Anti-inflammatory Library

Inflammation process as a part of the complex biological response is characterized with three phases, including alteration, exudation, proliferation and activation of the immune response. This is a standard behavior of all complex organisms in the course of a disease. In fact, modern treatment of all dangerous diseases includes usage of anti-inflammation medicines. Despite the natural origin of inflammation, it can seriously injure the patient with the body's depletion of resources and pain syndrome. Both effects are harmful for regeneration process and the nervous system.

Based on the cell types and molecules involved in the process, inflammation can be classified as either acute or chronic. Different studies of last decades revealed a group of the most important proteins and messengers that are responsible for the development of progressive and slow immune responses. Usually, treatment of more acute inflammation process is significantly easier because of its rapid and timely diagnosis. At the same time, chronic diseases often demonstrate typical features of a general disease and should be diagnosed at their early onset. Such disorders as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer showed involvement of similar pathways with a range of protein targets engaged in chronic inflammation development.

Taking into account the information about proteins interactions signaling pathways and the availability of protein structural data (RCSB protein data bank) along with active inhibitors (ChEMBL DB), Life Chemicals prepared its proprietary Anti-inflammatory Library. The following targets involved in activation of other modulators or strengthening defense mechanisms have been considered:

- Pi3KC
- PTP1B
- LTA4
- JAK3
- COX2
- SK1
- SPL1
- SYK
- RORγ
- LRRK2
- iNOS

Almost **1,400** small molecule potential anti-inflammatory agents were identified in the Life Chemicals HTS Compound Collection using docking screening method. The Library covers several stages of production and degradation of signaling intermediates (cytokines, phosphatidylinositol, prostaglandine, tromboxane, sphingosine-1-phosphate).

Phosphatidylinositol-4,5-bisphosphate 3-kinases (PI3K) Alpha and Beta Subunits

Phosphatidylinositol-4,5-bisphosphate 3-kinases (also called phosphatidylinositide 3-kinases, PI3K) are a family of enzymes involved in such cellular functions as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking. A disorder of each of the pathways may cause cancer development.

These intracellular signal transducer enzymes produce a signaling intermediate by phosphorylating the *3*-position hydroxyl group of the inositol ring of phosphatidylinositol. Class IA PI3K is composed of a heterodimer between a p110 catalytic subunit and a p85 regulatory subunit. Many of its functions relate to the ability of PI3-kinases to activate protein kinase B (PKB, aka Akt) as in the PI3K/AKT/mTOR pathway. It is also known that PI3Ks are involved in various immune responses and produced in different immune cells. A special role of p110δ and p110γ isoforms of PI3K consists in in regulation of different aspects of immune defence and inflammation.

This Library contains potential inhibitors of gamma and delta isoforms of PI3K from class Ia. Based on a crystallography data and spatial alignments, the most important ligand-protein interactions were determined. These data on pharmacophore sites were used in preparation of an *in silico* screening model.

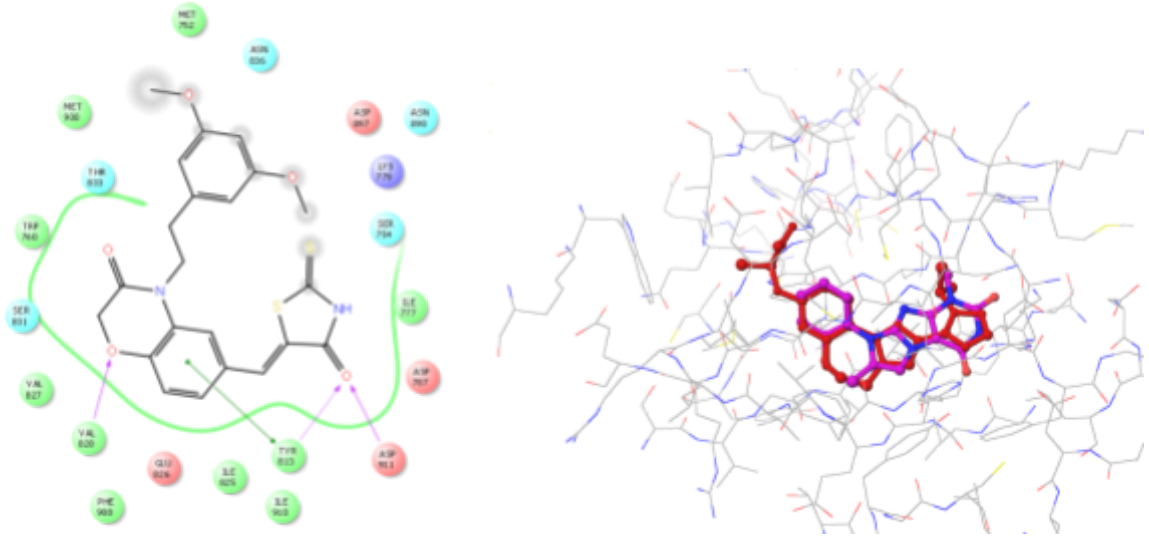

Fig. 1. Interaction map of the bound γ-subunit inhibitor CHEMBL394235 (IC50 = 11.8 nM) in the PI3K protein site (PDBID: 4EZK) during validation of the screening model (left). Superposed alignment of known PI3K inhibitor GDC-0326 (in red) and a compound from the Life Chemicals Stock Collection (right, in magenta) in the averaged structure of γ/δ subunits.

