Pain Chemogenomics Knowledgebase (PAIN-CKB) for Systems Pharmacology Target Mapping and PBPK Modeling Investigation of Opioid Drug-Drug Interactions

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Abstract

More than 50 million adults in America suffer from chronic pain. Opioids are commonly prescribed for their effectiveness in relieving many types of pain. However, excessive prescribing of opioids can lead to abuse, addiction, and death. Non-steroidal anti-inflammatory drugs (NSAIDs), another major class of analgesic, also have many problematic side effects including headache, dizziness, vomiting, diarrhea, nausea, constipation, reduced appetite, and drowsiness. There is an urgent need for the understanding of molecular mechanisms that underlie drug abuse and addiction to aid in the design of new preventive or therapeutic agents for pain management. To facilitate pain related small-molecule signaling pathway studies and the prediction of potential therapeutic target(s) for the treatment of pain, here we present a comprehensive platform of pain domain-specific chemogenomics knowledgebase (PAIN-CKB) with integrated data mining computing tools. Our new computing platform describes the chemical molecules, genes, proteins, and signaling pathways involved in pain regulation. PAIN-CKB is implemented with a friendly user-interface for the prediction of the relevant protein targets of the query compound and analysis and visualization of the outputs based on HTDocking, TargetHunter, BBB predictor, and Spider Plot. We performed three case studies to systematically validate the integrity and accuracy of PAIN-CKB and its algorithms/tools. First, system pharmacology target mapping was carried out for four FDA approved analgesics (acetaminophen,

diclofenac, fentanyl, and morphine) in order to identify the known targets and predict off-targets. Subsequently, the target mapping outcomes were applied to build physiologically based pharmacokinetic (PBPK) models for acetaminophen and fentanyl to explore the potential drug-drug interaction (DDI) between this pair of drugs. Finally, docking analysis was conducted to explore the detailed interaction pattern of acetaminophen reactive metabolite (NAPQI) and its hepatotoxicity target thioredoxin reductase (TrxR).

Key words: Pain, Knowledgebase, Opioids, NSAIDs, Computational Systems Pharmacology-Target Mapping, PBPK

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PREFACE

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1.0 INTRODUCTION

1.1 PAIN

1.1.1 Molecular mechanism of pain

Pain is defined by the International Association for the Study of Pain (IASP) as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.'[1] Briefly, it is an unpleasant sensation as a response to noxious stimuli from external or internal sources on the human body.[2]

The nociception process starts with the activation of sensory fibers, also known as the nociceptors, in the peripheral by chemical, mechanical, and thermal stimuli that go beyond the noxious range.[3] There are three main types of sensory fibers with different functionalities in the human body: Aβ-fibers, Aδ-fibers, and C-fibers.[4] All the A-fibers can respond to thermal and mechanical stimuli. The Aβ-fibers have relatively large diameters and low thresholds and are highly myelinated.[5-7] Thus they are mainly responsible for the quick conduction of light touch sensation.[8] The thin myelinated Aδ-fibers are relatively smaller in diameters and higher in thresholds compared to the Aβ-fibers.[4,9] They were believed to mediate fast and sharp pain caused by thermal or mechanical events.[10] As to the C-fibers, they have the slowest conducting rate because they are unmyelinated and have the smallest diameters among the three main nociception fibers.[11,12] These fibers have the highest thresholds and thus they only selectively respond to those noxious chemical, mechanical, and thermal stimuli.[13] It is widely accepted that the Aδ-fibers and C-fibers were together termed as the nociceptors for their selectivity on the

painful stimuli.[14] Once those nociceptors are evoked, electrical signals will be generated and transduced from the periphery to an area called dorsal horn located in the spinal cord (part of the central nervous system (CNS))[15], where various sensory and nociceptive signals are converged and modulated before being passed by neurotransmitters, such as the excitatory glutamate or inhibitory γ-aminobutyric acid (GABA), to the brain (**Figure 1**).[16]

Under normal circumstances, the ability to detect noxious signals is essential to the safety and wellbeing of individuals. Pain, or more specifically, acute pain, exhibits its protective role by motivating the body to keep away from damaging sources and adopting behaviors to promote the healing process of damaged body parts.[17,18] Acute pain typically lasts less than 3 to 6 months and can be relieved when the stimuli source no longer lasts.[19] For example, the pain from a healing wound can remind us to avoid further exposure to harmful sources and pain from inside of our body can be seen as a warning for certain diseases or internal organ damage. However, pain will be considered as chronic pain when it lasts longer than 12 weeks after the initial damage.[20,21] Chronic pain may occur as a result of alterations in the pain pathway. At this time, far from being beneficial, pain becomes both physiologically and psychologically debilitating.[22]

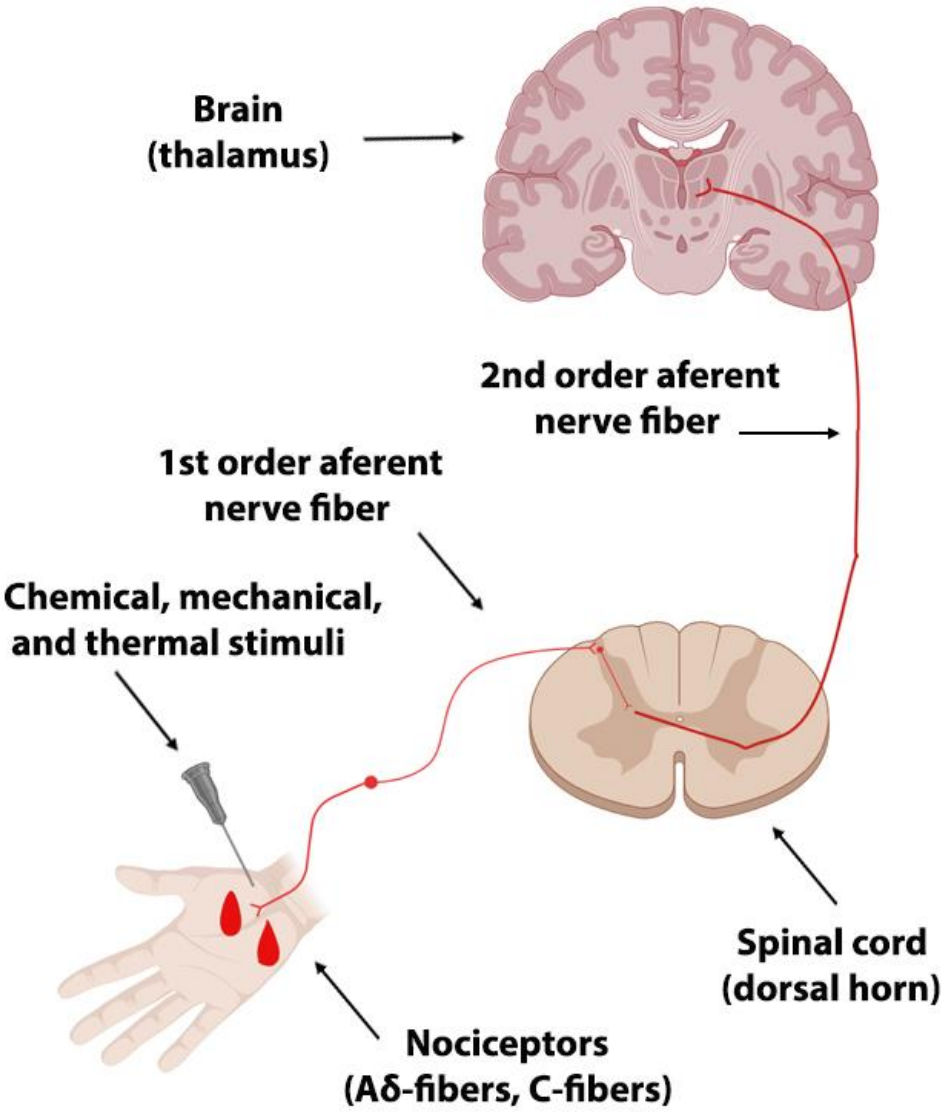


Figure 1 The diagram of pain pathway

1.1.2 Current statues of pain

Chronic pain is one of the most common reasons for people to seek medical help in the United States[23] and is often associated with restricted daily activities and lowered mood, which will ultimately lead to a decrease in quality of life. The consequence of chronic pain is far more server than acute pain. For example, it can cause listlessness, decreased appetite, sleep disturbance, weight loss, and immobility.[24] On the other hand, emotional changes can also occur in patients, especially the elderly with chronic pain[25], such as depression and anxiety and eventually lead to social withdrawal.

According to a previous National Health Interview Survey data-based estimation, around 50 million U.S. adults (20.4%) suffer from chronic pain.[26] With such high prevalence, pain-related healthcare costs are estimated to be $280 billion each year.[27] Other economic losses could result due to the loss of productivity caused by pain.[28] Yet still, approximately 79% of patients are not satisfied with their pain management medications they are receiving[29,30] due to limited effectiveness[31] and severe side effects caused by the analgesics currently available on the market.[32] Thus, efforts in the improvement of clinical guidelines about patient monitoring practices and the development of novel analgesics with less side effect and better therapeutic effect are urgently needed.[33]

1.1.3 Pain medications

Currently, there are many kinds of analgesics available on the market. Non-steroidal anti-inflammation drugs (NSAIDs), such as ibuprofen[34] and celecoxib[35], are some of the most used

classes of analgesics. These drugs are known to block the synthesis of prostanoids from arachidonic acids by inhibiting the prostaglandin G/H synthases (COX enzymes).[36] NSAIDs are usually used to treat mild to moderate pain[37] and their side effects include ulceration and bleeding in the GI tract, stomach pain, and renal failure[38,39] depending on the dose and the inhibition ratio between COX1 and COX2.[40]

When it comes to the relief of moderate to severe pain, the use of opioid analgesics like morphine, fentanyl, and codeine may be more dominant.[41] As agonists of opioid receptors, after binding, opioid analgesics can cause a conformational change and switch the opioids receptor to its active state and deliver signals to both block the voltage-gated dependent calcium channel (which will enable the calcium ions to flow into the neuron cells)[42,43] and activate the G protein-coupled inwardly-rectifying potassium channel (which will pump out the potassium ions from the neuron cells)[44]. This process will further hyperpolarize the neuronal cells[45] to inhibit the neural excitability and decrease the release of neurotransmitters to derive analgesia.[46] Opioids may have better therapeutic effects, however, compared with NSAIDs, they often have more severe side effects such as respiratory depression, dependence, and drug addiction.[32,47]

However, there are still critical problems associated with the analgesics available on the market. The most important aspect is the safety issue related to analgesics, especially opioids. Opioid poisoning is reported to be involved in nearly 40% of all the drug poisoning deaths[48] due to the synergistic effects caused by the combination use of opioids[49] (https://www.drugabuse.gov/related-topics/trends-statistics/overdose-death-rates).[50] Even under the recommended dosage, chronic use of opioids will still cause tolerance, addiction, and severe withdrawal syndrome.[51] Moreover, the lack of progress achieved by the pharmaceutical industry during the past few years is not reflective of the urgent need for new analgesics with better

efficacy and side effects profile.[52] Thus, the research into the pain process and treatment is of great importance and urgency.

1.2 DATABASE AND PILOT STUDIES

Herein, in order to accelerate the research of the pain-related areas, we constructed a pain domain-specific chemogenomics knowledgebase (PAIN-CKB) based on our previous established molecular information databases.[53-57] The knowledgebase assembled a large amount of analgesics' information and pain-related protein targets structure information extracted from the literature. The protein targets collected in PAIN-CKB are further divided into four major subgroups: G-protein coupled receptors (GPCRs), enzymes, ion channels, nuclear receptors, and others (if the target does not belong to the four major subgroups) based on the Drug Target Ontology (DTO) with slight modifications.[58]

1.2.1 Target classification

G-protein coupled receptors (GPCRs), which are consist of typical seven transmembrane domains and integral membrane G proteins, are the largest protein family of receptors in human body.[59] It regulates a wide range of physiologically important cell signaling pathways through the coupled G proteins and arrestins after activation.[60] As shown in **Figure 2** (this figure is adapted from the work of Daniel Hilger et al.)[60], when activated by extracellular signals, the receptor will go through a conformational change and further associate and activate the G protein (exchanging the GDP bound to the G protein for a GTP). The activation of G protein will cause

the Gα subunit and GTP disassociate from the Gβ and Gγ subunits. These subunits can then bind to its target receptors and exhibit the signaling function. Currently, there are around 34 percent drugs approved by the FDA target on different GPCRs.[61] For example, the nutrient sensors free fatty acid receptors (FFA2, FFA3) are potential targets for inflammatory diseases and metabolic dysfunction[62] and the formyl peptide receptors (FPR) can recognize pathogens and thus become drug targets related to the immune responses,[63] etc. Because of this wide participation, GPCRs has become a hot target for drug development.

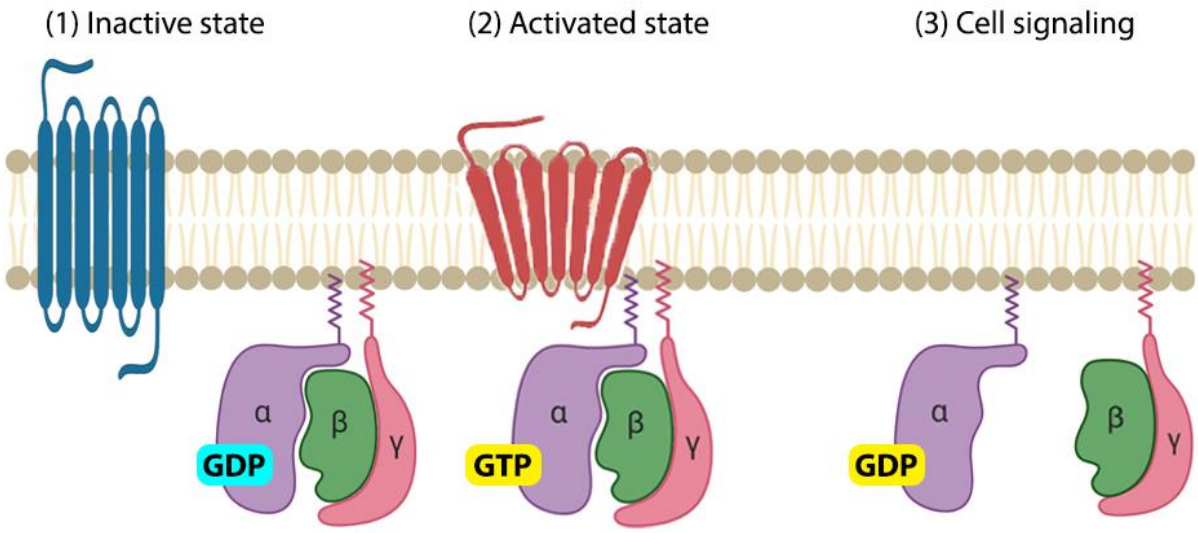


Figure 2 GPCR signaling mechanism

Enzymes are biological catalysts in organisms which accelerate the reactions in metabolic pathways to sustain life. Thus, the enzyme is another promising drug target.[64] Cases are abundant: the amine oxidase mainly catalyzes the oxidative deamination of biogenic amines such as 5-hydroxytryptamine (5-HT), norepinephrine and epinephrine.[65] Drugs targeting at amine oxidase

are thus used to treat the neurological disorders[66] like depressions and epilepsy or as anesthetics.[67] Another example is β-lactamase, which can provide resistance to β-lactam antibiotics in bacteria.[68] To overcome that, the β-lactamase inhibitor, tazobactam¸ was developed to restore the therapeutic effect of antibiotics such as Piperacillin.[69]

Ion channels are the third major drug target class. They are transmembrane proteins widely distributed in body with a pore that allows specific ions to pass through[70]. When activated by ligand binding, voltage changes or stretch, ions channels will go through conformational changes thus control the substance exchange between different sites of the membrane.[71] Ion channels are involved in a series of physiological processes such as nerve conduction, muscle contraction, and sensory reception[72] and they are the second biggest drug family accounting for approximately 13% of the known drugs so far.[73] For example, the anticonvulsant phenytoin works by blocking voltage-dependent of membrane sodium channels.[74] Verapamil, which is a non-dihydropyridine calcium channel blocker, is used for the treatment for angina, cardiac arrhythmias.[75]

The last subgroup, nuclear receptors, are proteins that can bind to the DNA and control the expression of genes. Therefore, nuclear receptors are often called as transcriptional factors.[76] Nuclear receptors can be divided into four different subgroups depending on the cellular distribution and dimerization state.[77,78] However, their mechanism of actions can be identical: ligand binding to the nuclear receptor can cause a conformational change to the receptor and further direct the nuclear receptor to the transcription regulation site to cause the up-regulation of down-regulation of the gene expression level.[79] The critical transcriptional regulation function of nuclear receptors have made them important therapeutic drug targets.[80] For example, the

peroxisome proliferator-activated receptors (PPARs) are popular targets for anti-inflammation[81] and anti-diabetic drugs,[82] orphan nuclear receptor 4A1 (NR4A1) is a target for breast cancer chemotherapy,[83] and the DAF-12 receptor in Caenorhabditis elegans is emerging as target for the treatment of parasitic diseases.[84]

Integrated with the algorithms previously developed by our lab, such as TargetHunter,[85] HTDocking, and Spider Plot, PAIN-CKB enables the pain-related target identification, drug repurposing analysis, small-molecule screening, and DDI predictions from the view of systems pharmacology. Therefore, PAIN-CKB is a valuable platform for information sharing and investigating in the pain domain in the hope of aiding the research of pain and analgesics. We also conducted a series of case studies based on four FDA-approved analgesics (acetaminophen, diclofenac, fentanyl, and morphine) to demonstrate the usage of our PAIN-CKB.

1.2.2 Four analgesics for pilot studies

Acetaminophen (N-acetyl-para-aminophenol), also known as paracetamol or Tylenol, is one of the most commonly used medication for the management of fever and mild to moderate pain.[86] It is widely used in combination with opioid analgesics to treat moderate to severe pain in order to reduce the dosage of opioids and avoid severe side effects.[87] Acetaminophen exhibits the therapeutic effect by interacting with the COX enzymes in the central. However, the COX enzyme inhibition effect is relatively weak in the peripheral[88] and this provides an explains why acetaminophen serves as analgesics as well as antipyretics but not anti-inflammation medication.[89] At its therapeutic dose, acetaminophen is mainly metabolized to acetaminophen glucuronide (52-57%), acetaminophen sulfate (30-44%), and N-acetyl-p-benzoquinone imine

(NAPQI, 5-10%) in the liver (**Figure 3**). Among them, NAPQI is a highly electrophilic metabolite produced by the cytochrome P-450 (CYP) enzymes and it is mainly responsible for the acetaminophen liver toxicity when overdosed.[90] According to Yi-Hua Jan et.al, NAPQI can covalently modify and inhibit the redox center of TrxR, which is an important cellular antioxidant involving in diverse physiological systems,[91] and cause enzyme inhibition and further hepatotoxicity.[92]

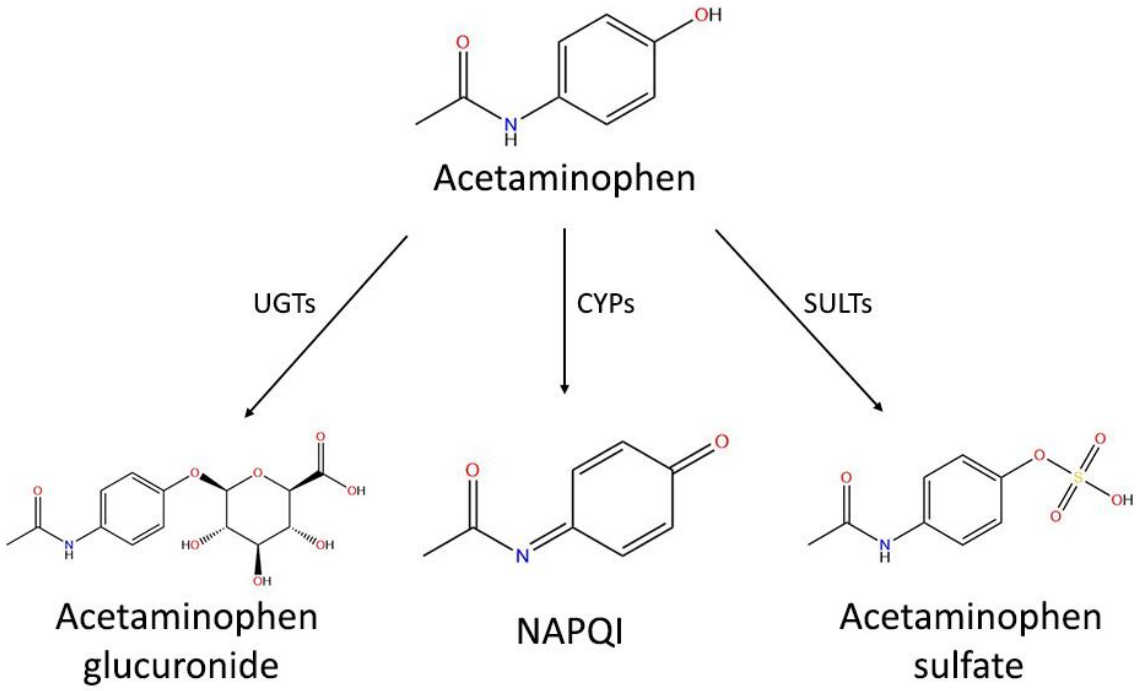


Figure 3 The metabolic pathways of acetaminophen

UGT: UDP-glucuronosyl transferases, main UGTs involved in the metabolic pathways of acetaminophen include UGT1A1, UGT1A6, UGT1A9 and UGT2B15. CYPs: cytochrome P450 enzymes, main CYPs involved in the metabolic pathways of acetaminophen include CYP3A4, CYP1A2, CYP2E1, CYP2D6, and CYP2A6. SULTs: sulfotransferases, main SULTs involved in the metabolic pathways of acetaminophen include SULT1E1, SULT1A1, SULT2A1, SULT1A3, and SULT1A4.

