RESEARCH ARTICLE

Virtual screening of zinc compounds similar to NSAIDS with better pharmacodynamic and pharmacokinetic profiles [version 1; peer review: awaiting peer review]

Faith Kamakia, Stephen Ouma, Richard Kagia

Pharmacology and Pharmacognosy, Kabarak University, Nakuru, Kenya

v1 **First published:** 26 Apr 2023, **12**:444
https://doi.org/10.12688/f1000research.132017.1
Latest published: 26 Apr 2023, **12**:444
https://doi.org/10.12688/f1000research.132017.1

Abstract

BACKGROUND: Pain is a common symptom that is managed in both outpatients and inpatients. There are many side effects associated with opioids such as respiratory depression, constipation, hyperalgesia, and tolerance. Non- steroidal anti-inflammatory drugs cause gastrointestinal tract (GIT) irritation and may be a risk factor for developing peptic ulcer disease. This study aimed to generate active analgesic agents from known analgesics, determine the docking scores of these agents to their receptors, determine the pharmacokinetic properties of these agents, and evaluate their toxicity profiles.

METHODS: PubChem was used to download smiles for ibuprofen, aspirin and celecoxib. Avogadro optimized the ligands. The smiles were copied to SwissSimilarity and were used as query compounds to generate zinc compounds. DrugBank and Protein Data Bank were used to download cyclooxygenase 1 and 2. Molecular docking was done using Chimera and Autodock Vina. Smiles for both query compounds and generated zinc compounds were pasted onto the Protox II webserver and SwissADME for toxicity and pharmacokinetics properties determination. The data was presented in tabular forms with textual descriptions of the contents in the tables.

RESULTS: Aspirin, ibuprofen and celecoxib's zinc compounds were generated and the first 20 compounds were docked to COX-1 and COX-2 enzymes. Seven, one, and four of the docked compounds showed better binding energies to COX-2 than COX-1. The zinc compounds were analyzed for toxicity profiles. ZINC01680731 and ZINC33823423 were predicted to have LD50 of 1240 mg/kg as compared to aspirin's 250mg/kg. Ibuprofen and ZINC39120409 showed LD50 of 299mg/kg and they were hepatoactive. Celecoxib and four of its zinc compounds showed LD50 of 1400mg/kg. All compounds had high GIT absorption and they conformed with Lipinski rule of five.

CONCLUSIONS: ZINC01680731 0.994 and ZINC00600558 0.988 were

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Approval Status *AWAITING PEER REVIEW*

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identified as the best compounds as they showed better binding affinities, toxicities and pharmacokinetics properties compared to standard compounds.

Keywords
Aspirin, Ibuprofen, Celecoxib, Ligand-based virtual screening, Structure-based virtual screening, SwissADME, Protox II

Corresponding author: Faith Kamakia (wfaithmuthoni578@gmail.com)

Author roles: Kamakia F: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Ouma S**: Conceptualization, Investigation, Methodology, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Kagia R**: Formal Analysis, Project Administration, Supervision, Validation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

How to cite this article: Kamakia F, Ouma S and Kagia R. **Virtual screening of zinc compounds similar to NSAIDS with better pharmacodynamic and pharmacokinetic profiles [version 1; peer review: awaiting peer review]** F1000Research 2023, **12**:444 https://doi.org/10.12688/f1000research.132017.1

First published: 26 Apr 2023, **12**:444 https://doi.org/10.12688/f1000research.132017.1

Abbreviations

COX: cyclooxygenase
NSAID: Nonsteroidal anti-inflammatory drugs
BBB: blood brain barrier
MMP: mitochondrial membrane potential
ER: estrogen receptor
Ahr: aryl hydrocarbon receptor

Introduction

Pain is part of normal body protective reflexes. Traditionally, pain has been referred to as a symptom and nowadays, pain is referred as a disease (Clauw *et al.*, 2019). Pain has many different aspects, which encompass both physical and mental aspects. These effects have destructive properties on the body. Pain and related diseases are the leading cause of disabilities and discomfort globally (Fitzmaurice, 2018).

Pain is described as irritable, emotional, and sensory activities that could be associated with damage to tissues according to the International Association for the Study of Pain (Cohen *et al.*, 2018). Most patients managed in the outpatient department have chief complaints associated with pain, which decreases the quality of life for many patients. Acute pain occurs in a period of fewer than three months while chronic pain occurs in a period greater than three months (Cohen *et al.*, 2018).

Management of pain involves both pharmacological and non-pharmacological approaches. Most pharmacological approaches involve the use of analgesics such as NSAIDS and opioids. NSAIDS are used in the management of conditions such as osteoarthritis and lower back p\in (Machado *et al.*, 2021). These medications involve serious toxicities that may worsen patients' clinical outcomes and recovery (Bindu *et al.*, 2020).

Non-selective NSAIDS provide relief from pain due to inhibition of both COX-1 and COX-2 (Ngo & Addison, 2018) in the body leading to decreased production of eicosanoids such as prostaglandins. Selective COX-2 agents lead to inhibition of COX-2 causing lack of eicosanoids. Aspirin exerts its pain modulation by blocking synthesis of prostaglandins (Loussert *et al.*, 2020).

Aspirin inhibits thromboxane A2 which causes irreversible blocking platelet aggregation, leading to persistent bleeding. Aspirin also causes hypersensitivity in patients which leads to anaphylaxis and bronchospasms, and can cause salicylism that manifests as tinnitus. Additionally, aspirin can cause Reye's syndrome in children with a past medical history of viral illness (Christiansen *et al.*, 2021).

NSAIDS have ceiling effects where no more analgesia is exerted despite increase in dose. Non-selective COX inhibitors cause renal failure, irritation and bleeding from gastric mucosa. Selective COX-2 inhibitors lead to both renal and liver failure on chronic use (Bindu *et al.*, 2020).

NSAIDS such as ibuprofen, when used during pregnancy, cause closing of the ductus arteriosus. Ductus arteriosus is important in the circulation of blood in neonates, especially those born with low birth weights. Ibuprofen use in neonates causes drug induced neonatal cholestasis which causes jaundice in the infants due to abnormalities in bile acid synthesis and conjugation, as well as disorders in urea cycle of metabolism (Shen *et al.*, 2021).

Due to the many unwanted side effects associated with cyclooxygenase inhibition, there is a need to develop new anti-inflammatory agents devoid of side effects. This study used aspirin, ibuprofen and celecoxib as query compounds to generate new compounds using the SwissSimilarity online tool. The compounds generated were docked to cyclooxygenase 1 and 2 enzymes (COX-1 and COX-2) and the docking scores were compared to those of aspirin, ibuprofen and celecoxib. Compounds with better docking scores to the query compounds were determined for their pharmacokinetics and toxicity profiles using SwissADME and Protox II webservers respectively.