Protein-tyrosine Phosphatase 1B (PTP1B)

Tyrosine-protein phosphatase non-receptor type 1 also known as protein-tyrosine phosphatase 1B (PTP1B) is a "primogenitor" member of the protein tyrosine phosphatase (PTP) family of enzymes. The two stage dephosphorylation mechanism provides engagement of the enzyme in different pathways. Among its substrates there are several tyrosine kinases (EGFR, c-SRC, JAK2 etc.) and some other tyrosine-phosphorylated proteins (BCAR1, DOK1). Several studies showed that over-expression of PTP1B decreases the level of TNF-α and IL-6 in macrophages. Its essential role in the development of diabetes and obesity was also proven, however, there are no evident data concerning the mechanism of such regulations. PTP1B plays a central role in producing pro-inflammatory cytokines in microglia through modulation of Src activity.

Due to a specific active site, the selectivity is one of the major issues in the development of PTP1B inhibitors as pharmaceutical drugs. To fully cover the prospective chemical space two binding modes were developed based on a list of crystal structures and interaction maps. Both screening models were validated with a set of potent reference compounds.

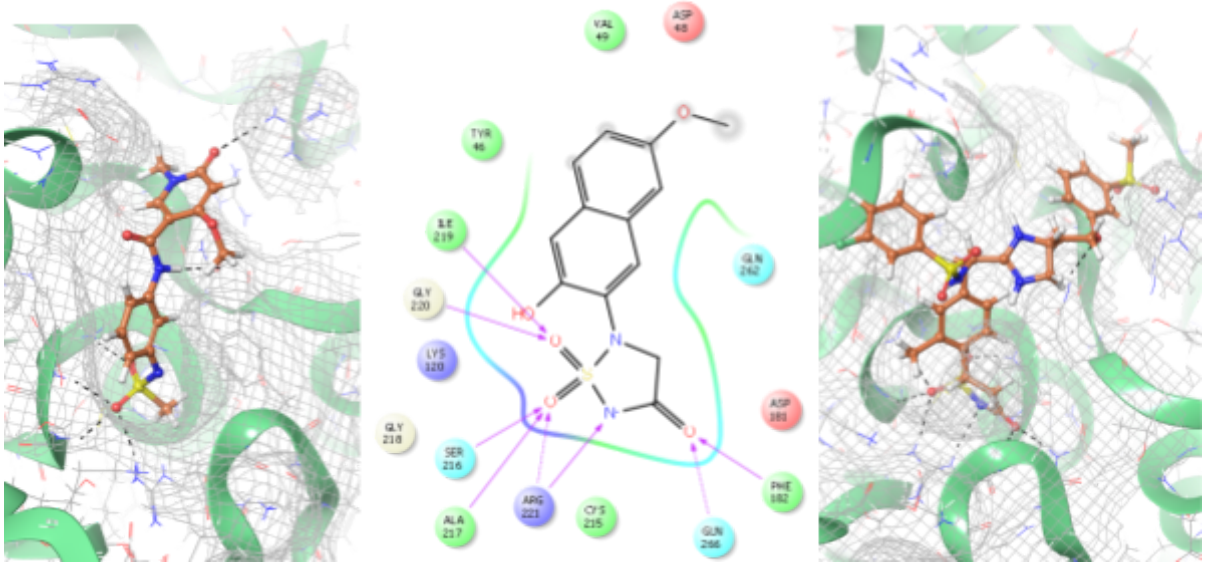

Fig. 2. Comparative demonstration of the docked Life Chemicals hit compound (left) and the reference compound from ChEMBL (right).

Leukotriene A4 (LTA4)

Leukotriene A4 (LTA4) hydrolase is a zinc-containing bifunctional enzyme that converts leukotriene A4 to leukotriene B4 and acts as an aminopeptidase (epoxide LTA4 to the diol leukotriene B4 (LTB4). The LTB4 plays a significant role in further development of many inflammatory disease conditions. As it was shown during the last 10-15 years, all therapeutic agents, which selectively inhibit LTA4 hydrolase and would inhibit the formation of LTB4, could be potentially useful for the inflammation process suppression. First inhibitors of LTA4 were based on the structure of a natural substrate. The next generation of peptide and non-peptide analogs was designed to mimic the substrate and contained potential zinc-chelating moieties, including thiols, hydroxamates and norstatines.