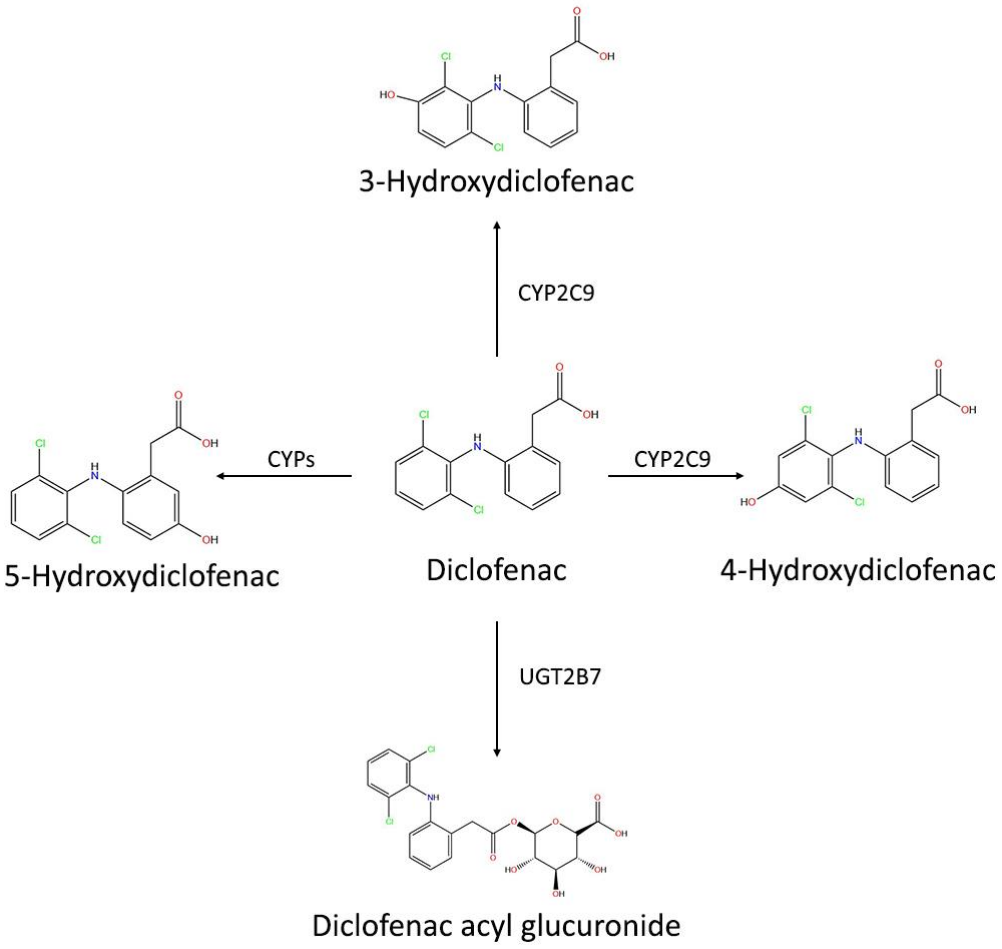


Figure 4 The metabolic pathways of diclofenac

CYPs: cytochrome P450 enzymes, main CYPs involved in the metabolic pathways of diclofenac to 5-hydroxydiclofenac include CYP2C8, CYP2C19, CYP3A4, CYP2B6, and CYP2C18.

Diclofenac (2-[2,6-dichloranilino]phenylacetic acid) is a NASID drug sold under the brand names Voltaren. It is used to treat pain and inflammatory disorders.[93] Diclofenac is confirmed to inhibit the COX enzymes (with a better inhibition effect on COX2 than COX1) and further reduce the synthesis of prostaglandin to achieve analgesia. There are also some publications discussing the possibility for diclofenac to inhibit the synthesis of leukotriene,

phospholipase A2, and simulate the peripheral nitric oxide–cyclic guanosine monophosphate–potassium channel pathways.[94] The metabolism of diclofenac mainly takes place in the liver and depends on the hydroxylation and glucuronidation pathways as shown in **Figure 4**. The main metabolite product of diclofenac is 4-hydroxydiclofenac.[95]

Fentanyl (1-phenethyl-4-N-propionylanilinopiperidine) is a potent (50 times more potent than heroin and 100 times more potent than morphine) synthetic opioids which is frequently used to treat severe chronic pain and cancer pain.[96] However, the strong potency of fentanyl has also contributed to its common appearance with the illicit drugs such as heroin.[97] According the data provided by the National Institute of Drug Abuse (https://www.drugabuse.gov/related-topics/trends-statistics/overdose-death-rates), the overdose death involving fentanyl is around 28,000 in 2017 and the number is still increasing dramatically.[98] Fentanyl is extensively metabolized in human liver by the CYP3A4 enzyme to norfentanyl (**Figure 5**).

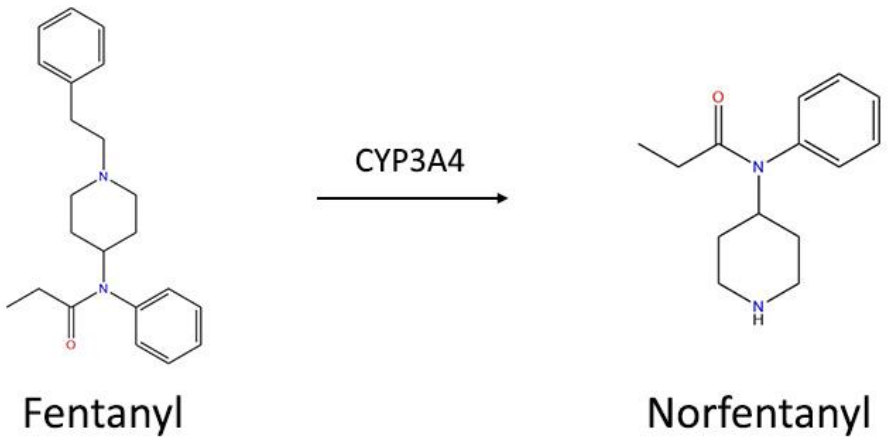


Figure 5 The metabolic pathway of fentanyl

Morphine ((5alpha,6alpha)-17-methyl-7,8-didehydro-4,5-epoxymorphinan-3,6-diol) is an opioid analgesics that naturally exists in the opium poppy.[99] It acts as an agonist for endogenous opioids receptors to achieve pain relief, sedation, euphoria and respiratory depression.[100] As shown in **Figure 6**, morphine is mainly metabolized through glucuronidation to morphine-3-glucuronide (around 60%) and morphine-6-glucuronide (5%-10%).[101,102] The remaining minor portion is metabolized by the CYP enzymes to normorphine.[103]

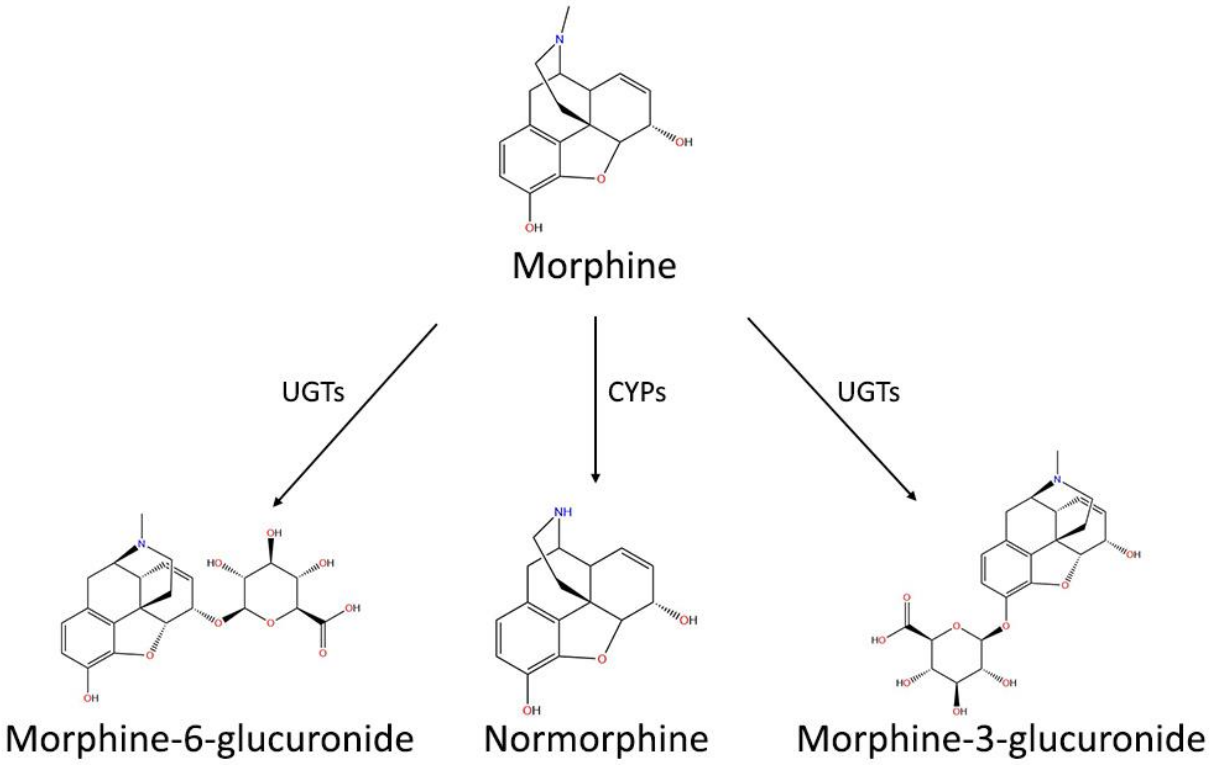


Figure 6 The metabolic pathways of morphine

UGT: UDP-glucuronosyl transferases, main UGTs involved in the metabolic pathways of morphine to morphine-6-glucuronide include UGT2B7, UGT2B4, UGT1A1, UGT1A3, and UGT1A8; main UGTs involved in the metabolic pathways of morphine to morphine-3-glucuronide include UGT2B7, UGT2B4, UGT1A1, UGT1A3, UGT2B15, UGT1A9, UGT1A10, and UGT2B17. CYPs: cytochrome P450 enzymes, main CYPs involved in the metabolic pathways of morphine to normorphine include CYP3A4 and CYP2C8.

Based on the newly established knowledgebase PAIN-CKB, firstly, a computational systems pharmacology target mapping was constructed to identify the known targets and potential off-targets based on the output of HTDocking and TargetHunter for the above four analgesics. Secondly, PBPK models were applied to quantify the DDI between acetaminophen and fentanyl from the PK aspect. Finally, we conducted docking studies to reveal the detailed interaction pattern between acetaminophen reactive metabolite and its reported liver toxicity target thioredoxin reductase 1 (TrxR1).

2.0 METHODS

2.1 PAIN-CKB CONSTRUCTION

2.1.1 Database infrastructure

PAIN-CKB (https://www.cbligand.org/g/pain-ckb) is a one-stop integrated cloud computing server containing analgesics profiles, pain-related protein targets information, and chemoinformatics tools. Users can submitted compounds using JSME Molecular Editor v2017-03-01.[104] PAIN-CKB was constructed on the base our established molecular database prototype DAKB-GPCRs (https://www.cbligand.org/dakb-gpcrs/)[56] using SQLite database management system (https://sqlite.org/) and Kestrel HTTP server (https://github.com/aspnet/KestrelHttpServer) with Apache HTTP server (https://httpd.apache.org/) as its reverse proxy server. To support this new pain-domain knowledgebase we refactored the architecture and added multi-domain support, keyword-based protein search, variable number of target structures support, new webpage layout, etc. The overall workflow of our PAIN-CKB is shown in **Figure 7**.

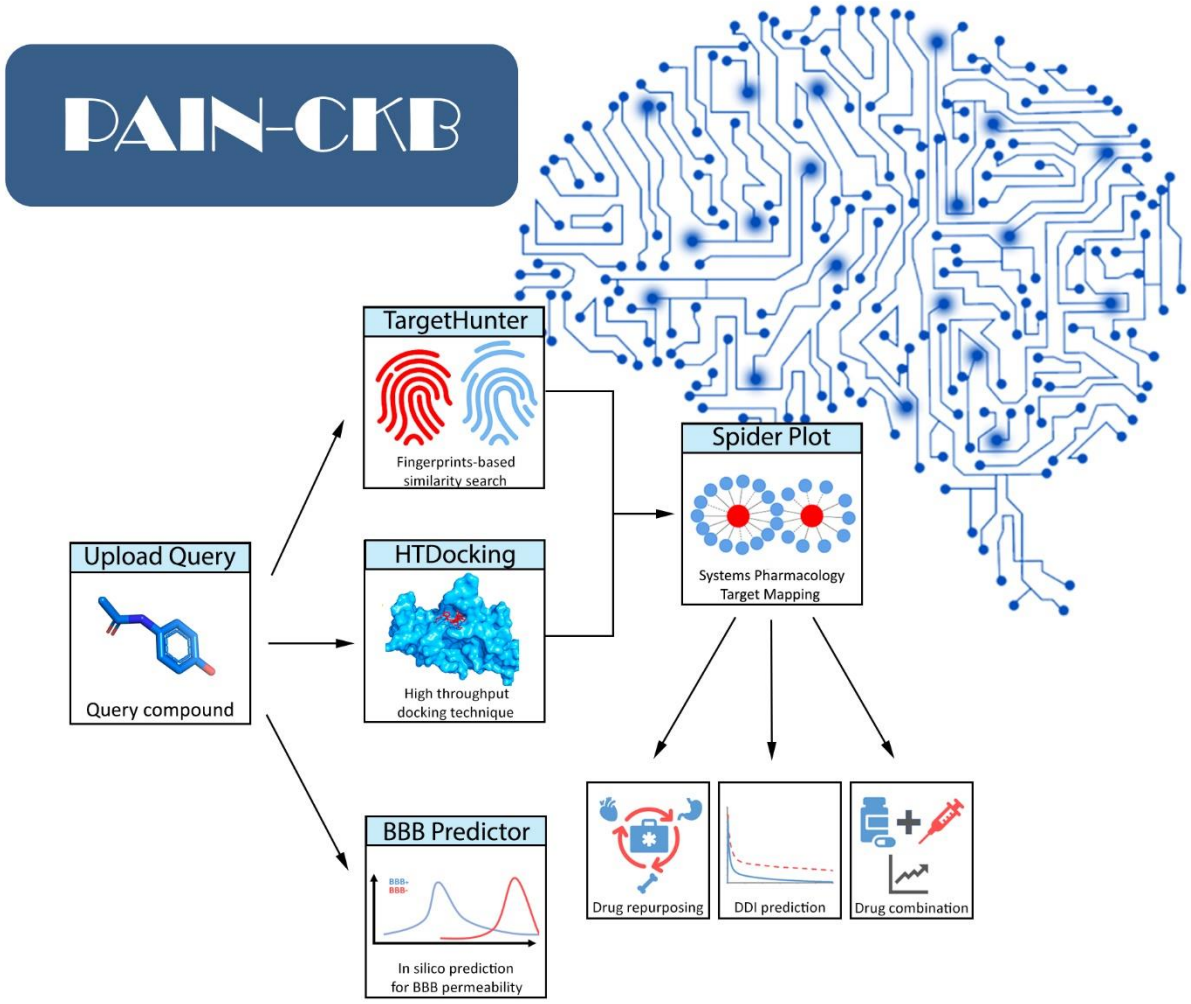


Figure 7 Overview of PAIN-CKB

PAIN-CKB is a one-stop integrated cloud computing server containing analgesics profiles, pain-related protein targets information and chemoinformatics tools with free access to the public. PAIN-CKB is designed to facilitate the research, for example, drug repurposing, DDI prediction, and drug combination studies in pain-related areas

The PAIN-CKB website is compatible with commonly used web browsers (such as Chrome, Firefox, Microsoft Edge, and Safari) provided JavaScript and cookies are enabled. Our PAIN-CKB is free to public users. The newest versions of these browsers are recommended for better user experience.

2.1.2 Genes and proteins

Genes or proteins that are related to pain were extracted from literature research and public databases such as Ensembl (https://useast.ensembl.org/index.html),[105] UniProt (http://www.uniprot.org/ uniprot/),[106] KEGG (https://www.genome.jp/kegg/),[107] GPCRDB (https://gpcrdb.org/),[108] and NCBI Protein Database (https://www.ncbi.nlm.nih.gov/protein/).[109] Protein targets were further divided into four major groups according to the Drug Target Ontology (DTO)[58] as introduced before.

Available 3D crystal structures of pain-related targets were collected from the Protein Data Bank (PDB) (https://www.rcsb.org/). PDB files of homo sapiens were chosen in priority. For those protein targets without homo sapiens' PDB files, homology modeling techniques were applied to replace the incorrect amino acid sequences. PDB files were further processed using PyMol to separate the target proteins and the small molecules binding in the corresponding binding pockets when crystalizing.[110] The binding pocket of the target proteins were then generated using the SYBYL software based on the coordinates of the separated small molecules. For PDB files without small molecules binding to the proteins, the binding pockets were generated automatically in SYBYL and manually selected based on important residues in the

binding pockets reported published literatures. If one protein target possesses more than one binding pocket such as glycine receptor subunit alpha-3 (GLRA3), which owns a ligand binding region and a modulator binding region,[111] different pockets will be defined based on both regions to fit specific purposes of different users.

2.1.3 Homology modeling

Homology modeling, also known as comparative modeling, is a technique to construct atomic models for a protein target based on the sequences and structures of homologous proteins.[112] For those protein targets without homo sapiens PDB files, we applied homology modeling method to build the 3D structure model using Modeler software.[113] The intact amino acid sequence from different species can be downloaded from the UniProt database (http://www.uniprot.org/uniprot/).

To build a homology model for a specific protein target, we start with providing the homo sapiens target sequence and a template structure from another species of that protein. Then the target sequence will be aligned with the sequence of the template structure before the 3D structure model was built. A brief graphic description of the overall workflow of homology modeling is shown in **Figure 8**.

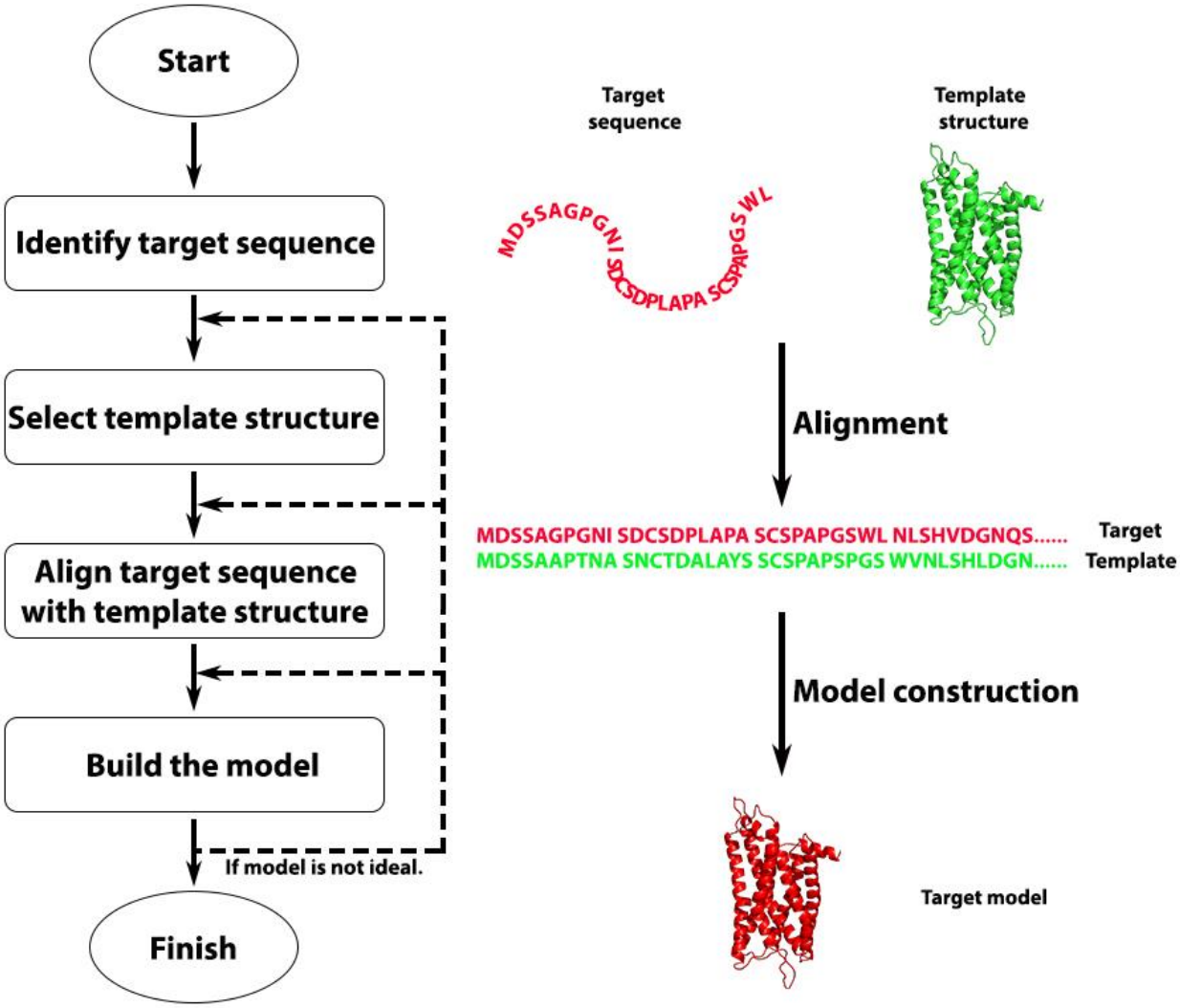


Figure 8 Brief workflow of homology modeling

2.1.4 Drugs and chemicals

All the drugs integrated in PAIN-CKB were collected from the DrugBank database (https://www.drugbank.ca/) filtered under drug categories using the keyword ‘analgesics’. The

drug targets, enzymes, carriers, and transporters information were also integrated in our knowledgebase for reference.

ChEMBL database (https://www.ebi.ac.uk/chembl/) (version 23)[114] was utilized in our work. The experimental data for each small-molecule against and their respective target proteins were collected using text mining techniques and cleaned up by manual inspection. Bioactivity data from different resources were normalized using the same standard.

2.1.5 Computational tools

In order to fulfil the potential of the collected target information and drug data in the PAIN-CKB, several chemoinformatics tools developed by our lab before were integrated in this knowledgebase to identify the therapeutic targets, potential off-targets, physiochemical properties, and ADME (absorption, distribution, metabolism, and excretion) profiles of the query compounds. Equipped with those tools, our PAIN-CKB can be used to facilitate the discovery and development of novel small-molecule analgesics and help investigate and understanding of possible DDIs between analgesics. The fundamental principles of these computational tools were briefly described as below:

HTDocking. PAIN-CKB is equipped with HTDocking, an online high-throughput molecular docking technique, for the identification of possible interactions between protein targets and the user-inputted small molecules. Three different conformations with the highest resolutions were processed as indicated before for each target/protein, depending on the availability of PDB files (if the currently available PDB files are less than three, then this number will shrink accordingly). Briefly, HTDocking will automatically dock each query compound into

the previously defined binding pockets of these different conformations and generate at most three independent docking scores. A higher the docking score indicates the more likely the query compound will be the ligand of the protein target of interest. The HTDocking algorithm is established based on the iDock algorithm[115], which can provide predicted binding affinity values (ΔG values) based on independent docking poses for each compound when interacting with different proteins. The HTDocking program only consider the best binding affinity value. Our PAIN-CKB can further transfer the affinity values to docking scores using the following equation: Docking score = -log10 (eΔG*4184/8.314/310.15).

TargetHunter. The second powerful web-interfaced chemoinformatics tool integrated in PAIN-CKB is called TargetHunter, which can be used to predict the potential of the submitted compounds to interact with the therapeutic targets of interest.[85] TargetHunter is constructed based on an fundamental principle of medicinal chemistry, that is compounds have similar physicochemical profiles or biological properties always share structural similarities. The query compounds will be first converted to molecular descriptors, which can be understood as numerical values that represent the structural information, physical properties, chemical reactivity, and biological activity of chemical compounds of interest, for these compounds to be treated mathematically by computers.[116]

More specifically, the molecular descriptors used in TargetHunter are various molecular fingerprints: Extended Connectivity Fingerprint 6 (ECFP6), ECFP4, and FP2 molecular fingerprints.[117,118] The detailed form of different molecular fingerprints can be different from each other but the overall fundamental concepts between these methods are identical. **Figure 9** provides a graphic explanation of the ECFP method. TargetHunter will automatically calculate the similarity score for each inputted compound based on the molecular fingerprints using the

Tanimoto coefficients (from 0.0-1.0, totally different to totally the same) with its known active compound's dataset collected from Drugs and Chemicals. For each target, the algorithm will give the highest similarity score between the query compound and all the known compounds interacting with that specific protein target.