Study Objectives

i. To determine similar zinc compounds to aspirin, ibuprofen and celecoxib using the SwissSimilarity online tool.

ii. To determine zinc compounds with better docking scores to cyclooxygenase 1 and 2 in reference to aspirin, ibuprofen and celecoxib.

iii. To determine the pharmacokinetic properties of zinc compounds in reference to aspirin, ibuprofen and celecoxib.

iv. To determine the toxicity profiles of zinc compounds in reference to aspirin, ibuprofen and celecoxib.

Methods

Ligands retrieval

The PubChem online tool was opened and aspirin, ibuprofen and celecoxib were searched. The summary button was clicked and this generated the smiles of the compounds. This also allowed the reference compounds (aspirin, ibuprofen and celecoxib) to be downloaded as SDF files. The downloaded files were saved to folders named aspirin, ibuprofen and celecoxib on the desktop of the laptop. Combined Zinc–Drug like was then clicked in SwissSimilarity, which automatically generated a red search button that was clicked on. Zinc compounds were generated.

Ligand-based virtual screening

Ligand-based virtual screening uses active ligands for lead identification and optimization rather than the structures of the protein. Using the SwissSimilarity online tool, the known compounds are used as queries in virtual screening to identify new active compounds. There must be similarities between the known ligands to the library of compounds in the form of geometrical measurements. Pharmacophore-based methods use molecular properties and then generate the similarity score between the known compounds and the ligand compounds. The molecular shape determines the overlap of shapes between the compounds and the known agents and creates the similarity scores. The assumptions of the molecular shape are that the overlap in structures of the known compounds and the ligands show activity (Hamza *et al.*, 2012), therefore the ligands with different shapes to the query compounds are missed in virtual screening. The compounds are then scored and differentiated as active or inactive (Hamza *et al.*, 2012).

In this study, aspirin, celecoxib, and ibuprofen were used as queries to generate zinc compounds based on the structures of the reference compounds (aspirin, celecoxib, and ibuprofen). The SwissSimilarity online tool was used. The SwissSimilarity tool generates agents with high drug-likeness to the agents by use of a vast database like ZINC (Bragina *et al.*, 2022) and this gives a pool of structures to be analysed.

Inclusion criteria for ZINC compounds

ZINC compounds with above 80% similarity scores to the query compounds ibuprofen and aspirin were chosen, while compounds with a similarity score above 70% to celecoxib were chosen. For the compounds that had the same similarity scores, only one compound was included. 20 compounds for ibuprofen, 20 compounds for aspirin and 20 compounds for celecoxib were included giving a total of 60 compounds that were included in this study.

Structure-based virtual screening

Structure-based virtual screening starts with protein structure identification through NMR spectroscopy or homology modelling. This is followed by binding site identification which considers druggability, stereochemistry, and tautomers as well as protonation of the binding site. Receptor preparation is done by modifying the tautomeric forms of histidine receptors, removing unnecessary side chains, determining flexibility of the compounds as well as solvation of the receptor binding sites. Ligand preparation follows by ensuring they are at as minimal energy state as possible which is done by Chimera software (Pettersen *et al.*, 2004). Docking of the ligands to the receptor is done using AutoDock Vina and scoring is done such that the higher the binding scores of the compounds, the higher the binding affinity (Eberhardt *et al.*, 2021). Post docking analysis of the compounds is completed to show the 2D or 3D interactions of the ligands to their receptors, such as by using Discovery studio (RRID: SCR_015651) (Lionta *et al.*, 2014).

In this study, cyclooxygenase 1 and 2 enzymes were prepared using Chimera (RRID: SCR_004097) software which eliminated the unwanted residues bound to the receptors. Ligand preparation was done using Avogadro software (RRID: SCR_011958). Molecular docking was done using AutoDock Vina (RRID: SCR_011958). Zinc compounds that showed higher docking scores in reference to aspirin, celecoxib and ibuprofen were further analyzed for pharmacokinetics as well as toxicity profiles.

Pharmacokinetics properties

The pharmacokinetics properties of the agents were analyzed using SwissADME, which lead to the discovery of agents with improved properties.

Toxicity predictions

Tox 21 (The Toxicology in the 21st century) has led to the development of computational methods for the toxicological assessment of compounds from the identified toxicological pathways (Banerjee *et al.*, 2018). ProTox II webserver is commonly used in toxicity predictions and predicts toxicities such as oral toxicity, organ toxicity (hepatoxicity), and toxicological endpoints which include mutagenicity, cytotoxicity, immunotoxicity, and immunogenicity. The molecular mechanism for predicting toxicities in the body is as well generated from the ProTox-II webserver (Banerjee *et al.*, 2018).

ProTox II (RRID: SCR_018506) webserver was used in this study to determine toxicity profiles of zinc compounds and were compared to the reference compounds.

Data analysis

Descriptive analysis was chosen since the data involved was numerical. Compounds that showed better docking scores to COX-2 and lower docking scores to COX-1 than aspirin, ibuprofen and celecoxib were identified and were chosen as promising compounds. The highlighted compounds were determined for their toxicity profiles and pharmacokinetics properties. Pharmacokinetic data were analyzed non-numerically since the data generated was qualitative.

Results

Docking results

Docking scores of aspirin and zinc compounds to Cox-1 and cox-2

Aspirin downloaded from PubChem was used as the reference against other zinc compounds. The ligands with better docking scores to COX-2 and lower docking scores to COX-1 in reference to aspirin are highlighted in Table 1 below (Faith *et al.*, 2023). ZINC00001050; 0.995, ZINC00409844; 0.992, ZINC01651927; 0.991, ZINC01680731; 0.994, ZINC19405119; 0.986, ZINC33823423; 0.996, and ZINC71771212; 0.994, were analysed further for their pharmacokinetics properties using the SwissADME online webserver and toxicity predictions using the ProTox II webserver.

All the zinc and aspirin compounds showed high gastrointestinal (GIT) absorption and permeation to the blood-brain barrier (BBB). The compounds were shown not to be P-glycoprotein substrates and to lack enzyme inhibition properties to CYP (cytochrome) 1A2, CYP 2C19, CYP2C9, CYP2D6 and CYP3A4 enzymes. The compounds further comply with the Lipinski rule of five. Exceptions to this were ZINC01651927; 0.991 and ZINC19405119; 0.986, where ZINC01651927; 0.991 showed inhibition of CYP1A2 enzyme while ZINC19405119; 0.986 showed no permeation to the BBB.

Docking scores of ibuprofen and zinc compounds to COX-1 and COX-2

The reference compound (ibuprofen) had better docking scores to both COX-1 and COX-2 than the zinc compounds except ZINC39120409; 0.996, which showed a higher docking score to COX-2 than ibuprofen and a lower docking score to COX-1 than ibuprofen as illustrated in Table 2. ZINC39120409; 0.996 was further analyzed for its pharmacokinetic properties and toxicity profile.