Taking into account literature data and activity of experimentally approved drugs, we applied both structure-based and ligand-based approaches to generate docking models. Metal-binding sites of the ligands, hydrophobic regions and H-bonds they are forming with a protein molecule were used to produce accurate and exhaustive docking.

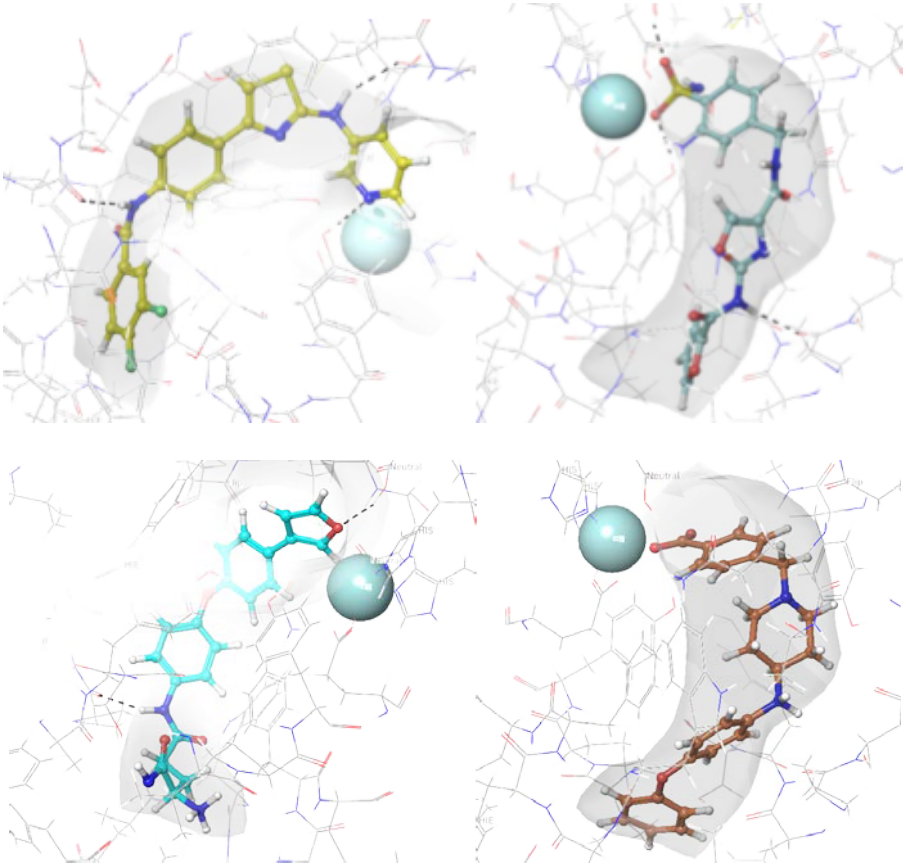

Fig. 3. Two hits from the Life chemicals Stock, bound to the Zn atom from the opposite direction in the active site of LTA4 (PDBID:3FH7) (upper row), and two similar docked inhibitors from ChEMBL database (CHEMBL515470, IC50 = 29 nM and CHEMBL481860 IC50 = 46 nM) in the same positions (bottom row).

Janus Kinase 3 (JAK3)

The Janus kinases (JAK 1,2,3) are a family of intracellular tyrosine kinases that play an important role in signaling cytokines that have been implicated in inflammatory process. All members of the family initiate binding of specific receptors with a broad array of cytokines. All of them are involved in activation of inflammation process. For example, they have a key function in Hemophagocytic lymphohistiocytosis (HLH).

While inhibition of JAK1 and JAK2 can cause drug-related adverse events (e.g., overt immunosuppression, anemia), Janus Kinase 3 (JAK3), a hematopoetic cell-restricted tyrosine kinase, represents an attractive target for immunosuppression due to its limited tissue distribution and specific role in lymphoid homeostasis. Such JAK3 inhibitor as CP-690 550 has shown efficacy as immunosuppressants.

Life Chemicals has prepared a library of potentially selective JAK3 inhibitors, identified by means of *in silico* screening and exhaustive docking procedures. Ligand efficiency was estimated by docking of the reference compounds.