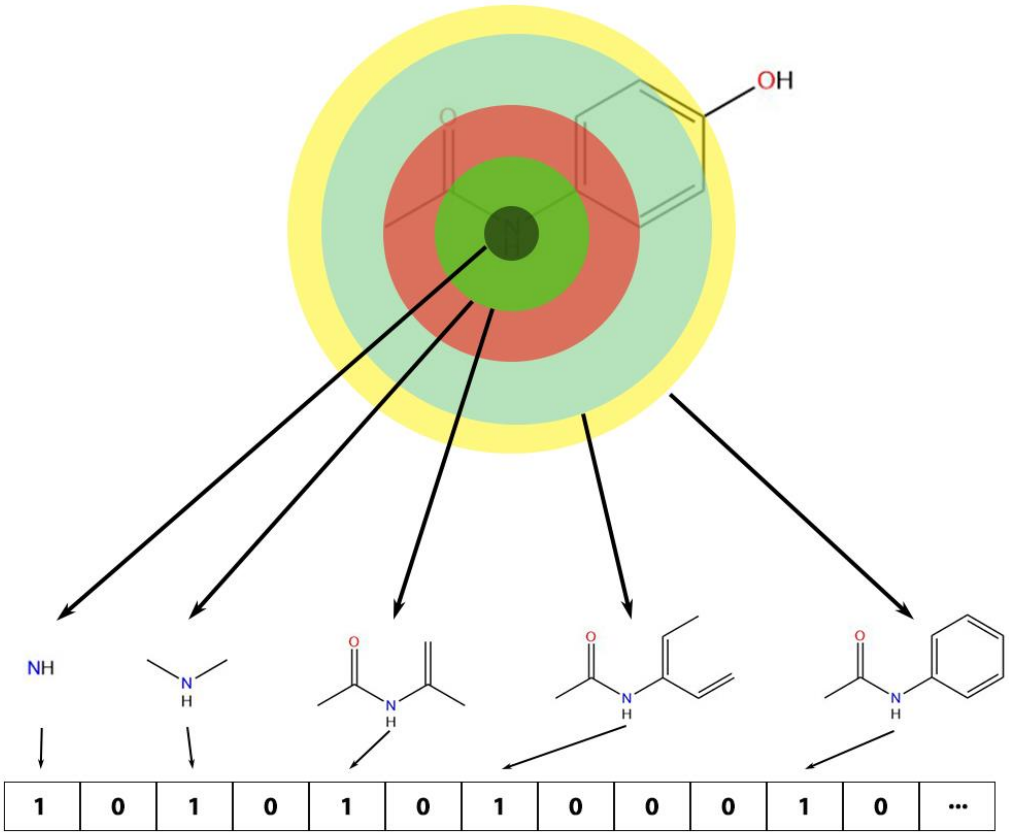


Figure 9 Graphic description of the principle of molecular fingerprints

BBB Predictor. We also integrated our blood-brain barrier (BBB) predictor into PAIN-CKB as well. This predictor was built based on the support vector machine (SVM) and

LiCABEDS[119,120] algorithms on four types of fingerprints of 1593 reported compounds.[121] It can predict the ability of a query compound to penetrate across the BBB to the central nervous system (CNS).[122] Besides our knowledgebase, the BBB predictor can also be visited at https://www.cbligand.org/BBB/.

Spider Plot. The last algorithm implanted in PAIN-CKB is Spider Plot, which is able to construct the interaction network map between molecules and proteins based on the output of HTDocking and TargetHunter. The averaged docking scores are shown as labels and the predicted protein targets of the query compounds are presented using circular dots. By default, the green nodes indicate targets with high similarity scores (> 0.7) comparing the best matched known compound and the query compound, while magenta ones denote the predicted off-targets. Users can customize the font sizes, node sizes, colors, border widths, node shapes as well as the layout completely within Spider Plot. Users can export the generated network maps as image files on particular browsers. Through the spider plots, users can easily get some primary hints about whether there are common targets between different molecules and combine other methods to investigate the possible DDIs.

2.2 TARGET MAPPING CONSTRUCTION

DDI is a very common yet life-threatening phenomenon in clinical situation.[123] To better detect and present the possible DDIs related to analgesics, we conducted a computational systems pharmacology mapping using Cytoscape (version 3.7.1)[124] for four commonly used analgesics (acetaminophen, diclofenac, fentanyl, and morphine) based on the results of HTDocking and TargetHunter. Cytoscape is an open source bioinformatics platform which can

be used to construct and visualize the interaction network between genes, proteins, and drugs. Users can easily modify the network to better cater their specific needs. In this study, drugs are indicated using circles, the nodes are used to represent the protein targets, and the interactions are denoted using lines. Specifically, the green nodes and the solid lines indicate those confirmed drug therapeutic targets and known interaction. The magenta nodes and dashed lines indicate those potential off-targets and predicted drug-protein interactions.

2.3 MOLECULAR DOCKING

Molecular docking is a commonly used method to predict the possible orientation and the detailed interaction pattern between a small molecule and its protein target.[125] The docking results can provide us information about the critical residues or functional groups responsible for the biding between the molecule and target and further guide the de-novo rational-based drug design process.[126] The docking algorithm is used in this study to reveal the interaction between the small-molecule analgesics (acetaminophen and fentanyl) and their common target (CYP3A4 (PDB ID: 4K9T) and ABCB1 (PDB ID: 6FN1)) as well as the active metabolite NAPQI and its reported hepatotoxicity target TrxR1 (PDB ID: 2ZZ0).

Docking analysis was conducted using Chimera (Version 1.13.1)[127] in this study. Chimera is a program used for visualizing and analyzing the protein and molecule structures. Energy minimization was first conducted on both the protein targets and small molecules analgesics using AMBER ff14SB force field.[128] After that, docking was ran using AutoDock Vina[129], which is integrated in Chimera. The docking workflow is shown in **Figure 10**. The output contains multiple possible docking poses. The final poses were manually selected based

on both the docking scores (the lower the better), the degree of overlap between the binding results and crystalized ligand, and the key residue contributions.

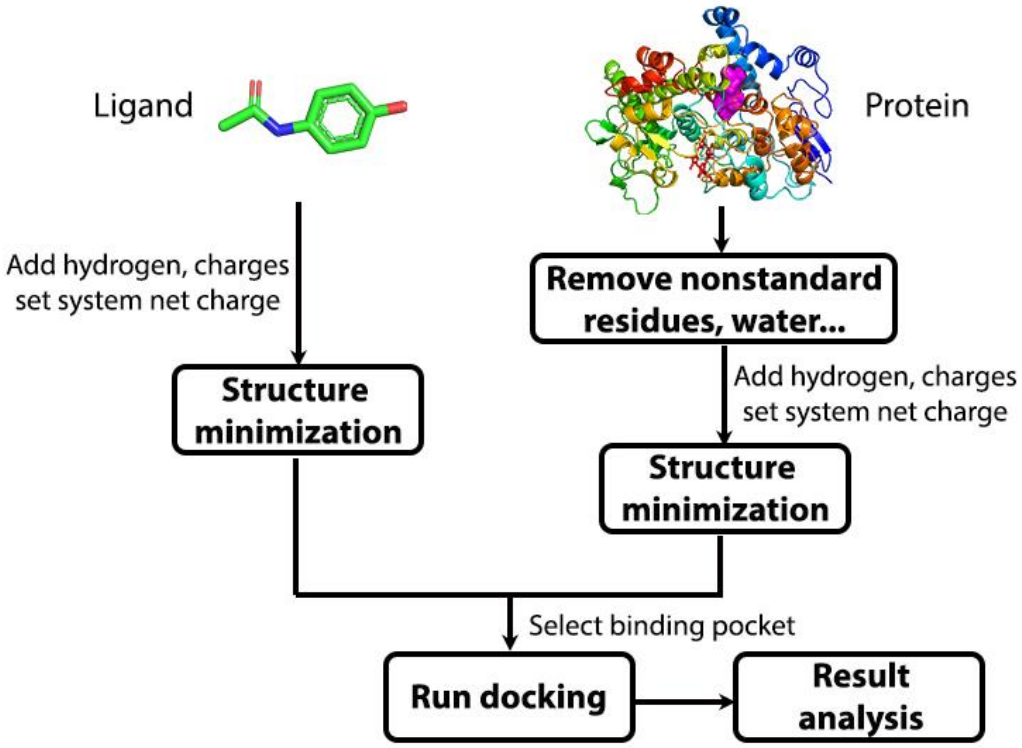


Figure 10 The docking workflow

2.4 PBPK MODELING

In this study, we tried to use Simcyp Population-based ADME Simulator[130] to construct PBPK models to investigate and explain the DDI between acetaminophen and fentanyl as indicated by our target mapping results from the PK aspect. Simcyp is an in-silico population-based simulation platform developed by the Simcyp Limited, Sheffield, UK.[131] It utilizes the

bottom-up method to build the mechanism-based PBPK models to predict the ADME profiles of drug compounds.

In Simcyp, the PBPK model is separated into systems data and compound/drug data. In this study, we used healthy volunteers as our simulation population. The parameters in systems data are kept default. As to the compound data, the required parameters of the PBPK model were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov)[132] or from previously published data if available and if not, we used Simcyp software to predict the missing parameters.

The simulations were conducted upon 10 trials of 10 virtual healthy volunteers. The mean value of systematic drug concentration in plasma were generated as well as the upper 95th percentile and the lower 5th percentile of the simulation range. The constructed models were validated using the PK profiles from previously published papers before being applied to investigate the possible DDI.

2.4.1 Full PBPK modeling

A full PBPK model consists of explicit compartments that indicated the organs of the human body critical to the ADME process of the drug for their intrinsic physiological functions. Organ compartments simulated include the lung, adipose tissue, bone, heart, kidney and blood. The separate organ compartments are linked by the circulating blood system. The parameters for each compartment include anatomy parameters such as the organ capacities, blood flow rates, and tissue/blood partition coefficients.[133] Based on the time-based differential equations, full PBPK models are able to simulate the drug concentrations in different organ compartments. The overall architecture of a typical full PBPK model is shown in **Figure 11**.

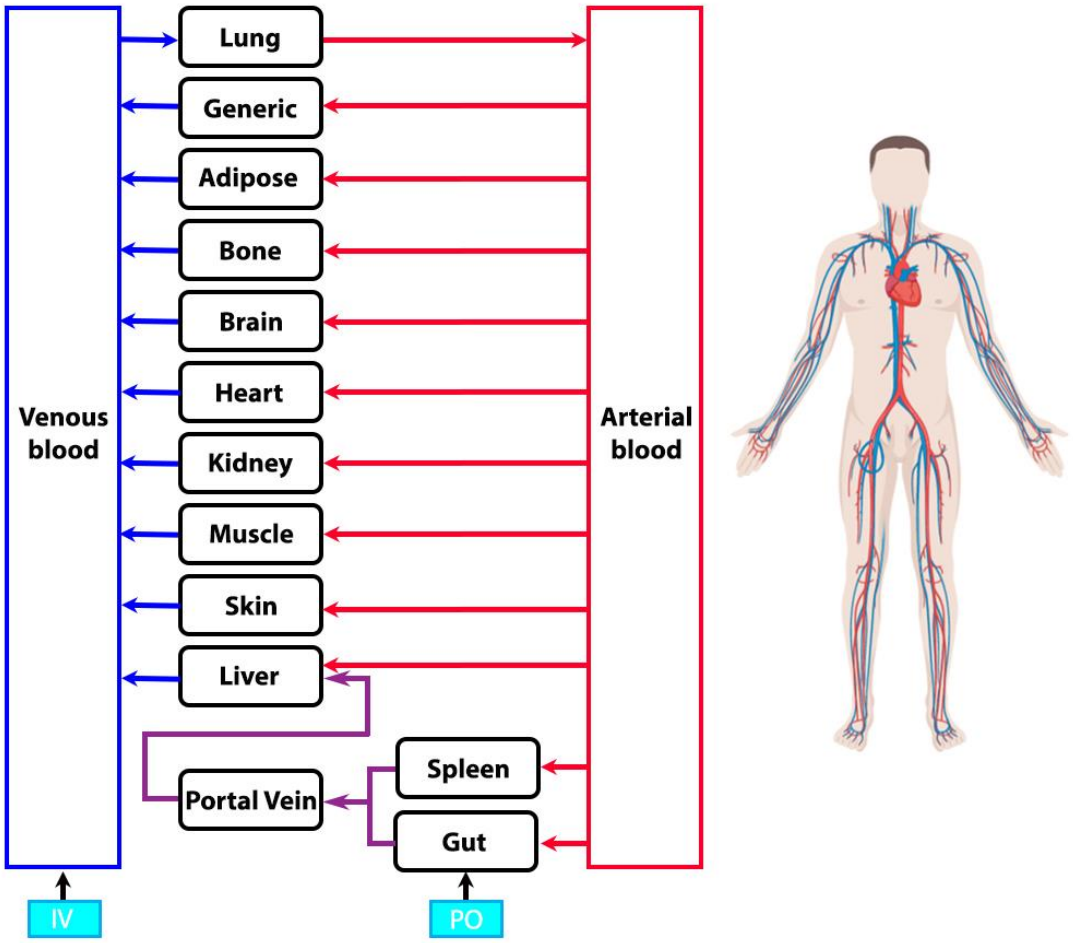


Figure 11 Full-PBPK model

Representation of the overall structure of a full PBPK model. The blue and red lines with arrows indicate the venous and arterial blood flow directions. IV: intravenous therapy; PO: oral administration.

In this project, we used the full PBPK modelling method to construct the fentanyl model in Simcyp. Factors like age, sex, height and body weight can contribute to the interindividual variability. According to the literature report, the metabolism of fentanyl in human body is mainly mediated by CYP3A4 pathway (around 90%). The renal clearance also accounted for less

than 10% of fentanyl elimination. Contribution from other clearance pathways are negligible, so this part of metabolism was not considered in this model.

2.4.2 Minimal PBPK model

The full PBPK model takes all the major compartments in the human body, which can better fit the experimental data but collecting all the parameters required can be a very demanding task. An alternative way is to use a minimal PBPK model. The major difference between full PBPK model and minimal PBPK model is that the full PBPK model considers the drug concentration in different compartments separately while the minimal PBPK model assumes that the drug concentration in plasma is identical to that of organ tissues (exclude liver).[134] The minimal PBPK model only considers the core compartments needed for constructing a PBPK model. Thus, the minimal PBPK model inherits the major advantages for being a mechanism-based model from whole-body PBPK models while requires relatively fewer input parameters.[134,135] **Figure 12** is the paradigm of a minimal PBPK model.

Acetaminophen is mainly metabolized in the liver, which is covered by the minimal PBPK model, and here we are mainly interested in the blood or plasma time-concentrations curves. Thus, the acetaminophen model was built using the minimal PBPK model with four basic compartments (central, liver, gut, and single adjusting compartment (SAC)) in Simcyp. The SAC is a virtual organ compartment with physiological parameters which can be adjusted arbitrarily to account for the influence caused by all the other organs on the drug PK profile.[136] The total systemic clearance was used to describe the metabolism of acetaminophen, and the percentage of drugs metabolized by CYP3A4 is around 5% to 10%.[137] In addition, the inhibition effect of

acetaminophen on the CYP3A4 enzyme is considered as the main reason for the DDI between acetaminophen and fentanyl in the PK level. The inhibition constant K_i (Ki = 2800 μM) of acetaminophen was collected from the literature.[138]

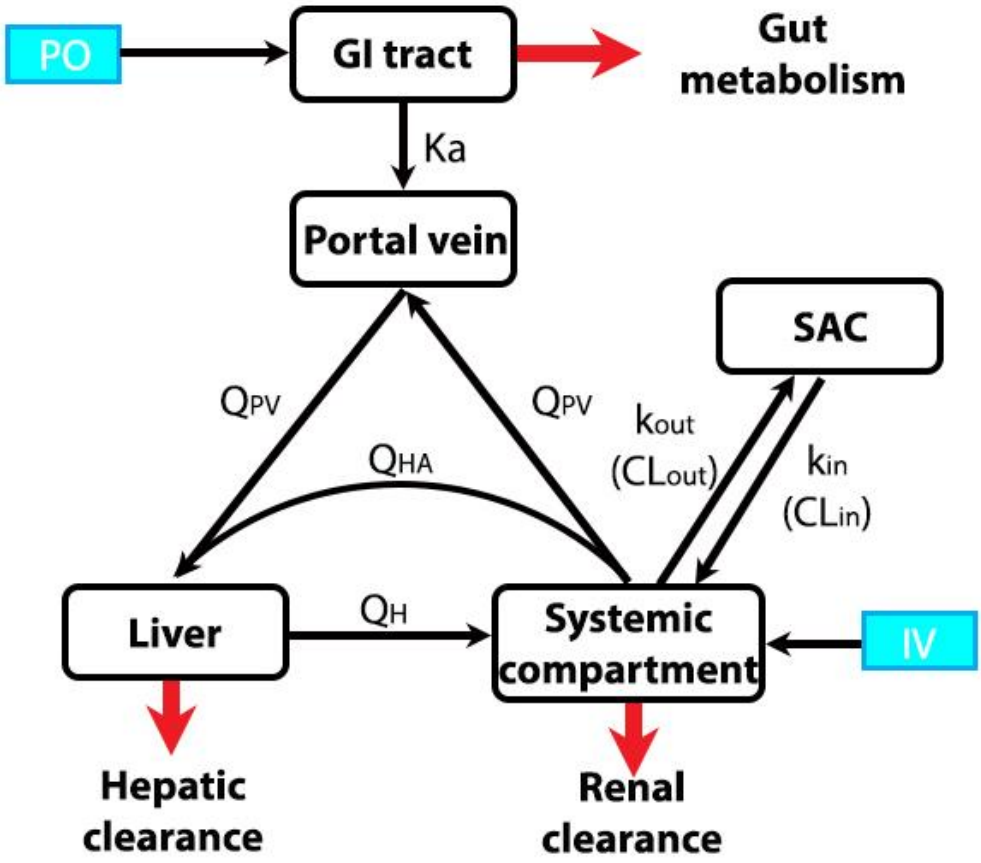


Figure 12 Minimal PBPK model

SAC: single adjusting compartment; Q_H: blood flows in the liver; Q_{PV}: blood flows in the portal vein; Q_{HA}: blood flows in the hepatic artery; k_{in} and k_{out} are first order rate constants of the masses of drug within the systemic compartment and the SAC; IV: intravenous therapy; PO: oral administration.

2.4.3 PBPK model validation

Before applying these models to investigate the possible DDI between acetaminophen and fentanyl, we first used experimental data to validate if the newly-constructed PBPK models can predict the PK profiles correctly. The experimentally observed plasma concentration-time data of acetaminophen and fentanyl were extracted from a previously published paper as a validation of the reliability of PBPK models. The simulation dosing regimen and administering route were selected based on the same literature where the experimental data comes from to wipe out unnecessary influence caused by those factors. The simulated plasma concentration profiles were generated upon 10 trials of 10 virtual healthy volunteers. The arithmetic mean prediction values and 95th/5th percentile values of simulated results were compared with the experimental data. The parameters related to absorption were ignored as all the drug administration routes selected in this study are iv bolus in order to keep consistency with previously published literature.

3.0 RESULTS

3.1 OVERVIEW OF PAIN-CKB

To date, PAIN-CKB archived 272 analgesics and 84 pain-related targets. All the collected pain-related targets are listed in **Table 7** in appendix. These targets were further divided into four main groups following the criteria introduced before. As shown in **Figure 13**, the targets collected were separated into: (1) 28 G-protein coupled receptors (GPCR), such as mu, kappa, delta-opioid receptor (OPRM, OPRK, OPRM), cannabinoid receptors (CNR1, CNR2); (2) 32 enzymes, such as Prostaglandin G/H synthases (PGH1, PGH2), angiotensin-converting enzyme (ACE); (3) 14 ion channels, such as transient receptor potential cation channels (TRPV1, TRPV2, TRPV3, TRPM4, TRPM8); and, (4) 2 nuclear receptors: progesterone receptor (PRGR) and estrogen receptor1 (ESR1). The PAIN-CKB will be continuously updated to keep the validity and comprehensiveness of the information collected. Also, we identified 169,321 chemical agents reported for these target proteins from ChEMBL database, which includes 39006 inactive compounds, 26704 intermediate compounds, and 103611 active compounds for further reference.

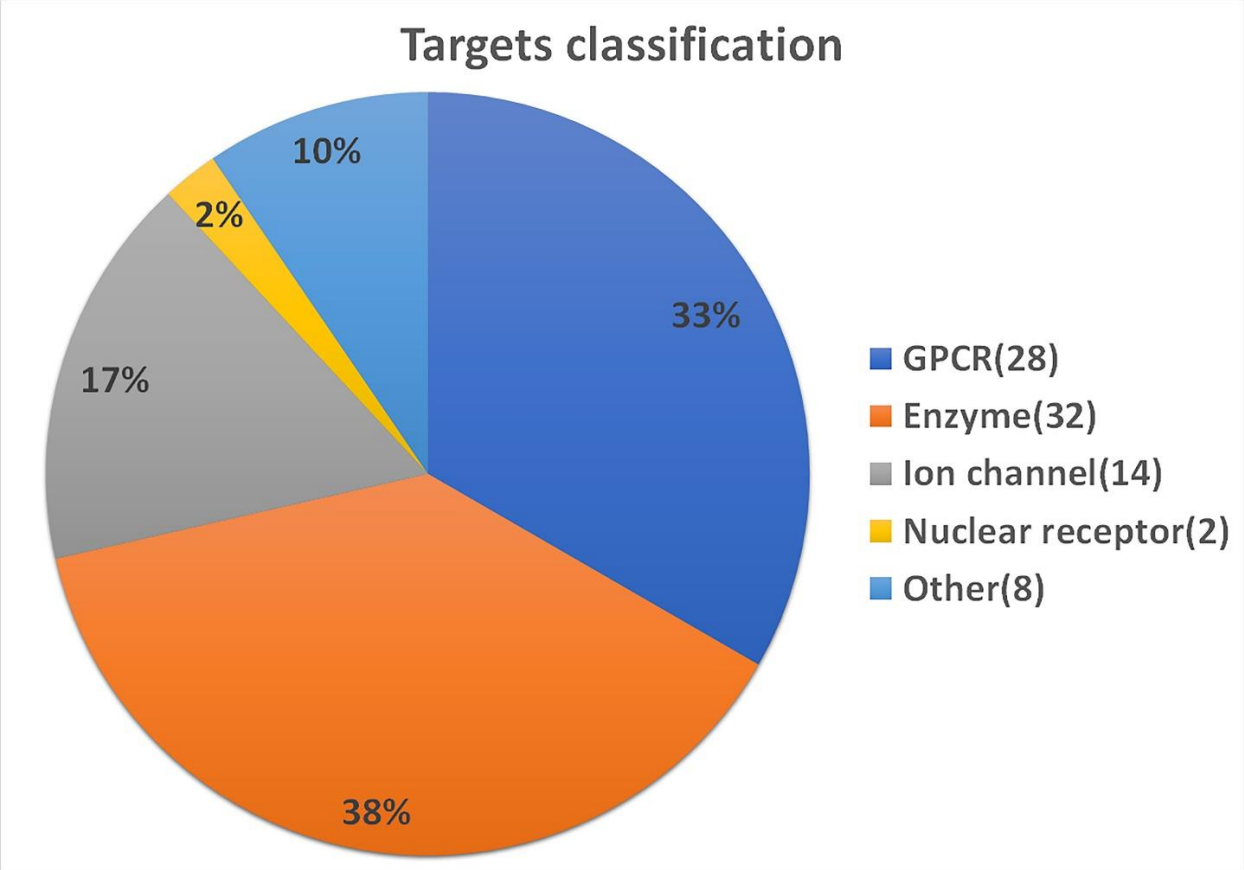


Figure 13 Summary of pain-related targets in PAIN-CKB

A total of 84 pain-related targets are collected in the PAIN-CKB: (1) 28 G-protein coupled receptors, (2) 32 enzymes, (3) 14 ion channels, (4) 2 nuclear receptors, and (5) 8 other targets categorize.

