IN SILICO APPROACHES FOR THE IDENTIFICATION OF NOVEL INHIBITORS FOR MITOCHONDRIAL PROSTAGLANDIN E SYNTHASE (mPGES) – 1

A Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY,

CHENNAI- 600 032

In partial fulfilment of the award of the degree of

MASTER OF PHARMACY

IN

Branch-II – PHARMACEUTICAL CHEMISTRY

Submitted by

Name: P. SARANYA

REG. No. 261715202

Under the Guidance of

Dr. P. SENTHIL KUMAR, M. Pharm., Ph. D.

PROFESSOR

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

J.K.K.NATTRAJA COLLEGE OF PHARMACY

KUMARAPALAYAM – 638 183

TAMILNADU

NOV – 2019

IN SILICO APPROACHES FOR THE IDENTIFICATION OF NOVEL INHIBITORS FOR MITOCHONDRIAL PROSTAGLANDIN E SYNTHASE (mPGES) – 1

A Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY,

CHENNAI - 600 032

In partial fulfilment of the award of the degree of

MASTER OF PHARMACY

IN

Branch-II – PHARMACEUTICAL CHEMISTRY

Submitted by

NAME: P. SARANYA

REG.No. 261715202

Under the Guidance of

Dr. P. SENTHIL KUMAR, M.Pharm., Ph.D.

PROFESSOR

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

J.K.K.NATTRAJA COLLEGE OF PHARMACY

KUMARAPALAYAM – 638 183

TAMILNADU

NOV – 2019

CERTIFICATES

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"*IN SILICO* APPROACHES FOR THE IDENTIFICATION OF NOVEL INHIBITORS FOR MITOCHONDRIAL PROSTAGLANDIN E SYNTHASE (mPGES) – 1",** submitted by the student bearing **Reg. No: 261715202** to "**The Tamil Nadu Dr. M.G.R. Medical University** – **Chennai**", in partial fulfilment for the award of Degree of **Master of Pharmacy** in **Pharmaceutical Chemistry** was evaluated by us during the examination held on……

Internal Examiner **External Examiner**

CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"*IN SILICO* APPROACHES FOR THE IDENTIFICATION OF NOVEL INHIBITORS FOR MITOCHONDRIAL PROSTAGLANDIN E SYNTHASE (mPGES) – 1",** submitted to "**The Tamil Nadu Dr. M.G.R. Medical University**- **Chennai**", in partial fulfilment and requirement of university rules and regulation for the award of Degree of **Master of Pharmacy** in **Pharmaceutical Chemistry**, is a bonafide work carried out by the student bearing Reg.No**. 261715202** during the academic year 2018-2019, under the guidance and supervision of **Dr. P. Senthil Kumar, M.Pharm., Ph.D.,** Professor, Department of Pharmaceutical Chemistry, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

Dr. P. Senthil Kumar, M.Pharm., PhD.,
Professor,
Department of Pharmaceutical Chemistry

Dr. M. Vijayabaskaran, M. Pharm., Ph.D.,
Professor & Head,
Department of Pharmaceutical Chemistry

CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"*IN SILICO* APPROACHES FOR THE IDENTIFICATION OF NOVEL INHIBITORS FOR MITOCHONDRIAL PROSTAGLANDIN E SYNTHASE (mPGES) – 1",** submitted to "**The Tamil Nadu Dr. M.G.R. Medical University** - **Chennai**", in partial fulfilment and requirement of university rules and regulation for the award of Degree of **Master of Pharmacy** in **Pharmaceutical Chemistry**, is a bonafide work carried out by the student bearing **Reg. No. 261715202** during the academic year 2018-2019, under my guidance and direct supervision in the Department of Pharmaceutical Chemistry, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

Place: Kumarapalayam

Date:

Dr. P. Senthil Kumar, M. Pharm., Ph.D.,
Professor,
Department of Pharmaceutical Chemistry

CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"*IN SILICO* APPROACHES FOR THE IDENTIFICATION OF NOVEL INHIBITORS FOR MITOCHONDRIAL PROSTAGLANDIN E SYNTHASE (mPGES) – 1",** submitted to "**The Tamil Nadu Dr. M.G.R. Medical University**- **Chennai**", in partial fulfilment and requirement of university rules and regulation for the award of Degree of **Master of Pharmacy** in **Pharmaceutical Chemistry**, is a bonafide work carried out by the student bearing Reg. No**. 261715202** during the academic year 2018-2019, under the guidance and supervision of **Dr. P. Senthil Kumar, M.Pharm., Ph.D.,** Professor, Department of Pharmaceutical Chemistry, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

Place: Kumarapalayam
Date:

Dr. R. Sambathkumar, M. Pharm., PhD.,
Professor & Principal,
J.K.K.Nattraja College of Pharmacy,
Kumarapalayam.

DECLARATON

I do hereby declared that the dissertation **"*IN SILICO* APPROACHES FOR THE IDENTIFICATION OF NOVEL INHIBITORS FOR MITOCHONDRIAL PROSTAGLANDIN E SYNTHASE (mPGES) – 1"** submitted to "**The Tamil Nadu Dr. M.G.R. Medical University - Chennai",** for the partial fulfilment of the degree of **Master of Pharmacy** in **Pharmaceutical Chemistry,** is a bonafide research work has been carried out by me during the academic year 2018-2019, under the guidance and supervision of **Dr. P. Senthil Kumar, M.Pharm., Ph.D.,** Professor, Department of Pharmaceutical Chemistry, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma, associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine to the best of my knowledge.

Place: Kumarapalayam **Ms. P. SARANYA**

Date: **Reg.no. 261715202**

Dedicated to Parents,

Teachers &

My Family

ACKNOWLEDGEMENT

ACKNOWLEDGEMENT

I am proud to dedicate my deep sense of gratitude to the founder, (Late) Thiru. **J.K.K. Nataraja Chettiar,** providing the historical institution to study.

My sincere thanks and respectful regards to our reverent Chairperson **Smt. N. Sendamaraai, B.Com.,** and Director **Mr. S. Omm Sharravana, B.Com., LLB.,** J.K.K.Nattraja Educational Institutions, Kumarapalayam, for their blessings, encouragement and support at all times.

It is most pleasant duty to thank for our beloved Principal, **Dr. R. Sambathkumar, M.Pharm., Ph.D.,** Principal, J.K.K.Nattraja College of Pharmacy, Kumarapalayam, for ensuring all the facilities were made available to me for smooth running of the project and tremendous encouragement at each and every step of this dissertation work. Without his critical advice and deep-rooted knowledge, this work would not have been a reality.

It is my privilege to express deepest sense of gratitude toward my guide, **Dr. P. Senthil Kumar, M.Pharm., Ph.D.,** Professor, Department of Pharmaceutical Chemistry, for his valuable suggestions and inspiration.

It is my privilege to express deepest sense of gratitude towards **Dr. M. Vijayabaskaran, M.Pharm., Ph.D.,** Professor & Head, Department of Pharmaceutical Chemistry, **Mrs. B. Vasuki, M.Pharm.,** Assistant Professor, **Mrs. B. Jayalakshmi, M.Pharm.,** Assistant Professor, and **Ms. P. Lekha,** Lecturer, for their valuable suggestions and inspiration.

I greatly acknowledge the help rendered by **Mrs. K. Rani,** Office Superintendent, **Miss. M. Venkateswari, M.C.A.,** typist**, Miss. S. Sudhalakshmi,** Typist, **Mrs. V. Gandhimathi, M.A., M.L.I.S.,** Librarian,

and **Mrs. S. Jayakala, B.A., B.L.I.S.,** Asst. Librarian for their co-operation. I owe my thanks to all the technical and non-technical staff members of the institute for their precious assistance and help.

Last, but nevertheless, I am thankful to my lovable parents and all my friends for their co-operation, encouragement and help extended to me throughout my project work.

Ms. P. SARANYA
Reg.no. 261715202

CONTENTS

CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

CHAPTER 1

INTRODUCTION

Pain produced by intense, potentially harmful stimuli is an important early warning sign that helps avoid tissue damage. This type of pain is known as nociceptive pain. Nociceptive pain signalling is initiated by peripheral terminals of sensory neurons, the nociceptors, which respond to heat, acids or severe mechanical stress resulting from direct pressure. Peripheral nociceptor terminals express molecules such as the transient receptor potential cation channel subtype V1 (TRPV1) and voltage-gated sodium channels (e.g. Nav1.8 or 1.9) to detect noxious stimuli and transducer them into electrical energy. Inflammatory pain develops when the sensitivity of the nociceptive system increases after the tissue integrity is disrupted by trauma, heat, infection, toxins, inadequate immune responses, tumors or other insults [Chen L, 2013]

Once tissue damage has occurred, multiple chemical mediators are released from the injured cells and the infiltrating immune cells to create an "inflammatory soup" that contains proinflammatory cytokines [such as interleukin 1β (IL-1β) and tumor necrosis factor (TNF)-α], chemokines [such as monocyte chemotactic protein-1 (MCP-1)], prostaglandins [such as prostaglandin (PG) E2 and prostacyclin (PGI2)], bradykinins, nerve growth factors, purines, amines, ions, and many others. The peripheral terminals of nociceptors express receptors for many of these inflammatory mediators. They act to lower the activation threshold of the TRPV1 and the Nav ion channels, for example, by inducing phosphorylation events at regulatory amino acid residues or by increasing expression levels. [Chen L, 2013]

Biosynthesis of prostanoids involves oxidation and subsequent isomerization of membrane-derived arachidonic acid (AA) via three sequential enzymatic reactions. The initial step of this metabolic pathway is the stimulus-induced liberation of AA from membrane

glycerophospholipids by phospholipase A2 (PLA2) enzymes. The released AA is sequentially metabolized to prostaglandin (PG) G2 and then to PGH2 by either cyclooxygenase (COX) -1 or COX-2. PGH2 is then converted to various bioactive PGs (thromboxane (TX) A2, PGD2, PGE2, PGF2 and PGI2) by the respective terminal prostanoid synthases, which have different structures and exhibit cell- and tissuespecific distributions. [Kudo I, 2005]

Prostaglandin (PG) endoperoxide synthase/cyclooxygenase (COX) is the rate limiting enzyme of PG synthesis from arachidonic acid. Two COX forms have been isolated; a constitutively produced COX-1 and an inducible COX-2. Normally, COX-1 is constitutively produced in the stomach and COX-1 derived PGE2 is considered involved in gastric mucosal protection, stimulating mucous bicarbonate barrier formation in the normal mucosa. [Gudis K, 2005]

There are three proteins that catalyze the conversion of PGH2 to PGE2; namely membrane-bound Prostaglandin E (PGE) Synthase (mPGES-1, mPGES-2), and cytosolic PGES (cPGES). The characteristic properties of three PGES enzymes (mPGES-1, mPGES-2 and cPGES) are summarized in Table 1. [Kudo I, 2005]

Table 1. Properties of PGES enzymes [Kudo I, 2005]

Enzymes	**Structural Properties**	**Expression**	**Subcellular Localizations**	**COX Preference**	**Specific Characteristics**	***In vivo* Functions**
mPGES-1	MAPEG family	Inducible Glucocorticoid-sensitive	Perinuclear Membrane	COX-2	Trimer formation	Inflammation, pain, fever, cancer
mPGES-2	Thioredoxin homology domain	Constitutive	Golgi, Cytosol	COX-1, COX-2	cleavage of N-terminal hydrophobic region dimer formation	Unknown
cPGES	Hsp90 cochaperone p23	Constitutive	Cytosol	COX-1	Complex formation with Hsp90 and CK2	Unknown

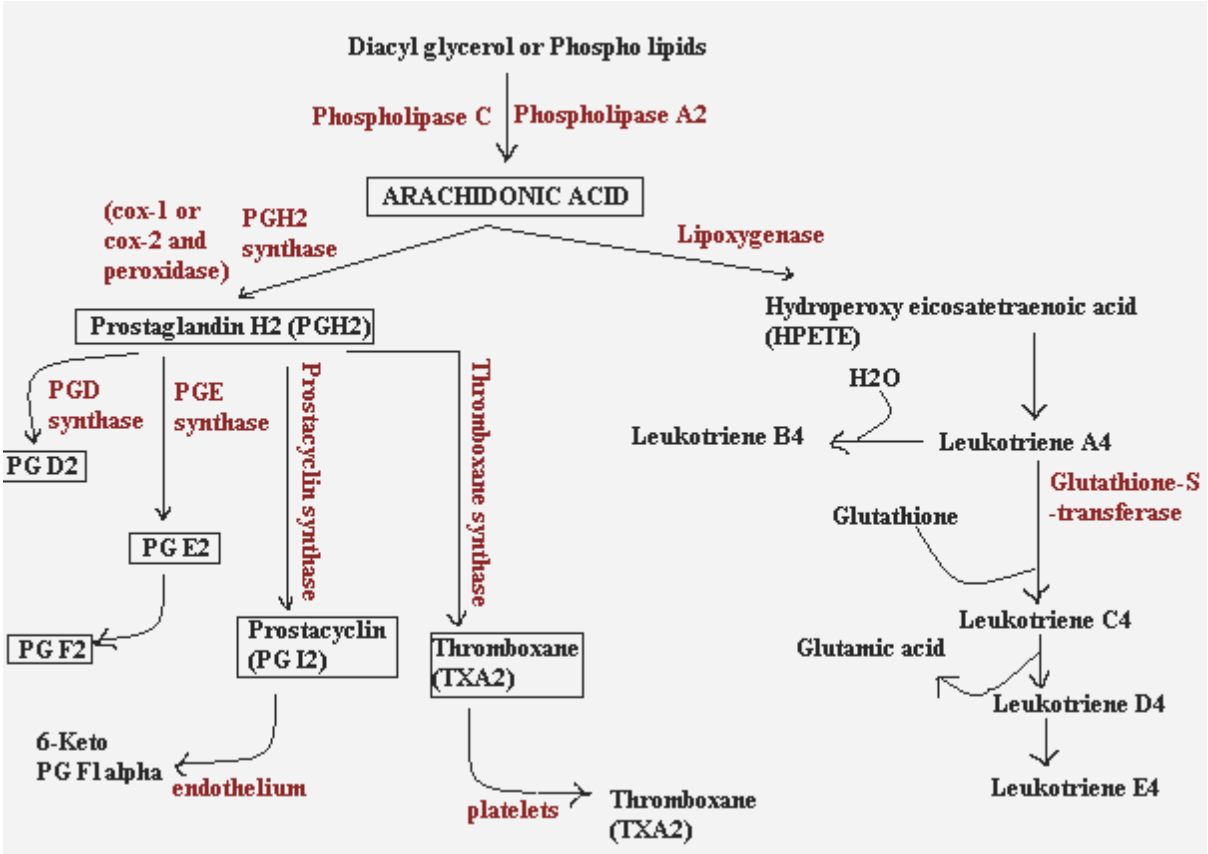


Figure 1: Biosynthesis of Eicosanoides

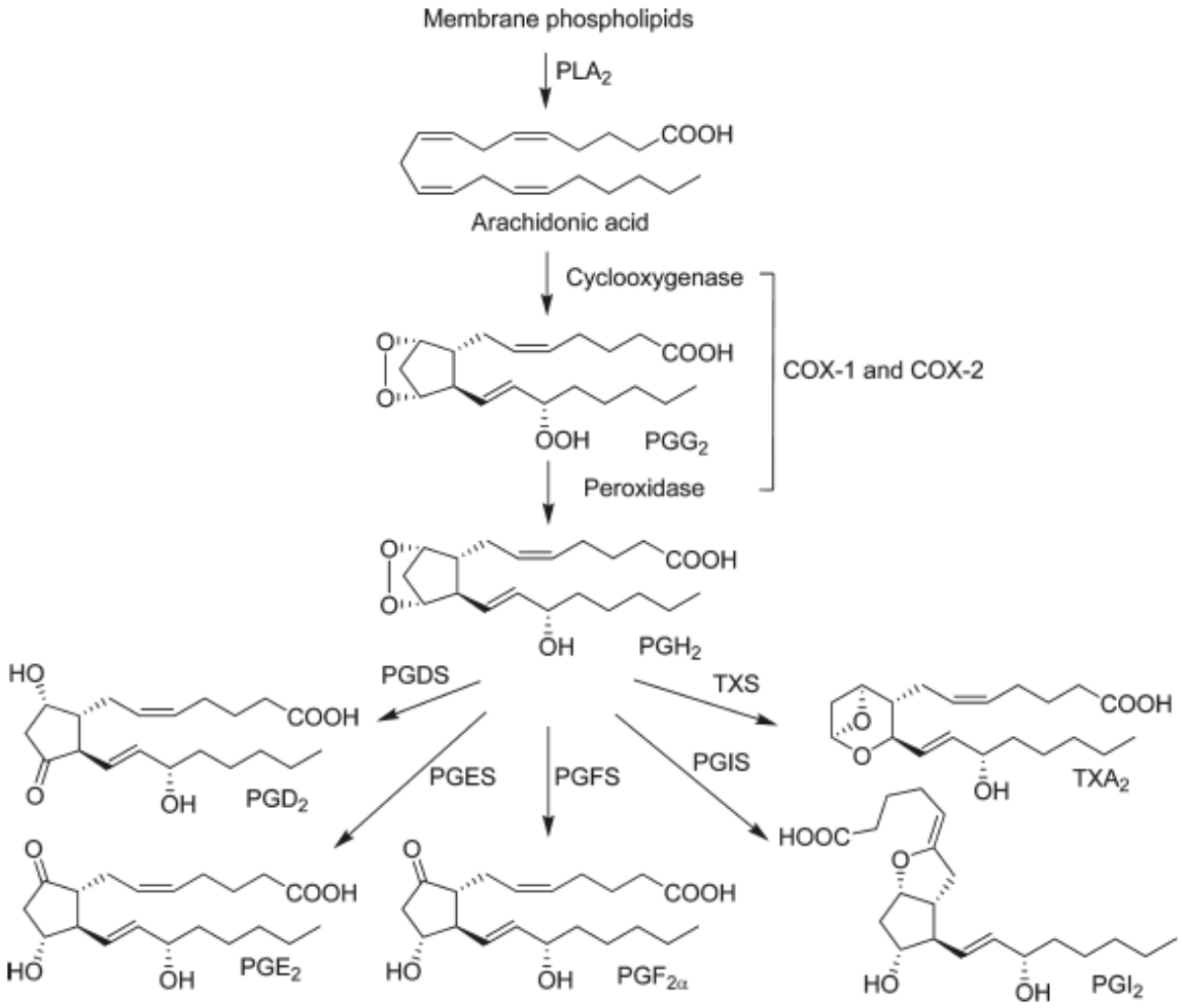


Figure 2: Prostaglandin biosynthetic pathway. PLA2, phospholipase A2; COX, cyclooxygenase; PG, prostaglandin; PGDS, prostaglandin D2 synthase; PGES, prostaglandin E2 synthase; PGFS, prostaglandin F2R synthase; PGIS, prostaglandin I2 synthase; TXS, thromboxane A2 synthase; TXA2, thromboxane A2.