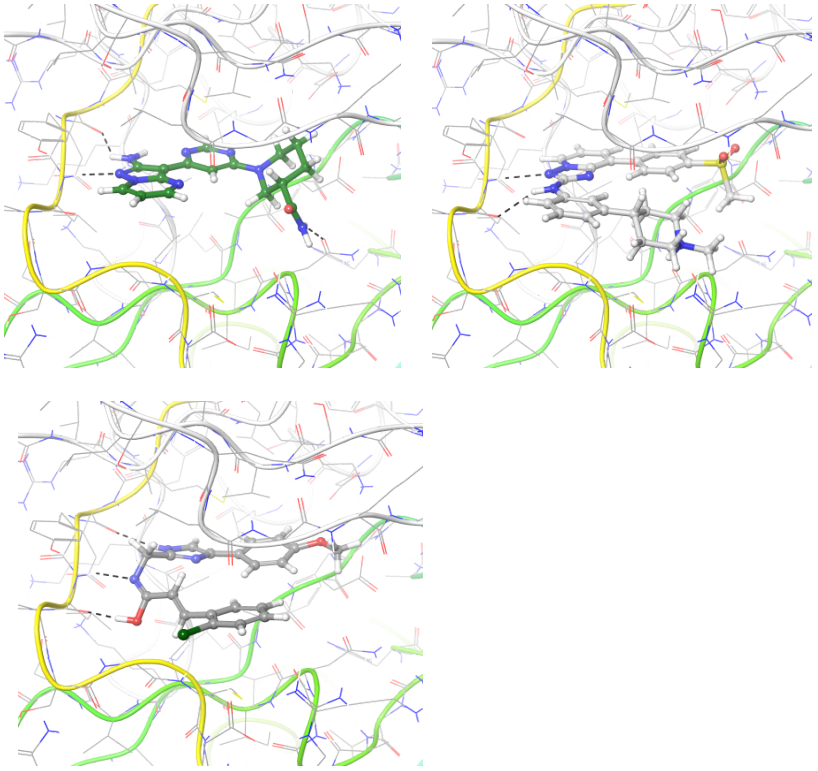

Fig. 4. Docked reference compounds CHEMBL577944 (IC_{50} = 500 nM) and CHEMBL2062809 (IC_{50} = 72 nM) (left) and a hit from Life Chemicals HTS Compound Collection (right).

Prostaglandin-endoperoxide Synthase 2, Cyclooxygenase-2 (COX-2)

Prostaglandin-endoperoxide synthase 2 (cyclooxygenase-2 or COX-2) is an enzyme that plays a key role in inflammatory processes by conversion of arachidonic acid to prostaglandin H2 which is an important precursor of prostaglandins (such as prostacyclin) and thromboxane A2. Hence, inhibition of COX can provide relief from inflammation and pain symptoms. This enzyme contains a short *N*-terminal epidermal growth factor (EGF) domain, an α-helical membrane-binding moiety and a C-terminal catalytic domain. Human PTGS2 (COX-2) functions as a conformational heterodimer, having a catalytic monomer and an allosteric monomer (E-cat) and E-allo. Heme binds only to the peroxidase site of catalytic part while substrates, as well as known inhibitors, bind to the COX site of catalytic part. E-cat is regulated by E-allo in a way dependent on a ligand to be bound to E-allo.

Several studies showed that inflammation proteins (like Prostaglandin endoperoxide H synthases) are also targets of COX-2. Inhibition of COX-2 that is selectively induced by pro-inflammatory cytokines at the inflammation site increases the anti-inflammatory properties effect. One of the benefits of targeting COX-2 is its usual specific localization in inflamed tissue, so it causes much less gastric irritation associated with COX-2 inhibitors. It was also reported that selective inhibition of PTGS2 (COX-2) shows fewer side-effects in comparison to COX-1 enzyme.

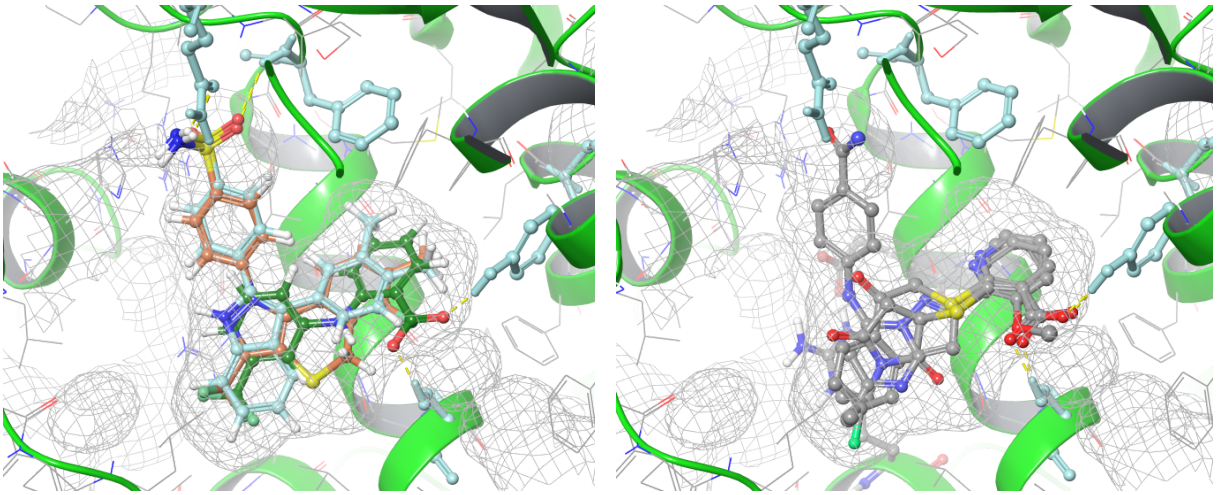

Fig. 5. Several docked reference compounds aligned in the active site of COX-2 protein (PDBID:5KIT) (left) and the same view for docked hits from the Life Chemicals Stock Collection (right).