The equipped chemoinformatics tools like TargetHunter, HTDocking, BBB predictor, and Spider Plot in PAIN-CKB provide public users with cloud computing services for target identification and systems pharmacology research. The knowledgebase enables two usages: protein search and query compound computation. The protein search is a keyword-based full-text search for the best-matched pain-domain targets in the database. The query compound computation accepts a small molecule (in SMILES or other common formats) and emits for each

pain-domain target in our database both the docking scores in the binding to the known X-ray / Cryo-EM structures and the most similar experiment-proved active compound. All ~90,000 known experimented compounds for the DAKB-GPCRs database[56] plus 3,000 compounds from Zinc database[139] were tested (~500,000 docking runs in total) before the server first released.

To start a new calculation job, users can click on the 'Create a new job' bottom from any page of PAIN-CKB. After naming the new job, users can submit the small-molecule structure by either draw the molecular structure or paste the MOL, SDF or SMILES format of the compound through the drop-down menu of the blue double-triangle icon located in the toolbar. Users can upload up to 5 small-molecules in one single job to explore the drug-target interaction network between them. After modifying and confirming your submitted job, hit on the 'Create Job' bottom then your job will be processed automatically (as shown in **Figure 14**).

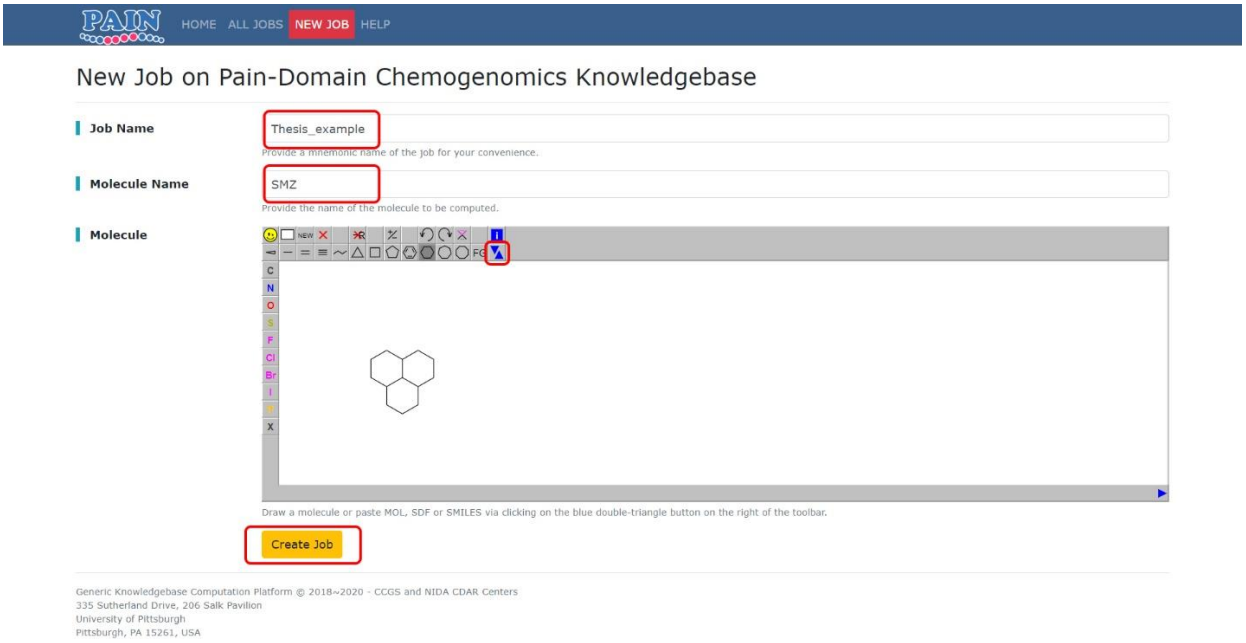


Figure 14 Create a new job in PAIN-CKB

While the new job is under processing, as shown in **Figure 15**, users can navigate the web page to see the ligand structure models, SMILES and various molecular fingerprints of the submitted small-molecule. Once the calculation is finished, the docking score and similarity score of the inputted ligand on each protein target will be listed near the respective protein. Users can switch between table format output (**Figure 16**) or card format output (**Figure 17**) for convenience. Also provided in the output page are some useful links to other webpages such as Uniport and PDBe[140] to information about the protein, to download models or to compare between best matched known molecule. The spider plot will be generated automatically based on the calculation result. In this case, our algorithm predicates that the example compound SMZ is not a potential ligand for any of the collected protein targets in the PAIN-CKB (**Figure 18**).

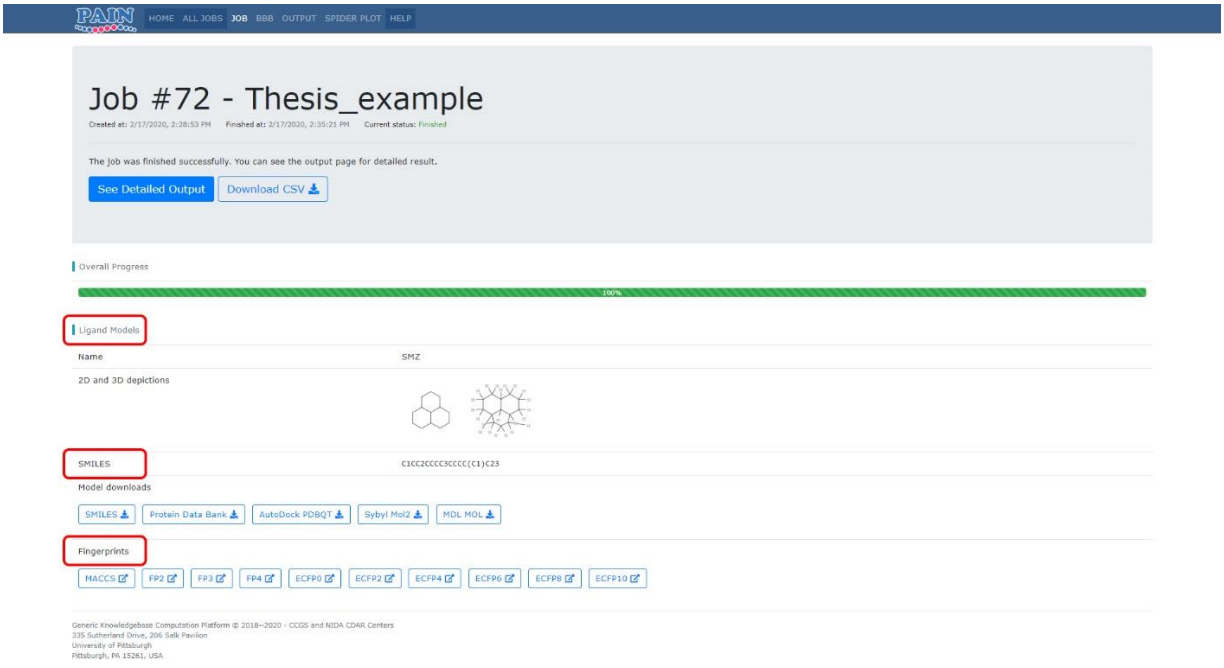


Figure 15 The processing page of PAIN-CKB

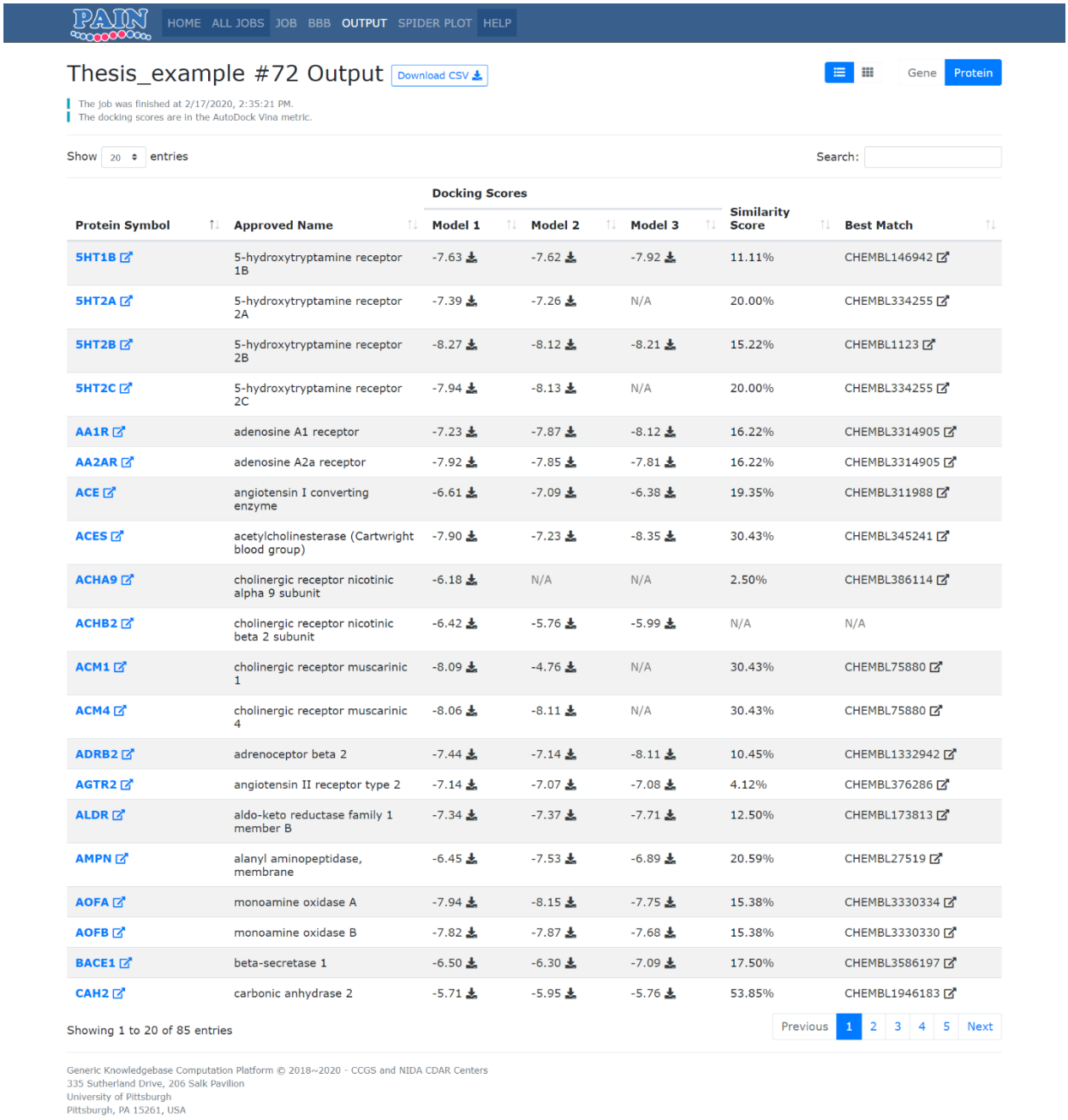


Figure 16 Example output of PAIN-CKB in table format

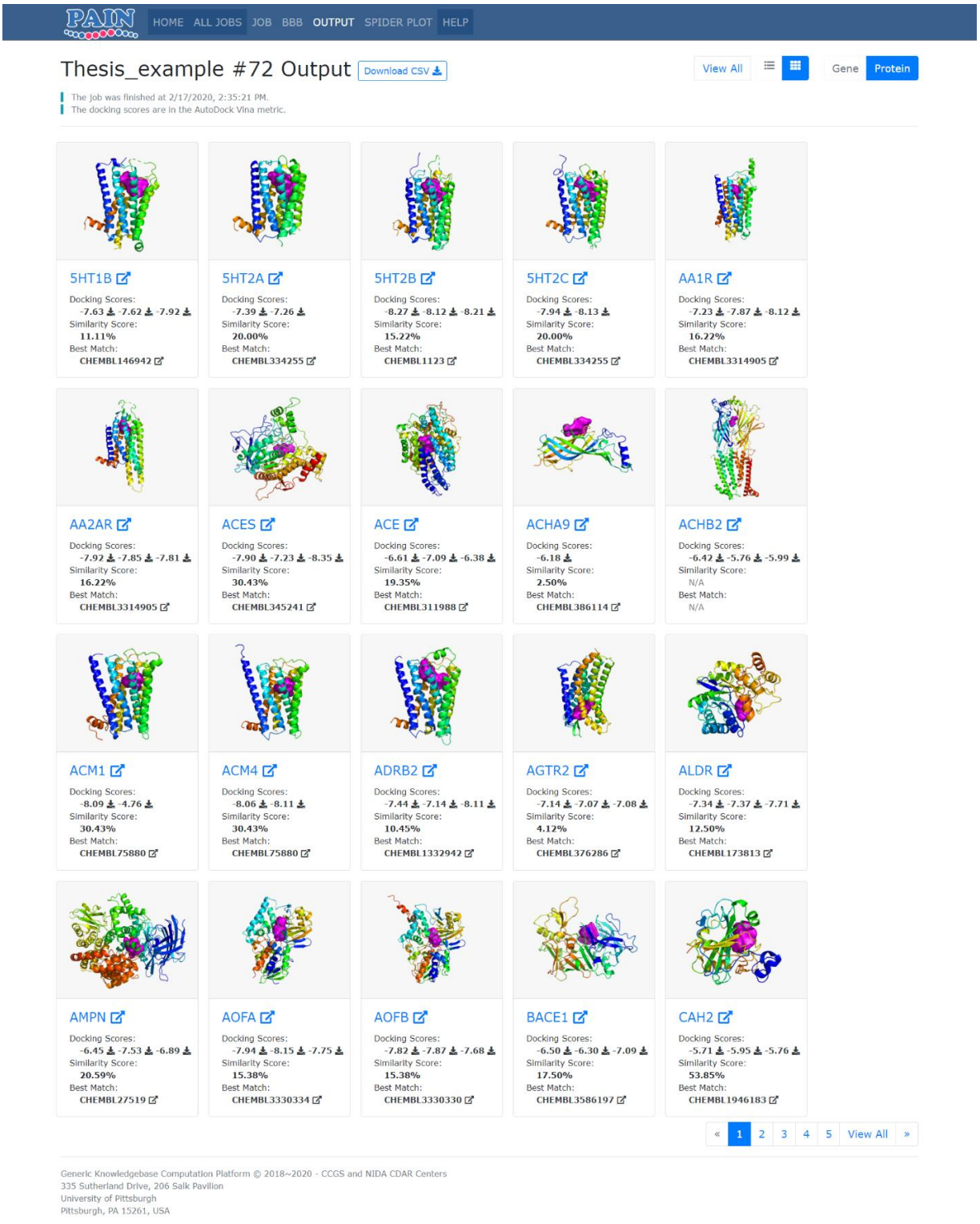


Figure 17 Example output of PAIN-CKB in card format

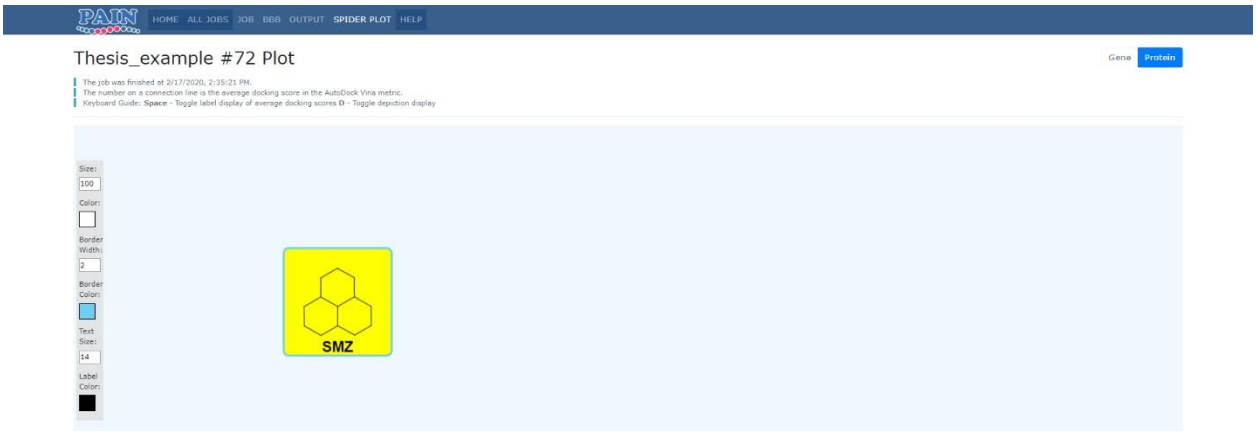


Figure 18 Example output of spider plot in PAIN-CKB

3.2 TARGET MAPPING FOR FOUR ANALGESICS

We conducted a series of pilot studies to demonstrate the functionality of our PAIN-CKB and how computation algorithms can aid the research in pain-related areas. First of all, we conducted a computational systems pharmacology-based study to generate a target mapping for four analgesics approved by the FDA (acetaminophen, diclofenac, morphine, and fentanyl). The prediction of polypharmacology of known drugs is a potential method for drug repurposing and DDI prediction.[141,142] Among those four drugs, though acetaminophen works by interacting with COX enzymes,[143] it is categorized as aniline analgesics because of the limited inhibition effect of COX enzymes[144] while diclofenac is a typical NSAID.[93] On the other hand, morphine and fentanyl are members of the opioid analgesics family which exhibit their pain-relieving effect by binding to opioid receptors to mimic the endogenous opioid peptides.[145] As shown in **Figure 19**,

after integrating the information included in the PAIN-CKB and the results of TargetHunter and HTDocking algorithm, we were able to generate a target mapping for these four drugs. The green dots and solid lines indicate the confirmed protein targets and interactions between the corresponding drugs and protein targets. The magenta dots and dashed lines represent the predicted protein targets with interactions.

As expected, our results showed clearly that the COX enzymes were targeted by both acetaminophen and diclofenac. For acetaminophen, transient receptor potential cation channel subfamily V member 1 (TRPV1), which is another known target for acetaminophen in the brain to produce antinociception,[146] is also listed in our result. Moreover, PAIN-CKB also predicted several acetaminophen off-targets such as nitric oxide synthase 1 (NOS1) and carbonic anhydrase 2 (CAH2). As to diclofenac, apart from the COX enzymes, possible off-targets like acetylcholinesterase (ACES) and C-X-C chemokine receptor type 1 (CXCR1), have been reported to interact with diclofenac analogs.[147,148]

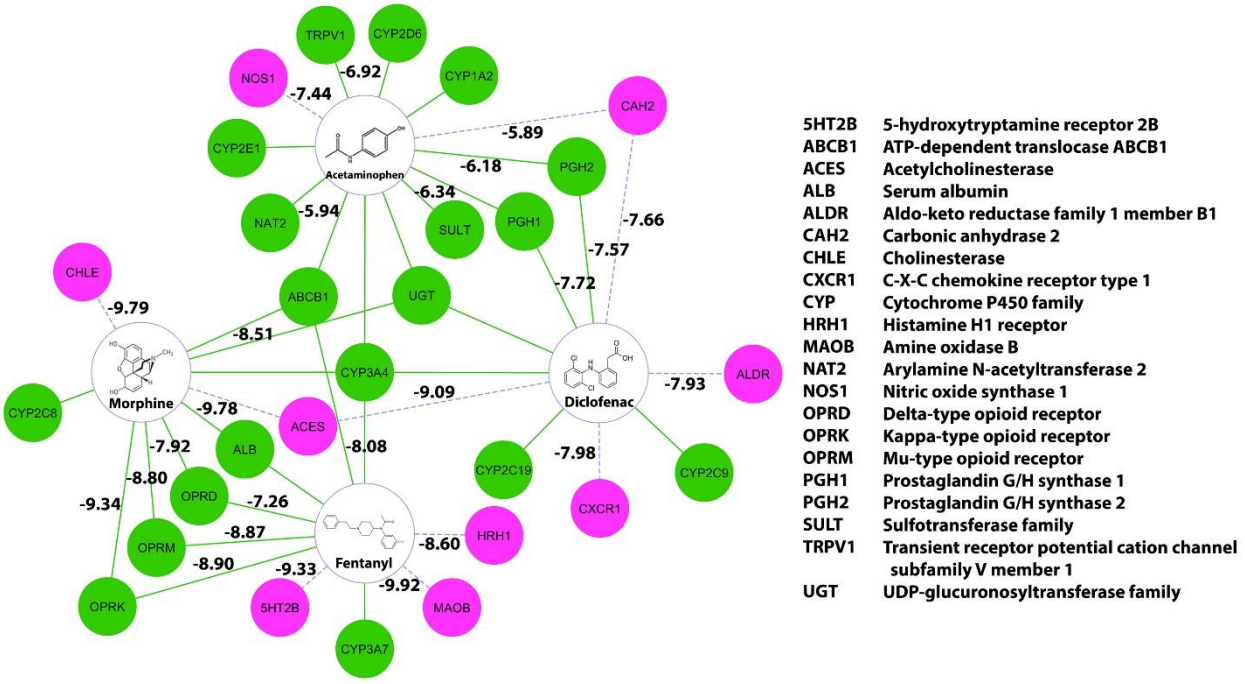


Figure 19 Target mapping for four analgesics

Computational systems pharmacology-target mapping (CSP-Target Mapping) for acetaminophen, diclofenac, morphine, and fentanyl. The green dots and solid lines indicate the confirmed targets and interaction. The magenta dots and dashed lines indicate the predicted targets and interaction.

For morphine and fentanyl, known targets such as mu, kappa, and delta-opioid receptors[149] were all successfully identified by PAIN-CKB. Our algorithm also predicted cholinesterase (CHLE) and ACES as potential targets for morphine. Surprisingly, both CHLE and ACES were reported to be inhibited by morphine[150] and its analogs[151] in the literature. Finally, the prediction that MAOB and HRH1 are potential targets for fentanyl is consistent with the ChEMBL database. This target map serves as a compelling example showing the reliability of our TargetHunter and HTDocking algorithm. Though at this time, other predicted targets in this target map have not be experimentally validated, we strongly believe they have the potential

to be confirmed as new targets for their corresponding drugs and here we highly encourage other peers interested in this result to validate our predictions through experiments.