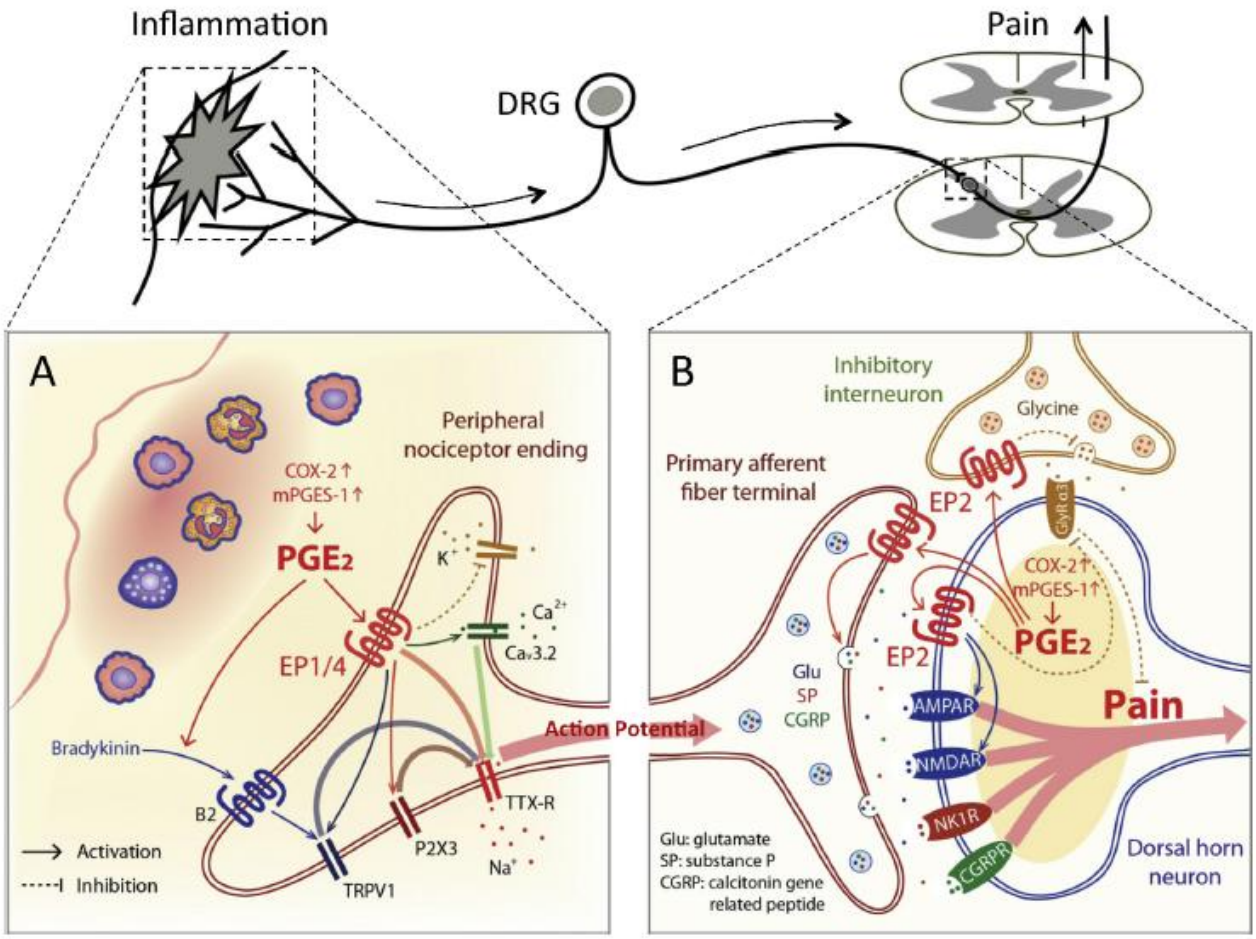


Figure 3: Molecular mechanisms that mediate PGE2 induced pain hypersensitivity in inflammation. [Chen L, 2013]

Microsomal prostaglandin E2 synthase-1 (mPGES-1)

Microsomal prostaglandin E2 synthase-1 belongs to the MAPEG (membrane-associated proteins involved in eicosanoid and glutathione metabolism) superfamily. mPGES-1 was initially reported in rat peritoneal macrophages, which produced TXA2 and PGD2 through COX-1 in the A23187-induced immediate response and PGE2 and PGI2 through COX-2 in the lipopolysaccharide (LPS)-induced delayed response. PGE2 production by osteoblasts occurred predominantly through COX-2 irrespective of the co-presence of COX-1. In a rat inflammatory model, COX-2-selective inhibitors reduced the accumulation of PGE2 but not

of other PGs. These observations predicted the presence of a particular terminal PGES that is coupled with COX-2 in marked preference to COX-1.

Studies employing co-transfection of mPGES-1 and either COX isozyme, as well as those with small interfering RNA or antisense technology to knockdown mPGES-1, have revealed that mPGES-1 is functionally coupled with COX-2 in marked preference to COX-1. Co-localization of COX-2 and mPGES-1 in the same perinuclear membrane may allow their efficient functional coupling. Nonetheless, Coupling between COX-1 and mPGES-1 can also occur if AA is abundantly supplied by explosive activation of cytosolic PLA2 (cPLA2). Crucial involvement of mPGES-1 in various pathophysiological events has been clarified by studies using mPGES-1 knockout mice.

mPGES-1-derived PGE2, in cooperation with VEGF, plays a crucial role in the development of inflammatory granulation and angiogenesis. PGE2 is known to be a mediator of inflammatory pain. mPGES-1 is functionally dissociated from the stress-induced hyperthermia, circadian temperature regulation, and the inflammation-induced activity depression.

mPGES-1 is involved in various types of pathology including inflammation, pain hyperalgesia, fever, and cancer. Notably, the absence of gross abnormalities in ductus arteriosus closure immediately after birth, which is markedly impaired in COX-1/COX-2-double and EP4 knockout mice, and in female reproduction, where EP2 is involved in the ovulation step, implies the compensatory participation of other PGESs in these physiological events. These facts, together with its inducible property during inflammation and other pathogenesis, agree well with the proposal that mPGES-1 represents a target for the treatment of various inflammatory diseases that will spare important physiological systems in which other PGs are involved. [Kudo I, 2005]

Real-time PCR analysis and Western blot analysis of gastric ulcer tissue clearly showed strong mPGES-1 gene and protein expression levels in open ulcers, lower mPGES-1 protein expression levels in closed ulcers, and no expression, whatsoever in gastritis mucosa. Furthermore, mPGES-1 enzyme is strongly expressed in macrophages and fibroblasts found exclusively between granulation and necrotic tissue of and around the gastric ulcer bed. This location corresponds precisely with that found for COX-2 expression on the human stomach. Confocal double immuno-staining of COX-2 and mPGES-1 confirmed mPGES-1 and COX-2 co-localization in fibroblast-like cells infiltrating into granulation tissue of the ulcer bed, raising the possibility that PGE2 is released in those cells. [Gudis K, 2005]

Regulation of mPGES-1

Similar to COX-2, mPGES-1 is highly up-regulated by proinflammatory stimuli and participates in the generation of elevated PGE2 in inflammation. In response to IL-1β and/or TNF-α, up-regulation of mPGES-1 expression is observed in rheumatoid arthritis synovial fibroblasts, as well as in many other cell types. Moreover, an antisense oligonucleotide blocking mPGES-1 expression inhibits PGE2 production, osteoclastogenesis, and bone resorption in mouse osteoblast coculture stimulated with IL-1β and TNF-α. The inhibitory actions of glucocorticoids also establish an important role for mPGES-1 in inflammation. In studies examining rheumatoid arthritis synovial cells and osteoarthritis chondrocytes, treatment with dexamethasone decreased mPGES-1 mRNA and protein expression mediated by proinflammatory cytokines. [Sampey AV, 2005]

mPGES-2

The second membrane-associated form of PGES, mPGES-2, has a catalytic glutaredoxin/thioredoxin-like domain and is activated by various thiol reagents. This enzyme is synthesized as a Golgi membrane-associated protein, and the proteolytic removal of the N-terminal hydrophobic domain leads to the formation of a mature cytosolic enzyme.

Expression of mPGES-2 is rather constitutive in various cells and tissues and is not elevated appreciably during inflammation or tissue damage. However, a considerable increase of mPGES-2 expression is observed in human colorectal cancer, in which mPGES-1 is also over expressed.

Crystallization of mPGES-2 reveals that it forms a dimer and is attached to lipid membrane by anchoring the N-terminal section. Two hydrophobic pockets connected to form a V shape are located in the bottom of a large cavity. The geometry suggests that the SH of Cys110 in the glutaredoxin/thioredoxin-like domain is most likely the catalytic site of mPGES-2. PGH2 fits well into the V-shaped pockets and its endoperoxide moiety interacts with the SH of Cys110. The fold of mPGES-2 is quite similar to those of GSH dependent hematopoietic PGD synthase, except for the two large loop sections. [Kudo I, 2005]

cPGES

Cytosolic PGES (cPGES) is a GSH-requiring enzyme constitutively expressed in a wide variety of cells and is identical to p23, a co-chaperone of heat shock protein 90 (Hsp90). The expression of cPGES is constitutive and is unaffected by pro-inflammatory stimuli in most cases, some exceptions have been reported. Cotransfection and antisense experiments indicated that cPGES is capable of converting COX-1-, but not COX-2-, derived PGH2 to PGE2 in cells, particularly during the immediate PGE2-biosynthetic response elicited by

Ca2+-evoked stimuli. Localization of cPGES in the cytosol may allow coupling with proximal COX-1 in the Endoplasmic Reticulum (ER) in preference to distal COX-2 in the perinuclear envelope. cPGES is directly associated with and phosphorylated by casein kinase 2 (CK2), resulting in marked reduction of Km for the substrate PGH2. [Kudo I, 2005]

There are multiple PGES enzymes in mammalian cells and that they display distinct functional coupling with upstream COX enzymes. Distinct PGES enzymes may control the spatial and temporal production of PGE2 in different aspects of pathophysiology in particular tissues and cells. Therefore, understanding the regulatory mechanisms for each PGES is of considerable importance. Although COX-2 inhibitors have reduced gastrointestinal toxicity as compared with traditional NSAIDs, there are also some adverse effects associated with this new group of drugs. For instance, specific inhibition of COX-2 blocks the production of renal and systemic PGI2, thereby causing altered excretion of sodium, edema, and elevated blood pressure. In addition, specific inhibition of COX-2 alters the balance between platelet-derived thromboxane A2 and endothelium-derived PGI2, leading to increases in the risk of thrombosis due to altered vascular tone. Thus, more selective modulation of the prostanoid pathway appears to be desirable. Thus, mPGES-1 represents an attractive novel target for therapeutic intervention for patients with various inflammatory diseases and cancer. [Kudo I, 2005]

Computer-Aided Drug Design

Computer-aided drug design (CADD) is a summarizing term for the methodology to design and develop new ligands with *in silico* techniques. It is used mainly for finding hit compounds and for lead optimization. CADD does not replace *in vitro* testing of ligand activities, but potentially saves on time and resources required by high-throughput screening.

The two major approaches for the search for new ligands are ligand- and structure-based approach. Ligand-based drug design has proven to be useful in drug discovery and lead optimization if the target structure is not resolved, but a set of known ligands exists. Prominent methods in ligand-based drug design are similarity search, (3D)-quantitative structure-activity relationship (QSAR) and pharmacophore modeling [Sliwoski G, 2014]. Structure-based approaches consume more computational power but result in more diverse hit molecules after virtual screening [McGaughey, G.B, 2007]. Starting from the crystal structure of a receptor and a putative co-crystallized ligand, the ligand interactions can be investigated in detail. Important methods of structure-based drug design are molecular docking, molecular dynamics and pharmacophore modelling, potentially also allowing for de-novo ligand design.

There is a vast amount of tools for computational drug design employing a set of different approaches and algorithms, ranging from command line tools to modelling suites with a graphical user interface. In this thesis, three methods of CADD were used primarily, namely molecular docking, molecular dynamics and pharmacophore modelling. A brief introduction into these topics will be given in the following paragraphs.

Similarity Search

The similarity search [Stumpfe D, 2011] is a computational approach that aims to find the similar compounds with the desired properties in a database. Since according to “Similarity Property Principle”, similar compounds are assumed to have similar properties. The similarity concept is defined according to different types of similarities, there are mainly six types of similarities [Maggiora G, 2014]. First types of similarity is the chemical similarity which is based on the physicochemical properties of the compound *e.g.* solubility and

molecular weight, second type is molecular similarity which assess the similarity according to structural features e.g. shared substructures. Third similarity type is the 3D similarity which depends on the 3D conformations of the compounds and its properties while fourth similarity type is biological similarity that evaluates the biological activities of the compounds. The last two types of similarity are global similarity and local similarity, where global similarity consider the similarity according to the whole molecule *e.g.* by using fingerprints to encode the molecule structure and properties while local similarity asses the similarity according to specific region of the molecule *e.g.* pharmacophore features.

Important to note that the similarity concept is subjective and in order to change it into an objective concept, a specific type of similarity, *e.g.* chemical similarity, is chosen with a precise molecular representation, *e.g.* a MACCS fingerprint, where the similarity is measured according to a particular similarity coefficient, *e.g.* Tanimoto coefficient, that will be discussed in the material and method section, so the result of the similarity search will be a meaningful efficient one.

Molecular Docking

Molecular docking tools employ algorithms to generate and score reasonable binding poses of a selected ligand to a specific binding site in a target protein. The goal of a docking experiment is an accurate *in silico* prediction of the ligand's binding mode. *In silico* experiments are advantageous in terms of time and material consumption: Assayed ligands do not have to be assessed in wet-lab experiments or even synthesized in the first place. Additionally, detailed information about ligand interactions and the binding conformation is provided. Numerous approaches to molecular docking have resulted in a vast amount of available algorithms, each coming with their very own strengths and weaknesses.

There are several approaches to simulate ligand-receptor interactions via molecular docking, which can roughly be divided into rigid and flexible docking simulations [Pagadala, N.S, 2017]. Both the receptor and the ligand can be treated as either rigid or flexible. Modeling the receptor as a rigid molecule saves on computational resources and therefore also time, while flexible receptor approaches tend to deliver more accurate results. Side-chain- and backbone flexibility can play an important role in the generation of a correct docked ligand conformation, which is frequently overlooked [Lexa, K.W., 2012].

Ligand (and in some circumstances also receptor) poses generated by the specified docking algorithm of each program are evaluated by a scoring function to determine the quality of the docking. Scoring functions take different aspects of the result into account, *e. g.* surface complementarity, solvent accessible surface area, solvation free energy, electrostatic interaction energy and/or total molecular mechanics energy and can be based either on force fields or empirical data [Pagadala, N.S, 2017]. The computational effort required to run the scoring function can differ a lot based on its complexity.

When a docking run is finished, it usually delivers several ligand poses as a result, ranked by the selected scoring function. The viability of the suggested ligand conformation and binding mode should be assessed by visual inspection. If several false ligand poses, which are top-ranked, are identified as false-positives, changing the scoring function or the docking tool is recommended. To determine whether the docking algorithm is suited for the molecule of interest, re-docking of a ligand, whose binding mode is known from a crystal structure, can be performed. If a low root-mean-square deviation of atomic positions (RMSD, expressed in Ångström (Å)) between the best docking solutions and the reference molecule from the crystal structure correlates well with the scoring function of the docking program, this indicates that the algorithm of choice is well-suited for the specific protein-ligand complex.