Sphingosine kinase 1 (SK1)

Sphingosine kinase 1 phosphorylates sphingosine to sphingosine-1-phosphate (S1P). Sphingosine kinase 1 is normally a cytosolic protein but is recruited to membranes rich in phosphatidate (PA), a product of Phospholipase D (PLD). Its substrate, Sphingosine-1-phosphate (S1P), is a novel lipid messenger with both intracellular and extracellular functions. Intracellularly, it regulates proliferation and survival of the cell, and extracellularly, it is a ligand for Sphingosine-1-phosphate receptor 1, providing an inflammation signalling.

Sphingosine kinases (SKs) and their lipid product S1P play essential roles in inflammatory signaling processes, as well as disease development and progression. Systemic lupus erythematosus, arthritis, ulcerative colitis and Crohn's disease are among the list of disorders, caused by activation of SK/S1P cascade. SKs can be activated by numerous growth factors and cytokines. Both isoforms of SK (SK1 and SK2) have the ability to phosphorylate sphingosine to form S1P, however, their substrate specificity is different.

This Library includes compounds selected by *in silico* screening and contains novel chemical structures with good predicted affinity to the active site.

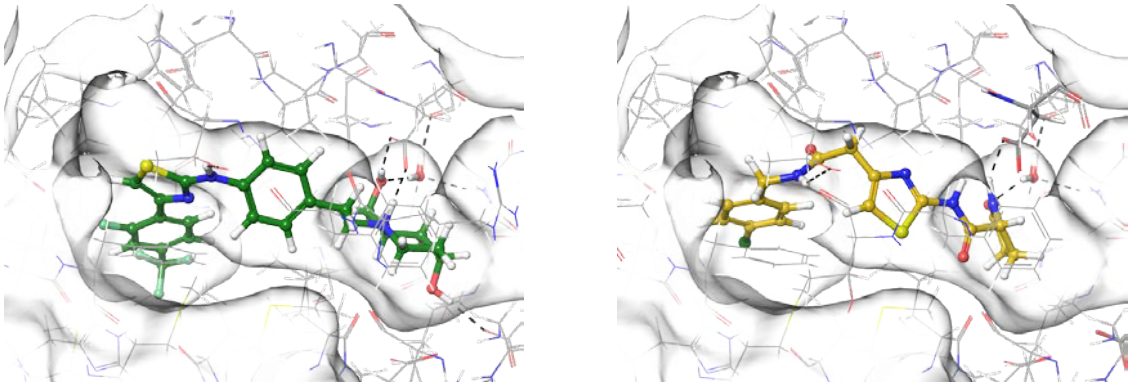

Fig. 6. Similar location and orientation of a reference compound CHEMBL2409849 (IC50 = 4 nM) (left) and one of the hits from the Life Chemicals Collection (right) in the binding site of Sphingosine Kinase 1 protein (PDBID: 4l02)

Sphingosine-1-phosphate Lyase (SPL1)

Sphingosine-1-phosphate lyase (SPL), a membrane-bound enzyme of sphingolipid metabolism, catalyzes irreversible degradation of sphingoid base phosphates. Its main substrate sphingosine-1-phosphate, a polar sphingolipid metabolite, acts both extracellularly by binding G protein-coupled receptors of the lysophospholipid receptor family, and inside the cell, as a second messenger. SPL is expressed in many mammalian tissues and S1P tissue level is usually low and kept under control through equilibrium between its synthesis mostly governed by sphingosine kinase-1 (SK1) and its degradation by sphingosine 1-phosphate lyase (SPL). Inhibitors of SPL may become useful therapeutic agents for a variety of diseases in which S1P is involved.

On the basis of the crystal structure of the ligand and its interaction map Life Chemicals has designed a library comprising compounds with excellent 3D pharmacophore matching.