3.3 INVESTIGATION OF THE DDI BETWEEN ACETAMINOPHEN AND FENTANYL

From the previous target mapping result, we found there are two common targets, cytochrome P450 3A4 (CYP3A4) and ATP-dependent translocase (ABCB1), between acetaminophen and fentanyl, suggesting there might exist a DDI between these two drugs. CYP3A4 is a well-known enzyme responsible for the phase I metabolism of many drugs and compounds such as steroids, fatty acids, and xenobiotics.[152] ABCB1 is a transmembrane active efflux pump for a wide range of drugs.[153] We then conducted literature research and found articles reporting that acetaminophen can inhibit the metabolism of fentanyl by CYP3A4 inhibition in vitro[138] and exhibit significant fentanyl-sparing effect in vivo.[154] However, there is no direct evidence indicating the DDI is related to ABCB1.

3.3.1 Docking analysis

To gain an insight into the detailed interaction between the small-molecule drugs and their protein targets at the molecular level, we first conducted docking studies between CYP3A4 and these two drugs. The binding poses and the energy contributions of each residue are shown in **Figure 20** and **Figure 21**, respectively. The key residues in the CYP3A4 ligand-binding pocket include Thr309, Phe304, Phe215, Phe241, Phe108, Ser119, Arg105, and HEME.

As acetaminophen is a relatively small-molecule, it is not likely to interact with most of the key residues. The binding pose of acetaminophen in the binding pocket of CYP3A4 allows the formation of two hydrogen bonds: one is observed between the hydroxyl group of acetaminophen and the HEME structure of CYP3A4 (2.8 Å) and another between the acetamide group of acetaminophen and Arg105 residue (2.9 Å). The benzene ring of fentanyl faces the HEME structure of the CYP3A4, forming one hydrophobic interaction between these two structures (4.6 Å). Other hydrophobic interactions can also be found between the drug and Thr309 (4.8 Å), Phe304 (4.8 Å), Phe215 (3.7 Å), and Phe241 (3.6 Å). One hydrogen bond with a binding distance of 3.3 Å formed between the oxygen on fentanyl and Phe108 residue of CYP3A4.

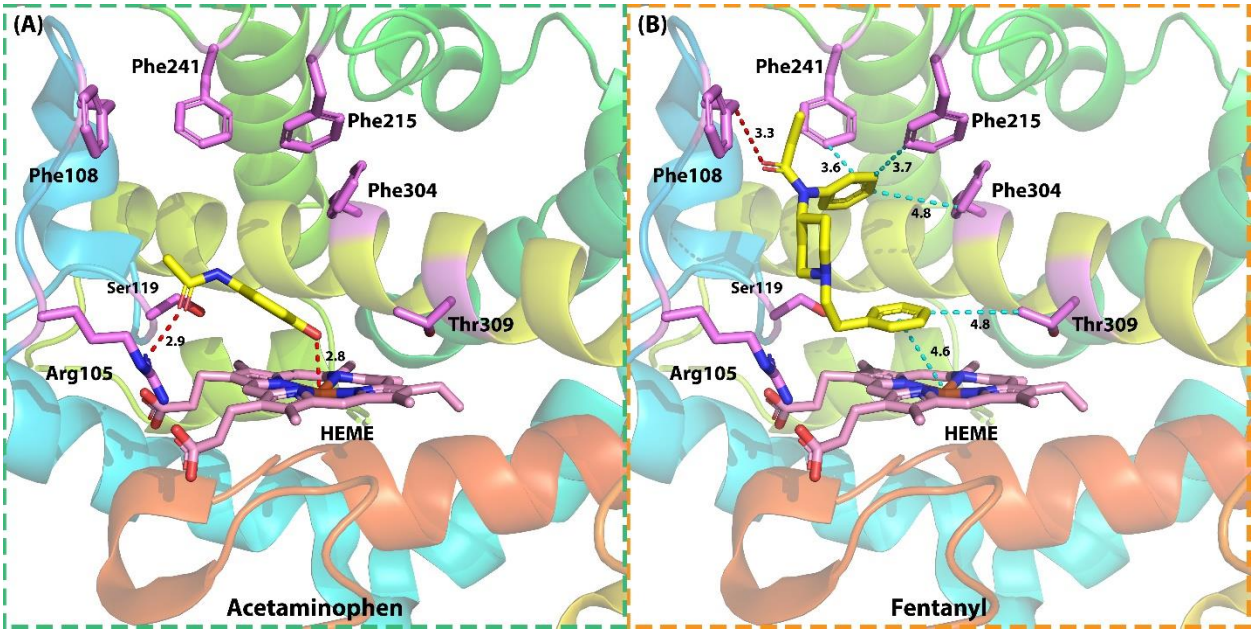


Figure 20 Interaction patterns of (A) acetaminophen and (B) fentanyl in CYP3A4

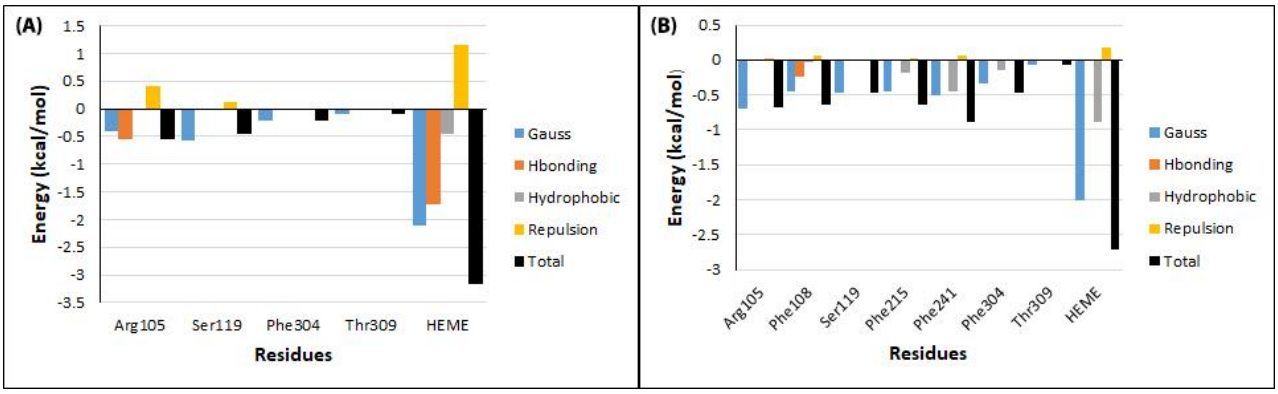


Figure 21 Residue energy contribution of (A) acetaminophen and (B) fentanyl in CYP3A4

Figure 21 and the following table provide some intuitive illustrations of the detailed energy contribution of the key residues in the binding between acetaminophen (**Table 1**), fentanyl (**Table 2**) and CYP3A4. The most important residues for acetaminophen to bind with CYP3A4 are Arg105 (-0.5604 kcal/mol) and HEME (-1.7225 kcal/mol through hydrogen bond and -0.4635 through hydrophobic interaction). Both of them can form hydrogen bond with the ligand. As for the binding of fentanyl, Phe108 contributes -0.238 kcal/mol mainly through forming the hydrogen bond with fentanyl. The binding energy contribution of Phe215, Phe241, Phe304, and HEME mainly comes from the hydrophobic interactions (-0.1902, -0.4416, -0.1401, and -0.8809 kcal/mol, respectively).

Table 1 Detailed residue energy contribution (kcal/mol) between CYP3A4 and acetaminophen

Residue	Gauss	H-bonding	Hydrophobic	Repulsion	Total
Arg105	-0.4094	-0.5604	0	0.4101	-0.5597
Ser119	-0.5689	0	0	0.1162	-0.4526
Phe304	-0.2081	0	0	0	-0.2081

Thr309	-0.0997	0	0	0	-0.0997
HEME	-2.1223	-1.7225	-0.4635	1.1446	-3.1636

Table 2 Detailed residue energy contribution (kcal/mol) between CYP3A4 and fentanyl

Residue	Gauss	H-bonding	Hydrophobic	Repulsion	Total
Arg105	-0.6988	0	0	0.0116	-0.6872
Phe108	-0.4497	-0.238	-0.0257	0.0678	-0.6456
Ser119	-0.4737	0	0	0	-0.4737
Phe215	-0.4568	0	-0.1902	0.0027	-0.6443
Phe241	-0.5166	0	-0.4416	0.0692	-0.889
Phe304	-0.3271	0	-0.1401	0	-0.4671
Thr309	-0.0651	0	-0.0135	0	-0.0786
HEME	-2.0051	0	-0.8809	0.1797	-2.7064

For the other overlapped target ABCB1 between acetaminophen and fentanyl, the docking results for the important residues on this target protein and their energy contributions are shown in **Figure 22** and **Figure 23**. Key residues in the ABCB1 binding pocket are Tyr306, Tyr309, Phe335, Phe342, Gln724, Phe977, and Val981. Acetaminophen formed two hydrogen bonds between the hydroxyl group and Tyr306 (3.1 Å), Tyr 309 (2.8 Å) residues. Three hydrophobic interactions can also be observed at Phe335 (4.2 Å), Phe977 (3.8 Å) and Val981 (3.6 Å). For fentanyl, during its binding with ABCB1, one hydrogen bond was formed with oxygen on the amide group of Gln724 (3.3 Å), What's more, five hydrophobic interactions can also be detected between the drug molecule and Phe335 (5.0 Å), Tyr306 (3.4 Å), Phe342 (3.7 Å), Phe977 (3.5 Å), and Val981 (4.0 Å) as well.

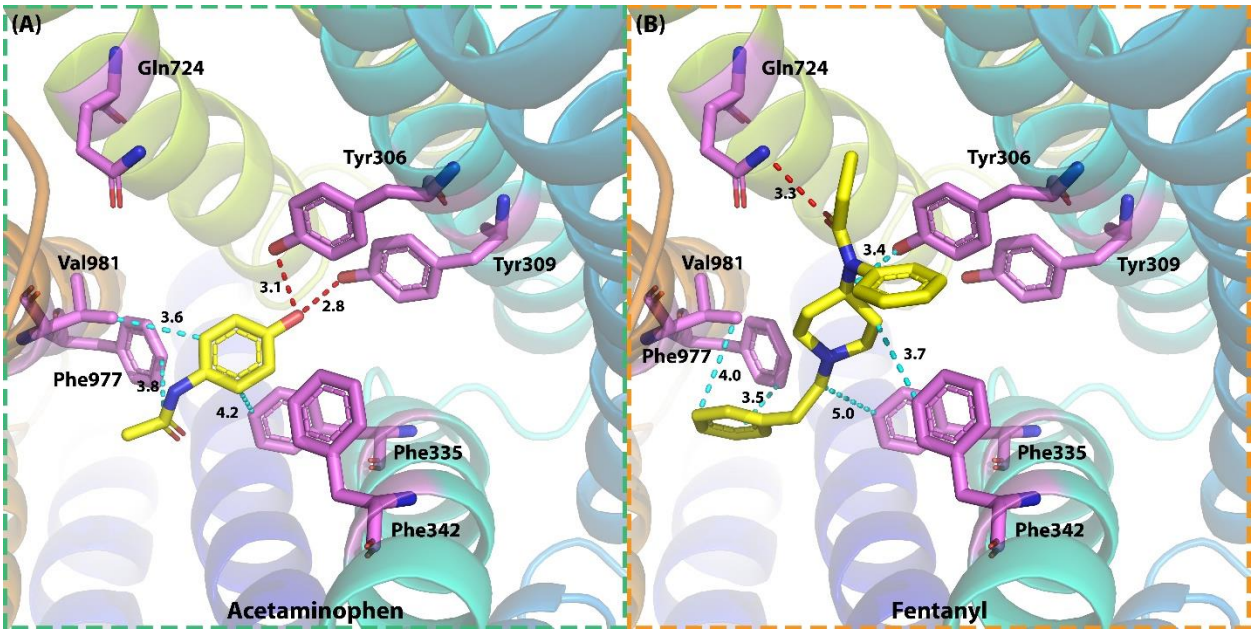


Figure 22 Interaction patterns of (A) acetaminophen and (B) fentanyl in P-glycoprotein (ABCB1)

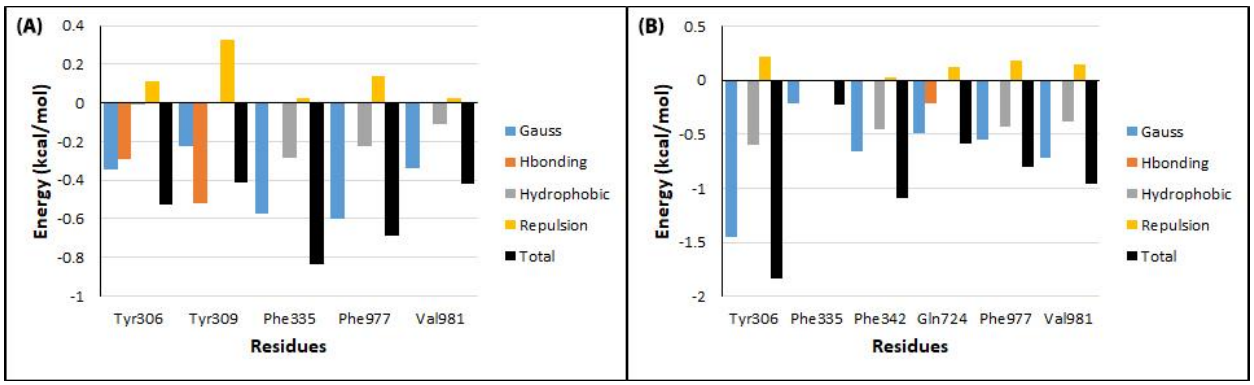


Figure 23 Residue energy contribution of (A) acetaminophen and (B) fentanyl in ABCB1

Table 3 and **Table 4** are the detailed key residue energy contribution tables between ABCB1 and the two analgesics of interest. The hydrogen bond between acetaminophen and ABCB1 contributed -0.293 (Tyr306) and -0.5189 (Tyr309) kcal/mol and the hydrophobic

interaction contributes -0.2831 (Phe335), -0.2259 (Phe977), and -0.1086 (Val981). On the other hand, the hydrogen bond between Gln724 and fentanyl contributes -0.2186 kcal/mol energy and the energy contribution of hydrophobic interactions are -0.6008 (Tyr306), -0.4554 (Phe342), -0.4307 (Phe977), and -0.3883 (Val981) kcal/mol, respectively.

Table 3 Detailed residue energy contribution (kcal/mol) between ABCB1 and acetaminophen

Residue	Gauss	H-bonding	Hydrophobic	Repulsion	Total
Tyr306	-0.3445	-0.293	-0.0058	0.1137	-0.5296
Tyr309	-0.223	-0.5189	0	0.3303	-0.4116
Phe335	-0.5739	0	-0.2831	0.022	-0.835
Phe977	-0.6023	0	-0.2259	0.1378	-0.6904
Val981	-0.335	0	-0.1086	0.0251	-0.4185

Table 4 Detailed residue energy contribution (kcal/mol) between ABCB1 and fentanyl

Residue	Gauss	H-bonding	Hydrophobic	Repulsion	Total
Tyr306	-1.4522	0	-0.6008	0.2207	-1.8322
Phe335	-0.2176	0	-0.0093	0	-0.2269
Phe342	-0.6583	0	-0.4554	0.0272	-1.0865
Gln724	-0.4892	-0.2186	0	0.1234	-0.5844
Phe977	-0.5563	0	-0.4307	0.1848	-0.8021
Val981	-0.7219	0	-0.3883	0.1476	-0.9626

3.3.2 PBPK models

Furthermore, we built two physiologically based pharmacokinetic (PBPK) models to quantitively explore the metabolism changes of these two drugs caused by the drug interaction

on CYP3A4 when administrated simultaneously from a pharmacometrics point of view. The final parameters used to build the PBPK models for acetaminophen and fentanyl are listed in **Table 5** and **Table 6**.

Using the optimized acetaminophen PBPK model, we simulated the drug plasma concentration profile in healthy volunteers after administered a single 1000 mg dose through iv bolus. As shown in **Figure 24**, our simulation is highly consistent with the observed data reported by previous papers.[155] The PBPK model for fentanyl was built based on the model published by our lab before.[156] The simulated drug plasma concentration profile after a single 7.0 mg iv bolus in healthy individuals overlaps with the experimental data well[157] (**Figure 25**). In conclusion, our PBPK model is highly consistent with the clinical data, thus supporting the further utilization of these PBPK models to study the DDI between acetaminophen and fentanyl.

Table 5 Input parameters for acetaminophen PBPK model

Parameter [a]	Value	Source
PhysChem and Blood Binding		
MW (g/mol)	151.16	b
log P	0.46	b
pK_a	9.46	Ref[158], c
B/P	0.98	Ref[159], c
f_u	0.82	Ref[160], c
Distribution		
V_{ss} (L/kg)	1	Ref[161], c
Elimination		
Cl_{APAP} (L/h)	19.7	Ref[162], c
Interaction		
K_i(μM)	2800	Ref[138], c

[a] Abbreviations: MW, molecule weight; log P, log of the octanol−water partition coefficient for the neutral compound; pKa, dissociation constant; B/P, blood/plasma concentration ratio; fu,

fraction of drug unbound in plasma; Vss, steady-state volume of distribution; ClAPAP, in vivo clearance of acetaminophen; Ki, inhibition constant of acetaminophen on CYP3A4. [b] From PubChem (PubChem CID: 1983) (https://pubchem.ncbi.nlm.nih.gov). [c] Derived from published data and then optimized based on observed data.

Table 6 Input parameters for fentanyl PBPK model

Parameter [a]	Value	Source
PhysChem and Blood Binding		
MW (g/mol)	336.47	b
log P	2.8	b
pK_a	8.06	b
B/P	0.963	Ref[163], c
f_u	0.297	d
Distribution		
V_{ss} (L/kg)	4.089	d
Elimination		
Cl_{int} 3A4 (μL/min/pmol)	0.496	Ref[164], c
Cl_R (L/h)	4.6	Ref[165], c
Interaction		
K_i(μM)	24.2	Ref[166], c

[a] Abbreviations: MW, molecule weight; log P, log of the octanol−water partition coefficient for the neutral compound; pKa, dissociation constant; B/P, blood/plasma concentration ratio; fu, fraction of drug unbound in plasma; Vss, steady-state volume of distribution; ClAPAP, in vivo clearance of acetaminophen; Ki, inhibition constant of acetaminophen on CYP3A4. [b] From PubChem (PubChem CID: 3345) (https://pubchem.ncbi.nlm.nih.gov). [c] Derived from published data and then optimized based on observed data.

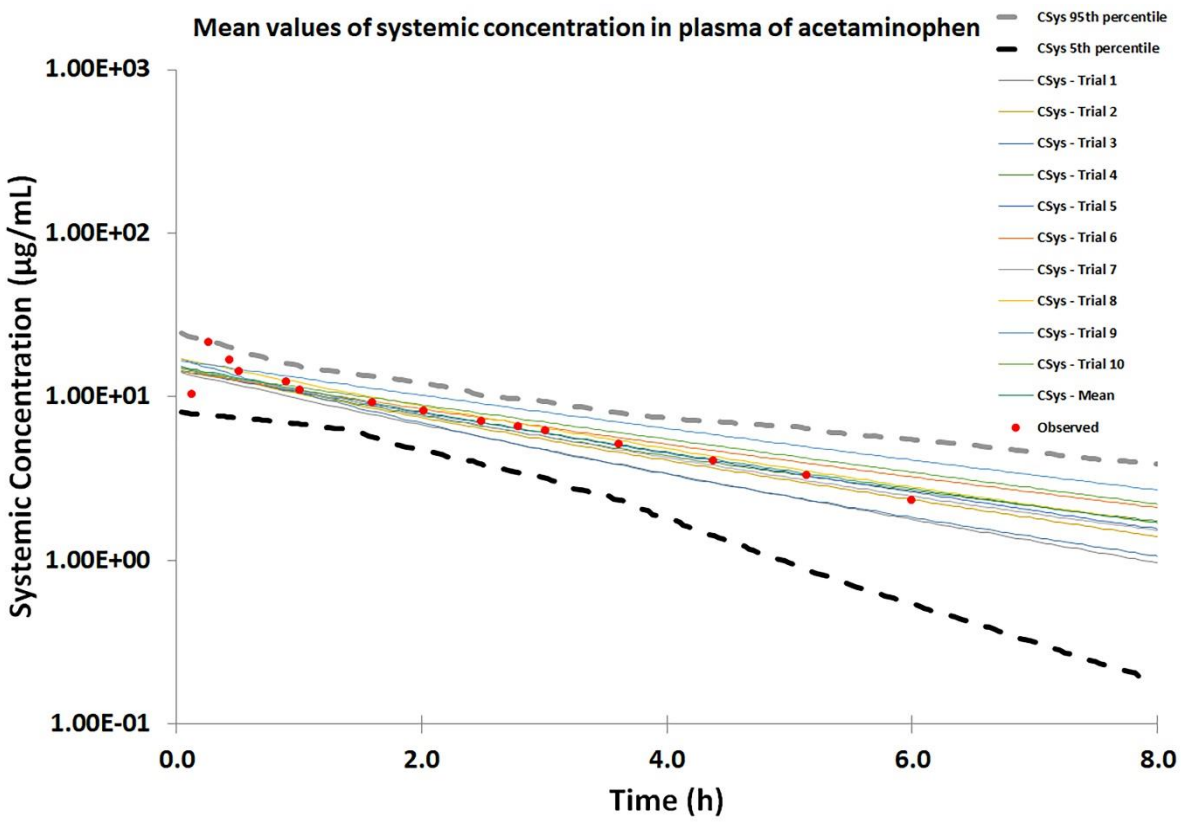


Figure 24 PBPK model simulated and experimentally observed concentration−time profiles of acetaminophen after 1000 mg iv bolus dosing

The simulated results for acetaminophen were generated using 10 trials of 10 virtual healthy volunteers. Observed data were highlighted in red dots while the simulated results, mean value, and the 95th/5th percentile of the simulation were shown by corresponding lines.