In the chart below, five of the best-known docking tools are listed together with their operating principle.

Table 2: Five popular docking methods with their operating principles briefly explained.

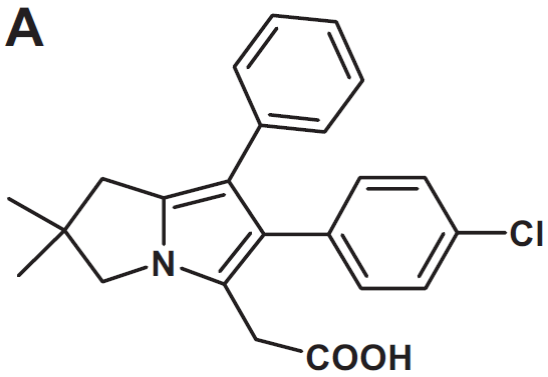
Docking Method	Working methodology
AutoDock [Osterberg, F, 2002]	Flexible docking into a rigid receptor.
DOCK [Venkatachalam, C. M., 2003]	Rigid, shaped-based docking, key-into-lock principle.
FlexX [Rarey, M., 1996]	Fragment-based docking approach.
GOLD [Jones, G., 1997]	Genetic algorithm to dock flexible ligand into a semi-rigid receptor.
Glide [Friesner, R., 2004; Friesner, R., 2006]	Systematically docking a ligand into a receptor; offers induced-fit docking.

LITERATURE REVIEW

CHAPTER 2

LITERATURE REVIEW

Andreas Koeberle, *et al.*, 2008, examined the direct interference of licofelone with enzymes participating in PGE2 biosynthesis, that is, cyclooxygenase (COX-1 and COX-2) as well as microsomal PGE2 synthase (mPGES-1). It was concluded that licofelone suppresses inflammatory PGE2 formation preferentially by inhibiting mPGES-1 at concentrations that do not affect COX-2, implying an attractive and thus far unique molecular pharmacological dynamics as inhibitor of COX-1, the 5-lipoxygenase pathway, and of mPGES-1. [Koeberle A, 2008]

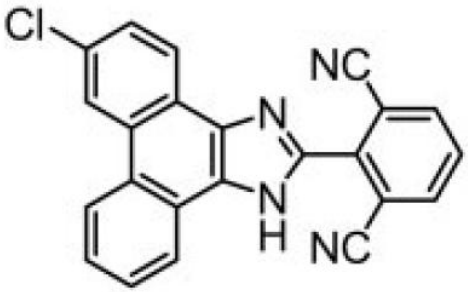


licofelone

1

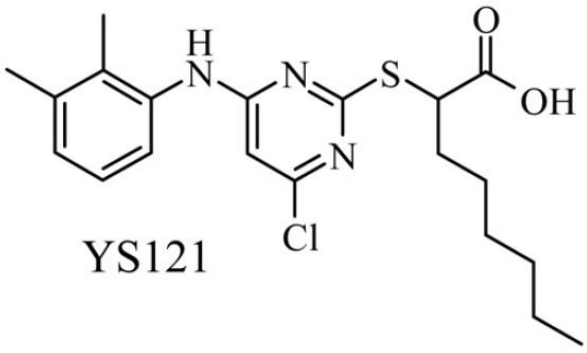
Daigen Xu, *et al.*, 2008, demonstrated that mPGES-1 inhibition leads to effective relief of both pyresis and inflammatory pain in preclinical models of inflammation and may be a useful approach for treating inflammatory diseases. The authors also evaluated the antipyretic and analgesic properties of a novel and selective mPGES-1 inhibitor, compound **2** [2-(6-chloro-1H-phenanthro-[9,10-d]imidazol-2-yl)isophthalonitrile], in animal models of inflammation. Compound **2** potently inhibited the human mPGES-1 enzyme (IC50 = 1.3 nM), with a high degree (>1000 fold) of selectivity over other prostanoid synthases. In rodent

species, MF63 strongly inhibited guinea pig mPGES-1 (IC50 = 0.9 nM) but not the mouse or rat enzyme. When tested in the guinea pig and a knock-in (KI) mouse expressing human mPGES-1, the compound selectively suppressed the synthesis of PGE2, but not other prostaglandins inhibitable by nonsteroidal anti-inflammatory drugs (NSAIDs), yet retained NSAID-like efficacy at inhibiting lipopolysaccharide-induced pyresis, hyperalgesia, and iodoacetate-induced osteoarthritic pain. [Xu D, 2008]



2

Andreas Koeberle, *et al.*, 2010, investigated the mechanism of mPGES-1 inhibition, the selectivity profile, and the in vivo activity of α-(n-hexyl)-substituted pirinixic acid [YS121; 2-(4-chloro-6-(2,3-dimethylphenylamino)pyrimidin-2-ylthio)octanoic acid)] as a lead compound. In cell-free assays, compound **3** inhibited human mPGES-1 in a reversible and noncompetitive manner (IC50 = 3.4 µM), and surface plasmon resonance spectroscopy studies using purified in vitro-translated human mPGES-1 indicated direct, reversible, and specific binding to mPGES-1 (KD = 10–14 µM). [Koeberle A, 2010]

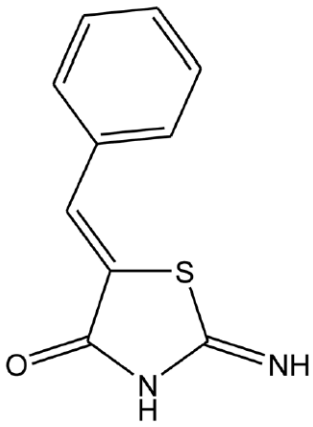


YS121

3

Takako A, *et al.*, 2010, analysed and studied the role of microsomal prostaglandin E synthase-1 (mPGES-1) in the facilitation of angiogenesis and the healing of gastric ulcers. It was found that mPGES-1 enhances the ulcer-healing processes and the angiogenesis indispensable to ulcer healing, and that a selective mPGES-1 inhibitor should be used with care in patients with gastric ulcers. [Ae T, 2010]

Adel Hamzaa, *et al.*, 2011, identified novel mPGES-1 inhibitors through a combination of large-scale structure-based virtual screening, flexible docking, molecular dynamics simulations, binding free energy calculations, and *in vitro* assays on the actual inhibitory activity of the computationally selected compounds. The combined computational and experimental studies have led to identification of (Z)-5-benzylidene-2-iminothiazolidin-4-one as a promising novel scaffold for further rational design and discovery of new mPGES-1 inhibitors. [Hamza A, 2011]

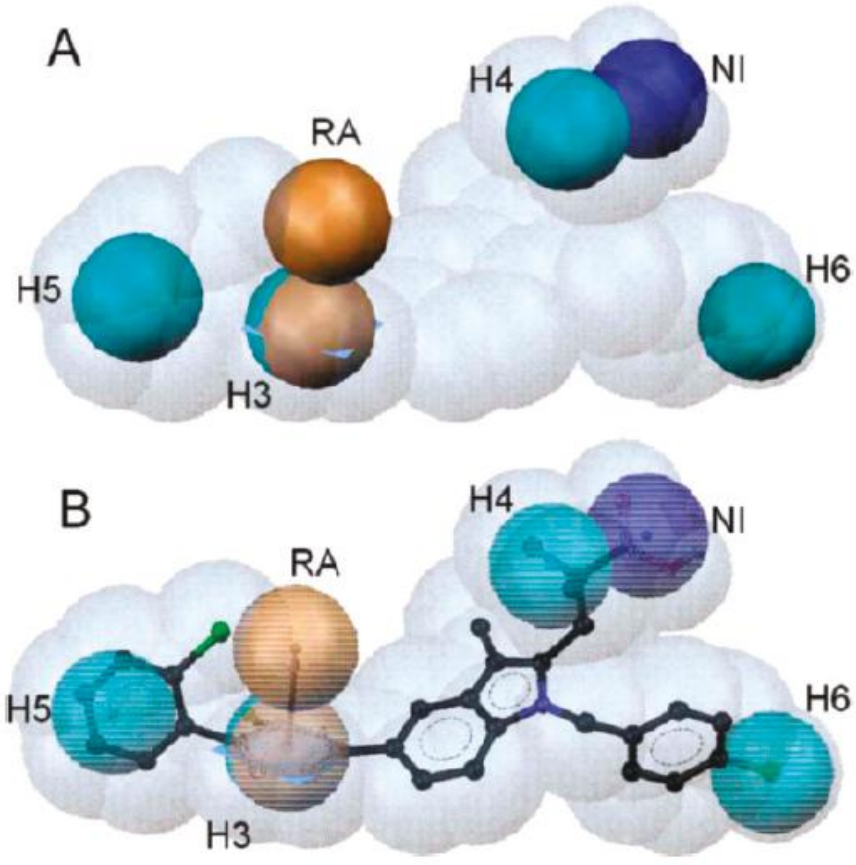


scaffold

(Z)-5-benzylidene-2-iminothiazolidin-4-one scaffold

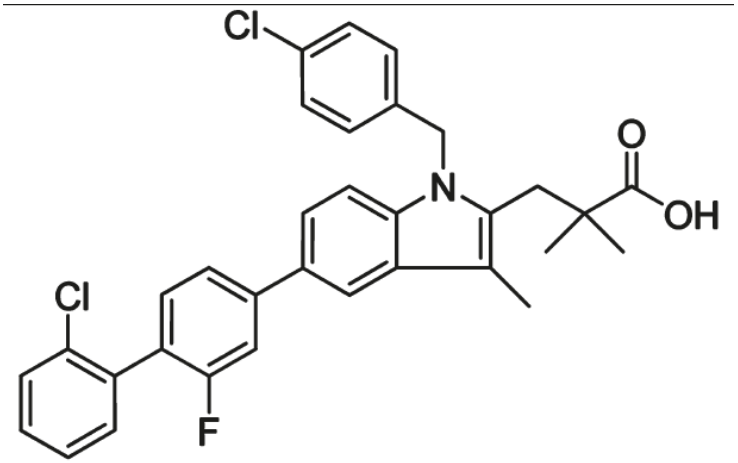
4

Birgit Waltenberger, *et al.*, 2011, developed and theoretically validated two pharmacophore models for acidic mPGES-1 inhibitors using information on mPGES-1 inhibitors from literature and identified novel chemical scaffolds active on this enzyme. Out of 29 compounds selected for biological evaluation, nine chemically diverse compounds caused concentration-dependent inhibition of mPGES-1 activity in a cell-free assay with IC50 values between 0.4 and 7.9 μM, respectively. Further pharmacological characterization revealed that 5-lipoxygenase (5-LO) was also inhibited by most of these active compounds in cell-free and cell-based assays with IC50 values in the low micromolar range. Together, nine novel chemical scaffolds inhibiting mPGES-1 are presented that may possess anti-inflammatory properties based on the interference with eicosanoid biosynthesis. [Waltenberger B, 2011]



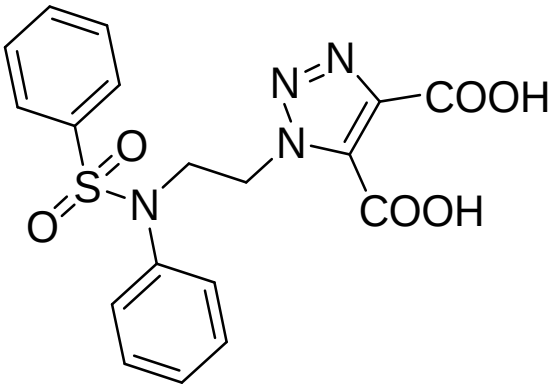
(A) Pharmacophore model for acidic mPGES-1 inhibitors consisting of one aromatic ring (RA, brown), one negatively ionisable group (NI, dark blue), four hydrophobic

features (H36, cyan), and a shape of the most potent inhibitor from the training set (compound 5). (B) Compound 5 mapped to the pharmacophore model.



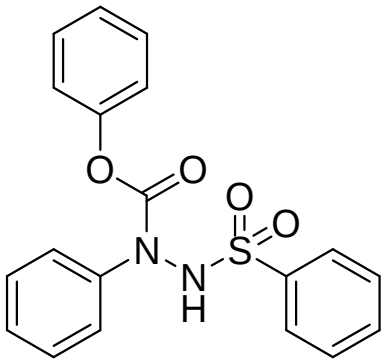
5

Lee, K., *et al.*, 2012, discovered sulfonamido-1,2,3-triazole-4,5-dicarboxylic derivatives as a novel class of mPGES-1 inhibitors identified through fragment-based virtual. 1-[2-(NPhenylbenzenesulfonamido) ethyl]-1H-1,2,3-triazole-4,5-dicarboxylic acid (**6**) inhibited human mPGES-1 (IC50 of 1.1 µM) with high selectivity (ca.1000-fold) over both COX-1 and COX-2 in a cell-free assay. [Lee K, 2013]



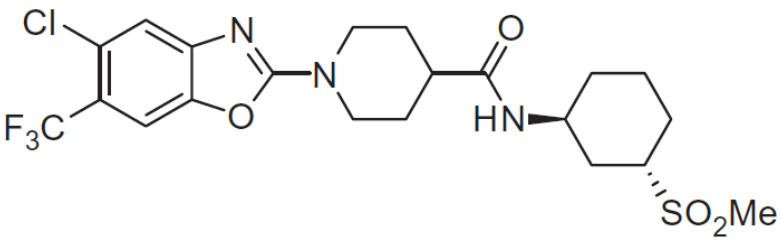
6

Sung-Jun Park, *et al.*, 2012, identified mPGES-1inhibitors through screening of a chemical library. Initial screening of 1841 compounds out of 200,000 in a master library resulted in 9 primary hits. From the master library, 387 compounds that share the scaffold structure with the 9 primary hit compounds were selected, of which 3 compounds showed strong inhibitory activity against mPGES-1 having IC50 values of 1–3 μM. Notably, a derivative of sulfonyl hydrazide, compound **7**, inhibited the LPS-induced PGE2 production in RAW 264.7 cells. This compound showed novel scaffold structure compared to the known inhibitors of mPGES-1. [Park SJ, 2012]



7

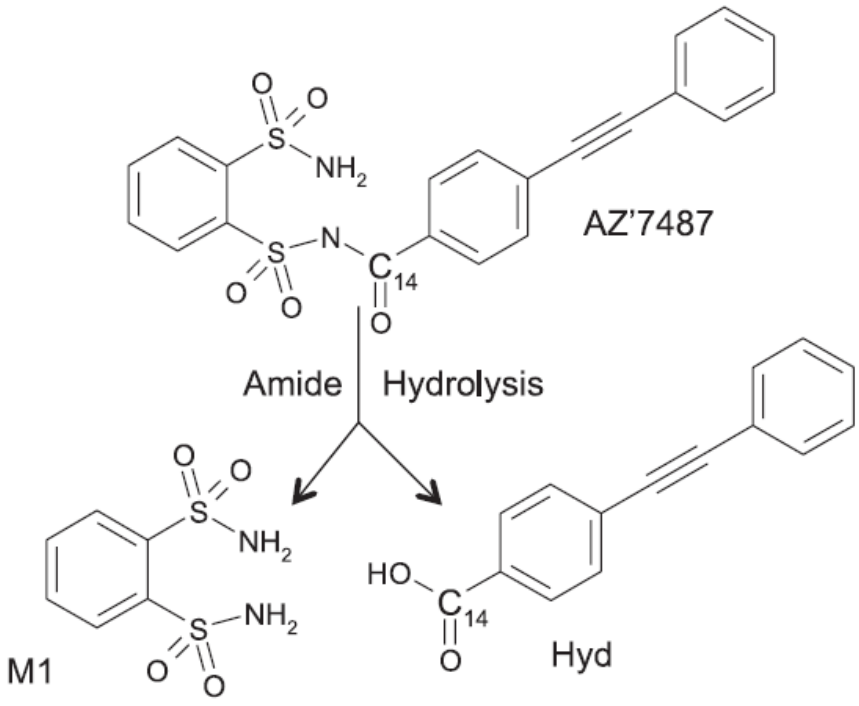
Daniel P. Walker, *et al.*, 2013, designed and synthesized novel set of substituted benzoxazoles and tested for their mPGES-1 inhibitory property and also studied *in vivo* PK. Among all the synthesized compounds, compound **8** displayed the overall profile. [Walker DP, 2013]



8

Gaia Corso *et al.*, 2013, elucidated the structure of mPGES-1 and predicted the binding mode prediction of inhibitors by Homology Modeling and Site-Directed Mutagenesis. The work could provide grounds for a rational structure-based drug design aimed to identify new inhibitors active against both human and murine mPGES-1. [Corso G, 2013]