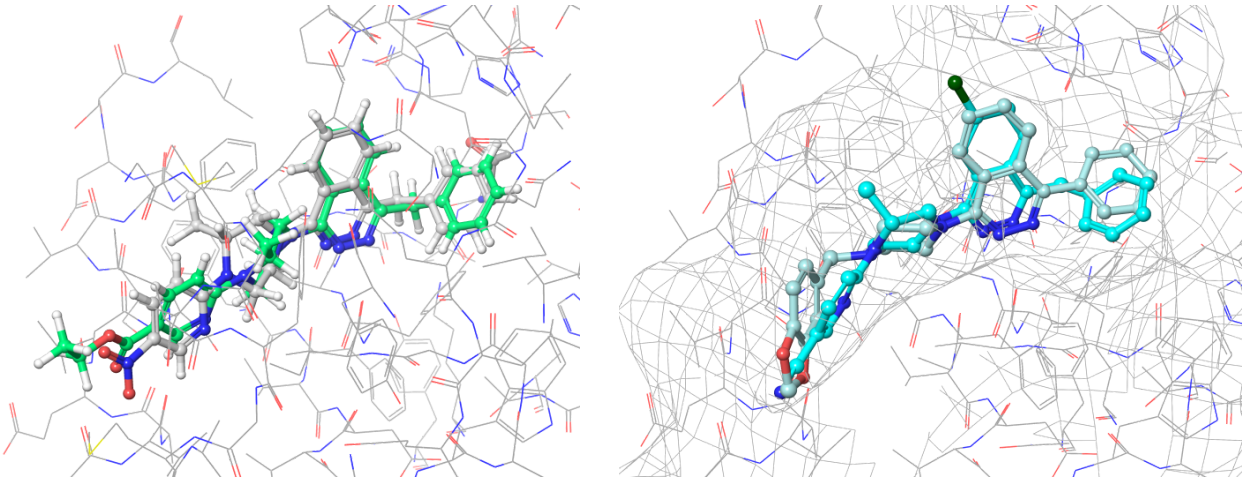

Fig. 7. Reference compounds docked into the active site (left) and one of the hits aligned to the co-crystalized ligand of SPL (PDBID: 4Q6R)

Spleen Tyrosine Kinase (SYK)

Spleen tyrosine kinase (SYK) is a member of the SYK family of tyrosine kinases. Basically, tyrosine kinases are enzymes that catalyze phosphorylation of tyrosine residues on protein substrates, involved in signaling pathways that drive an array of cellular responses including proliferation, differentiation, migration and survival.

The structure of these non-receptor cytoplasmic tyrosine kinases shares a characteristic dual SH2 domain separated by a linker domain. SH2 domains typically bind to phosphorylated tyrosine residues within a motif of a target protein. This interaction initiates a cascade of events inducing several cellular responses. SYK couples immune cell receptors to intracellular signalling pathways that regulate cellular responses on initiated inflammation process. Due to its overexpression in hematopoietic cells SYK is considered to be a pro-survival factor for several hematological and non-hematological cancers. SYK kinases are good therapeutic targets for such disorders as arthritis, allergic conditions, idiopathic thrombocytopenic purpura, systemic lupus erythematosus, B-cell lymphoma.

The structural and functional features of the target open up an opportunity to use small-molecule inhibitors of SYK for treatment of both immune disorders and cancer.

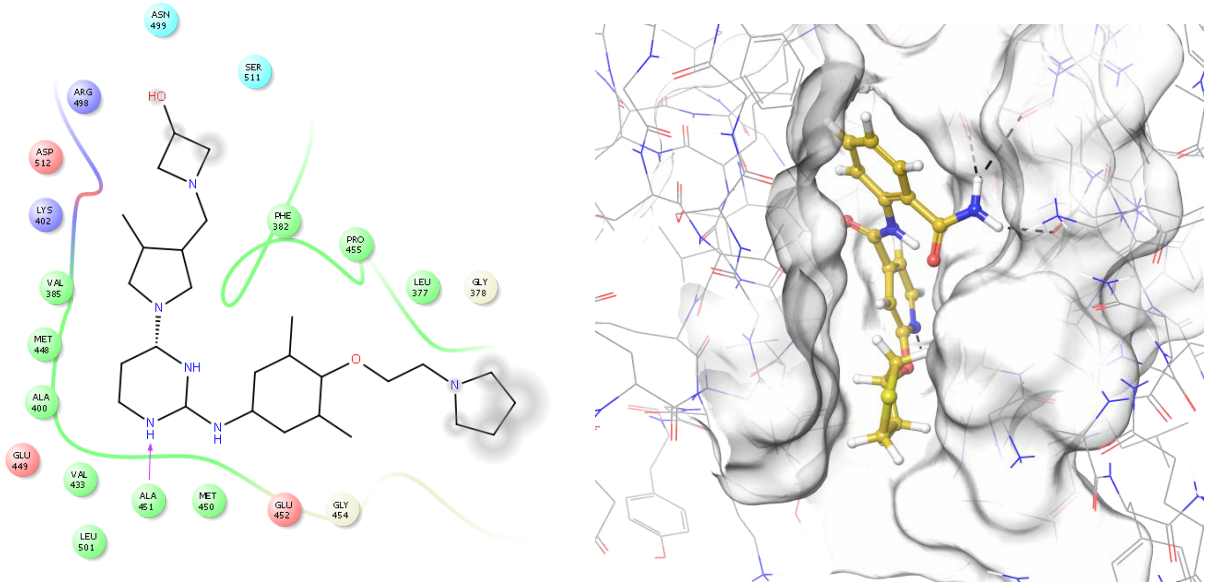

Fig. 8. Interaction map of the known SYK inhibitor and one of the screened compounds in the active site of the kinase (PDBID: 4XG9)