ZINC39120409; 0.996 and ibuprofen showed high GIT absorption and permeation to the blood-brain barrier. The compounds shown not to be P-glycoprotein substrates and lack enzyme inhibition properties to CYP 1A2, CYP 2C19, CYP2C9, CYP2D6 and CYP3A4 enzymes. The compounds further complied with Lipinski rule of five.

Celecoxib and zinc compounds docking scores to COX-2 and 1

The highlighted compounds in Table 3 showed better docking scores to the COX-2 enzyme than celecoxib and lower docking scores to COX-1 than celecoxib. ZINC00600558; 0.988; ZINC34660892; 0.988; and ZINC43464372; 0.750; were further analyzed for their pharmacokinetics properties as well as toxicity parameters.

Celecoxib showed high GIT absorption and showed inhibition to CYP1A2 and CYP 2C9. Celecoxib lacks inhibition to CYP2C19, CYP2D6, and CYP3A4. Celecoxib is not a substrate to P-glycoprotein and lacks BBB permeation.

ZINC00600558; 0.988, ZINC34660892 and ZINC43464372; 0.750 showed high GIT permeability, and a lack of CYP2D6 and CYP3A4 inhibition. These zinc compounds are not substrates of P-glycoprotein, and they lack permeation to the BBB. ZINC00600558; 0.988 and ZINC43464372; 0.750 however, showed enzyme inhibition activity to CYP1A2, CYP2C19, and CYP2C9. ZINC34660892 showed a lack of enzyme inhibition to CYP1A2 and the presence of CYP2C9 and CYP2C19 inhibition. They all complied with Lipinski rule of five.

Table 1. Aspirin and zinc compounds docking scores to COX-1 and 2.

Serial no.	Similarity score	Compound	Binding scores to COX-1	Binding scores to Cox2
1.		Aspirin	-6.3	-6
2.	99.50%	ZINC00001050; 0.995;	-6.3	-6.4
3.	99.60%	ZINC00001221; 0.996;	-5.6	-5.5
4.	99.50%	ZINC00149023; 0.995;	-6.4	-6.5
5.	99.20%	ZINC00409844; 0.992;	-7.2	-8.2
6.	99.50%	ZINC01604684; 0.995;	-6.1	-5.6
7.	99.10%	ZINC01651927; 0.991;	-6.8	-7.8
8.	99.50%	ZINC01676317; 0.995;	-5.3	-5.4
9.	99.40%	ZINC01680731; 0.994;	-6.7	-7
10.	99.40%	ZINC02548021; 0.994;	-6.5	-6.8
11.	99.20%	ZINC14488787; 0.992;	-6.3	-5.9
12.	98.60%	ZINC19405119; 0.986;	-10.6	-11.3
13.	98.70%	ZINC26469982; 0.987;	-6.8	-8
14.	99.50%	ZINC32627943; 0.995;	-6.5	-6.4
15.	99.60%	ZINC33823423; 0.996;	-6.5	-7.6
16.	99.60%	ZINC34515547; 0.996;	-6.6	-5.8
17.	99.00%	ZINC39214688; 0.990;	-6.1	-6
18.	99.00%	ZINC39402339; 0.990;	-6.2	-6.1
19.	98.80%	ZINC57339565; 0.988;	-6	-6.1
20.	99.40%	ZINC71771212; 0.994;	-5.6	-7.1

Toxicity

Oral toxicity

ZINC01680731; 0.994 and ZINC01680731; 0.994 were predicted to be the safest with oral LD50 of 1240 mg/kg as compared to aspirin which was predicted to have oral LD50 of 250 mg/kg. ZINC00409844; 0.992, ZINC01651927; 0.991 and ZINC19405119; 0.986 were predicted to have the same oral LD50 as aspirin of 250 mg/kg. ZINC00001050; 0.995 and ZINC71771212; 0.994 were predicted to have oral LD50 of 650mg/kg (see Table 4).

Ibuprofen and ZINC39120409; 0.996 were predicted to have similar oral LD50 of 299 mg/kg.

ZINC00600558; 0.988, ZINC34660892; 0.988 and ZINC43464372; 0.750 showed similar oral LD50 of 1400mg/g as celecoxib.

Hepatotoxicity and immunotoxicity

ZINC00001050; 0.995, ZINC71771212; 0.994 and ZINC33823423; 0.996 were predicted to be hepatotoxic with probability scores of 0.56, 0.56 and 0.55 respectively in relation to aspirin, which is inactive. Ibuprofen and ZINC39120409; 0.996 were predicted to be hepatotoxic with probability scores of 0.66 and 0.56 respectively. ZINC43464372; 0.750 was predicted to be hepatoactive with probability score of 0.58 in relation to celecoxib. None of the compounds was predicted to be immunotoxic (see Table 5).

Genotoxicity

Celecoxib, ZINC34660892; 0.988 and ZINC43464372; 0.750 were predicted to be carcinogenic with probability scores of 0.56, 0.59 and 0.67 respectively. The rest of the compounds were inactive for carcinogenic, mutagenic and cytogenic properties. ZINC00600558; 0.988 was predicted to be inactive for carcinogenesis as compared to celecoxib (see Table 6).

Table 2. Docking scores of ibuprofen to COX-1 and 2 enzymes.

Seral no.	Similarity score	Compounds	Binding scores to COX-1	Binding scores to Cox2
1.		Ibuprofen	-7.5	-7.6
2.	99.80%	ZINC00002647; 0.998;	-5.7	-6.6
3.	99.80%	ZINC00113398; 0.998;	-6.6	-6.5
4.	99.60%	ZINC03750842; 0.996;	-6	-6.5
5.	99.70%	ZINC07023266; 0.997;	-7	-6.9
6.	99.50%	ZINC22063333; 0.995;	-7	-6
7.	99.50%	ZINC22063336; 0.995;	-7	-6.9
8.	99.80%	ZINC28472777; 0.998;	-7.5	-7.3
9.	99.70%	ZINC28472784; 0.997;	-7.4	-7.1
10.	99.70%	ZINC28472788; 0.997;	-7	-7.4
11.	99.70%	ZINC28472792; 0.997;	-7.2	-6.9
12.	99.60%	ZINC36157909; 0.996;	-7.3	-6.3
13.	99.50%	ZINC36157911; 0.995;	-7.1	-6.6
14.	99.80%	ZINC38141758; 0.998;	-6.7	-6.5
15.	99.80%	ZINC38141759; 0.998;	-7.2	-6.8
16.	99.60%	ZINC39120409; 0.996;	-6	-7.9
17.	99.60%	ZINC39120410; 0.996;	-7	-6.5
18.	99.50%	ZINC39120416; 0.995;	-6.7	-6.3
19.	99.50%	ZINC39120418; 0.995;	-7	-6.8
20.	99.50%	ZINC39120419; 0.995;	-7	-6.7
21.	99.60%	ZINC55241870; 0.996;	-7.1	-6.3

Pathway toxicity

ZINC00409844; 0.992 and ZINC01651927; 0.991 were predicted to have activity on aryl hydrocarbon receptors with probability scores of 0.5 as compared to aspirin, which was predicted to be inactive. Celecoxib, ZINC00600558; 0.988 and were predicted to be active for estrogen receptor with probability scores of 1.0, 0.7 and 0.93 respectively (see Table 7).