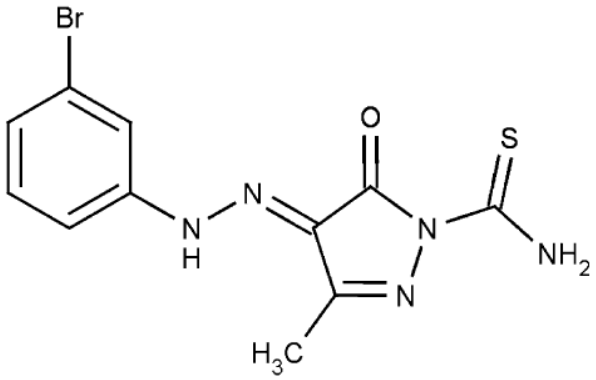
Johan Bylund, *et al.*, 2013, identified that a new chemical series of sulfonamide-containing compounds, a potential microsomal prostaglandin E2 synthase-1 (mPGES-1) inhibitors, intended for pain treatment, induced kidney injury at exposures representing less than 4 times the anticipated efficacious exposure in humans. *In vitro* and *in vivo* metabolic profiling generated a working hypothesis that a bis-sulfonamide metabolite (determined M1) formed by amide hydrolysis caused this toxicity. [Bylund J, 2013]



9

Patrick Leclerca, *et al.*, 2013, characterize the new mPGES-1 inhibitor compound **10**, a pyrazolone that has similar potency on rat and human recombinant mPGES-1, in experimental models of inflammation. In cell culture, compound **10** inhibited PGE2 production in synovial fibroblasts from patients with rheumatoid arthritis (RA) and in rat

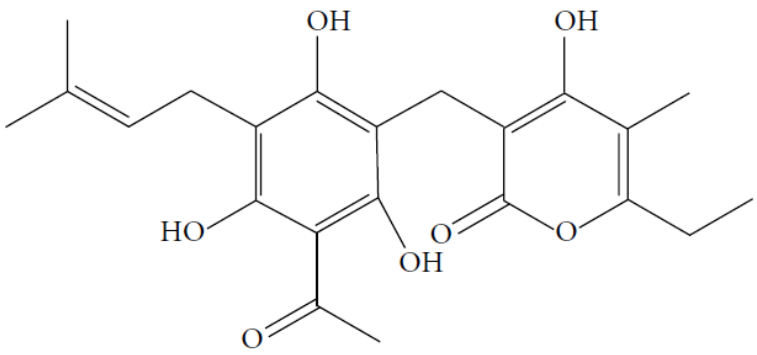
peritoneal macrophages. In vivo, compound **10** was first characterized in the rat air pouch model of inflammation where treatment inhibited intra-pouch PGE2 production. Compound **10** was also investigated in a rat adjuvant-induced arthritis model where it attenuated both the acute and delayed inflammatory responses. [Leclerc P, 2013]



(4E)-4-[2-(3-bromophenyl) hydrazin-1-ylidene]-1-ethanethioyl-3-methyl-4,5-dihydro-1H-pyrazol-5-one

10

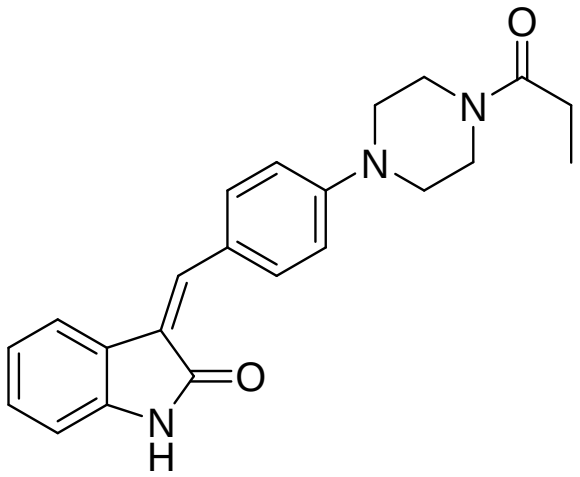
Pankaj S. Kothavade, *et al.,* 2013, studied the chemistry, structure-activity relationship, and pharmacological activities of arzanol. Arzanol is a novel phloroglucinol α-pyrone, isolated from a Mediterranean plant *Helichrysum italicum* (Roth) Don ssp. *microphyllum* which belongs to the family Asteraceae. [Kothavade PS, 2013]



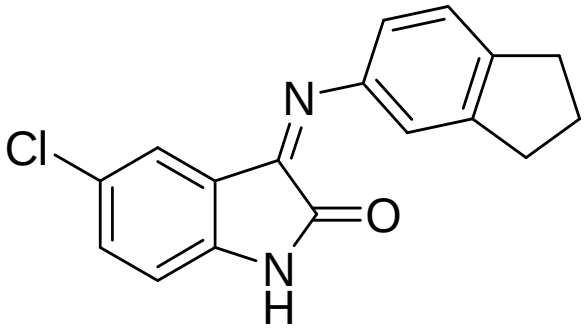
Arzanol

11

Gaozhi Chen, *et al.*, 2014, designed and synthesized thirty indole-2-one and 7-aza-2-oxindole derivatives based on the skeleton of tenidap, and determined their anti-inflammatory activity by evaluating the inhibitory potency against lipopolysaccharide (LPS)-stimulated tumor necrosis factor (TNF)-α and interleukin (IL)-6 release in RAW264.7 macrophages. Quantitative structure–activity relationship analysis revealed that high molecular polarizability and low lipid/water partition coefficient (ALogP) in indole-2-one are beneficial for anti-inflammatory activity. Moreover, compounds **12** and **13** inhibited the expression of TNF-α, IL-6, COX-2, PGES, and iNOS in LPS-stimulated macrophages, and **12** exhibited a significant protection from LPS-induced septic death in mouse models. [Chen G, 2014]



12

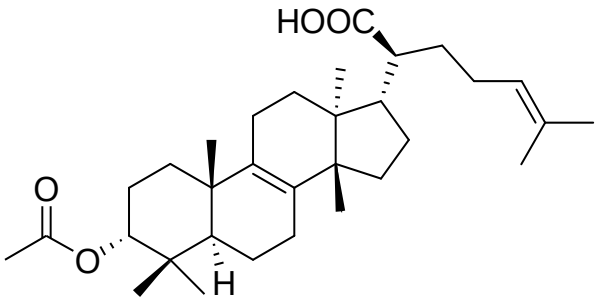


13

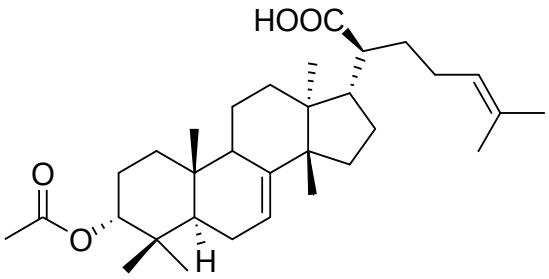
Amide hydrolysis of AZ'7847. Amide hydrolysis results in the formation of two metabolites: M1 and Hyd.

Moritz Verhoff, *et al.*, 2014, isolated triterpene acids (i.e., tircuallic, lupeolic, and roburic acids) from frankincense and tested as mPGES-1 inhibitors. 3α-acetoxy-8,24-dienetirucallic acid (**14**) and 3α-acetoxy-7,24-dienetirucallic acid (**15**) inhibited mPGES-1 activity in a cell-free assay with IC50 = 0.4 μM, each. Structure−activity relationship studies and docking

simulations revealed concrete structure-related interactions with mPGES-1 and its cosubstrate glutathione. [Verhoff M, 2014]

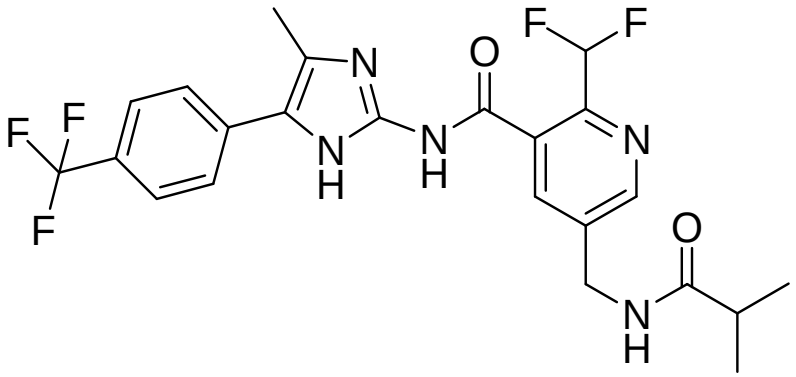


14



15

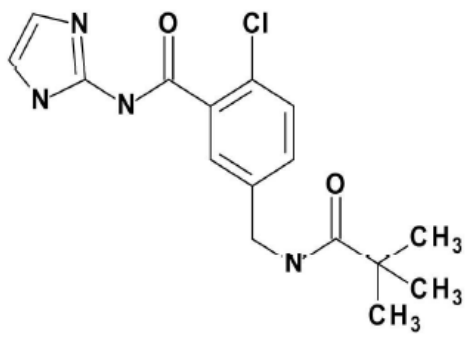
Jin Y, *et al.*, 2016, conducted multiple ascending dose study to assess the safety, tolerability, and pharmacology of compound **16**, a microsomal prostaglandin E synthase 1 (mPGES1) inhibitor. Compared with placebo, compound **16** inhibited *ex vivo* lipopolysaccharide-stimulated prostaglandin E2 (PGE2) synthesis 91% and 97% on days 1 and 28, respectively, after 30-mg dosing, comparable to celecoxib's effect (82% inhibition compared to placebo). Unlike celecoxib, which also inhibited prostacyclin synthesis by 44%, compound **16** demonstrated a maximal increase in prostacyclin synthesis of 115%. Transient elevations of serum aminotransferase were observed in one subject after 30-mg compound **16** dosing (10× upper limit of normal (ULN)), and one subject after 15-mg dosing (about 1.5× ULN).



16

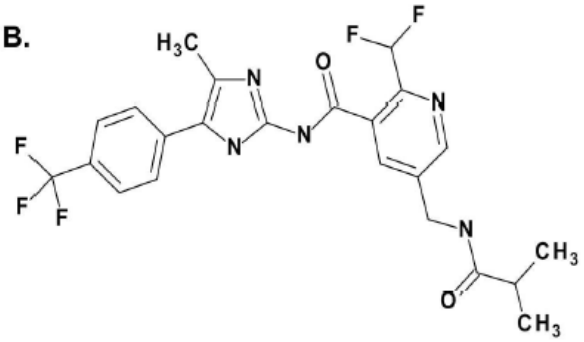
Srinivasan Chandrasekhar, *et al.*, 2016, showed that the compounds **17** and **18** are potent against human, dog, and guinea pig mPGES-1 enzymes and bind to the human enzyme in a reversible manner. They were highly selective and show no discernible activity versus mPGES-2, COX-1, and COX-2 enzymes. Both molecules were effective in blocking PGE2 production in IL-1 stimulated A549 cells, as well as in LPS stimulated human whole blood. Finally, they demonstrated efficacy in a guinea pig monoiodoacetate (MIA) model of pain. [Chandrasekhar S, 2016]

A.



17

B.



18

AIM AND OBJECTIVE

CHAPTER 3

AIM AND OBJECTIVE

Non-steroidal anti-inflammatory drugs (NSAIDs) relieve inflammation, fever and pain by suppressing the formation of prostanoids through interference with COX. Long-term treatment of chronic diseases like rheumatoid arthritis with NSAIDs is associated with severe gastrointestinal, renal and cardiovascular side effects because COX derived prostanoids possess important homeostatic functions. Gastrointestinal bleeding, ulceration and perforation upon treatment with classical NSAIDs have been ascribed to the inhibition of constitutively expressed homeostatic COX-1 and the subsequently decreased formation of cytoprotective PGE2 and prostacyclin (PGI2) by epithelium.

The urgent need for potent and safe anti-inflammatory drugs led to the propagation of mPGES-1 as drug target. Inhibition of mPGES-1 promises selective reduction of disease-promoting PGE2 without compromising the requirements for producing homeostatic PGE2 and other prostanoids.

The objectives of the present work can be summarized as follows:

- Microsomal prostaglandin E2 synthase-1 involved in the target pain.
- Computational approaches in medicinal chemistry
- Literature analysis of mPGES-1
- Similarity search against Enamine Database
- Structure analysis of mPGES-1
- Molecular docking of hit molecules obtained from Similarity Search.

PLAN OF WORK

CHAPTER 4

PLAN OF WORK

The plan of present work is

- Download Enamine database and analyse the molecules
- On the basis of selected query molecule (potent inhibitor of mPGES-1), similarity search against the downloaded database
- Learning of Glide docking from Schrodinger.
- Docking of the hit molecules obtained from similarity search using Glide-docking
- Analysing the interactions of molecules with the amino acids in the binding pocket of mPGES-1
- Identifying potential molecules for purchase/synthesis and test against mPGES-1.

EXPERIMENTAL WORK

CHAPTER 5

EXPERIMENTAL WORKS

Enamine Database

The Enamine REAL database covers rigorously validated chemical space over 700 million compounds. The compounds in the database comply with Lipinksi rule of 5 and Verber criteria of Molecular Weight < 500, SlogP < 5, Hydrogen Bond Acceptor (HBA) < 10, Hydrogen Bond Donor (HBD) < 5, rotatable bonds < 10 and Total Polar Surface Area (TPSA) < 140. In addition to this database, Enamine provides 8.5 million diverse set of compounds called as "Diverse REAL drug-like" that represent REAL drug-like space. In addition to Lipinski rule of 5 and Verber criteria the compounds in Diverse REAL drug-like dataset avoid compounds which are toxic and PAINS. Diverse REAL drug-like dataset contains compounds that have no analogs with Tanimoto similarity more than 0.6 (Morgan 2 fingerprint, 512 bit) within the set and from Enamine stock screening compound collection.

Similarity Search using MOE

Similarity search using MOE software, which is based on generating a precise fingerprint for the molecules and calculating the similarity according to a specific threshold using a specific measuring metric [Stumpfe, D., 2011]. There are different ways to measure similarity but in this thesis only tanimoto coefficient will be used. Tanimoto coefficient is a type of symmetric similarity metrics that is calculated using fingerprints in a bit form, Tanimoto coefficient is define as:

$$TC(A,B) = \frac{\mathrm{c}}{\mathrm{a} + \mathrm{b} - \mathrm{c}}$$

Where A and B are the two molecules to measure their similarity regarding to each other, 'a' and 'b' is the fingerprint features, represented in bits, in molecule A and molecule B respectively while c is the common features fingerprints bits between the molecule A and molecule B. The result of Tanimoto coefficient ranges between 0 and 1, where 0 means that the molecules are not similar since there is no common fingerprints shared between them while by increasing the value, the similarity increases where finally a value of 1 means that the molecules are similar and that the two molecules have identical fingerprints. Basically the concept of using a specific fingerprint and calculating the Tanimoto coefficient will be used in the similarity searching in this thesis.

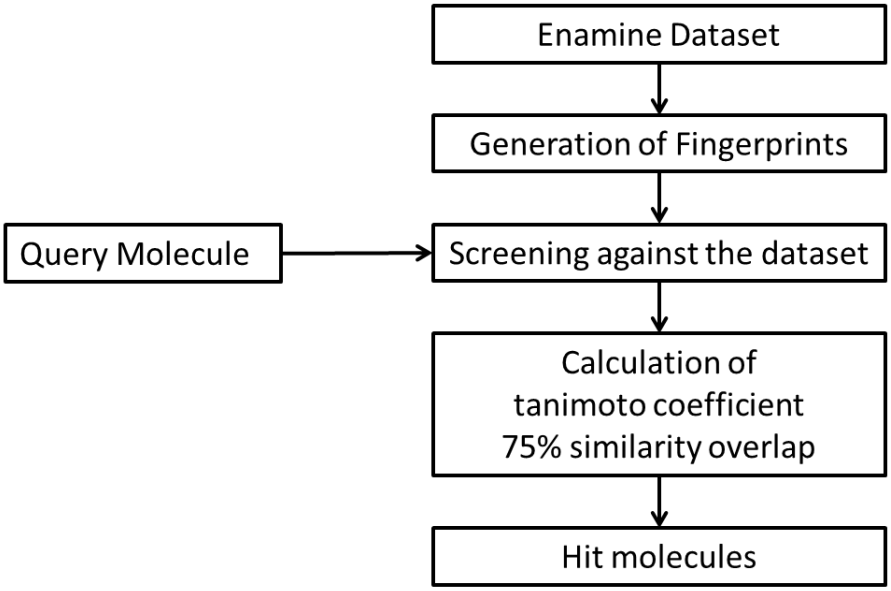


Figure 4. Workflow of similarity search process

Regarding the steps done for the similarity search using MOE. As seen in **Figure 4**, BIT MACCS fingerprint were generated for all the ligands then the similarity search process is done. Then the similarity is calculated using Tanimoto coefficient for both processes independently, with setting the overlap similarity threshold to 75%. Then the results of the process will be analyzed.