RAR-related orphan receptor gamma (RORγ)

RAR-related orphan receptor gamma (RORγ or RORC) is a member of a nuclear receptor family that is specifically expressed in T cell compartments. Two isoforms RORγt and RORγ are encoded by a single gene called Rorg, the difference between these isoforms is in the length of N-terminus and more restricted distribution of the second one. One of the most interesting functions of RORγ suggests a conversion of $CD4^+$ immune cells into pro-inflammatory Th17 cells. Interleukin-17 (IL-17) and T helper 17 (TH17) cells play a key role in tissue inflammation. So, prevention of cytokines' overproduction can regulate immune responses.

It was shown that RORγ takes part in many autoimmune disorders, like psoriasis, psoriatic arthritis, ankylosing spondylitis, inflammatory bowel disease and multiple sclerosis. Despite a number of novel medicines, which were developed and tested during the last decade, a large molecular weight of antibodies makes them unsuitable for usage as topical medicines because they cannot diffuse across the skin barrier. Another way to reduce symptoms involves modulation of RORγ transcriptional activity by interfering with RORγ-DNA binding to decrease the level of cytokine transcription.

Therefore, a local inhibition of RORγ/RORγt with small molecular weight inhibitors represents a unique approach to selective inhibition of aberrant IL-17 cytokine production.

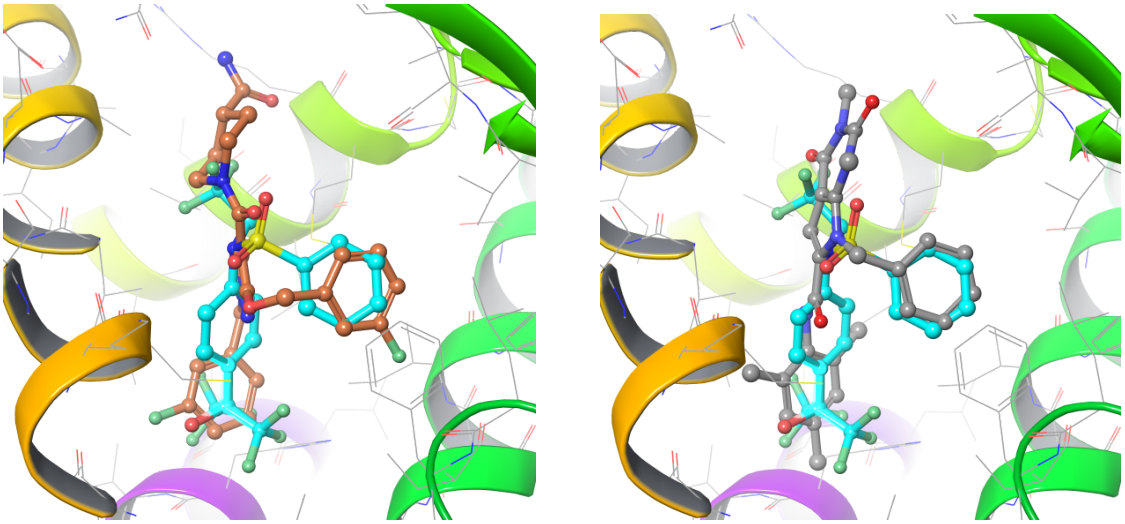

Fig. 9. Evaluation of the docking model by alignment and scoring of reference compounds (left) and demonstration of pharmacophore matching to the hit compound from the Life Chemicals Stock (right). The template taken from the crystal structure is colored with cyan.

Leucine-rich repeat kinase 2 (LRRK2)

Leucine-rich repeat kinase 2 (LRRK2), also known as dardarin, belongs to the Ras of complex protein family, which is characterized by the presence of a Ras-like G-domain (Roc), a C-terminal of Roc domain (COR), and a kinase domain. Its active form is a dimer, whose functions are similar to serine/threonine specific kinase. Expression of LRRK2 mutants has been found to be the most frequent cause of Parkinson's disease with a shortening and simplification of the dendritic tree.

LRRK2 takes place in some pathways involved in neuronal damage, including the microtubule network, actin cytoskeleton reorganization, autophagy, mitochondria, vesicular trafficking and protein quality control. It is also a novel regulator of Nuclear factor of activated T-cells (NFAT) that modulates severity of inflammatory bowel disease. The mechanism of its action is not fully investigated, but the knockout or pharmacological inhibition of LRRK2 results in defects in synaptic vesicle endocytosis, altered synaptic morphology and impairments in neurotransmission. It is suggested that because of the kinase nature of LRRK2, it might modulate the phenotype of microglia through hyperpolymerization and hyperphosphorylation of cytoskeleton and vesicle components, thus pushing these cells toward a pro-inflammatory state.