ZINC00409844; 0.992 and ZINC01651927; 0.991; were predicted to be active for mitochondrial membrane potential with a probability score of 0.67. ZINC43464372; 0.750 was also predicted to be active for mitochondrial membrane potential with a probability score of 0.52,relative to celecoxib that was predicted inactive (see Table 8).

Pharmacokinetics profile

BBB permeation

ZINC19405119; 0.986, celecoxib, ZINC00600558; 0.988, ZINC34660892; 0.988 and ZINC43464372; 0.750 lack blood brain barrier penetration while aspirin has blood brain barrier penetration (see Table 9).

Metabolism

ZINC01651927; 0.991, celecoxib, ZINC00600558; 0.988 and ZINC43464372; 0.750 were all predicted to be inhibitors of CYP1A2. ZINC00600558; 0.988, ZINC34660892; 0.988 and ZINC43464372; 0.750 were predicted to be inhibitors of both CYP2C9 and CYP2C19 in comparison to celecoxib, which lacks CYP2C9 and CYP2C19 inhibition (see Table 10).

Table 3. **Celecoxib and zinc compounds docking scores to COX-1 and 2 receptors.**

Serial no.	Similarity score	Compound	Binding scores to COX-1	Binding scores to Cox2
1.		Celecoxib	-7.7	-9
2.	97.10%	ZINC00598952; 0.971;	-7.5	-8.3
3.	98.80%	ZINC00600558; 0.988;	-7.6	-9.2
4.	70.00%	ZINC01028122; 0.700;	-9.1	-9
5.	76.50%	ZINC01028131; 0.765;	-9.2	-9.7
6.	92.80%	ZINC01044570; 0.928;	-6.9	-8
7.	84.70%	ZINC01414060; 0.847;	-7.2	-8
8.	99.20%	ZINC02047040; 0.992;	-7.6	-8.5
9.	71.00%	ZINC02150297; 0.710;	-7	-8
10.	99.70%	ZINC02570895; 0.997;	-7.7	-8.7
11.	99.50%	ZINC13761811; 0.995;	-7.8	-9.1
12.	88.20%	ZINC13761818; 0.882;	-7.9	-8.3
13.	80.60%	ZINC13761819; 0.806;	-7.9	-8.7
14.	78.30%	ZINC13761871; 0.783;	-6.9	-8.3
15.	96.20%	ZINC22054185; 0.962;	-7.6	-8.4
16.	77.30%	ZINC26673721; 0.773;	-8.2	-8.1
17.	86.20%	ZINC33413600; 0.862;	-7.2	-8.6
18.	98.80%	ZINC34660892; 0.988;	-7.5	-9
19.	93.50%	ZINC40450106; 0.935;	-7.6	-8.2
20.	75.00%	ZINC43464372; 0.750;	-7.6	-9.2
21.	93.10%	ZINC77291217; 0.931;	-7.7	-8.3

Table 4. **Oral acute toxicity prediction, class toxicity and accuracy percentage of different compounds.**

Serial number	Compound name	Oral LD50 (mg/kg)	Predicted toxicity class	Prediction Accuracy (%)
1.	Aspirin	250	III	100
2.	ZINC00001050; 0.995;	650	IV	100
3.	ZINC00409844; 0.992;	250	III	72.9
4.	ZINC01651927; 0.991;	250	III	72.9
5.	ZINC01680731; 0.994;	1240	III	70.97
6.	ZINC71771212; 0.994;	650	III	70.97
7.	ZINC19405119; 0.986;	250	III	69.2
8.	ZINC01680731; 0.994;	1240	III	70.97
9.	Ibuprofen	299	III	100
10.	ZINC39120409; 0.996;	299	III	100
11.	Celecoxib	1400	IV	54.26
12.	ZINC00600558; 0.988;	1400	IV	54.26
13.	ZINC34660892; 0.988;	1400	IV	54.26
14.	ZINC43464372; 0.750;	1400	IV	67.38

Table 5. **Hepatotoxicity and Immunotoxicity predictions.**

Serial number	Compound name	Hepatotoxicity	Probability	Immunotoxicity	Probability2
1.	Aspirin	I	0.51	I	0.99
2.	ZINC00001050; 0.995;	A	0.56	I	0.99
3.	ZINC00409844; 0.992;	I	0.61	I	0.96
4.	ZINC01651927; 0.991;	I	0.61	I	0.99
5.	ZINC01680731; 0.994;	I	0.57	I	0.97
6.	ZINC71771212; 0.994;	A	0.56	I	0.99
7.	ZINC19405119; 0.986;	I	0.63	I	0.97
8.	ZINC33823423; 0.996;	A	0.55	I	0.98
9.	Ibuprofen	A	0.66	I	0.99
10.	ZINC39120409; 0.996;	A	0.56	I	0.99
11.	Celecoxib	I	0.6	I	0.99
12.	ZINC00600558; 0.988;	I	0.59	I	0.96
13.	ZINC34660892; 0.988;	I	0.6	I	0.95
14.	ZINC43464372; 0.750;	A	0.58	I	0.98

Table 6. **End points genetic toxicities predictions.**

Serial number	Compound name	Carcinogenic	P	Mutagenic	P2	Cytotoxic	P3
1.	Aspirin	I	0.86	I	0.97	I	0.94
2.	ZINC00001050; 0.995;	I	0.73	I	0.87	I	0.66
3.	ZINC00409844; 0.992;	I	0.63	I	0.57	I	0.91
4.	ZINC01651927; 0.991;	I	0.63	I	0.57	I	0.91
5.	ZINC01680731; 0.994;	I	0.78	I	0.97	I	0.82
6.	ZINC71771212; 0.994;	I	0.73	I	0.87	I	0.66
7.	ZINC19405119; 0.986;	I	0.75	I	0.84	I	0.81
8.	ZINC33823423; 0.996;	I	0.82	I	0.91	I	0.59
9.	Ibuprofen	I	0.74	I	0.99	I	0.85
10.	ZINC39120409; 0.996;	I	0.59	I	0.94	I	0.84
11.	Celecoxib	A	0.56	I	0.75	I	0.91
12.	ZINC00600558; 0.988;	I	0.5	I	0.78	I	0.87
13.	ZINC34660892; 0.988;	A	0.59	I	0.78	I	0.89
14.	ZINC43464372; 0.750;	A	0.67	I	0.5	I	0.64