Glide Docking

The hit compounds obtained from similarity search were subjected to molecular docking using Glide docking from Schrodinger Inc. The steps involved in Glide docking are as follows:

- **Ligand structure:** The chemical structure of each ligand was drawn using build.
- **Ligand preparation:** In order to prepare high quality, all-atom 3D structures for large numbers of drug-like molecules, starting with the 3D structures in SD Maestro format, LigPrep was used. LigPrep produced a single, low-energy, 3D structure with corrected chiralities for each successfully processed input structure.
- **Preparation of protein:** The structure file from the PDB is not suitable for immediate use in molecular modelling calculations. A typical PDB structure file consist only of heavy atoms and may include a co-crystallized ligand, water molecules, metal ions, cofactors. Some structures are polymeric and may need to be reduced to a single unit. Because of the limited resolution of X-ray experiments, it can be difficult to distinguish between NH and O, and the place of these groups must be checked. PDB structures may be missing information on connectivity, which must be assigned, along with bond orders and formal charges. This was done using the Protein Preparation Wizard.
- **Receptor Grid Generation:** Receptor grid generation requires a "prepared" structure: an all atom structure with appropriate bond orders and formal charges. Glide searches for favourable interactions between one or more ligand molecules and a receptor molecule, usually a protein. The shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. The options in each tab of the Receptor Grid Generation panel allow defining the receptor structure by excluding any co-crystallized ligand that may be

present, determine the position and size of the active site as it will be represented by receptor grids, and set up Glide constraints.

- **Ligand Docking:** This is carried out using GLIDE DOCK. Glide searches for favourable interactions between one or more ligand molecules and a receptor molecule, usually a protein. Each ligand acts as single molecule, while the receptor may include more than one molecule, *e.g.*, a protein and a cofactor. Glide was run in rigid or flexible docking modes; the latter automatically generated conformations for each input ligand. The combination of position and orientation of a ligand relative to the receptor, along with its conformation in flexible docking, is referred to as a ligand pose. The ligand poses that Glide generates pass through a series of hierarchical filters that evaluate the ligand's interaction with the receptor. The initial filters test the spatial fit of the ligand to the defined active site and examine the complementarity of ligand-receptor interactions using a grid based method patterned after the empirical ChemScore function. Poses that passed these initial screens entered the final stage of the algorithm, which involves evaluation and minimization of a grid approximation to the OPLS-AA non bonded ligand-receptor interaction energy. Final scoring is then carried out on the energy minimized poses.
- **Glide Extra-Precision Mode (XP):** The extra-precision (XP) mode of Glide combines a powerful sampling protocol with the use of a custom scoring function designed to identify ligand poses that would be expected to have unfavourable energies, based on well-known principles of physical chemistry. The presumption is that only active compounds will have available poses that avoid these penalties and also receive favourable scores for appropriate hydrophobic contact between the protein and the ligand, hydrogen binding interactions, and so on. The chief purposes of the XP method are to week out false positives and to provide a better correlation between good poses

and good scores. Extra-precision mod is a refinement tool designed for use only on good ligand poses. Finally, the minimized poses are re-scored using Schrodinger's proprietary Glide Score scoring function. GlideScore is based on ChemScore, but includes a steric-clash term and adds buried polar terms devised by Schrodinger to penalize electrostatic mismatches.

RESULT AND DISCUSSION

CHAPTER 6

RESULTS AND DISCUSSION

Query Molecule

LY3023703 is one of the potent inhibitor for mPGES-1. LY30223703 is designed to inhibit the mPGES-1 enyzme thus affecting the metabolism of eicosanoids and glutathiones. The compound is 17-fold more potent than celecoxib. Also it decreased urinary PGEM and increased urinary PGIM and 11-dehydro TXB2, and there was no increase in TXB2 formation in serum.

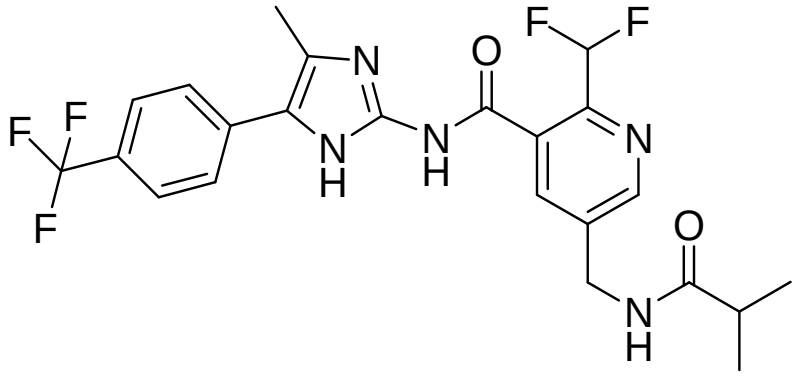


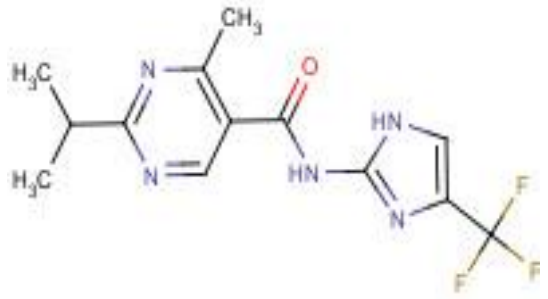
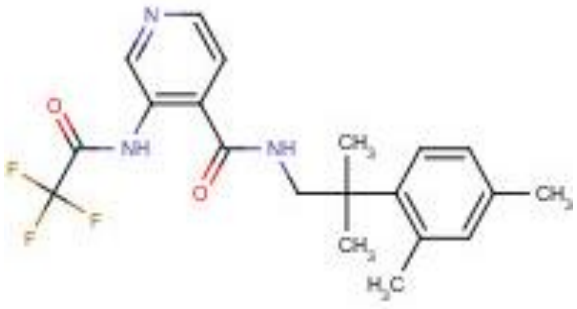
Figure 5. Structure of LY30223703, a potent mPGES-1 inhibitor used as query molecule.

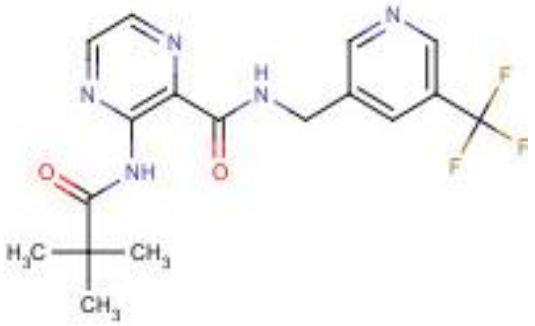
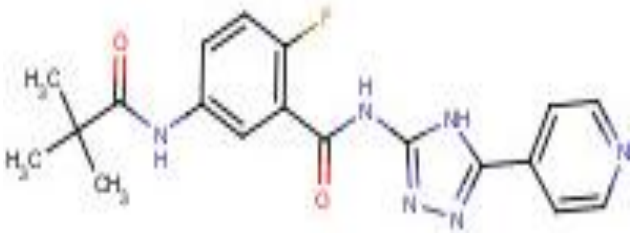
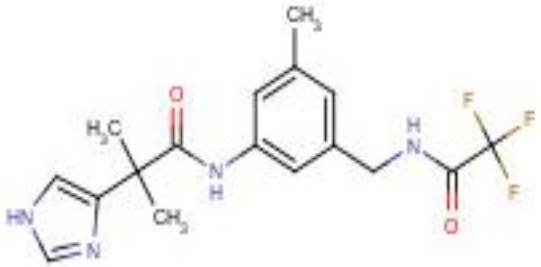
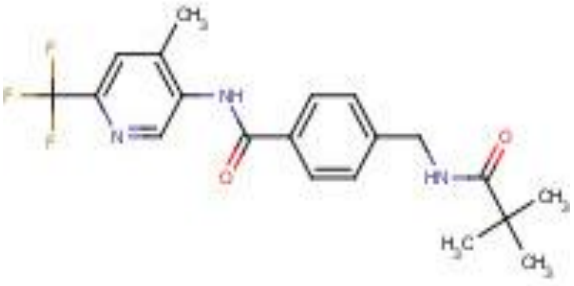
Similarity search

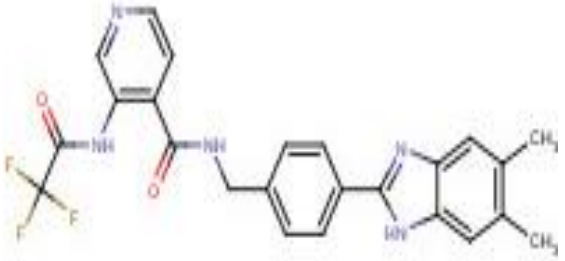
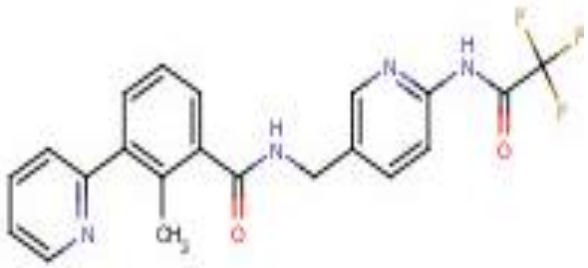
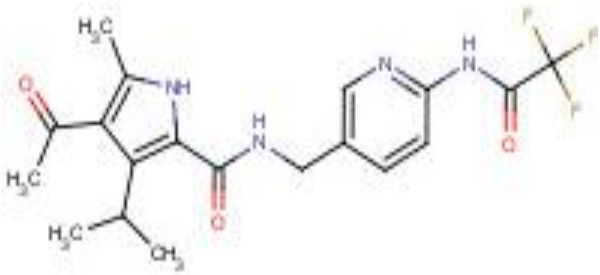
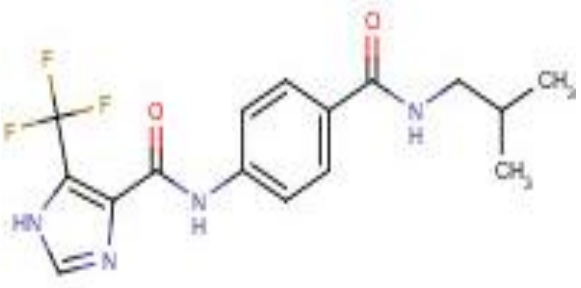
The goal of the similarity search is to perform similarity search by screening the Enamine database using the query molecule to get similar compounds, aiming to have similar activities that inhibit mPGES. First, similarity searching was applied in which 2D fingerprint 'MACCS' was calculated for our selected query molecule shown in Figure X and systematically compared to compounds from Enamine Database via MOE software. Each query-target pair had a specific similarity threshold at which the agonist showed number of hits. Fingerprint overlap is quantified as a measure of molecular similarity using similarity

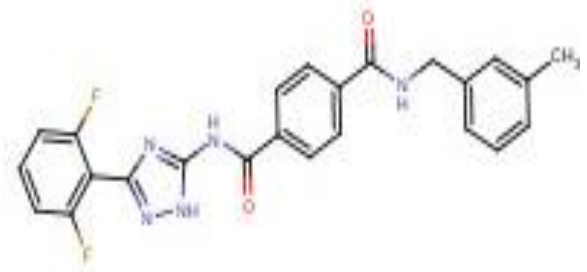
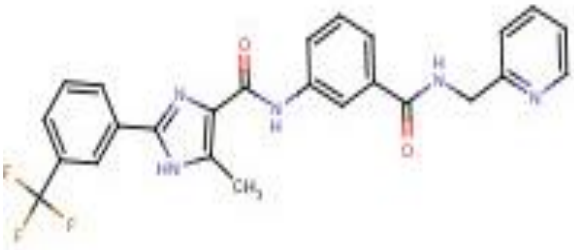
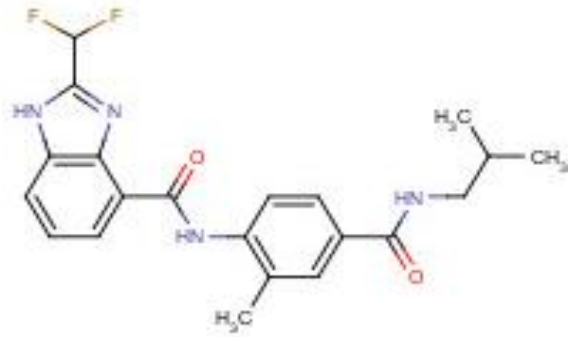
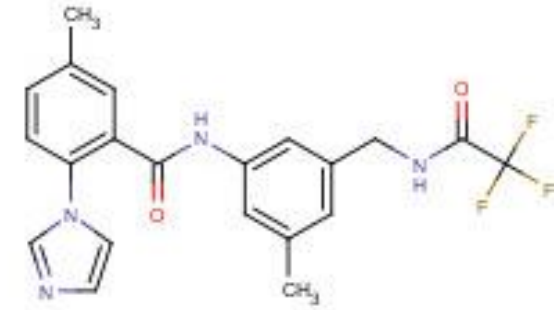
coefficient 'Tanimoto coefficient'. For our similarity search we selected the threshold value of 75 % similarity overlap. The search against the Enamine Database has resulted 306 molecules as hits. When the threshold value was increased to 80% the number of hit molecules is reduced from 306 to 24. This shows that high number of molecules was identified as hits within the range from 75 to 80% similarity overlap. The highly similar 24 molecules were shown in **Table 3**. However for our docking calculation we considered all the 306 molecules.

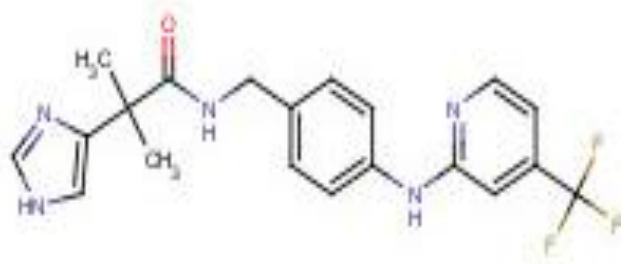
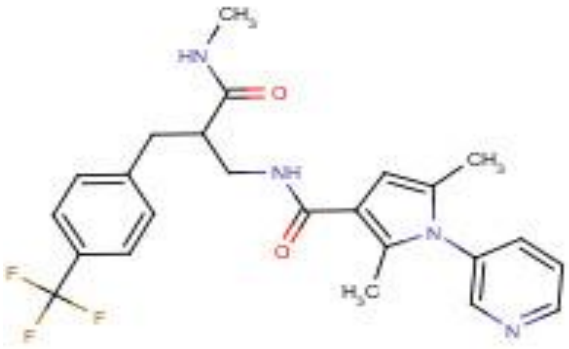
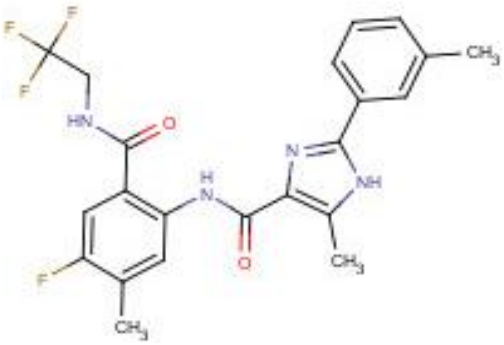
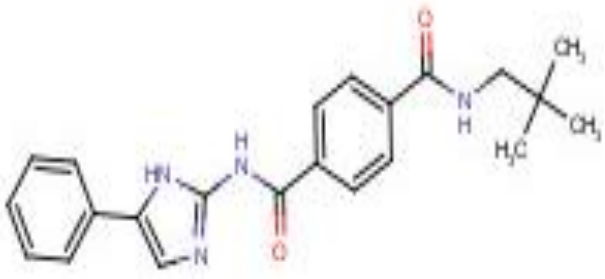
Table 3. Hit molecules identified using similarity search with the threshold of 80% similarity overlap.

