoring functions (Chemgauss2, Chemscore, PLP, Screengscore, Shapegauss, Zapbind ta Consensus). Consensus scoring function was selected to analyze the results because of its property to result in ranking (compound ranking) that include the means of all scoring functions.

Analysis and ranking of the molecular docking results obtained using the selected compounds and two crystallographic models of COX-2 (6COX and 1CX2) with cumulative scoring function (consensus) allowed to select 20 compounds from 4-thiazolidinone group (Fig. 6 and 7) which probably exhibit selective inhibition of COX-2 on level of Celecoxib.

**Figure 6.** Structural formulas of potential COX-2 inhibitors obtained with molecular docking (crystallographic model 1CX2).

**Figure 7.** Structural formulas of potential COX-2 inhibitors obtained with molecular docking (crystallographic model 6COX).
Below are shown an interactions between COX-2 active site and two “hit compounds” (Les-942 and Les-1009) (Fig. 8 and 9) selected in a result of docking results analysis. Data visualized with VIDA (Open Eye Scientific Software). Compound Les-942 and Compound Les-1009 docked in the active site of COX-2 in comparison with the selective inhibitor SC-558. This will enable the compounds to interact strongly on the selective targets.

CONCLUSION
Preliminary docking studies of in-house library could be considered as a first stage of long term project dedicated to rational design of COX-2-inhibitors among thiazolidinone-based compounds. Adapted elements of rational methods for searching selective inhibitors of COX-2 as potential anticancer drugs on the basis of molecular docking that allows to predict affinity of some compounds to its certain biotarget.

Figure 8. Compound Les-942 docked in the active site of COX-2 (a) in comparison with selective inhibitor SC-558 (b) (crystallographic model 1CX2).

Figure 9. Compound Les-1009 docked in the active site of COX-2 (a) in comparison with selective inhibitor SC-558 (b) (crystallographic model 6COX).
Based on the affinity studies, the compound Les-942 and compound Les-1009 docked in the active site of COX-2 in comparison with the selective inhibitor SC-558. As a result, this will allow the compounds to interact strongly on the selective targets & exerts the favorable results. Molecular docking for 145 compounds (4-thiazolidinone derivatives and related heterocyclic systems) that has shown in vitro anticancer activity into the active site of enzyme COX-2 using crystallographic models 1CX2 and 6COX. In silico investigations were performed stage-by-stage with OpenEye Scientific Software program package. As a result, 20 compounds were thiopyran [2,3-d] thiazole-2-ones, 5-arylidene-4-thioxo-2-thiazolidinone and 2-thioxo-(heteryl, imino or o xo)-4-thiazolidinone derivatives that can show the selective COX-2 inhibition with blockage of COX-2 pathway in cancerogenesis, which determines the role of selective COX-2 inhibitors in prevention and treatment of cancer. Consequently, molecular docking of 20 derivatives of 4-thiazolidinone that exhibit anticancer activity were selected for further investigations of this compound as selective COX-2 inhibitors.

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