Post-docking analysis (Faith *et al.*, 2023)

Aspirin and ZINC01680731; 0.994 interactions with COX-2 receptor (Figure 1)

In both aspirin and ZINC01680731; 0.994, the phenyl ring is involved in pi-pi binding with histidine at chain A of the receptor at position 388. The conventional hydrogen bond in aspirin is at tryptophan of chain A of position 387 while in ZINC01680731; 0.994, three conventional hydrogen bonds are at histidine at positions 207 of chain A, tryptophan at position 387 of chain A and threonine at position 206 of chain A. Van der Waals forces at positions 391 and 390 of leucine, 199 of alanine, 203 of glycine, and 206 and 385 of threonine in chain B of COX-2 receptor in aspirin are different from the van der Waals forces in ZINC01680731; 0.994 at positions 199, 202 and 385. These differences in binding of the

Table 7. **Tox 21 Nuclear receptor signaling pathway toxicity prediction.**

Compounds	Aryl hydrocarbon receptor	P
Aspirin	I	0.99
ZINC00409844; 0.992;	A	0.5
ZINC01651927; 0.991;	A	0.5
Compounds	**ER (estrogen receptor)**	**P**
Celecoxib	A	1
ZINC00600558; 0.988;	A	0.7
ZINC34660892; 0.988;	A	0.93

Table 8. **Tox 21 stress response pathway toxicity predictions.**

Serial no.	Compound	Mitochondrial membrane potential	P
1.	Aspirin	I	0.97
2.	ZINC00409844; 0.992;	A	0.67
3.	ZINC01651927; 0.991;	A	0.67
4.	Celecoxib	I	0.87
5.	ZINC43464372; 0.750;	A	0.52

Table 9. **Compounds that lack BBB permeation compared to standards.**

Serial no.	Compound	Blood brain barrier permeation
1.	Aspirin	Yes
2.	ZINC19405119; 0.986;	No
3.	Celecoxib	No
4.	ZINC00600558; 0.988;	No
5.	ZINC34660892; 0.988;	No
6.	ZINC43464372; 0.750;	No

Table 10. **Enzyme inhibition.**

Compound	CYP2C9 Inhibition	Compound2	CYP2C19 Inhibition
Celecoxib	No	Celecoxib	No
ZINC00600558; 0.988;	Yes	ZINC00600558; 0.988;	Yes
ZINC34660892; 0.988;	Yes	ZINC34660892; 0.988;	Yes
ZINC43464372; 0.750;	Yes	ZINC43464372; 0.750;	Yes
Compound	**CYP1A2 Inhibition**		**CYP1A2 INHIBITION**
Aspirin	No		
ZINC01651927; 0.991;	Yes	ZINC00600558; 0.988;	Yes
Celecoxib	Yes	ZINC43464372; 0.750;	Yes

compounds to the COX-2 receptor could have been attributed to ZINC01680731; 0.994 having a higher binding score compared to aspirin (Figure 1).

Ibuprofen and ZINC39120409; 0.996 2D-interactions with receptors (Figure 2)

Both ibuprofen and ZINC39120409; 0.996 showed similar modes of interactions in COX-1 as compared to their binding to COX-2. Ibuprofen showed similar interactions to COX-2 as ZINC39120409; 0.996 with the exemptions that ibuprofen's pharmacophore also bonded with pi-pi binding to active sites in COX-2 which was absent from ZINC39120409; 0.996 binding to COX-2 (Figure 2).

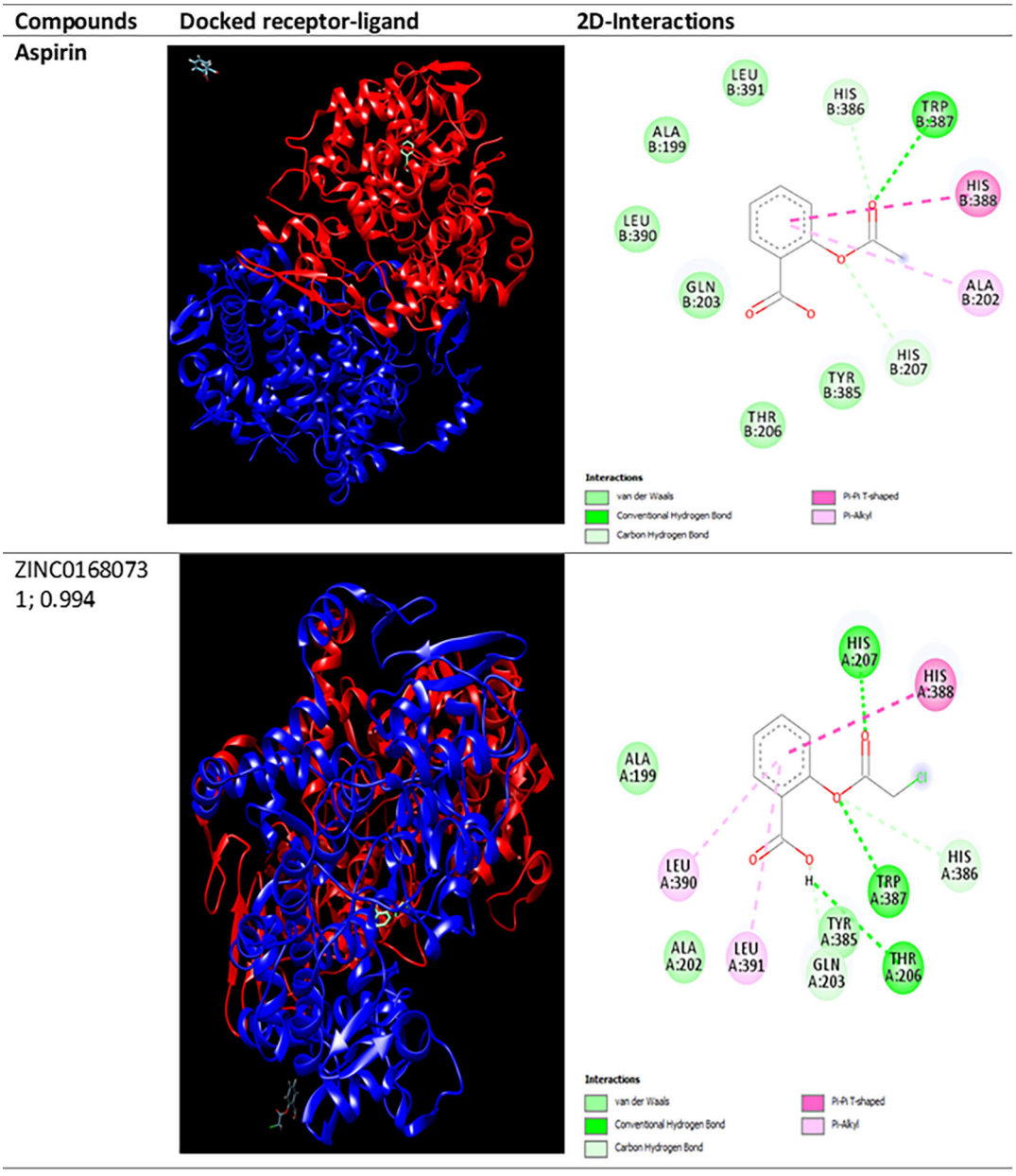

Figure 1. Aspirin and zinc compounds 2D-interactions with receptors.