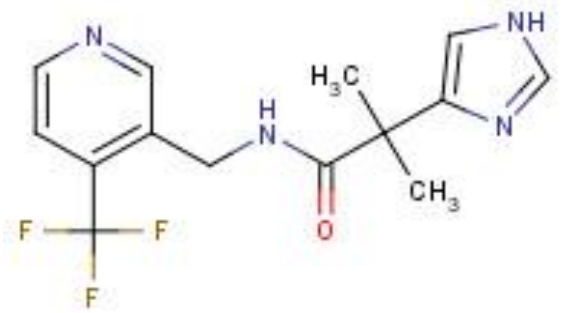
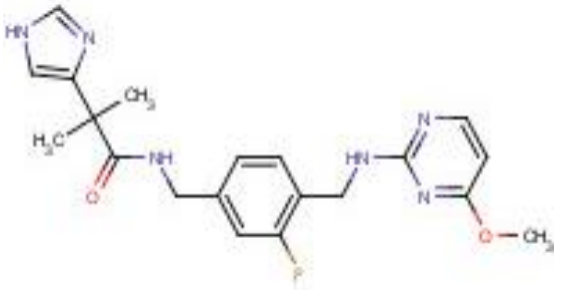
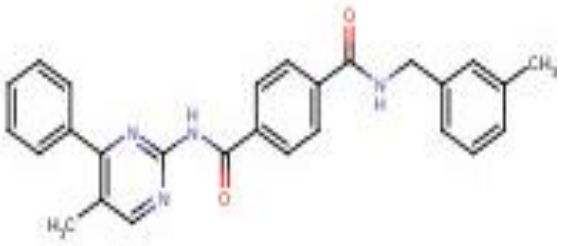
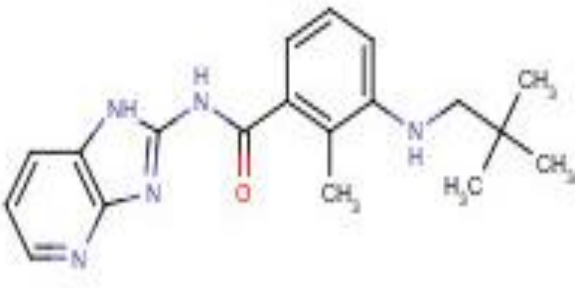
S. No.	Enamine ID	Structure
1	Z2838499575	
2	PV-001850922195	

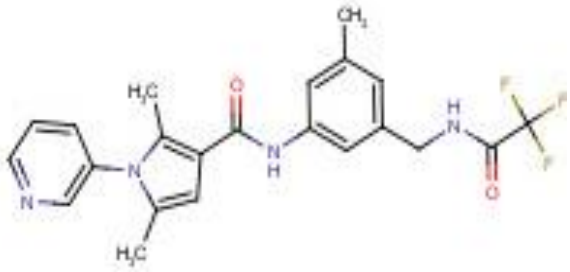
3	Z2966876803	
4	Z2215736428	
5	Z2297880587	
6	Z2192853546	

7	PV-002441033516	
8	PV-001853071310	
9	PV-001853072258	
10	Z2642548869	

11	PV-001886592085	
12	PV-002362919606	
13	PV-002557603090	
14	PV-002203981798	

15	PV-002224286147	
16	PV-002331049037	
17	PV-001851635920	
18	Z2120882817	

19	Z2650112315	
20	PV-002681752702	
21	PV-001805218556	
22	Z1452908463	

23	PV-002204066739	
24	Z768814574	

Structure Analysis of mPGES-1

Three different subtypes of PGES are available and among them mPGES-1 is one of the potential target for pain. Currently, there are 17 crystal structures available for mPGES-1 in protein data bank (**Table 4**) and the structures are complexed with different inhibitors. Furthermore, the structures were crystallized in high resolution.

Table 4. List of crystal structure available for mPGES-1

PDB ID	Structure Title	Release Date	Resolution (Å)
1Z9H	Microsomal prostaglandin E synthase type-2	2005	2.60
2PBJ	GSH-heme bound microsomal prostaglandin E synthase	2008	2.80
4AL0	Crystal structure of Human PS-1	2013	1.16

4AL1	Crystal structure of Human PS-1 GSH-analog complex	2013	1.95
4BPM	Crystal structure of a human integral membrane enzyme	2014	2.08
4WAB	Crystal structure of mPGES1 solved by native-SAD phasing	2014	2.70
4YK5	Crystal Structures of mPGES-1 Inhibitor Complexes	2015	1.42
4YL0	Crystal Structures of mPGES-1 Inhibitor Complexes	2015	1.52
4YL1	Crystal Structures of mPGES-1 Inhibitor Complexes	2015	1.41
4YL3	Crystal Structures of mPGES-1 Inhibitor Complexes	2015	1.41
5BQG	Crystal Structure of mPGES-1 Bound to an Inhibitor	2016	1.44
5BQH	Discovery of a Potent and Selective mPGES-1 Inhibitor for the Treatment of Pain	2016	1.60
5BQI	Discovery of a Potent and Selective mPGES-1 Inhibitor for the Treatment of Pain	2016	1.88
5K0I	mpges1 bound to an inhibitor	2016	1.30
5T36	Crystal structure of mPGES-1 bound to inhibitor	2017	1.40
5T37	crystal structure of mPGES-1 bound to inhibitor	2017	1.76
5TL9	crystal structure of mPGES-1 bound to inhibitor	2017	1.20

Among the crystal structures for the docking study the mPGES-1 in complex with one of the potent inhibitor MF63 with 6nM was considered. The compound MF63 is a substituted phenanthrene imidazole and occupies the extreme upper region of the binding pocket which is place above GSH. The planar chlorophenanthrene of MF63 was extended over a flat surface of α-helica 4 of monomer 1 which included the amino acid residues Pro124, Ser127, and Val128 of monomer 1. Additionally, one face of the aromatic tetracycle facing towards the solvent. Similar to the bis-o-chlorofluorophenyl of 5, a slightly larger 2,6-dicyanophenyl

points inward, clamped between the two protein chains, with one nitrile on the backside directed pointed toward and forming van der Waals contact with the Cβ of Ala123 (monomer 1) at a distance of 3.6 Å and interaction with the side chain hydroxyl of Ser127 (monomer 1) located at a distance of 3.2 Å. The second nitrile packs against the L39 side chain (monomer 2) while engaging a network of structured waters in the front of the binding site. Carbons of the 2,6-dicyanophenyl form hydrophobic contacts with the side chains of Arg38, Leu39, Phe44, and Asp49 from monomer 2, while the imidazole forms H-bonds with His53 from monomer 2 and a structured water.

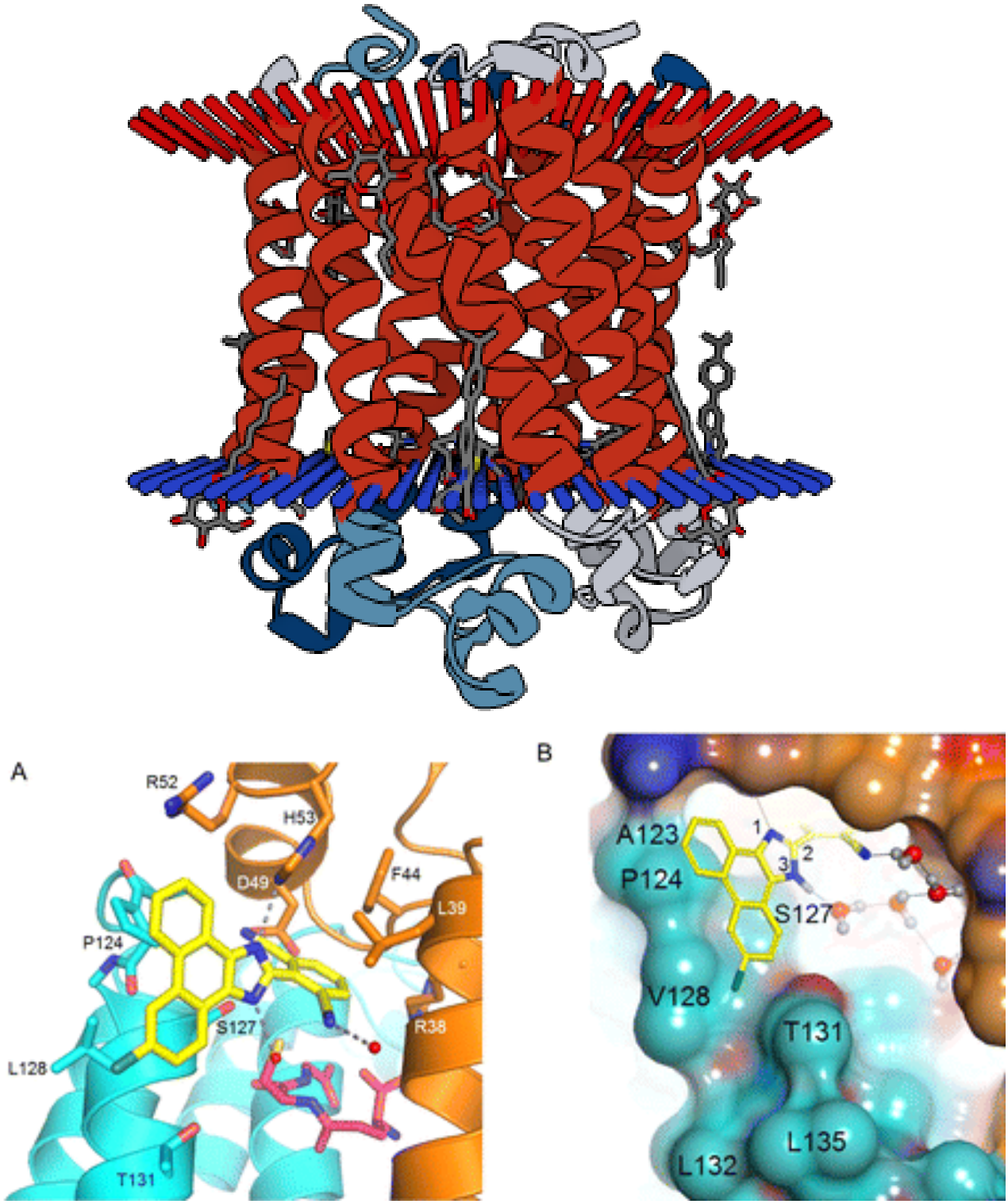


Figure 6. Crystal structure of mPGES in complex with MF63 (Luz J.G, 2015)

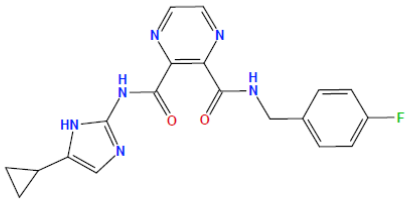
Molecular Docking

The 306 molecules identified as hit molecules from similarity search using MOE were subjected to molecular docking. The molecules were docked using Glide docking module implemented in Schrodinger package. For docking the crystal structure with PDB Id: 2YL1 was selected. The 306 molecules were prepared using ligprep module. The structure obtained from Protein Data Bank as a single monomer, however the mPGES-1 is a homomeric trimer. Hence, initially the enzyme is fixed as a trimer on the basis of the single monomer. The enzyme was prepared using protein preparation wizard where the amino acid residues were optimized. After the protein preparation wizard application, the prepared enzyme is then used in Grid generation. The co-crystallized ligand MF63 is considered as the binding site and grid is generated around the binding site for docking the ligands. Then the prepare trimer is subjected to molecular docking using Glide docking tool from Schrodinger.

The docking studies show that the docking score was obtained in the range from -7.097 to -1.776. The top 20 molecules obtained from docking studies with their docking score greater than -6.0 kcal/mol are shown in **Figure 7.**

1

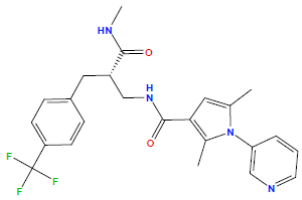
PV-001857177657



-7.0968

2

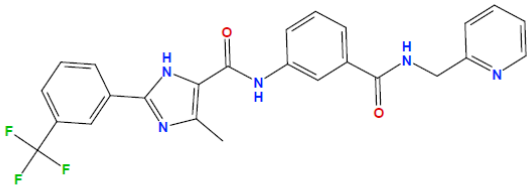
PV-002331049037



-7.0118

3

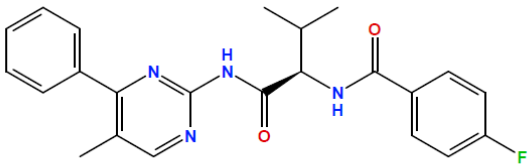
PV-002362919606



-6.8185

4

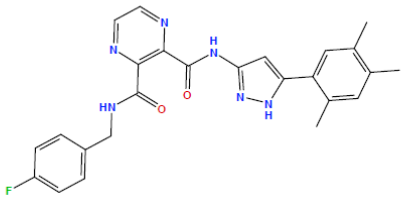
PV-002562830538



-6.7406

5

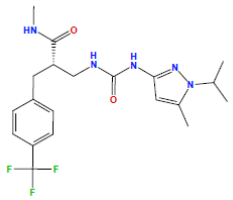
PV-001869139330



-6.7180

6

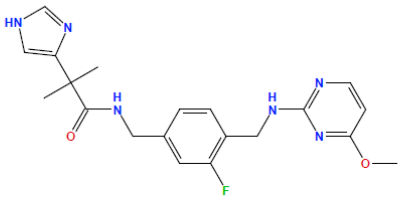
PV-002147811234



-6.6782

7

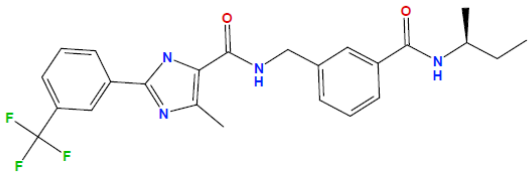
PV-002681752702



-6.5635

8

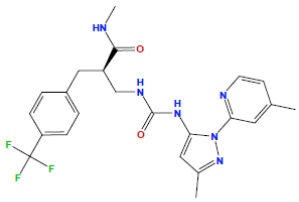
PV-001825146795



-6.4811

9

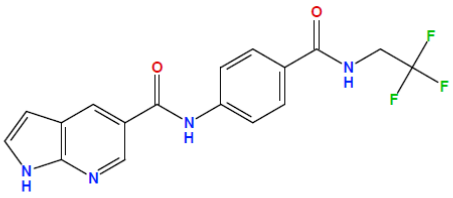
PV-002214872868



-6.4245

10

PV-001823779730



-6.3861

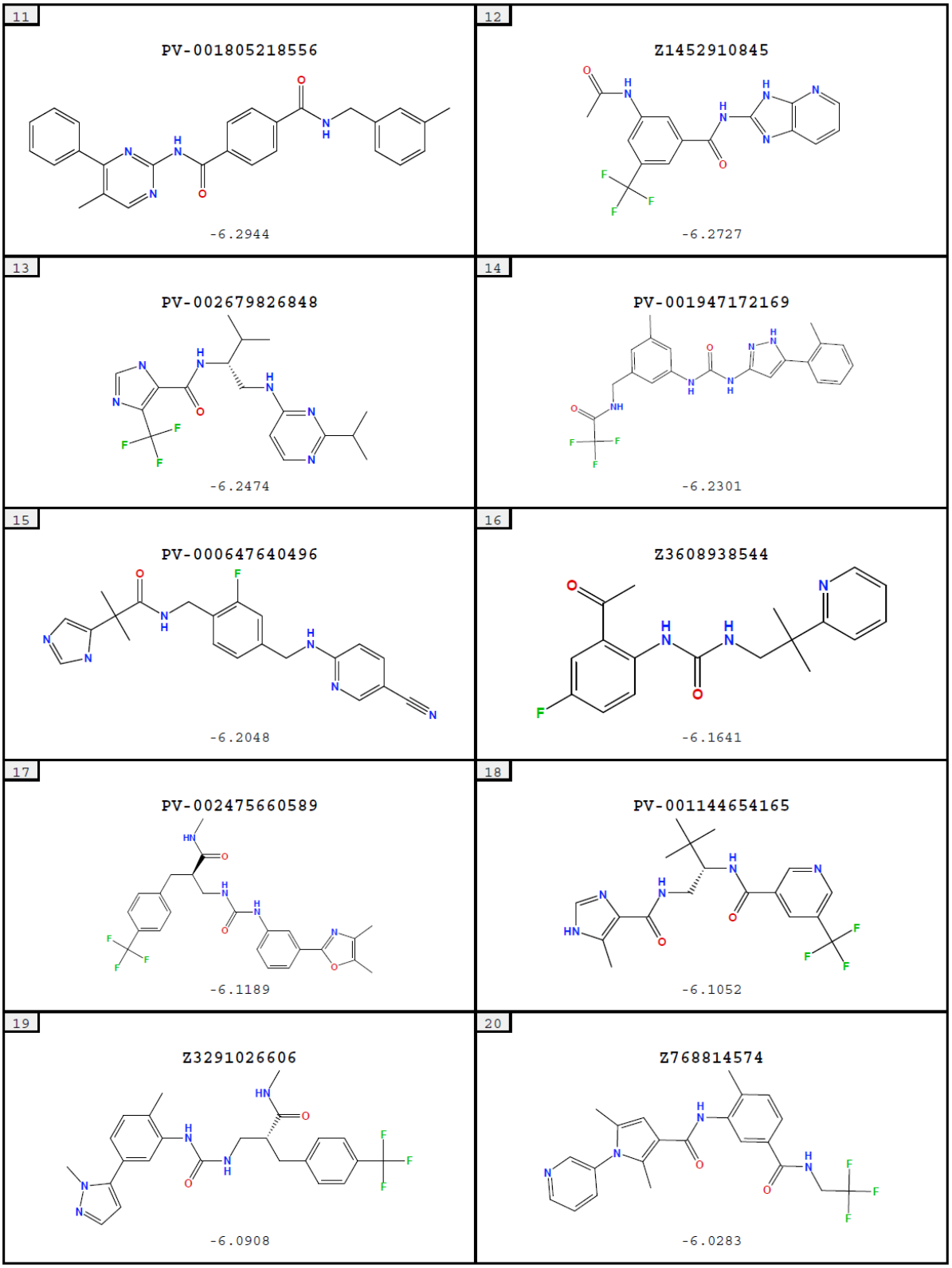
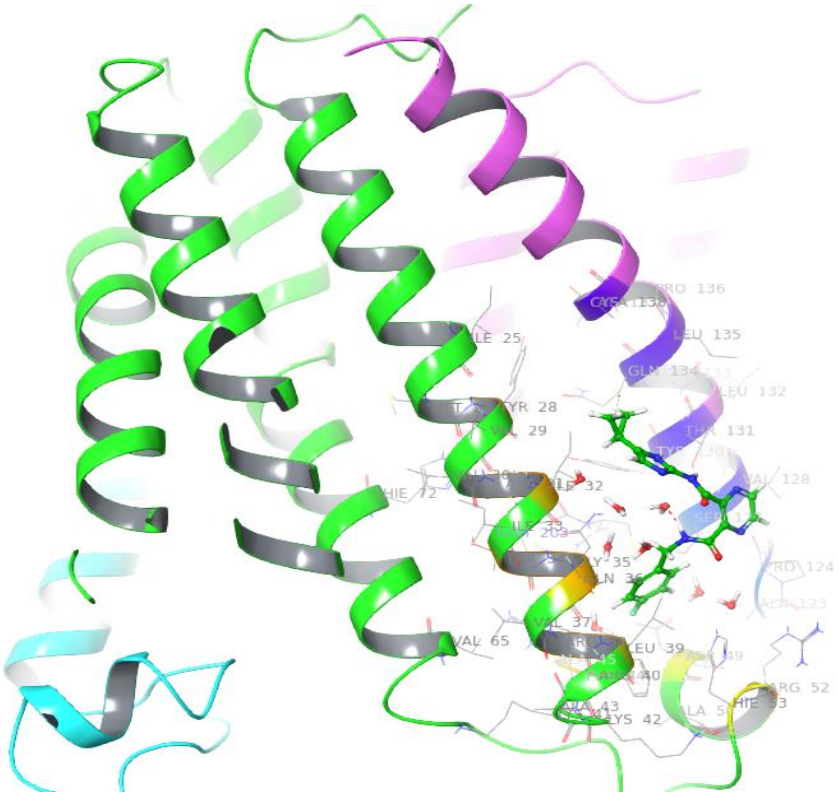
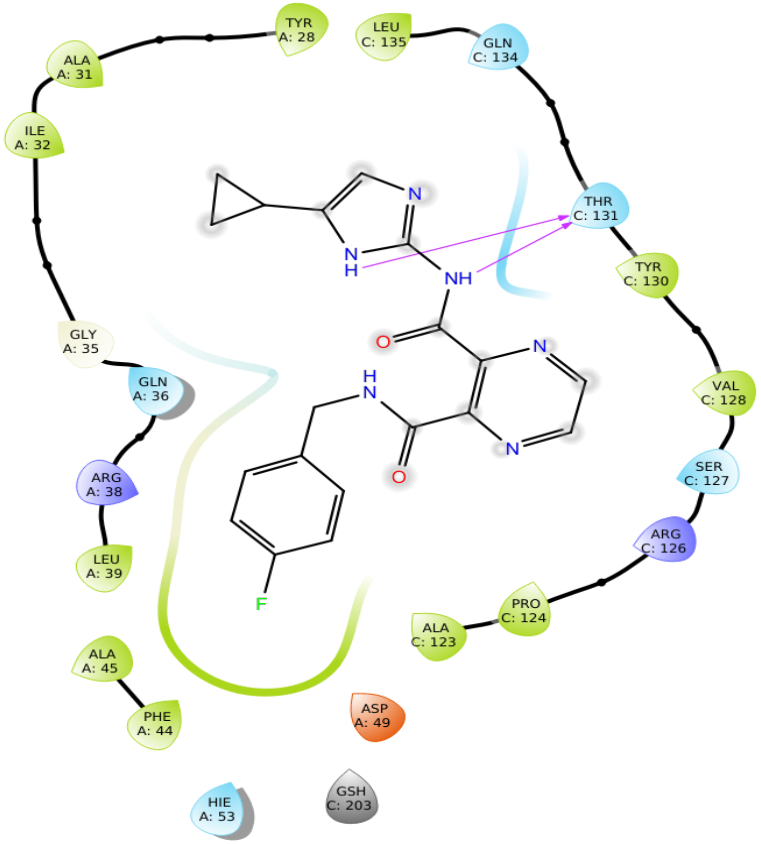