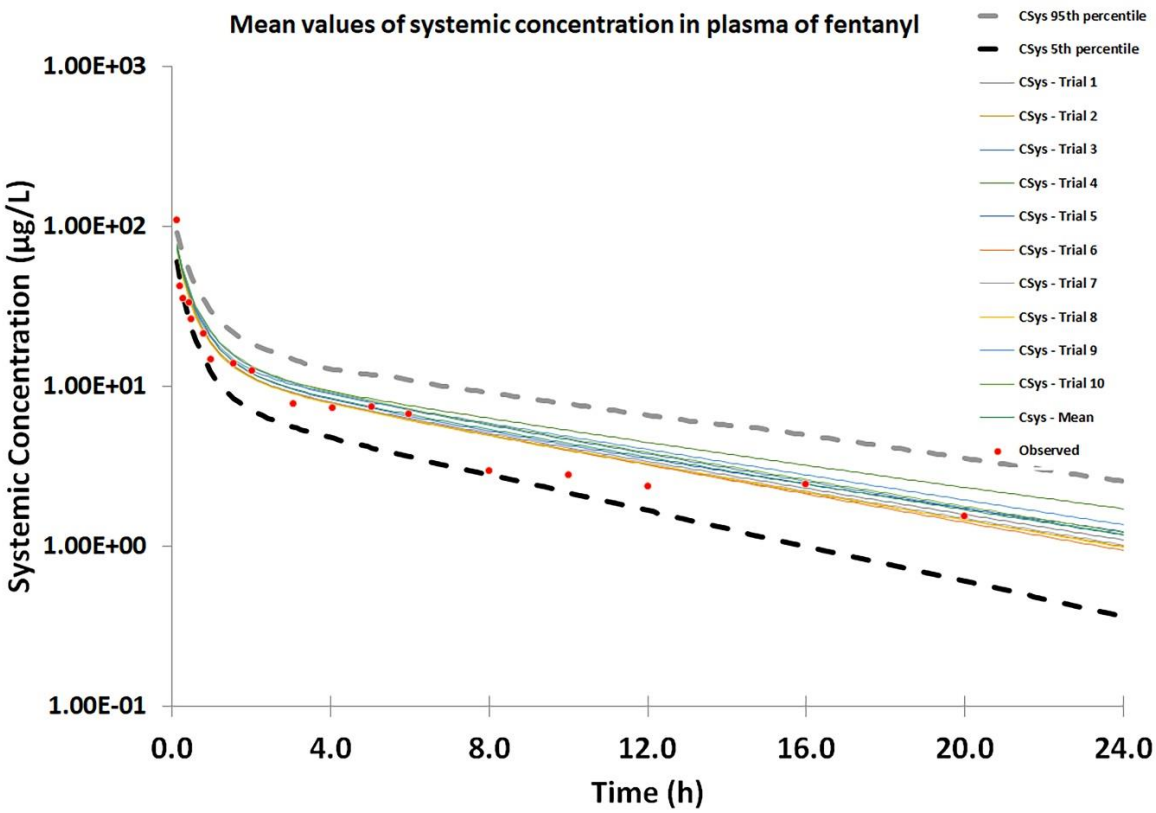


Figure 25 PBPK model simulated and experimentally observed concentration−time profiles of fentanyl after 7 mg iv bolus dosing

The simulated results for fentanyl were generated using 10 trials of 10 virtual healthy volunteers. Observed data were highlighted in red dots while the simulated results, mean value, and the 95th/5th percentile of the simulation were shown by corresponding lines.

Next, the virtual study of the DDI between acetaminophen and fentanyl was carried out based on the established PBPK models. Acetaminophen was chosen as the inhibitor substrate of fentanyl metabolism as reported by the literature.[138] We first used the therapeutic doses of acetaminophen (4000 mg)[167] and fentanyl (0.003 mg/kg)[168] to see if metabolism inhibition may occur under this dosage. As shown in **Figure 26a**, the systematic concentration of fentanyl with or without acetaminophen is almost identical. The AUC ratio (**Figure 26b**) is around 1.02, also

indicating there are no significant changes in body exposure to fentanyl due to the enzyme inhibition effect of acetaminophen.

We then used acetaminophen concentration around one order of magnitude greater than the therapeutic concentration to run the simulation, where potential fentanyl–paracetamol drug interactions have been reported to occur.[138] When the dosage of acetaminophen reaches 80,000 mg, which is 20-fold the therapeutic dose, we observed a significant elevation (the mean AUC ratio between administering fentanyl with acetaminophen and fentanyl alone is 1.27) in the systematic concentration of fentanyl (**Figure 27a, 27b**). The simulated results indicate that when used in combination with acetaminophen at this concentration, fentanyl metabolism by the CYP3A4 is likely to be affected.

The dose for acetaminophen to exhibit the significant DDI is incredibly high and not very like to reach in clinical usage. We believe the DDI observed in clinical scenario[154] is more likely to related to the PD aspect. However, considering that the lethal dose of fentanyl can be as low as 2 mg according to the drug enforcement administration (DEA), more attention should be paid when administrating it with very high doses of acetaminophen. Our results not only provide a possible explanation to the real-world observed data, but it also builds more confidence for users to utilize our PAIN-CKB along with pharmacometrics and systems pharmacology tools to explore DDI problems.

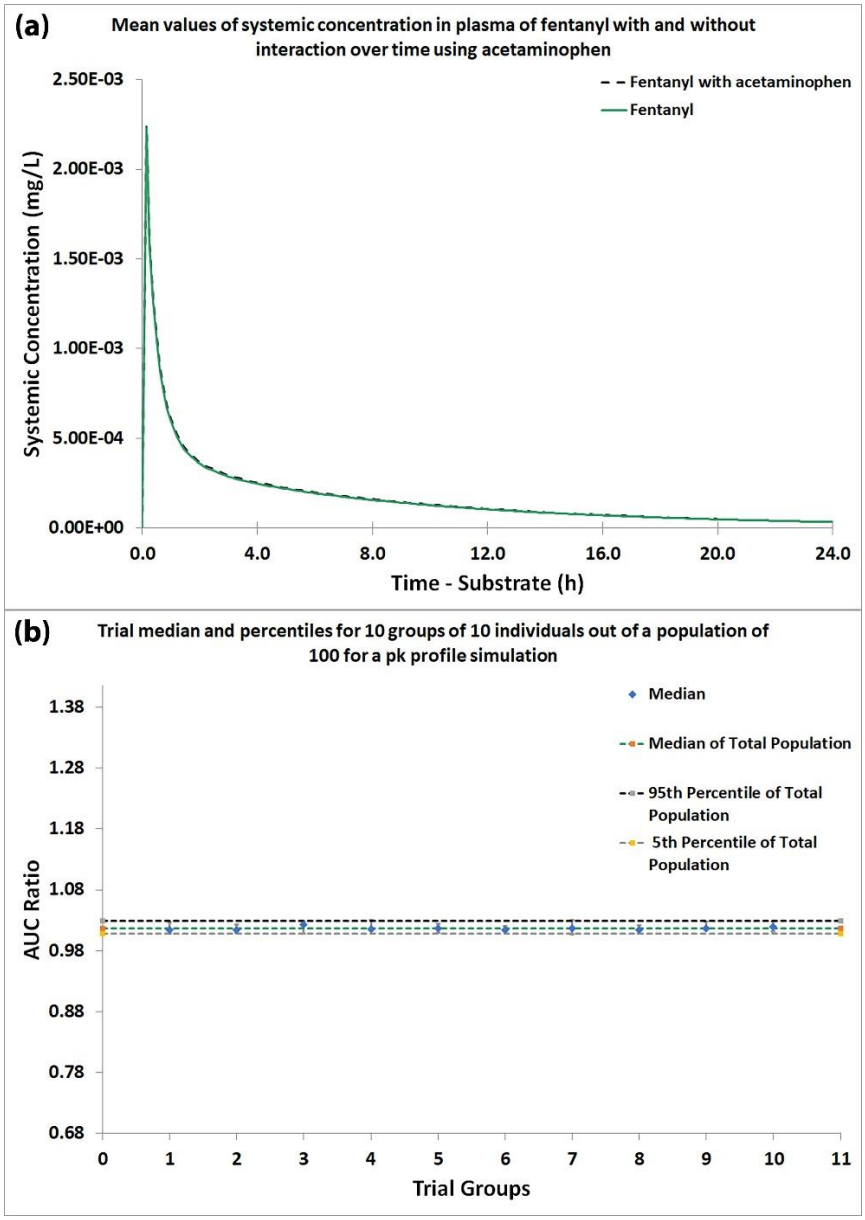


Figure 26 Virtual PBPK model DDI studies between acetaminophen and fentanyl under therapeutic dosing

26(a): Fentanyl systemic plasma time-concentration profile with and without 4000 mg acetaminophen. 26(b) The mean value and standard deviation for 10 groups of 10 healthy volunteers of fentanyl simulated PK profiles with and without 4000 mg acetaminophen.

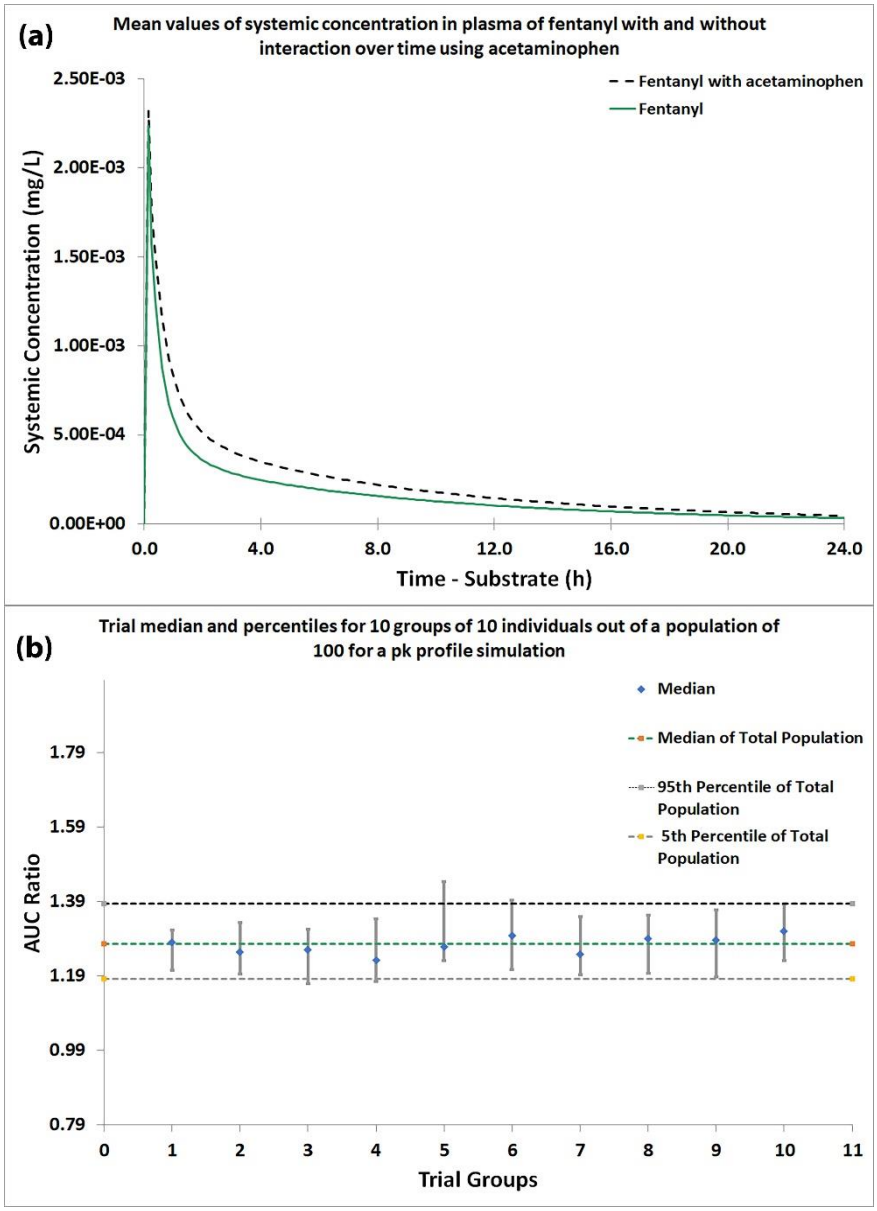


Figure 27 Virtual PBPK model DDI studies between acetaminophen and fentanyl under acetaminophen overdosing

27(a): Fentanyl systemic plasma time-concentration profile with and without 80000 mg acetaminophen. 26(b) The mean value and standard deviation for 10 groups of 10 healthy volunteers of fentanyl simulated PK profiles with and without 4000 mg acetaminophen.

3.3.3 NAPQI hepatotoxicity

Acetaminophen is, currently, one of the most common causes of acute liver failure (ALF) in the United States.[169] At its therapeutic dose, around 90% of acetaminophen is metabolized in the human body into inactive glucuronide and sulfate conjugates, while the rest of the unmetabolized drug is converted to a highly reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI)[137] primarily by CYP2E1 and CYP3A4.[170] However, the supratherapeutic dose of acetaminophen will cause the conjugation pathways to become saturated and increase the amount of NAPQI in the body, which can cause hepatotoxicity.[171,172]

NAPQI has been reported to target thioredoxin reductase (TrxR) to induce oxidative stress and liver toxicity. In detail, LC-MS/MS analysis confirmed that NAPQI can modify the cysteine 59, cysteine 497, and selenocysteine 498 residues in the redox centers of TrxR to cause the enzyme inhibition.[92] TrxR has been collected as a potential pain target[173] in our PAIN-CKB. We were excited to find that in our previous attempt to create the target map for the four drugs, our platform suggested that acetaminophen is very much likely to interact with TrxR. Considering the structural similarity between acetaminophen and NAPQI, it is highly possible that if the inputted compound was NAPQI instead of acetaminophen, our algorithms would successfully predict the toxicity target for NAPQI. We again validated the powerful functionality of our PAIN-CKB, and the algorithms embedded in it.

To study the interaction pattern between NAPQI and TrxR1, a docking study was conducted between this receptor-ligand pair. In perfect agreement with the observed results,[92] as shown in **Figure 28**, NAPQI is likely to interact with TrxR1 in three different ways. First, the carbonyl group of NAPQI can form a strong hydrogen bond with the Cys497 residue with a

distance of 2.9 Å. Another possible hydrogen bond interaction is observed between the N-acyl amides functional group and Sec498 residue with a distance of 3.1 Å. Finally, NAPQI can also form a steric interaction with the Cys59 residue (3.4 Å) because of the special spatial structure of the Cys59 residue. These possible binding poses provide a suitable spatial condition which can then facilitate the alkylation reaction on these residues and cause enzyme inhibition and further hepatotoxicity.

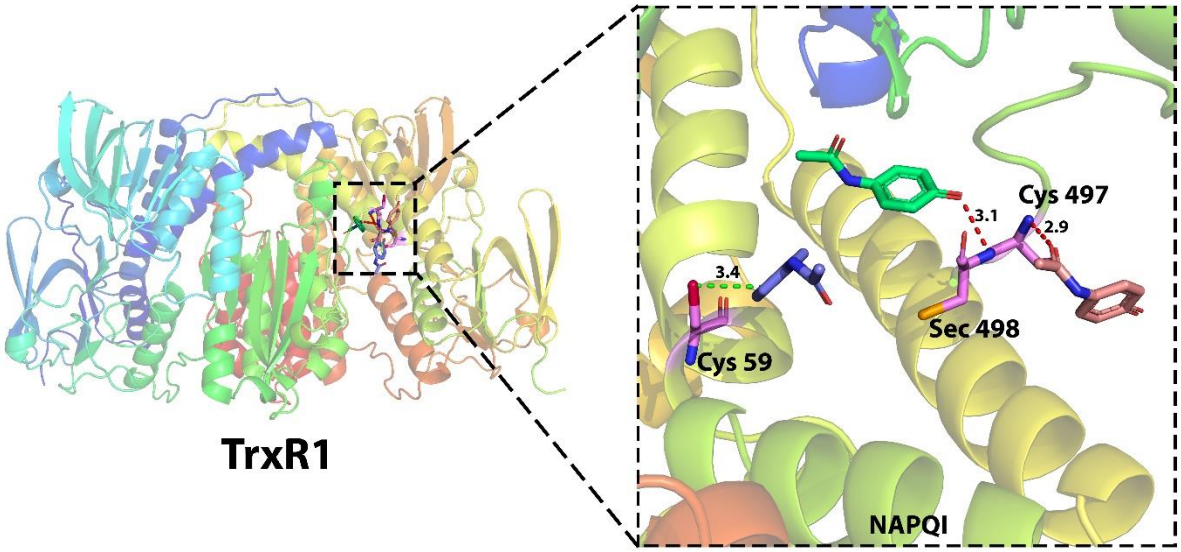


Figure 28 Detailed binding pattern between NAPQI and TrxR1

4.0 DISCUSSION

Pain is a highly complex phenomenon[174] which can cause severe challenges to patients' daily life.[175] Efforts have been devoted to improving the pain management, yet limited improvements have been achieved.[176] Several databases related to pain such as PainNetworks database have been built to study the pain-related genes and their network associations.[120] Several other specific disease-related databases have already been developed and implemented as novel ways to explore the molecular mechanisms and pathways of the disease to facilitate the studies in related areas.[177,178] Here, our PAIN-CKB serves as a pain domain-specific chemogenomics knowledgebase with free access to the public and user-friendly web interface and powerful algorithms implemented, such as the target structural-based HTDocking and ligand structural-based TargetHunter. Our database has the potential to pave the gap between biology and chemistry in the hope of facilitating the discovery of new analgesics.

The pilot studies presented here validated the ability of our PAIN-CKB to map the network between different drugs and their targets based on the results of docking score (HTDocking) and similarity score (TargetHunter). The target map precisely identified known targets for the inputted analgesics. It can also make predictions based on the known target structure as well as some small-molecule structures that have been confirmed to bind to the target. As shown in the present work, our platform successfully predicted targets that are not widely recognized but have been reported by previously published papers. The ability for PAIN-CKB to identify drug targets allows users to conduct drug repurpose studies by looking for potential off-targets for a known compound or scan new compounds as a starting point for new

analgesics development. In addition, based on the common targets mapped between certain drug pairs, studies can be carried out by using the molecular simulation methods (molecular docking, molecular dynamic simulation, etc.) and pharmacometrics models to explore the potential DDIs and synergistic drug pairs. Given the fact that DDIs related to analgesics can cause severe adverse effects[179] and the synergistic drug pairs with greater therapeutic effects and fewer side effects are of great research interest to the pain-related area,[180] PAIN-CKB can be extremely beneficial for future researches.

However, our PAIN-CKB has its limitations. First, although we conducted a thorough literature research to implement as many pain-related targets as we could, potential pain related targets that are not included can still exist. To minimize this limitation, we will continuously update our PAIN-CKB database with future literature research. Secondly, the performance of the HTDocking algorithm relies largely on the quality and availability of the 3D structure of the target protein. Luckily, PAIN-CKB is also equipped with TargetHunter, therefore, this ligand-based algorithm can predict the targets for small-molecules based on the structure of ligands known to bind to that specific protein target. Combining these tools, PAIN-CKB can handle most of the analysis work.

5.0 CONCLUSION

Currently, chronic pain is one of the main challenges in the modern medication area. The annual cost of pain medication is burdensome and there are still many unsatisfying features in the analgesics available in the market such as the limited pain-relieving effect, severe side effects, DDIs between different medications, and drug addiction. The high prevalence of chronic pain makes this problem even more urgent to solve.

Computer-aided drug discovery (CADD) may provide a new aspect to solve this problem. CADD is a newly emerging technique in the drug discovery area. With the help of the amazing calculation speed of modern computers, CADD is more efficient and economic compared to the traditional drug discovery methods. The typical methods used in CADD includes molecular docking, molecular dynamic simulation, machine learning, PBPK models and etc.

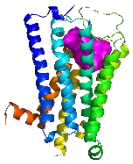
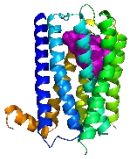
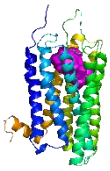
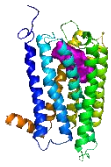
In this study, we constructed a pain-domain specific knowledgebase containing the information of current analgesics and pain-related protein targets files. Equipped with cutting-edge computational tools, the PAIN-CKB is able to identify the potential protein targets of the input small molecule. The pilot studies presented here demonstrated the functionality and validity of this newly-built database. Future users are encouraged, but should not be restricted, to follow the principle procedures of the presented case studies to make more breakthroughs in the pain-related area.

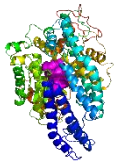
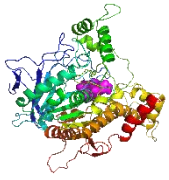
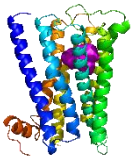
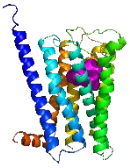
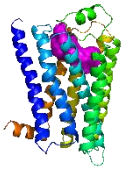
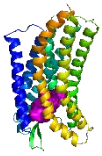
All in all, PAIN-CKB is an integrative pain-domain specific knowledgebase with full access for public use. It includes pain-related targets data and tools for target identification and

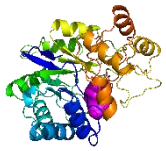
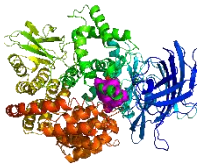
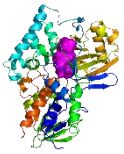
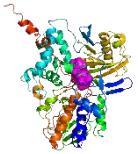
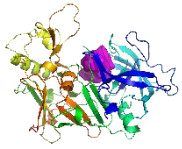
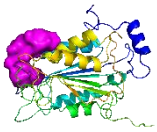
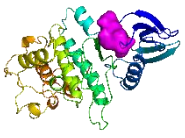
systems pharmacology research. This knowledgebase will benefit the pain research area by bridging the knowledge barrier between computational, chemical, and biological to facilitate the identification of new DDIs and to accelerate the development of new analgesics.