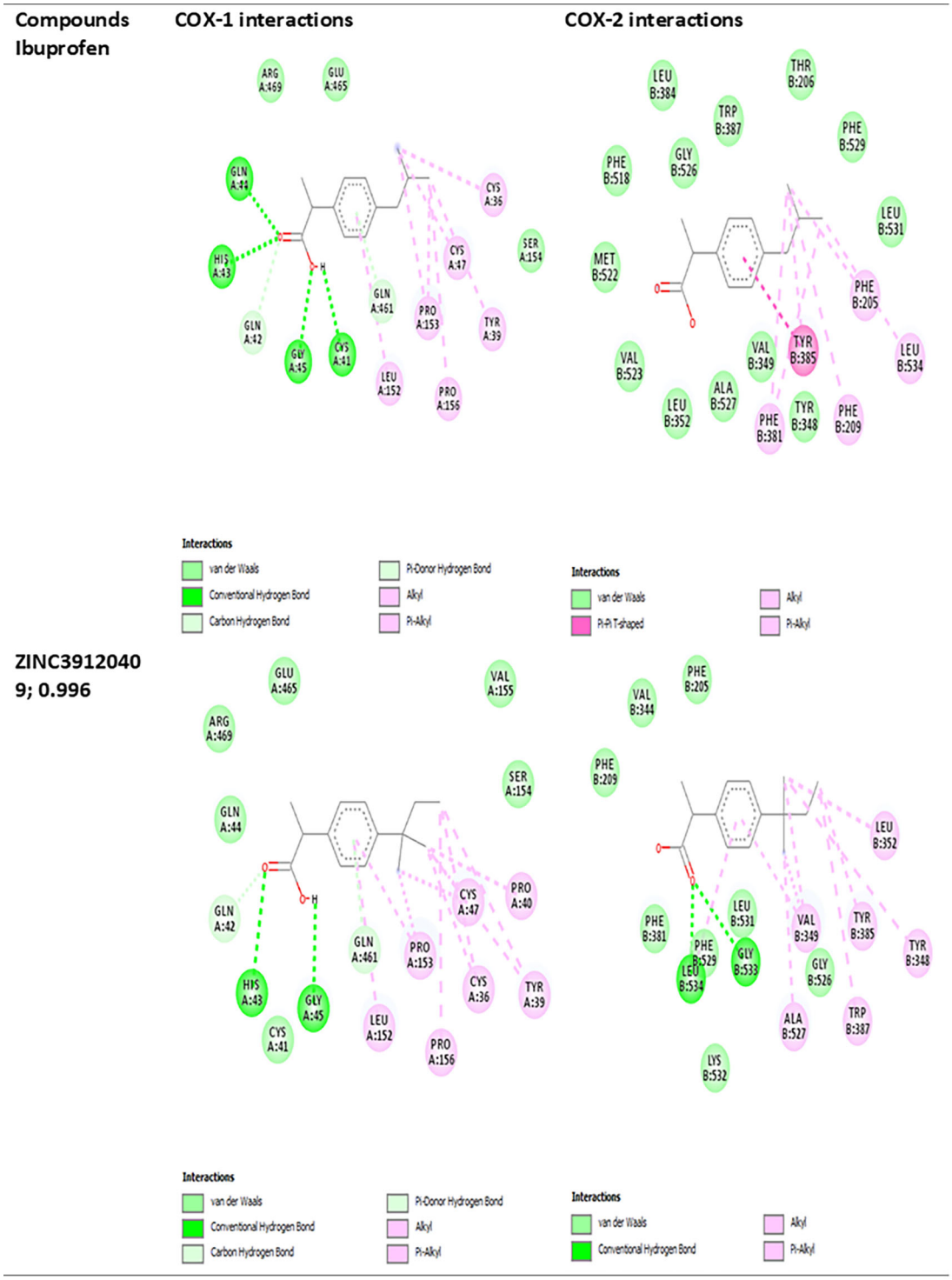

Figure 2. Ibuprofen and zinc compounds 2D-interactions with receptors.

Celecoxib and its zinc compounds 2D-visualization (Figures 3 and 4)

Celecoxib's pharmacophore binds similarly to active sites in COX-1 and COX-2 with fluorine binding to active sites in both receptors (Figure 3). A halogen bond was noted in all the zinc compounds. Covalent bonding, van der Waals forces,