Figure 7. The top 20 molecules obtained from docking with the docking score.

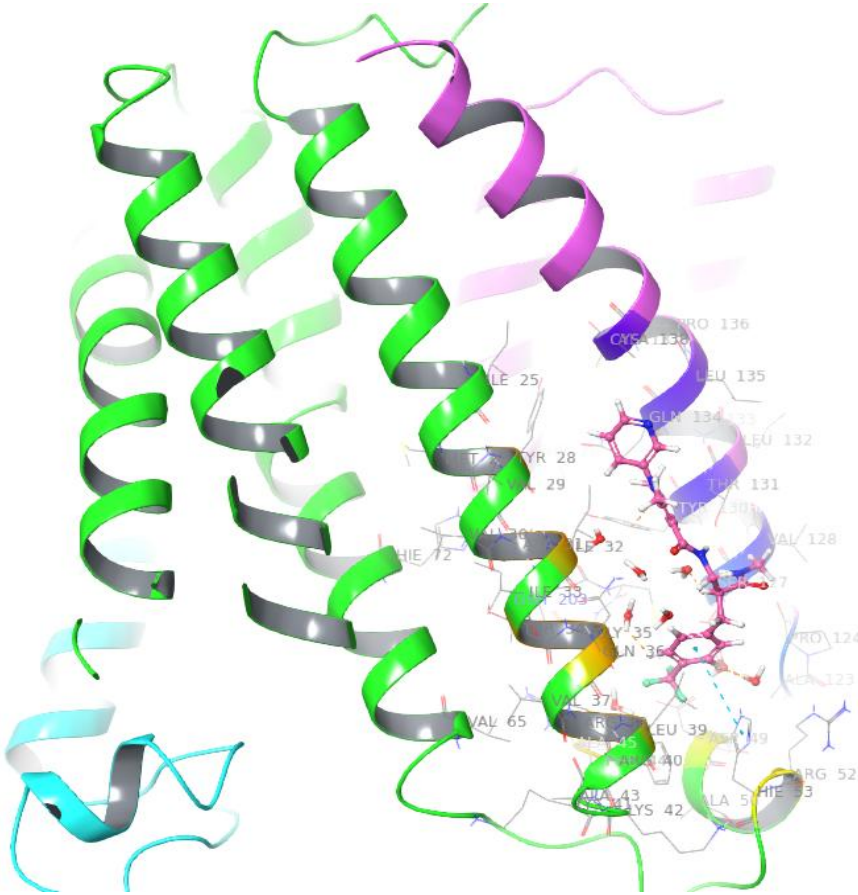
The selected molecules occupied the same binding site of MF63 and form interactions with the amino acid residues in the binding site. This shows that the molecules form interaction similar to the query molecule. In particular, most of the ligands form interaction with Thr131 and His53. The binding pose and the 2D interaction of the top 5 molecules in shown in **Figure 8**.

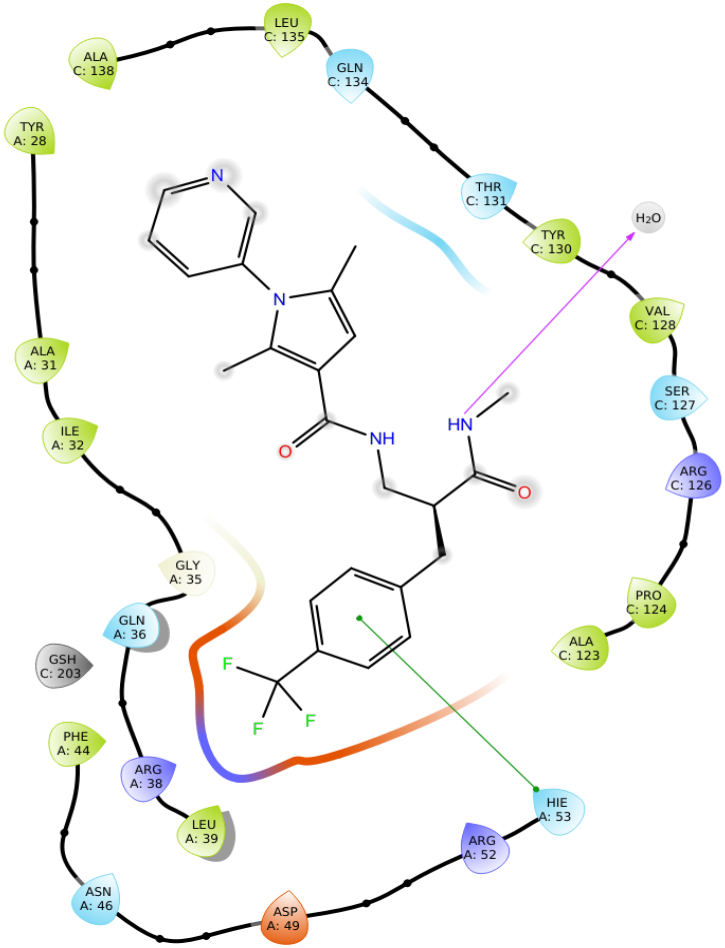
A)



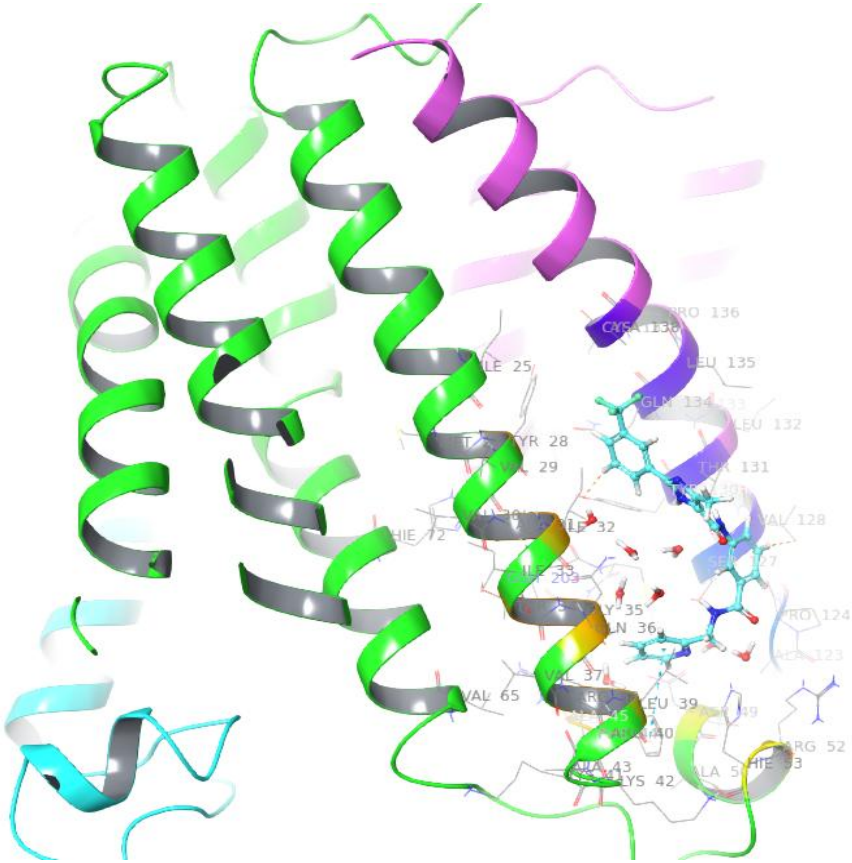

TYR A: 28
LEU C: 135
GLN C: 134
ALA A: 31
ILE A: 32
THR C: 131
TYR C: 130
GLY A: 35
GLN A: 36
VAL C: 128
SER C: 127
ARG A: 38
ARG C: 126
LEU A: 39
PRO C: 124
ALA C: 123
ALA A: 45
ASP A: 49
PHE A: 44
HIE A: 53
GSH C: 203
F
N
NH
O
H

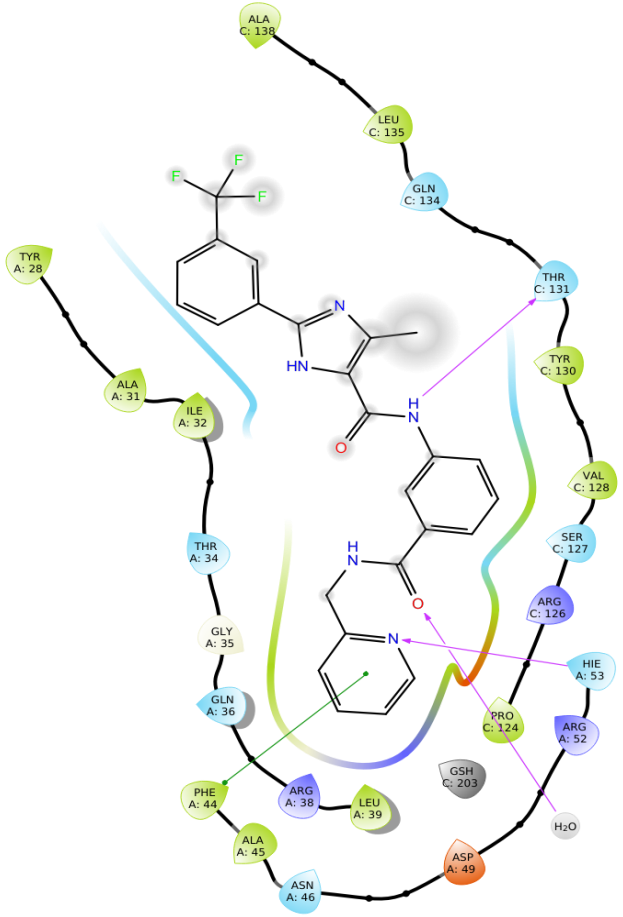
B)



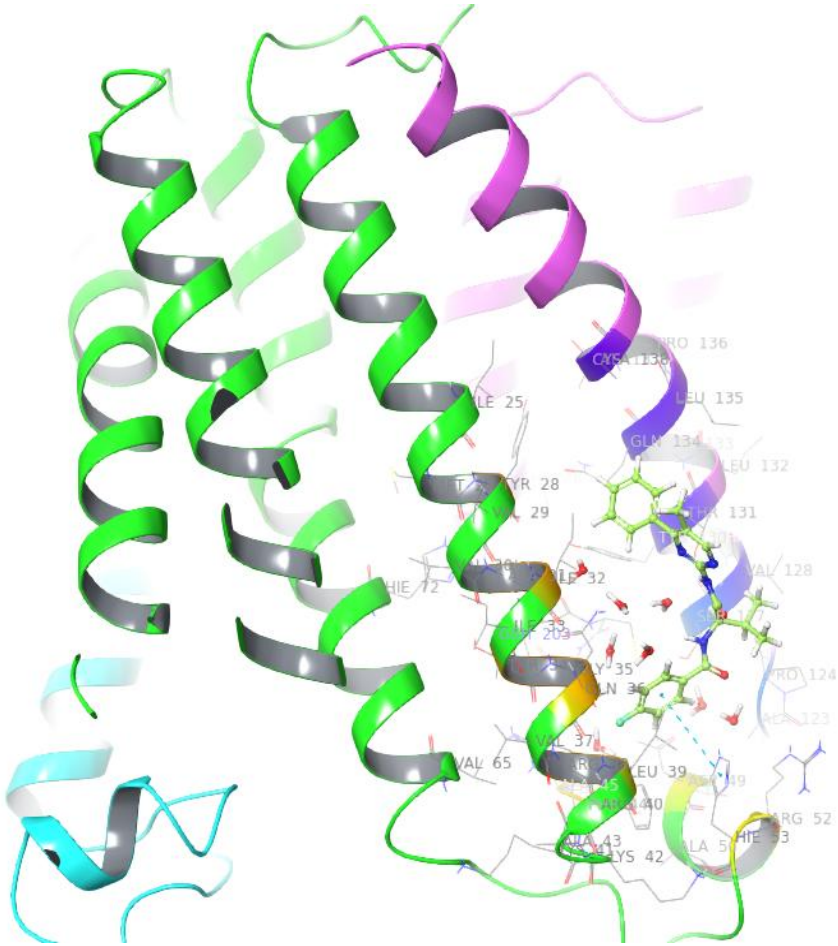

LEU C: 135
ALA C: 138
GLN C: 134
TYR A: 28
THR C: 131
TYR C: 130
H2O
VAL C: 128
ALA A: 31
SER C: 127
ILE A: 32
ARG C: 126
GLY A: 35
PRO C: 124
GLN A: 36
ALA C: 123
GSH C: 203
PHE A: 44
ARG A: 38
HIE A: 53
LEU A: 39
ARG A: 52
ASN A: 46
ASP A: 49
N
NH
HN
O
F