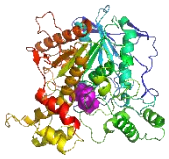
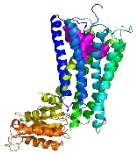
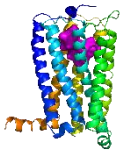
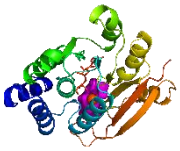
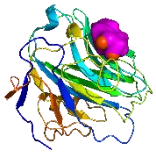
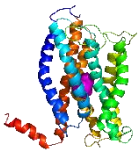
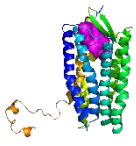
APPENDIX

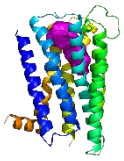
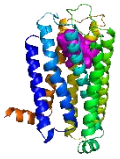
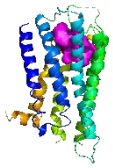
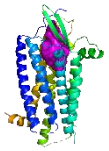
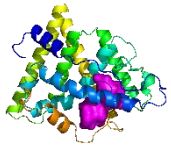
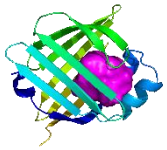
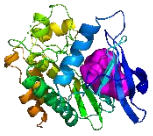
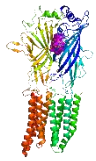
Table 7 The pain-related targets collected in PAIN-CKB

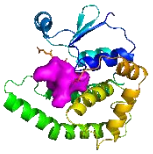
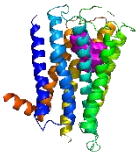
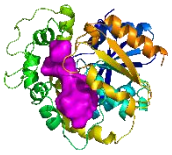
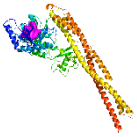
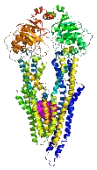
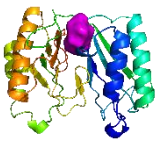
Gene name	Protein name	Uniport ID	3D structure
5HT1B	5-hydroxytryptamine receptor 1B	P28222	
5HT2A	5-hydroxytryptamine receptor 2A	P28223	
5HT2B	5-hydroxytryptamine receptor 2B	P41595	
5HT2C	5-hydroxytryptamine receptor 2C	P28335	
AA1R	Adenosine receptor A1	P30542	
AA2AR	Adenosine receptor A2a	P29274	

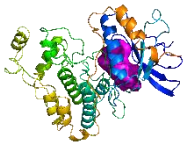
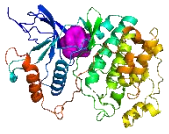
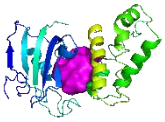
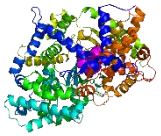
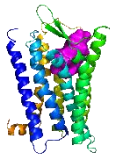
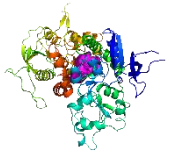
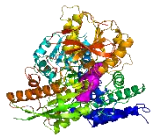
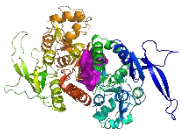
ACE	Angiotensin-converting enzyme	P12821	
ACES	Acetylcholinesterase	P22303	
ACHA9	Neuronal acetylcholine receptor subunit alpha-9	Q9UGM1	
ACHB2	Neuronal acetylcholine receptor subunit beta-2	P17787	
ACM1	Muscarinic acetylcholine receptor M1	P11229	
ACM4	Muscarinic acetylcholine receptor M4	P08173	
ADRB2	Beta-2 adrenergic receptor	P07550	
AGTR2	Type-2 angiotensin II receptor	P50052	

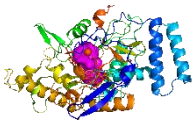
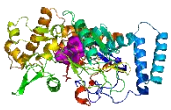
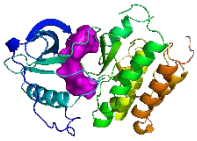
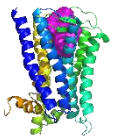
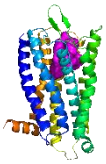
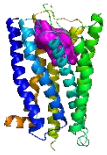
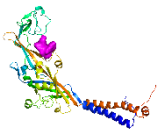
ALDR	Aldo-keto reductase family 1 member B1	P15121	
AMPN	Aminopeptidase N	P15144	
AOFA	Amine oxidase [flavin-containing] A	P21397	
AOFB	Amine oxidase [flavin-containing] B	P27338	
BACE1	Beta-secretase 1	P56817	
CAH2	Carbonic anhydrase 2	P00918	
CASP1	Caspase-1	P29466	
CDK5	Cyclin-dependent-like kinase 5	Q00535	

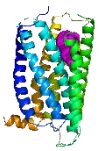
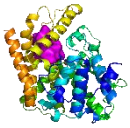
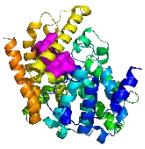
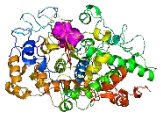
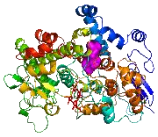
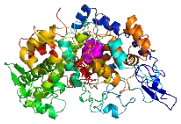
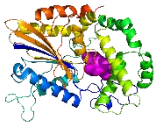
CHLE	Cholinesterase	P06276	
CNR1	Cannabinoid receptor 1	P21554	
CNR2	Cannabinoid receptor 2	P34972	
COLI	Pro-opiomelanocortin	P01189	
COMT	Catechol O-methyltransferase	P21964	
CRP	C-reactive protein	P02741	
CXCR1	C-X-C chemokine receptor type 1	P25024	
CXCR4	C-X-C chemokine receptor type 4	P61073	

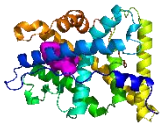
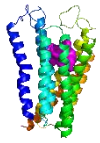
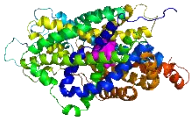
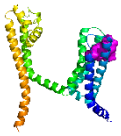
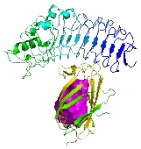
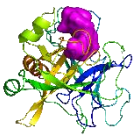
DRD2	D (2) dopamine receptor	P14416	
DRD3	D (3) dopamine receptor	P35462	
DRD4	D (4) dopamine receptor	P21917	
EDN1	Endothelin-1	P05305	
ESR1	Estrogen receptor	P03372	
FABP5	Fatty acid-binding protein 5	Q01469	
FYN	Tyrosine-protein kinase Fyn	P06241	
GLRA3	Glycine receptor subunit alpha-3	O75311	

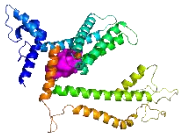
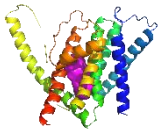
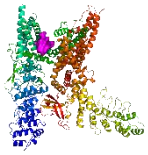
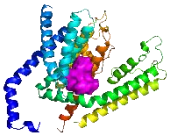
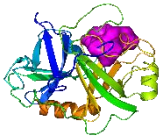
GLRA3 modulator	Glycine receptor subunit alpha-3	O75311	
GRM5	Metabotropic glutamate receptor 5	P41594	
HPGDS	Hematopoietic prostaglandin D synthase	O60760	
HRH1	Histamine H1 receptor	P35367	
HYES	Bifunctional epoxide hydrolase 2	P34913	
IKKB	Inhibitor of nuclear factor kappa-B kinase subunit beta	O14920	
MDR1	ATP-dependent translocase ABCB1	P08183	
MIF	Macrophage migration inhibitory factor	P14174	

MK01	Mitogen-activated protein kinase 1	P28482	
MK03	Mitogen-activated protein kinase 3	P27361	
MTOR	Serine/threonine-protein kinase mTOR	P42345	
NEP	Neprilysin	P08473	
NK1R	Substance-P receptor	P25103	
NMDE1	Glutamate receptor ionotropic epsilon-1	Q12879	
NMDE2	Glutamate receptor ionotropic epsilon-2	Q13224	
NMDZ1	Glutamate receptor ionotropic zeta-1	Q05586	

NOS1	Nitric oxide synthase 1	P29475	
NOS2	Nitric oxide synthase 2	P35228	
NTRK1	High affinity nerve growth factor receptor	P04629	
OPRD	Delta-type opioid receptor	P41143	
OPRK	Kappa-type opioid receptor	P41145	
OPRM	Mu-type opioid receptor	P35372	
OPRX	Nociceptin receptor	P41146	
P2RX3	P2X purinoceptor 3	P56373	

P2Y12	P2Y purinoceptor 12	Q9H244	
PAR1	Proteinase-activated receptor 1	P25116	
PDE4B	cAMP-specific 3',5'-cyclic phosphodiesterase 4B	Q07343	
PDE5A	cGMP-specific 3',5'-cyclic phosphodiesterase 5A	O76074	
PERM	Myeloperoxidase	P05164	
PGH1	Prostaglandin G/H synthase 1	P23219	
PGH2	Prostaglandin G/H synthase 2	P35354	
PPAP	Prostatic acid phosphatase	P15309	

PPARG	Peroxisome proliferator-activated receptor gamma	P37231	
PRGR	Progesterone receptor	P06401	
PTGES	Prostaglandin E synthase	O14684	
SC6A4	Solute carrier family 6 member 4	P31645	
SCN9A	Sodium channel protein type 9 subunit alpha	Q15858	
TLR4	Toll-like receptor 4	O00206	
TNFA	Tumor necrosis factor	P01375	
TRBM	Thrombomodulin	P07204	

TRPM4	Transient receptor potential cation channel subfamily M member 4	Q8TD43	
TRPM8	Transient receptor potential cation channel subfamily M member 8	Q7Z2W7	
TRPV1	Transient receptor potential cation channel subfamily V member 1	Q8NER1	
TRPV2	Transient receptor potential cation channel subfamily V member 2	Q9Y5S1	
TRPV3	Transient receptor potential cation channel subfamily V member 3	Q8NET8	
TRY1	Trypsin-1	P07477	

BIBLIOGRAPHY

1 Bonica, J. J. The need of a taxonomy. *Pain* **6**, 247-248 (1979).

2 Garland, E. L. Pain processing in the human nervous system: a selective review of nociceptive and biobehavioral pathways. *Prim Care* **39**, 561-571, doi:10.1016/j.pop.2012.06.013 (2012).

3 Basbaum, A. I. The perception of pain. *Principles of neural science* (2000).

4 D'Mello, R. & Dickenson, A. H. Spinal cord mechanisms of pain. *BJA: British Journal of Anaesthesia* **101**, 8-16, doi:10.1093/bja/aen088 (2008).

5 Koltzenburg, M., Stucky, C. L. & Lewin, G. R. Receptive properties of mouse sensory neurons innervating hairy skin. *Journal of neurophysiology* **78**, 1841-1850 (1997).

6 Djouhri, L. & Lawson, S. N. Aβ-fiber nociceptive primary afferent neurons: a review of incidence and properties in relation to other afferent A-fiber neurons in mammals. *Brain Research Reviews* **46**, 131-145, doi:https://doi.org/10.1016/j.brainresrev.2004.07.015 (2004).

7 Devor, M. Ectopic discharge in Aβ afferents as a source of neuropathic pain. *Experimental brain research* **196**, 115-128 (2009).

8 Fleming, M. S. & Luo, W. The anatomy, function, and development of mammalian Aβ low-threshold mechanoreceptors. *Frontiers in Biology* **8**, 408-420, doi:10.1007/s11515-013-1271-1 (2013).

9 Adriaensen, H., Gybels, J., Handwerker, H. O. & Hees, J. V. Response properties of thin myelinated (A-delta) fibers in human skin nerves. *Journal of Neurophysiology* **49**, 111-122, doi:10.1152/jn.1983.49.1.111 (1983).

10 GOPIKRISHNA, V., PRADEEP, G. & VENKATESHBABU, N. Assessment of pulp vitality: a review. *International Journal of Paediatric Dentistry* **19**, 3-15, doi:10.1111/j.1365-263X.2008.00955.x (2009).

11 Sundar, S. & Gonzalez-Cueto, J. A. in *2006 International Conference of the IEEE Engineering in Medicine and Biology Society*. 2908-2911 (IEEE).

12 Clark, D., Hughes, J. & Gasser, H. S. Afferent function in the group of nerve fibers of slowest conduction velocity. *American Journal of Physiology-Legacy Content* **114**, 69-76 (1935).

13 Perl, E. R., Kumazawa, T., Lynn, B. & Kenins, P. in *Progress in Brain Research* Vol. 43 (eds A. Iggo & O. B. Ilyinsky) 263-277 (Elsevier, 1976).

14 Bove, G. M. & Swenson, R. S. in *Pain Management* (eds Steven D. Waldman & Joseph I. Bloch) 1081-1092 (W.B. Saunders, 2007).

15 Brown, A. G. REVIEW ARTICLE THE DORSAL HORN OF THE SPINAL CORD. *Quarterly Journal of Experimental Physiology* **67**, 193-212, doi:10.1113/expphysiol.1982.sp002630 (1982).

16 Todd, A. J. Neuronal circuitry for pain processing in the dorsal horn. *Nature Reviews Neuroscience* **11**, 823-836, doi:10.1038/nrn2947 (2010).

17 Swift, A. Understanding the effect of pain and how the human body responds. *Nursing times [online]* **114**, 22-26 (2018).

18 Cervero, F. Understanding Pain : Exploring the Perception of Pain. (2012).

19 Merskey, H. E. Classification of chronic pain: Descriptions of chronic pain syndromés and definitions of pain terms. *Pain* (1986).
20 Chapman, C. R. & Vierck, C. J. The Transition of Acute Postoperative Pain to Chronic Pain: An Integrative Overview of Research on Mechanisms. *The Journal of Pain* **18**, 359.e351-359.e338, doi:https://doi.org/10.1016/j.jpain.2016.11.004 (2017).
21 Schneiderhan, J., Clauw, D. & Schwenk, T. L. Primary Care of Patients With Chronic Pain. *JAMA* **317**, 2367-2368, doi:10.1001/jama.2017.5787 (2017).
22 Basbaum, A. I., Bautista, D. M., Scherrer, G. & Julius, D. Cellular and molecular mechanisms of pain. *Cell* **139**, 267-284, doi:10.1016/j.cell.2009.09.028 (2009).
23 Schappert, S. M. & Burt, C. W. Ambulatory care visits to physician offices, hospital outpatient departments, and emergency departments: United States, 2001-02. *Vital and Health Statistics. Series 13, Data from the National Health Survey*, 1-66 (2006).
24 Beers, M., Porter, R., Jones, T., Kaplan, J. & Berkwits, M. Approach to the patient with anemia: Hematology and Oncologyin the Merck Manual of Diagnosis and Therapy. 18. Chapter 3. Merck Research Laboratories, Division of Merck & Co. *Inc. Whitehouse Station NJ*, 1031-1033 (2006).
25 Persons, A. P. o. P. P. i. O. The management of persistent pain in older persons. *Journal of the American Geriatrics Society* **50**, S205 (2002).
26 Dahlhamer, J. *et al.* Prevalence of chronic pain and high-impact chronic pain among adults—United States, 2016. *Morbidity and Mortality Weekly Report* **67**, 1001 (2018).
27 Gaskin, D. J. & Richard, P. The Economic Costs of Pain in the United States. *The Journal of Pain* **13**, 715-724, doi:10.1016/j.jpain.2012.03.009 (2012).
28 van Leeuwen, M. T., Blyth, F. M., March, L. M., Nicholas, M. K. & Cousins, M. J. Chronic pain and reduced work effectiveness: The hidden cost to Australian employers. *European Journal of Pain* **10**, 161-161, doi:10.1016/j.ejpain.2005.02.007 (2006).
29 Melnikova, I. (Nature Publishing Group, 2010).
30 Geurts, J. W. *et al.* Patient expectations for management of chronic non-cancer pain: A systematic review. *Health Expectations* **20**, 1201-1217 (2017).
31 Innis, J., Bikaunieks, N., Petryshen, P., Zellermeyer, V. & Ciccarelli, L. Patient satisfaction and pain management: an educational approach. *Journal of Nursing Care Quality* **19**, 322-327 (2004).
32 Ricardo Buenaventura, M., Rajive Adlaka, M. & Nalini Sehgal, M. Opioid complications and side effects. *Pain physician* **11**, S105-S120 (2008).
33 Dunwoody, C. J., Krenzischek, D. A., Pasero, C., Rathmell, J. P. & Polomano, R. C. Assessment, Physiological Monitoring, and Consequences of Inadequately Treated Acute Pain. *Pain Management Nursing* **9**, 11-21, doi:https://doi.org/10.1016/j.pmn.2007.11.006 (2008).
34 Busson, M. Update on Ibuprofen: Review Article. *Journal of International Medical Research* **14**, 53-62, doi:10.1177/030006058601400201 (1986).
35 O'Connor, J. P. & Lysz, T. Celecoxib, NSAIDs and the skeleton. *Drugs of today* **44**, 693 (2008).
36 Cashman, J. N. The Mechanisms of Action of NSAIDs in Analgesia. *Drugs* **52**, 13-23, doi:10.2165/00003495-199600525-00004 (1996).
37 Wiebalck, C. A. & Van Aken, H. 4 Paracetamol and propacetamol for post-operative pain: contrasts to traditional NSAIDs. *Baillière's Clinical Anaesthesiology* **9**, 469-482, doi:https://doi.org/10.1016/S0950-3501(95)80017-4 (1995).

38 Henry, D. A. Side-effects of non-steroidal anti-inflammatory drugs. *Baillière's Clinical Rheumatology* **2**, 425-454, doi:https://doi.org/10.1016/S0950-3579(88)80021-9 (1988).
39 Bjarnason, I., Hayllar, J., Macpherson, A. N. d. J. & Russell, A. N. t. S. Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine in humans. *Gastroenterology* **104**, 1832-1847, doi:https://doi.org/10.1016/0016-5085(93)90667-2 (1993).
40 Rainsford, K. D. Profile and mechanisms of gastrointestinal and other side effects of nonsteroidal anti-inflammatory drugs (NSAIDs). *The American Journal of Medicine* **107**, 27-35, doi:https://doi.org/10.1016/S0002-9343(99)00365-4 (1999).
41 Carr, D. B. & Goudas, L. C. Acute pain. *The Lancet* **353**, 2051-2058, doi:https://doi.org/10.1016/S0140-6736(99)03313-9 (1999).
42 Altier, C. & Zamponi, G. W. Targeting Ca2+ channels to treat pain: T-type versus N-type. *Trends in pharmacological sciences* **25**, 465-470 (2004).
43 Altier, C. in *GPCR Signalling Complexes–Synthesis, Assembly, Trafficking and Specificity* 241-262 (Springer, 2012).
44 Yamada, M., Inanobe, A. & Kurachi, Y. G protein regulation of potassium ion channels. *Pharmacological reviews* **50**, 723-757 (1998).
45 Al-Hasani, R. & Bruchas, M. R. Molecular mechanisms of opioid receptor-dependent signaling and behavior. *Anesthesiology: The Journal of the American Society of Anesthesiologists* **115**, 1363-1381 (2011).
46 Chahl, L. A. Opioids–mechanism of action. *Aust Prescr* **19**, 63-65 (1996).
47 Manglik, A. *et al.* Structure-based discovery of opioid analgesics with reduced side effects. *Nature* **537**, 185, doi:10.1038/nature19112 (2016).
48 Warner, M., Chen, L. H. & Makuc, D. M. Increase in fatal poisonings involving opioid analgesics in the United States, 1999-2006. (2009).
49 Shah, N. G., Lathrop, S. L., Reichard, R. R. & Landen, M. G. Unintentional drug overdose death trends in New Mexico, USA, 1990–2005: combinations of heroin, cocaine, prescription opioids and alcohol. *Addiction* **103**, 126-136, doi:10.1111/j.1360-0443.2007.02054.x (2008).
50 Seth, P., Scholl, L., Rudd, R. A. & Bacon, S. Overdose deaths involving opioids, cocaine, and psychostimulants—United States, 2015–2016. *Morbidity and Mortality Weekly Report* **67**, 349 (2018).
51 Christie, M. J. Cellular neuroadaptations to chronic opioids: tolerance, withdrawal and addiction. *British Journal of Pharmacology* **154**, 384-396, doi:10.1038/bjp.2008.100 (2008).
52 Woolf, C. J. Overcoming obstacles to developing new analgesics. *Nature Medicine* **16**, 1241-1247, doi:10.1038/nm.2230 (2010).
53 Liu, H. *et al.* AlzPlatform: an Alzheimer's disease domain-specific chemogenomics knowledgebase for polypharmacology and target identification research. *Journal of chemical information and modeling* **54**, 1050-1060 (2014).
54 Zhang, Y. *et al.* StemCellCKB: an integrated stem cell-specific chemogenomics knowledgebase for target identification and systems-pharmacology research. *Journal of chemical information and modeling* **56**, 1995-2004 (2016).
55 Zhang, H. *et al.* Cardiovascular disease chemogenomics knowledgebase-guided target identification and drug synergy mechanism study of an herbal formula. *Scientific reports* **6**, 33963 (2016).

56 Chen, M., Jing, Y., Wang, L., Feng, Z. & Xie, X.-Q. DAKB-GPCRs: An Integrated Computational Platform for Drug Abuse Related GPCRs. *Journal of Chemical Information and Modeling* **59**, 1283-1289, doi:10.1021/acs.jcim.8b00623 (2019).
57 Xu, X. *et al.* Chemogenomics knowledgebase and systems pharmacology for hallucinogen target identification—Salvinorin A as a case study. *Journal of Molecular Graphics and Modelling* **70**, 284-295 (2016).
58 Lin, Y. *et al.* Drug target ontology to classify and integrate drug discovery data. *Journal of Biomedical Semantics* **8**, 50, doi:10.1186/s13326-017-0161-x (2017).
59 Hanlon, C. D. & Andrew, D. J. Outside-in signaling – a brief review of GPCR signaling with a focus on the Drosophila GPCR family. *Journal of Cell Science* **128**, 3533-3542, doi:10.1242/jcs.175158 (2015).
60 Hilger, D., Masureel, M. & Kobilka, B. K. Structure and dynamics of GPCR signaling complexes. *Nature Structural & Molecular Biology* **25**, 4-12, doi:10.1038/s41594-017-0011-7 (2018).
61 Hauser, A. S., Attwood, M. M., Rask-Andersen, M., Schiöth, H. B. & Gloriam, D. E. Trends in GPCR drug discovery: new agents, targets and indications. *Nature Reviews Drug Discovery* **16**, 829-842, doi:10.1038/nrd.2017.178 (2017).
62 Richards, J. L., Yap, Y. A., McLeod, K. H., Mackay, C. R. & Mariño, E. Dietary metabolites and the gut microbiota: an alternative approach to control inflammatory and autoimmune diseases. *Clinical & Translational Immunology* **5**, e82, doi:10.1038/cti.2016.29 (2016).
63 Bufe, B. *et al.* Recognition of Bacterial Signal Peptides by Mammalian Formyl Peptide Receptors: A NEW MECHANISM FOR SENSING PATHOGENS. *Journal of Biological Chemistry* **290**, 7369-7387, doi:10.1074/jbc.M114.626747 (2015).
64 Robertson, J. G. Mechanistic Basis of Enzyme-Targeted Drugs. *Biochemistry* **44**, 5561-5571, doi:10.1021/bi050247e (2005).
65 Herraiz, T., Flores, A. & Fernández, L. Analysis of monoamine oxidase (MAO) enzymatic activity by high-performance liquid chromatography-diode array detection combined with an assay of oxidation with a peroxidase and its application to MAO inhibitors from foods and plants. *Journal of Chromatography B* **1073**, 136-144, doi:https://doi.org/10.1016/j.jchromb.2017.12.004 (2018).
66 Binda, C., Newton-Vinson, P., Hubálek, F., Edmondson, D. E. & Mattevi, A. Structure of human monoamine oxidase B, a drug target for the treatment of neurological disorders. *Nature Structural Biology* **9**, 22-26, doi:10.1038/nsb732 (2002).
67 Stack, C. G., Rogers, P. & Linter, S. P. K. MONOAMINE OXIDASE INHIBITORS AND ANAESTHESIA: A Review. *British Journal of Anaesthesia* **60**, 222-227, doi:https://doi.org/10.1093/bja/60.2.222 (1988).
68 Majiduddin, F. K., Materon, I. C. & Palzkill, T. G. Molecular analysis of beta-lactamase structure and function. *International Journal of Medical Microbiology* **292**, 127-137, doi:https://doi.org/10.1078/1438-4221-00198 (2002).
69 Bryson, H. M. & Brogden, R. N. Piperacillin/tazobactam. *Drugs* **47**, 506-535 (1994).
70 Jentsch, T. J., Hübner, C. A. & Fuhrmann, J. C. Ion channels: Function unravelled by dysfunction. *Nature Cell Biology* **6**, 1039-1047, doi:10.1038/ncb1104-1039 (2004).
71 Talwar, S. & Lynch, J. W. Investigating ion channel conformational changes using voltage clamp fluorometry. *Neuropharmacology* **98**, 3-12, doi:https://doi.org/10.1016/j.neuropharm.2015.03.018 (2015).