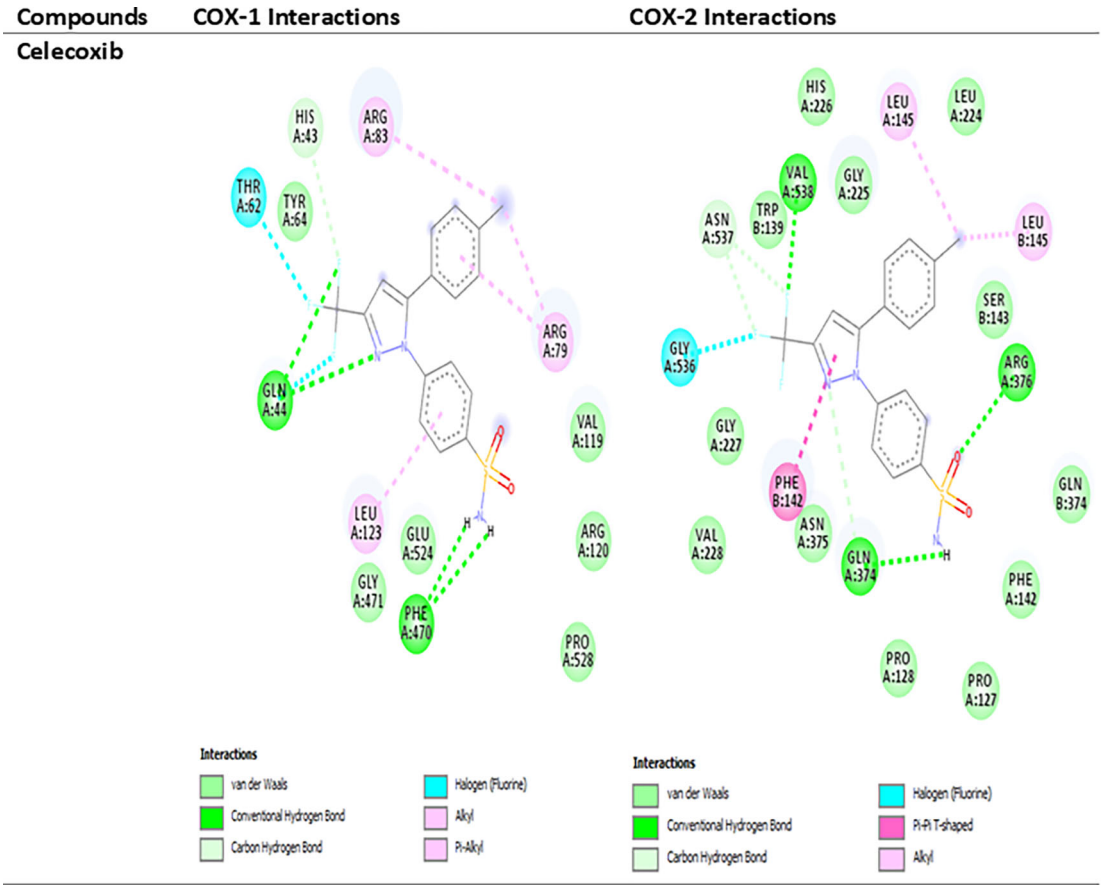

Figure 3. Celecoxib 2D-interactions with receptors.

alkyl, and pi-alkyl bonds were noted in all the compounds' pharmacophores in binding with COX-1 active sites. Celecoxib's pharmacophore interacted with active sites in COX-2 using van der Waals forces, covalent bonding, and pi-pi bonds, Pi-anion and pi-sigma bonds were noted in ZINC00600558; 0.988, and ZINC01028131; 0.765; respectively (Figure 4).

Discussion

The drug molecules used as standards in this study were assessed for similar zinc compounds using SwissSimilarity online tool. NSAIDs were analysed for their docking scores with those having better binding energies to COX-2 than the standard known compounds and lower docking energies to COX-1 than the reference compounds being preferred.

Cox breaks down arachidonic acid into prostaglandin H2 and prostaglandin G2. Prostaglandin GH2 rapidly decomposes into thromboxane, prostacyclin, and other forms of prostaglandin such as PGE and PGF2*a*. Thromboxane effects involve platelet aggregation and vasoconstriction, PG12 inhibits platelet aggregation and vasoconstriction, PGE2 regulates renal function and PGF2a is used to indicate oxidative stress (Majed & Khalil, 2012). COX-1 is expressed constitutively in human cells such as somatic cells of the ovary and therefore maintains normal physiological functions (Kirkby *et al.*, 2012) such as normal GIT integrity by maintaining mucus and bicarbonate secretion. COX-2 is highly inducible by factors such as hypoxia (Wang *et al.*, 2019) and inflammation. Conditions such as pre-eclampsia occur due to dysregulation of thromboxane/prostaglandin 12 causing an increase in maternal thromboxane.

In Table 1, ZINC00001050; 0.995; ZINC00409844; 0.992; ZINC01651927; 0.991; ZINC01680731; 0.994 ZINC19405119; 0.986; ZINC33823423; 0.996; and ZINC71771212; 0.994; showed higher docking scores to COX-1 and COX-2in reference to aspirin. These compounds, therefore, would produce fewer GIT side effects such as ulcers than aspirin. ZINC39120409; 0.996 showed better binding energies to COX-2 than COX-1 than ibuprofen as demonstrated in Table 2. Table 3 demonstrated that ZINC00600558; 0.988; ZINC34660892; 0.988; and ZINC43464372; 0.750; have