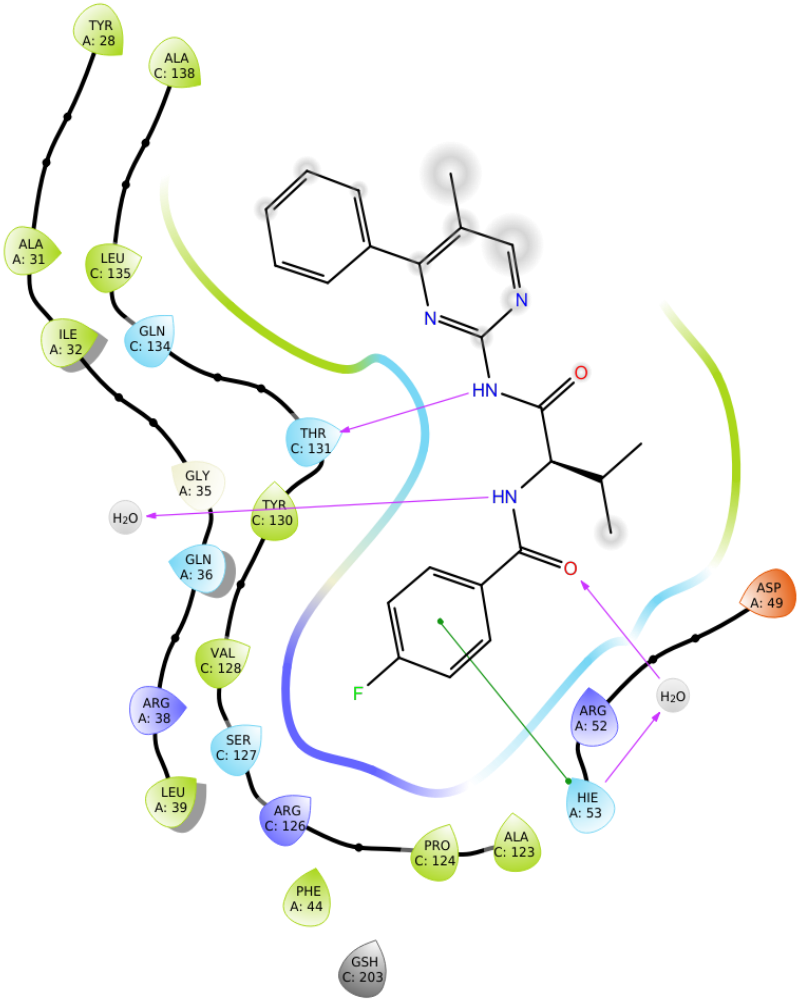
C)




ALA C: 138
LEU C: 135
GLN C: 134
TYR A: 28
THR C: 131
TYR C: 130
ALA A: 31
ILE A: 32
VAL C: 128
SER C: 127
THR A: 34
ARG C: 126
GLY A: 35
HIE A: 53
GLN A: 36
PRO C: 124
ARG A: 52
GSH C: 203
PHE A: 44
ARG A: 38
LEU A: 39
H2O
ALA A: 45
ASP A: 49
ASN A: 46

D)




TYR A: 28
ALA C: 138
ALA A: 31
LEU C: 135
ILE A: 32
GLN C: 134
THR C: 131
GLY A: 35
TYR C: 130
H2O
GLN A: 36
VAL C: 128
ARG A: 38
SER C: 127
LEU A: 39
ARG C: 126
PRO C: 124
ALA C: 123
PHE A: 44
GSH C: 203
HIE A: 53
ARG A: 52
H2O
ASP A: 49
F
N
N
HN
O
HN
O

E)

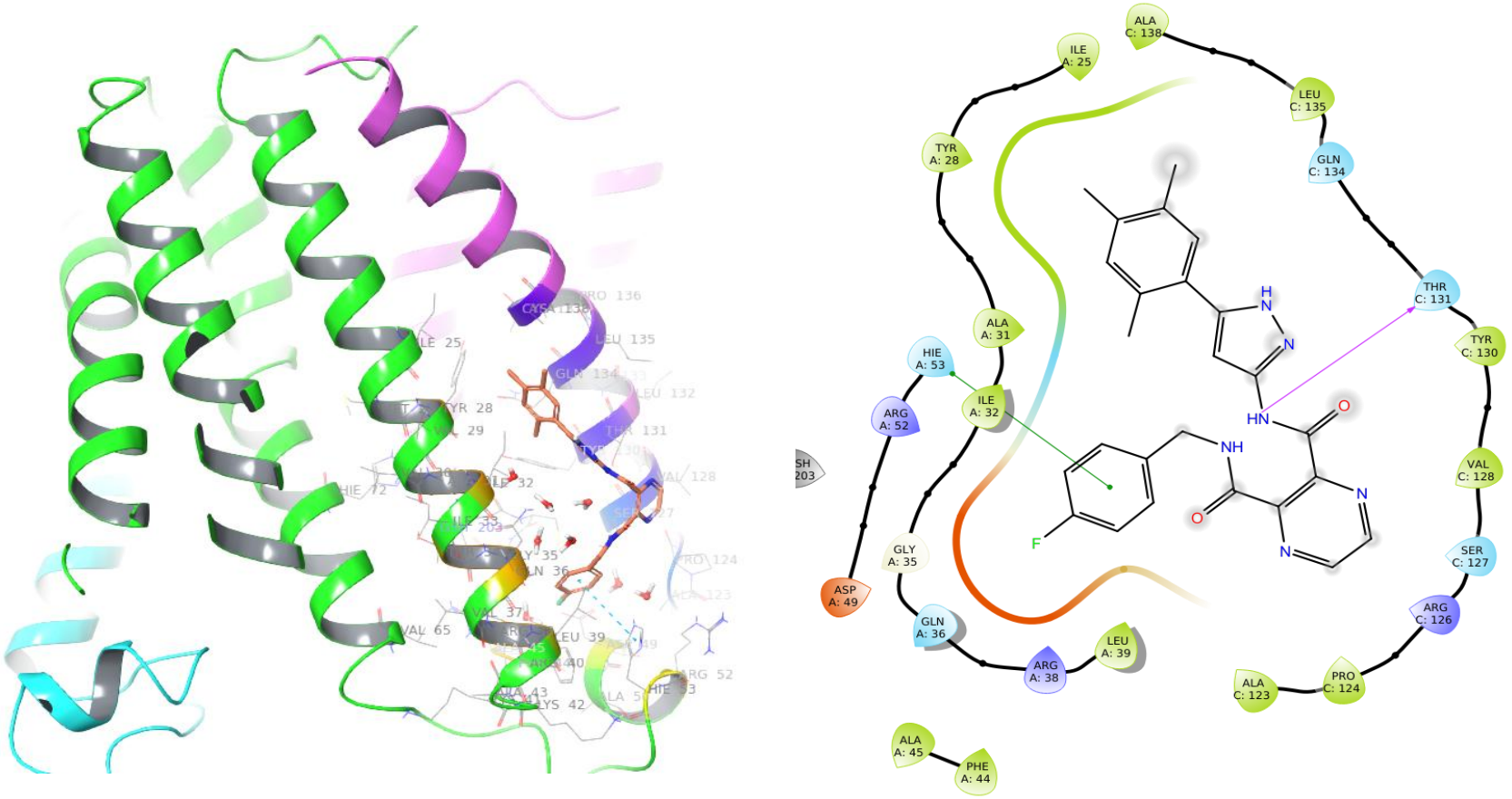


Figure 8. The binding pose of the top five molecules (A) PV-001857177657 (B) PV-002331049037 (C) PV-002362919606 (D) PV-002562830538 (E) PV-001869139330 are shown with their amino acid residues in the binding pocket and their 2D interaction diagrams.

CONCLUSION

CHAPTER 7

CONCLUSION

In the present study, an *in silico* approach using similarity search and molecular docking methods are utilized to identify new inhibitors of mPGES-1. The initial study revealed from these two approaches top 5 molecules found to occupy the binding pocket of mPGES-1 and form electrostatic interaction with amino acids and water molecules. The selected 5 molecules also showed favourable docking score and interaction with the enzyme. These compounds can be further considered *in-vitro* testing. The approach opens up new scaffolds as inhibitors of mPGES-1 .

REFERENCES

CHAPTER 8

REFERENCES

- Ae T, Ohno T, Hattori Y, Suzuki T, Hosono K, Minamino T, Sato T, Uematsu S, Akira S, Koizumi W, Majima M. Role of microsomal prostaglandin E synthase-1 in the facilitation of angiogenesis and the healing of gastric ulcers. *Am J Physiol Gastrointest Liver Physiol.* **2010** Nov;299(5):G1139-1146.
- Bylund J, Annas A, Hellgren D, Bjurström S, Andersson H, Svanhagen A. Amide hydrolysis of a novel chemical series of microsomal prostaglandin E synthase-1 inhibitors induces kidney toxicity in the rat. *Drug Metab Dispos.* **2013** Mar;41(3):634-641.
- Chandrasekhar S, Harvey AK, Yu XP, Chambers MG, Oskins JL, Lin C, Seng TW, Thibodeaux SJ, Norman BH, Hughes NE, Schiffler MA, Fisher MJ. Identification and Characterization of Novel Microsomal Prostaglandin E Synthase-1 Inhibitors for Analgesia. *J Pharmacol Exp Ther*. **2016** Mar;356(3):635-644.
- Chen L, Yang G, Grosser T. Prostanoids and inflammatory pain. *Prostaglandins Other Lipid Mediat.* **2013** Jul-Aug;104-105:58-66.
- Chen G, Jiang L, Dong L, Wang Z, Xu F, Ding T, Fu L, Fang Q, Liu Z, Shan X, Liang G. Synthesis and biological evaluation of novel indole-2-one and 7-aza-2-oxindole derivatives as anti-inflammatory agents. *Drug Des Devel Ther*. **2014** Oct 13;8:1869-1892.
- Corso G, Coletta I, Ombrato R. Murine mPGES-1 3D structure elucidation and inhibitors binding mode predictions by homology modeling and site-directed mutagenesis. *J Chem Inf Model.* **2013** Jul 22;53(7):1804-1817.

- Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. Glide. A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J Med Chem.* **2004**, 47, 1739–1749.
- Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. Extra precision glide. Docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. *J Med Chem.* **2006**, 49, 6177–6196.
- Gudis K, Tatsuguchi A, Wada K, Futagami S, Nagata K, Hiratsuka T, Shinji Y, Miyake K, Tsukui T, Fukuda Y, Sakamoto C. Microsomal prostaglandin E synthase (mPGES)-1, mPGES-2 and cytosolic PGES expression in human gastritis and gastric ulcer tissue. *Lab Invest.* **2005** Feb;85(2):225-236.
- Hamza A, Zhao X, Tong M, Tai HH, Zhan CG. Novel human mPGES-1 inhibitors identified through structure-based virtual screening. *Bioorg Med Chem.* **2011** Oct 15;19(20):6077-6086.
- Jin Y, Smith CL, Hu L, Campanale KM, Stoltz R, Huffman LG Jr, McNearney TA, Yang XY, Ackermann BL, Dean R, Regev A, Landschulz W. Pharmacodynamic comparison of LY3023703, a novel microsomal prostaglandin e synthase 1 inhibitor, with celecoxib. *Clin Pharmacol Ther*. **2016** Mar;99(3):274-84.
- Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Development and validation of a genetic algorithm for flexible docking. *J Mol Biol.* **1997**, 267, 727–748.
- Koeberle A, Siemoneit U, Bühring U, Northoff H, Laufer S, Albrecht W, Werz O. Licofelone suppresses prostaglandin E2 formation by interference with the inducible

microsomal prostaglandin E2 synthase-1. *J Pharmacol Exp Ther.* **2008** Sep;326(3):975-982.

- Koeberle A, Rossi A, Zettl H, Pergola C, Dehm F, Bauer J, Greiner C, Reckel S, Hoernig C, Northoff H, Bernhard F, Dötsch V, Sautebin L, Schubert-Zsilavecz M, Werz O. The molecular pharmacology and in vivo activity of 2-(4-chloro-6-(2,3-dimethylphenylamino)pyrimidin-2-ylthio)octanoic acid (YS121), a dual inhibitor of microsomal prostaglandin E2 synthase-1 and 5-lipoxygenase. *J Pharmacol Exp Ther.* **2010** Mar;332(3):840-848.
- Koeberle A, Werz O. Perspective of microsomal prostaglandin E2 synthase-1 as drug target in inflammation-related disorders. *Biochem Pharmacol.* **2015** Nov 1;98(1):1-15.
- Kothavade PS, Nagmoti DM, Bulani VD, Juvekar AR. Arzanol, a potent mPGES-1 inhibitor: novel anti-inflammatory agent. *ScientificWorldJournal.* **2013** Oct 1;2013:986429.
- Kudo I, Murakami M. Prostaglandin E synthase, a terminal enzyme for prostaglandin E2 biosynthesis. *J Biochem Mol Biol.* **2005** Nov 30;38(6):633-638.
- Leclerc P, Pawelzik SC, Idborg H, Spahiu L, Larsson C, Stenberg P, Korotkova M, Jakobsson PJ. Characterization of a new mPGES-1 inhibitor in rat models of inflammation. *Prostaglandins Other Lipid Mediat.* **2013** Apr-May;102-103:1-12.
- Lee K, Pham VC, Choi MJ, Kim KJ, Lee KT, Han SG, Yu YG, Lee JY. Fragment-based discovery of novel and selective mPGES-1 inhibitors Part 1: identification of sulfonamido-1,2,3-triazole-4,5-dicarboxylic acid. *Bioorg Med Chem Lett.* **2013** Jan 1;23(1):75-80.
- Lexa, K. W.; Carlson, H. A. Protein flexibility in docking and surface mapping. *Q Rev Biophys.* **2012**, 45, 301–343.

- Maggiora, G.; Vogt, M.; Stumpfe, D.; Bajorath, J. "Molecular similarity in medicinal chemistry.," *J. Med. Chem.*, vol. 57, no. 8, pp. 3186–3204, **2014**.
- McGaughey, G. B.; Sheridan, R. P.; Bayly, C. I.; Culberson, J. C.; Kreatsoulas, C.; Lindsley, S.; Maiorov, V.; Truchon, J.-F.; Cornell, W. D. Comparison of topological, shape, and docking methods in virtual screening. *J Chem Inf Model.* **2007**, 47, 1504–1519.
- Osterberg, F.; Morris, G. M.; Sanner, M. F.; Olson, A. J.; Goodsell, D. S. Automated docking to multiple target structures. Incorporation of protein mobility and structural water heterogeneity in AutoDock. *Proteins.* **2002**, 46, 34–40.
- Pagadala, N. S.; Syed, K.; Tuszynski, J. Software for molecular docking. A review. *Biophys Rev.* **2017**, 9, 91–102.
- Park SJ, Han SG, Ahsan HM, Lee K, Lee JY, Shin JS, Lee KT, Kang NS, Yu YG. Identification of novel mPGES-1 inhibitors through screening of a chemical library. *Bioorg Med Chem Lett.* **2012** Dec 15;22(24):7335-7339.
- Rarey, M.; Kramer, B.; Lengauer, T.; Klebe, G. A fast flexible docking method using an incremental construction algorithm. *J Mol Biol.* **1996**, 261, 470–489.
- Sampey AV, Monrad S, Crofford LJ. Microsomal prostaglandin E synthase-1: the inducible synthase for prostaglandin E2. *Arthritis Res Ther.* **2005**;7(3):114-117.
- Sliwoski, G.; Kothiwale, S.; Meiler, J.; Lowe, E. W. Computational methods in drug discovery. *Pharmacol Rev.* **2014**, 66, 334–395.
- Stumpfe, D.; Bajorath, J. "Similarity searching," WIREs *Comput. Mol. Sci.*, vol. 1, no. 2, pp. 260–282, **2011**.
- Venkatachalam, C. M.; Jiang, X.; Oldfield, T.; Waldman, M. LigandFit. A novel method for the shape-directed rapid docking of ligands to protein active sites. *J Mol Graph Model.* **2003**, 21, 289–307.

- Verhoff M, Seitz S, Paul M, Noha SM, Jauch J, Schuster D, Werz O. Tetra- and pentacyclic triterpene acids from the ancient anti-inflammatory remedy frankincense as inhibitors of microsomal prostaglandin E(2) synthase-1. *J Nat Prod.* **2014** Jun 27;77(6):1445-1451.
- Walker DP, Arhancet GB, Lu HF, Heasley SE, Metz S, Kablaoui NM, Franco FM, Hanau CE, Scholten JA, Springer JR, Fobian YM, Carter JS, Xing L, Yang S, Shaffer AF, Jerome GM, Baratta MT, Moore WM, Vazquez ML. Synthesis and biological evaluation of substituted benzoxazoles as inhibitors of mPGES-1: use of a conformation-based hypothesis to facilitate compound design. *Bioorg Med Chem Lett.* **2013** Feb 15;23(4):1120-126.
- Waltenberger B, Wiechmann K, Bauer J, Markt P, Noha SM, Wolber G, Rollinger JM, Werz O, Schuster D, Stuppner H. Pharmacophore modeling and virtual screening for novel acidic inhibitors of microsomal prostaglandin E_2 synthase-1 (mPGES-1). *J Med Chem.* **2011** May 12;54(9):3163-3174.
- Xu D, Rowland SE, Clark P, Giroux A, Côté B, Guiral S, Salem M, Ducharme Y, Friesen RW, Méthot N, Mancini J, Audoly L, Riendeau D. MF63 [2-(6-chloro-1H-phenanthro[9,10-d]imidazol-2-yl)-isophthalonitrile], a selective microsomal prostaglandin E synthase-1 inhibitor, relieves pyresis and pain in preclinical models of inflammation. *J Pharmacol Exp Ther.* **2008** Sep;326(3):754-763.