72 Bagal, S. K. *et al.* Ion channels as therapeutic targets: a drug discovery perspective. *Journal of medicinal chemistry* **56**, 593-624 (2012).
73 Clare, J. J. Targeting ion channels for drug discovery. *Discovery medicine* **9**, 253-260 (2010).
74 Yaari, Y., Selzer, M. E. & Pincus, J. H. Phenytoin: Mechanisms of its anticonvulsant action. *Annals of Neurology* **20**, 171-184, doi:10.1002/ana.410200202 (1986).
75 McTavish, D. & Sorkin, E. M. Verapamil. *Drugs* **38**, 19-76 (1989).
76 Evans, R. M. The steroid and thyroid hormone receptor superfamily. *Science* **240**, 889-895, doi:10.1126/science.3283939 (1988).
77 Novac, N. & Heinzel, T. Nuclear receptors: overview and classification. *Curr Drug Targets Inflamm Allergy* **3**, 335-346, doi:10.2174/1568010042634541 (2004).
78 Mangelsdorf, D. J. *et al.* The nuclear receptor superfamily: the second decade. *Cell* **83**, 835-839, doi:10.1016/0092-8674(95)90199-x (1995).
79 Tata, J. R. Signalling through nuclear receptors. *Nature Reviews Molecular Cell Biology* **3**, 702-710, doi:10.1038/nrm914 (2002).
80 Chen, T. Nuclear receptor drug discovery. *Current Opinion in Chemical Biology* **12**, 418-426, doi:https://doi.org/10.1016/j.cbpa.2008.07.001 (2008).
81 Rizzo, G. & Fiorucci, S. PPARs and other nuclear receptors in inflammation. *Current Opinion in Pharmacology* **6**, 421-427, doi:https://doi.org/10.1016/j.coph.2006.03.012 (2006).
82 Saltiel, A. R. & Olefsky, J. M. Thiazolidinediones in the treatment of insulin resistance and type II diabetes. *Diabetes* **45**, 1661-1669 (1996).
83 Hedrick, E., Lee, S.-O., Doddapaneni, R., Singh, M. & Safe, S. Nuclear receptor 4A1 as a drug target for breast cancer chemotherapy. *Endocr Relat Cancer* **22**, 831-840 (2015).
84 Wang, Z., Schaffer, N. E., Kliewer, S. A. & Mangelsdorf, D. J. Nuclear receptors: emerging drug targets for parasitic diseases. *The Journal of Clinical Investigation* **127**, 1165-1171, doi:10.1172/JCI88890 (2017).
85 Wang, L. *et al.* TargetHunter: An In Silico Target Identification Tool for Predicting Therapeutic Potential of Small Organic Molecules Based on Chemogenomic Database. *The AAPS Journal* **15**, 395-406, doi:10.1208/s12248-012-9449-z (2013).
86 Lee, W. M. Acetaminophen (APAP) hepatotoxicity—Isn't it time for APAP to go away? *Journal of Hepatology* **67**, 1324-1331, doi:https://doi.org/10.1016/j.jhep.2017.07.005 (2017).
87 Michna, E., Duh, M. S., Korves, C. & Dahl, J. L. Removal of opioid/acetaminophen combination prescription pain medications: assessing the evidence for hepatotoxicity and consequences of removal of these medications. *Pain medicine* **11**, 369-378 (2010).
88 ANDERSON, B. J. Paracetamol (Acetaminophen): mechanisms of action. *Pediatric Anesthesia* **18**, 915-921, doi:10.1111/j.1460-9592.2008.02764.x (2008).
89 Jóźwiak-Bebenista, M. & Nowak, J. Z. Paracetamol: mechanism of action, applications and safety concern. *Acta poloniae pharmaceutica* **71**, 11-23 (2014).
90 Dahlin, D. C., Miwa, G. T., Lu, A. Y. & Nelson, S. D. N-acetyl-p-benzoquinone imine: a cytochrome P-450-mediated oxidation product of acetaminophen. *Proceedings of the National Academy of Sciences* **81**, 1327-1331, doi:10.1073/pnas.81.5.1327 (1984).
91 Arnér, E. S. J. & Holmgren, A. Physiological functions of thioredoxin and thioredoxin reductase. *European Journal of Biochemistry* **267**, 6102-6109, doi:10.1046/j.1432-1327.2000.01701.x (2000).

92 Jan, Y.-H. *et al.* Acetaminophen reactive intermediates target hepatic thioredoxin reductase. *Chemical research in toxicology* **27**, 882-894 (2014).
93 Todd, P. A. & Sorkin, E. M. Diclofenac Sodium. *Drugs* **35**, 244-285, doi:10.2165/00003495-198835030-00004 (1988).
94 Gan, T. J. Diclofenac: an update on its mechanism of action and safety profile. *Current Medical Research and Opinion* **26**, 1715-1731, doi:10.1185/03007995.2010.486301 (2010).
95 Boerma, J. S., Vermeulen, N. P. E. & Commandeur, J. N. M. One-electron oxidation of diclofenac by human cytochrome P450s as a potential bioactivation mechanism for formation of 2′-(glutathion-S-yl)-deschloro-diclofenac. *Chemico-Biological Interactions* **207**, 32-40, doi:https://doi.org/10.1016/j.cbi.2013.11.001 (2014).
96 Clark, A. J. *et al.* Efficacy and safety of transdermal fentanyl and sustained-release oral morphine in patients with cancer and chronic non-cancer pain. *Current Medical Research and Opinion* **20**, 1419-1428, doi:10.1185/030079904X2114 (2004).
97 Raffa, R. B. *et al.* The fentanyl family: A distinguished medical history tainted by abuse. *Journal of Clinical Pharmacy and Therapeutics* **43**, 154-158, doi:10.1111/jcpt.12640 (2018).
98 Vohra, V., Hodgman, M., Marraffa, J., Barba, K. & Stoppacher, R. Fentanyl- and fentanyl analog-related deaths across five counties in Central New York between 2013 and 2017. *Clinical Toxicology* **58**, 112-116, doi:10.1080/15563650.2019.1613548 (2020).
99 Ziegler, J. *et al.* Evolution of morphine biosynthesis in opium poppy. *Phytochemistry* **70**, 1696-1707, doi:https://doi.org/10.1016/j.phytochem.2009.07.006 (2009).
100 Pacifici, G. M. Metabolism and pharmacokinetics of morphine in neonates: A review. *Clinics* **71**, 474-480 (2016).
101 Lötsch, J. *et al.* Pharmacokinetics of morphine and its glucuronides after intravenous infusion of morphine and morphine-6-glucuronide in healthy volunteers. *Clinical Pharmacology & Therapeutics* **60**, 316-325, doi:10.1016/s0009-9236(96)90058-2 (1996).
102 Ohno, S., Kawana, K. & Nakajin, S. Contribution of UDP-Glucuronosyltransferase 1A1 and 1A8 to Morphine-6-Glucuronidation and Its Kinetic Properties. *Drug Metabolism and Disposition* **36**, 688-694, doi:10.1124/dmd.107.019281 (2008).
103 Projean, D., Morin, P. E., Tu, T. M. & Ducharme, J. Identification of CYP3A4 and CYP2C8 as the major cytochrome P450 s responsible for morphine N -demethylation in human liver microsomes. *Xenobiotica* **33**, 841-854, doi:10.1080/0049825031000121608 (2003).
104 Ertl, P. Molecular structure input on the web. *Journal of cheminformatics* **2**, 1 (2010).
105 Zerbino, D. R. *et al.* Ensembl 2018. *Nucleic acids research* **46**, D754-D761 (2017).
106 UniProt Consortium, T. UniProt: the universal protein knowledgebase. *Nucleic acids research* **46**, 2699-2699, doi:10.1093/nar/gky092 (2018).
107 Kanehisa, M. & Goto, S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic acids research* **28**, 27-30 (2000).
108 Isberg, V. *et al.* GPCRdb: an information system for G protein-coupled receptors. *Nucleic Acids Res* **45**, 2936 (2017).
109 Coordinators, N. R. Database resources of the national center for biotechnology information. *Nucleic acids research* **45**, D12 (2017).
110 DeLano, W. L. Pymol: An open-source molecular graphics tool. *CCP4 Newsletter on protein crystallography* **40**, 82-92 (2002).

111 Huang, X., Chen, H. & Shaffer, P. L. Crystal Structures of Human GlyRα3 Bound to Ivermectin. *Structure* **25**, 945-950.e942, doi:https://doi.org/10.1016/j.str.2017.04.007 (2017).
112 Cavasotto, C. N. & Phatak, S. S. Homology modeling in drug discovery: current trends and applications. *Drug Discovery Today* **14**, 676-683, doi:https://doi.org/10.1016/j.drudis.2009.04.006 (2009).
113 Webb, B. & Sali, A. Comparative protein structure modeling using MODELLER. *Current protocols in bioinformatics* **47**, 5.6. 1-5.6. 32 (2014).
114 Gaulton, A. *et al.* ChEMBL: a large-scale bioactivity database for drug discovery. *Nucleic acids research* **40**, D1100-D1107 (2011).
115 Li, H., Leung, K.-S. & Wong, M.-H. in *2012 IEEE Symposium on Computational Intelligence in Bioinformatics and Computational Biology (CIBCB).* 77-84 (IEEE).
116 Roy, K., Kar, S. & Das, R. N. in *Understanding the Basics of QSAR for Applications in Pharmaceutical Sciences and Risk Assessment* (eds Kunal Roy, Supratik Kar, & Rudra Narayan Das) 47-80 (Academic Press, 2015).
117 Rogers, D. & Hahn, M. Extended-Connectivity Fingerprints. *Journal of Chemical Information and Modeling* **50**, 742-754, doi:10.1021/ci100050t (2010).
118 Vandermeersch, T. & Hutchison, G. Open Babel: AN Open Chemical Toolbox. *Journal of Chemoinformatics* **3** (2011).
119 Ma, C., Wang, L. & Xie, X.-Q. Ligand classifier of adaptively boosting ensemble decision stumps (LiCABEDS) and its application on modeling ligand functionality for 5HT-subtype GPCR families. *Journal of chemical information and modeling* **51**, 521-531 (2011).
120 Perkins, J. R. *et al.* PainNetworks: A web-based resource for the visualisation of pain-related genes in the context of their network associations. *PAIN®* **154**, 2586.e2581-2586.e2512, doi:https://doi.org/10.1016/j.pain.2013.09.003 (2013).
121 Zhao, Y. H. *et al.* Predicting penetration across the blood-brain barrier from simple descriptors and fragmentation schemes. *Journal of chemical information and modeling* **47**, 170-175 (2007).
122 Kousik, S. M., Napier, T. C. & Carvey, P. M. The effects of psychostimulant drugs on blood brain barrier function and neuroinflammation. *Frontiers in pharmacology* **3**, 121 (2012).
123 Peyriere, H. *et al.* Adverse Drug Events Associated with Hospital Admission. *Annals of Pharmacotherapy* **37**, 5-11, doi:10.1345/aph.1C126 (2003).
124 Shannon, P. *et al.* Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* **13**, 2498-2504, doi:10.1101/gr.1239303 (2003).
125 Lengauer, T. & Rarey, M. Computational methods for biomolecular docking. *Curr Opin Struct Biol* **6**, 402-406, doi:10.1016/s0959-440x(96)80061-3 (1996).
126 McConkey, B. J., Sobolev, V. & Edelman, M. The performance of current methods in ligand–protein docking. *Current Science*, 845-856 (2002).
127 Pettersen, E. F. *et al.* UCSF Chimera—a visualization system for exploratory research and analysis. *Journal of computational chemistry* **25**, 1605-1612 (2004).
128 Maier, J. A. *et al.* ff14SB: improving the accuracy of protein side chain and backbone parameters from ff99SB. *Journal of chemical theory and computation* **11**, 3696-3713 (2015).

129 Trott, O. & Olson, A. J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry* **31**, 455-461, doi:10.1002/jcc.21334 (2010).
130 Jamei, M. *et al.* The Simcyp® population-based ADME simulator. *Expert opinion on drug metabolism & toxicology* **5**, 211-223 (2009).
131 Jamei, M. *et al.* The Simcyp population-based ADME simulator. *Expert Opin Drug Metab Toxicol* **5**, 211-223, doi:10.1517/17425250802691074 (2009).
132 Kim, S. *et al.* PubChem substance and compound databases. *Nucleic acids research* **44**, D1202-D1213 (2015).
133 Zhuang, X. & Lu, C. PBPK modeling and simulation in drug research and development. *Acta Pharmaceutica Sinica B* **6**, 430-440, doi:https://doi.org/10.1016/j.apsb.2016.04.004 (2016).
134 Cao, Y. & Jusko, W. J. Applications of minimal physiologically-based pharmacokinetic models. *Journal of Pharmacokinetics and Pharmacodynamics* **39**, 711-723, doi:10.1007/s10928-012-9280-2 (2012).
135 Sager, J. E., Yu, J., Ragueneau-Majlessi, I. & Isoherranen, N. Physiologically Based Pharmacokinetic (PBPK) Modeling and Simulation Approaches: A Systematic Review of Published Models, Applications, and Model Verification. *Drug Metab Dispos* **43**, 1823-1837, doi:10.1124/dmd.115.065920 (2015).
136 Ando, H., Izawa, S., Hori, W. & Nakagawa, I. Utility of a single adjusting compartment: a novel methodology for whole body physiologically-based pharmacokinetic modelling. *Theoretical biology & medical modelling* **5**, 19-19, doi:10.1186/1742-4682-5-19 (2008).
137 Mazaleuskaya, L. L. *et al.* PharmGKB summary: pathways of acetaminophen metabolism at the therapeutic versus toxic doses. *Pharmacogenet Genomics* **25**, 416-426, doi:10.1097/FPC.0000000000000150 (2015).
138 Feierman, D. E. The effect of paracetamol (acetaminophen) on fentanyl metabolism in vitro. *Acta Anaesthesiologica Scandinavica* **44**, 560-563, doi:10.1034/j.1399-6576.2000.00513.x (2000).
139 Irwin, J. J. & Shoichet, B. K. ZINC− a free database of commercially available compounds for virtual screening. *Journal of chemical information and modeling* **45**, 177-182 (2005).
140 Armstrong, D. R. *et al.* PDBe: improved findability of macromolecular structure data in the PDB. *Nucleic acids research* **48**, D335-D343 (2020).
141 Anighoro, A., Bajorath, J. & Rastelli, G. Polypharmacology: Challenges and Opportunities in Drug Discovery. *Journal of Medicinal Chemistry* **57**, 7874-7887, doi:10.1021/jm5006463 (2014).
142 Keiser, M. J. *et al.* Predicting new molecular targets for known drugs. *Nature* **462**, 175-181, doi:10.1038/nature08506 (2009).
143 Graham, G. G. & Scott, K. F. Mechanism of Action of Paracetamol. *American Journal of Therapeutics* **12**, 46-55 (2005).
144 Bertolini, A. *et al.* Paracetamol: New Vistas of an Old Drug. *CNS Drug Reviews* **12**, 250-275, doi:10.1111/j.1527-3458.2006.00250.x (2006).
145 Trescot, A. M., Datta, S., Lee, M. & Hansen, H. Opioid pharmacology. *Pain physician* **11**, S133-153 (2008).
146 Mallet, C. *et al.* TRPV1 in brain is involved in acetaminophen-induced antinociception. *PLoS one* **5**, e12748 (2010).

147 Young, S. *et al.* Peripheral site acetylcholinesterase inhibitors targeting both inflammation and cholinergic dysfunction. *Bioorganic & Medicinal Chemistry Letters* **20**, 2987-2990, doi:https://doi.org/10.1016/j.bmcl.2010.02.102 (2010).
148 Sablone, M. R. *et al.* Structure–Activity Relationship of novel phenylacetic CXCR1 inhibitors. *Bioorganic & Medicinal Chemistry Letters* **19**, 4026-4030, doi:https://doi.org/10.1016/j.bmcl.2009.06.027 (2009).
149 Raynor, K. *et al.* Pharmacological characterization of the cloned kappa-, delta-, and mu-opioid receptors. *Molecular Pharmacology* **45**, 330 (1994).
150 Wright, C. I. & Sabine, J. C. THE INACTIVATION OF CHOLINESTERASE BY MORPHINE, DILAUDID, CODEINE AND DESOMORPHINE. *Journal of Pharmacology and Experimental Therapeutics* **78**, 375 (1943).
151 Galli, A. *et al.* Reversible inhibition of acetylcholinesterase by eseroline, an opioid agonist structurally related to physostigmine (eserine) and morphine. *Biochemical Pharmacology* **31**, 1233-1238, doi:https://doi.org/10.1016/0006-2952(82)90009-0 (1982).
152 Keshava, C., McCanlies, E. C. & Weston, A. CYP3A4 Polymorphisms—Potential Risk Factors for Breast and Prostate Cancer: A HuGE Review. *American Journal of Epidemiology* **160**, 825-841, doi:10.1093/aje/kwh294 (2004).
153 Leschziner, G. D., Andrew, T., Pirmohamed, M. & Johnson, M. R. ABCB1 genotype and PGP expression, function and therapeutic drug response: a critical review and recommendations for future research. *The Pharmacogenomics Journal* **7**, 154-179, doi:10.1038/sj.tpj.6500413 (2007).
154 Hong, J.-Y., M.D. *et al.* Fentanyl-sparing Effect of Acetaminophen as a Mixture of Fentanyl in Intravenous Parent-/Nurse-controlled Analgesia after Pediatric Ureteroneocystostomy. *Anesthesiology: The Journal of the American Society of Anesthesiologists* **113**, 672-677, doi:10.1097/ALN.0b013e3181e2c34b (2010).
155 Singla, N. K. *et al.* Plasma and Cerebrospinal Fluid Pharmacokinetic Parameters After Single-Dose Administration of Intravenous, Oral, or Rectal Acetaminophen. *Pain Practice* **12**, 523-532, doi:10.1111/j.1533-2500.2012.00556.x (2012).
156 Cheng, J. *et al.* Computational Systems Pharmacology-Target Mapping for Fentanyl-Laced Cocaine Overdose. *ACS chemical neuroscience* (2019).
157 Björkman, S. Reduction and lumping of physiologically based pharmacokinetic models: prediction of the disposition of fentanyl and pethidine in humans by successively simplified models. *Journal of pharmacokinetics and pharmacodynamics* **30**, 285-307 (2003).
158 Lorphensri, O. *et al.* Sorption of acetaminophen, 17α-ethynyl estradiol, nalidixic acid, and norfloxacin to silica, alumina, and a hydrophobic medium. *Water Research* **40**, 1481-1491, doi:https://doi.org/10.1016/j.watres.2006.02.003 (2006).
159 Taylor, R. R. *et al.* Comparison of the quantification of acetaminophen in plasma, cerebrospinal fluid and dried blood spots using high-performance liquid chromatography–tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis* **83**, 1-9, doi:https://doi.org/10.1016/j.jpba.2013.04.007 (2013).
160 Mutlib, A. E. *et al.* Kinetics of Acetaminophen Glucuronidation by UDP-Glucuronosyltransferases 1A1, 1A6, 1A9 and 2B15. Potential Implications in Acetaminophen−Induced Hepatotoxicity. *Chemical Research in Toxicology* **19**, 701-709, doi:10.1021/tx050317i (2006).

161 Peterson, R. G. & Rumack, B. H. Pharmacokinetics of acetaminophen in children. *Pediatrics* **62**, 877-879 (1978).
162 Jiang, X.-L., Zhao, P., Barrett, J., Lesko, L. & Schmidt, S. Application of Physiologically Based Pharmacokinetic Modeling to Predict Acetaminophen Metabolism and Pharmacokinetics in Children. *CPT: Pharmacometrics & Systems Pharmacology* **2**, 80, doi:10.1038/psp.2013.55 (2013).
163 Mather, L. Clinical pharmacokinetics of fentanyl and its newer derivatives. *Clinical pharmacokinetics* **8**, 422-446 (1983).
164 Feierman, D. E. & Lasker, J. M. Metabolism of fentanyl, a synthetic opioid analgesic, by human liver microsomes. Role of CYP3A4. *Drug Metabolism and Disposition* **24**, 932-939 (1996).
165 Ariano, R. E., Duke, P. C. & Sitar, D. S. Population pharmacokinetics of fentanyl in healthy volunteers. *The Journal of Clinical Pharmacology* **41**, 757-763 (2001).
166 Oda, Y. *et al.* Fentanyl inhibits metabolism of midazolam: competitive inhibition of CYP3A4 in vitro. *British journal of anaesthesia* **82**, 900-903 (1999).
167 Dart, R. C. & Bailey, E. Does Therapeutic Use of Acetaminophen Cause Acute Liver Failure? *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy* **27**, 1219-1230, doi:10.1592/phco.27.9.1219 (2007).
168 Peng, Philip W. H., MBBS, FRCPC & Sandler, Alan N., MBChB, MSc, FRCPC. A Review of the Use of Fentanyl Analgesia in the Management of Acute Pain in Adults *Anesthesiology: The Journal of the American Society of Anesthesiologists* **90**, 576-599 (1999).
169 Chun, L. J., Tong, M. J., Busuttil, R. W. & Hiatt, J. R. Acetaminophen Hepatotoxicity and Acute Liver Failure. *Journal of Clinical Gastroenterology* **43**, 342-349, doi:10.1097/MCG.0b013e31818a3854 (2009).
170 Manyike, P. T., Kharasch, E. D., Kalhorn, T. F. & Slattery, J. T. Contribution of CYP2E1 and CYP3A to acetaminophen reactive metabolite formation. *Clinical Pharmacology & Therapeutics* **67**, 275-282, doi:10.1067/mcp.2000.104736 (2000).
171 Streeter, A., Dahlin, D., Nelson, S. & Baillie, T. The covalent binding of acetaminophen to protein. Evidence for cysteine residues as major sites of arylation in vitro. *Chemico-biological interactions* **48**, 349-366 (1984).
172 Hart, S. G. E., Cartun, R. W., Wyand, D. S., Khairallah, E. A. & Cohen, S. D. Immunohistochemical Localization of Acetaminophen in Target Tissues of the CD-1 Mouse: Correspondence of Covalent Binding with Toxicity. *Fundamental and Applied Toxicology* **24**, 260-274, doi:https://doi.org/10.1006/faat.1995.1029 (1995).
173 Xiao, W. H. & Bennett, G. J. Effects of mitochondrial poisons on the neuropathic pain produced by the chemotherapeutic agents, paclitaxel and oxaliplatin. *Pain* **153**, 704-709 (2012).
174 Stephenson, R. The complexity of pain: part 1. No pain without gain: the augmentation of nociception in the CNS. *Physical Therapy Reviews* **4**, 105-116, doi:10.1179/ptr.1999.4.2.105 (1999).
175 Eccleston, C., Morley, S. J. & Williams, A. C. d. C. Psychological approaches to chronic pain management: evidence and challenges. *BJA: British Journal of Anaesthesia* **111**, 59-63, doi:10.1093/bja/aet207 (2013).
176 Gordon, D. B. & Dahl, J. L. Quality improvement challenges in pain management. *PAIN* **107**, 1-4, doi:10.1016/s0304-3959(03)00267-7 (2004).

177 Wang, L. *et al.* HLungDB: an integrated database of human lung cancer research. *Nucleic acids research* **38**, D665-D669 (2009).
178 Gu, J., Gui, Y., Chen, L., Yuan, G. & Xu, X. CVDHD: a cardiovascular disease herbal database for drug discovery and network pharmacology. *Journal of cheminformatics* **5**, 51 (2013).
179 Hersh, E. V., Pinto, A. & Moore, P. A. Adverse drug interactions involving common prescription and over-the-counter analgesic agents. *Clinical Therapeutics* **29**, 2477-2497, doi:https://doi.org/10.1016/j.clinthera.2007.12.003 (2007).
180 Raffa, R. B. Pharmacology of oral combination analgesics: rational therapy for pain. *Journal of Clinical Pharmacy and Therapeutics* **26**, 257-264, doi:10.1046/j.1365-2710.2001.00355.x (2001).