Based on a reference set of compounds (ChEMBL DB, K_I and IC_{50} values), which were clustered by structure, five diverse scaffolds were identified. A conformer search calculation and cross-alignment were carried out to identify a possible active conformation for core structures of each cluster. Five different QSAR models were built and validated for subsequent prediction of potential compounds from the Life Chemicals HTS Compound Database.

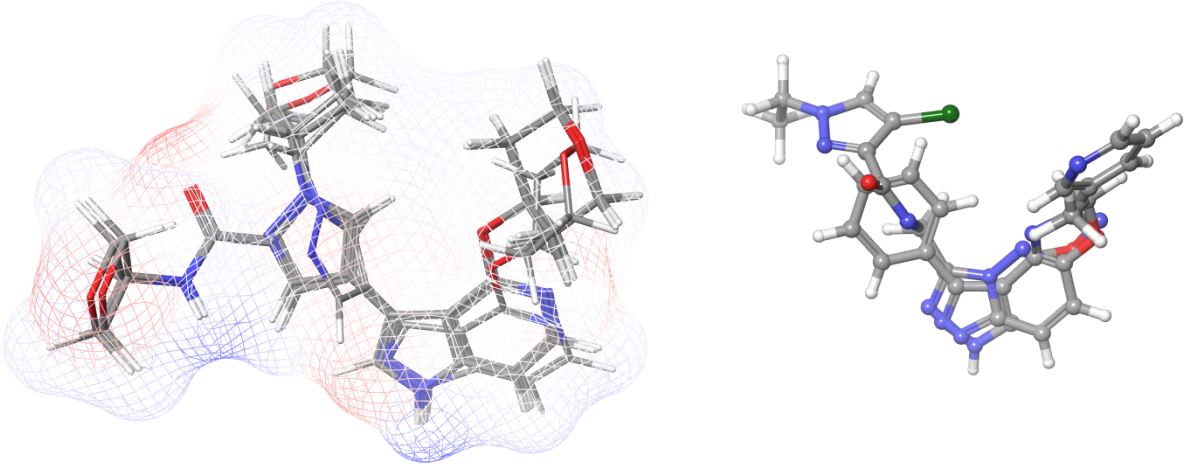

Fig. 10. An example of conformational search pull with an electrostatic surface (left) and randomly chosen hits from the Life Chemicals HTS Compound Collection.

Nitric Oxide Synthase Inducible (iNOS)

Nitric oxide synthases (NOSs) are a family of enzymes catalyzing the production of nitric oxide (NO) from L-arginine. It helps modulate vascular tone, insulin secretion, airway tone, and peristalsis, and is involved in angiogenesis and neural development. It may function as a retrograde neurotransmitter and it is an important cellular signaling molecule.

There are three known isoforms in mammals, two are constitutive (cNOS) and the third is inducible (iNOS). iNOS has been described as calcium-insensitive due to its tight non-covalent interaction with calmodulin (CaM) and Ca^{2+}. The expression of the inducible nitric oxide synthase (iNOS) is one of the markers of an inflammatory process, contributing to local tissue destruction during chronic inflammation.

Life Chemicals HTS Compound Collection was screened using the model which contains obligatory interactions with two key-role residues in the active site and/or metal-binding ability.

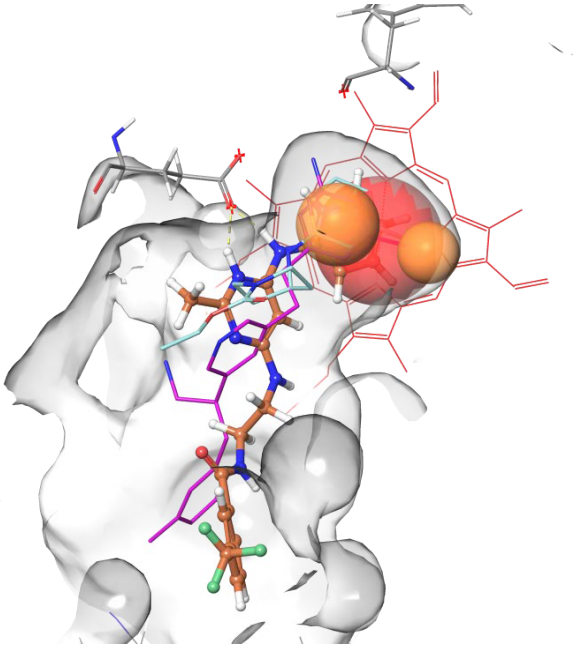

Fig. 11. iNOS binding site surface with docking constraints (spheres and asterisks) covers both re-docked inhibitors form crystal structures (wireframe) and one of the compounds from the Life Chemicals HTS Compound Collection (balls and sticks).

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