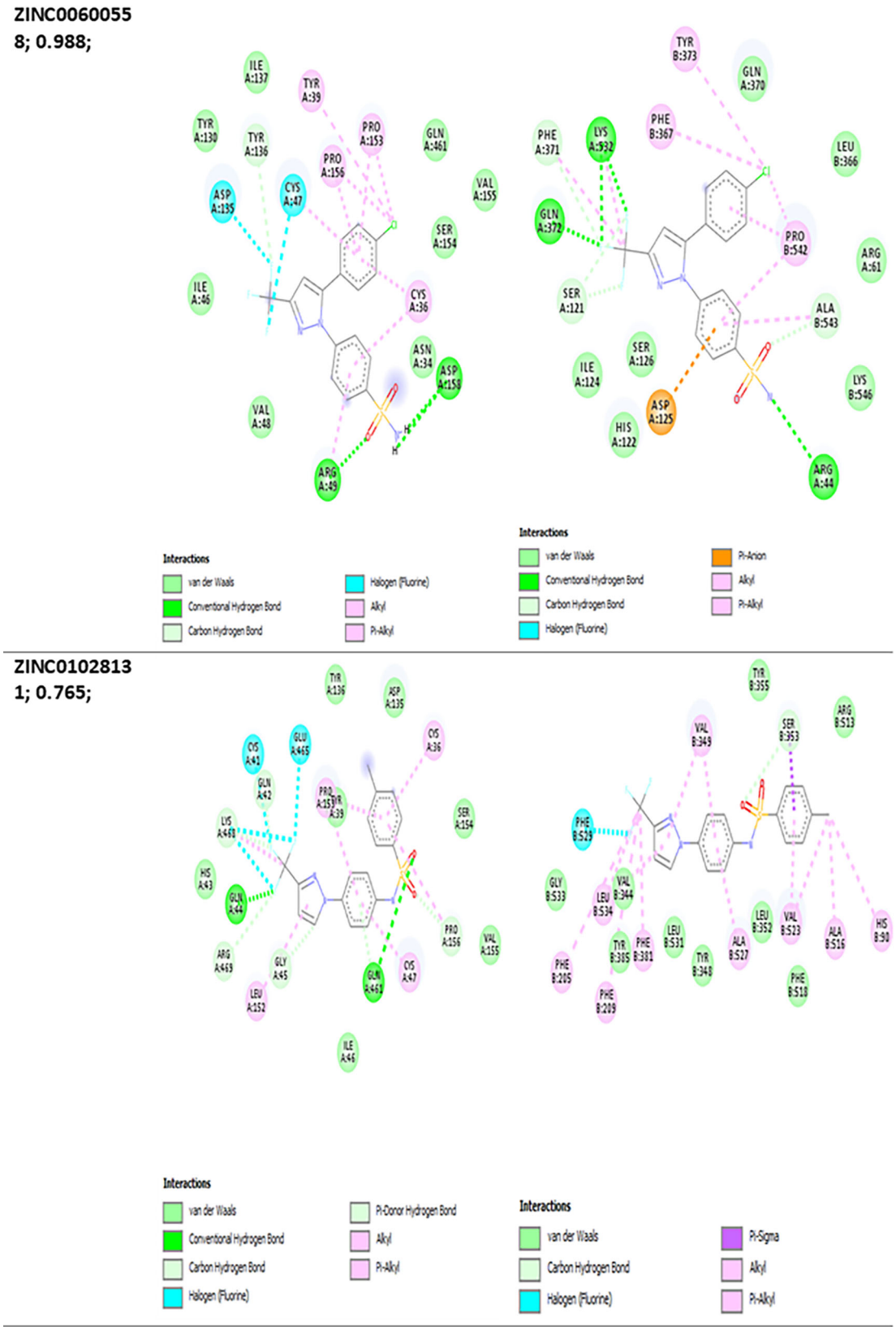

Figure 4. **Zinc compounds' 2D-interactions with receptors.**

higher docking scores to COX-2 than COX-1 in reference to celecoxib and therefore, create promising results to the discovery of COX-2 selective agents.

ProTox II webserver was used to predict toxicities of the compounds. In Table 4, oral toxicities of the compounds were highlighted. ZINC00409844; 0.992; ZINC01651927; 0.991, ZINC19405119; 0.986 showed LD50 of 250mg/kg similar to aspirin. ZINC00001050; 0.995 and ZINC71771212; 0.994; showed LD50 of 650mg/kg therefore, they were considered safer than aspirin. ZINC01680731; 0.994 and ZINC33823423; 0.996; showed LD50 of 1240mg/kg and therefore, they were considered the safer zinc compounds than aspirin. Celecoxib and its zinc compounds showed LD50 of 1400mg/kg and therefore, they were considered harmful when swallowed as indicated in Table 4. Ibuprofen and its zinc compound showed an LD50 of 299mg/kg.

Table 5 indicated the organ toxicity and immunotoxicity predictions. ZINC00001050; 0.995, ZINC71771212 and ZINC33823423; 0.996; were predicted to be active in causing hepatotoxicity with probability scores of 0.56 and 0.55 as compared to aspirin which was predicted to be inactive. Ibuprofen and its zinc compound ZINC39120409; 0.996 were predicted to be active in causing hepatotoxicity with probabilities of 0.66 and 0.56 respectively. Ibuprofen-induced hepatotoxicity has been demonstrated by other researchers (Zoubek *et al.*, 2020). ZINC43464372; 0.750 was predicted to be hepatotoxic with a probability value of 0.58 as compared to celecoxib which was predicted to be inactive with a probability of 0.60.

Celecoxib and its zinc compounds, namely ZINC00600558; 0.988; and ZINC34660892; were predicted to be active in signalling estrogen receptors with probability scores of 1.0 and 0.70 respectively. These predictions have been supported by other researchers such as (Bafna *et al.*, 2020) where celecoxib was proven to inhibit estrogen receptor alpha positive.

In Tables 9 and 10, the pharmacokinetics profiles of the NSAIDs were outlined. Compounds which lack cytochrome P450 system inhibition are ideal since they lack the potential to interact with food and other drugs. ZINC01714507; 0.986, celecoxib, ZINC00600558; 0.988; ZINC34660892; 0.988 and ZINC43464372; 0.750 showed CYP2C9 inhibition as outlined in Table 10. Inhibition of CYP2C9 could affect the metabolism of drugs such as S-warfarin and CYP2C19 enzyme inhibition affects the metabolism of drugs such as R-warfarin. ZINC00600558; 0.988; ZINC34660892; 0.988 and ZINC43464372; 0.750 showed CYP2C19 inhibition as indicated in Table 10. ZINC01714506; 0.986; ZINC00600558; 0.988; ZINC43464372; 0.750 and ZINC01714507; 0.986 showed CYP1A2 inhibition as indicated in Table 10. All NSAIDs and their zinc compounds lack CYP2D6 and CYP3A4 inhibition.

All NSAIDS in Table 9 and their zinc compounds showed permeation to BBB with the exception of ZINC19405119; 0.986; celecoxib, ZINC00600558; 0.988; ZINC34660892; 0.988, ZINC43464372; 0.750, ZINC01557001; 0.987, ZINC19281575; 0.992; and ZINC00294715; 0.980; which showed lack of BBB permeation.

All the NSAIDs and their zinc compounds were not substrates for the P-glycoprotein efflux system, and this showed promising inferences in terms of influencing the bioavailability of the molecules and resistance. All NSAIDs as well as their zinc compounds showed high GIT absorption, and this is important in the absorption of the molecules and achieving high bioavailability.

Conventional hydrogen bonding and van der Waals forces were predominantly between the compounds pharmacophore and cyclooxygenase's enzyme binding sites. These bonds were manifested by aspirin, ZINC01680731; 0.994, and ibuprofen among other compounds. Other bonding forces were pi-pi stacking of the ligands phenyl ring with the targets such as seen in ibuprofen and celecoxib binding modes in Figures 2 and 3-4 respectively.

Conclusion

The objectives of this study were achieved. Compounds with better docking scores to reference compounds were highlighted and their pharmacokinetic profiles were assessed.

This study gave results that are highly promising in the discovery of drugs with better pharmacokinetic profiles and fewer side effects. Compounds with better docking scores to receptors and their pharmacokinetics profile were identified. ZINC01680731; 0.994 was considered the safest among all ofaspirin's zinc compounds since it showed the highest LD50 as well as a lack of hepatoactivity. ZINC39120409; 0.996 and ibuprofen showed similar toxicity profiles since they both showed activity in hepatotoxicity. ZINC00600558; 0.988; was predicted to be carcinoinactive as compared to all the zinc compounds of celecoxib and therefore, it was considered the best compound.

In summary, ZINC01680731; 0.994, ZINC00600558; 0.988 were considered the safest considering their better docking score, better pharmacokinetics and better toxicity profiles. Further studies such as quantitative structure-activity

relationship for ZINC01680731; 0.994, ZINC00600558; 0.988, and ZINC39120409; 0.996 should be done to improve on toxicity and pharmacokinetic properties. in vitro studies of these compounds should be done to assess further pharmacological activities.

Data availability

Underlying data

Harvard DataVerse: Virtual screening of Ibuprofen, Celecoxib and Aspirin. https://doi.org/10.7910/DVN/XMFF8N (Faith *et al.*, 2023).

This project contains the following underlying data:

a. ADME for NSAIDs

b. Docking scores

c. Oral toxicity

d. Post Docking analysis

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