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IN-SILICO COMBINATORIAL INHIBITION EFFECT ANALYSIS OF NSAIDS AGAINST MMP-9 FOR THE TREATMENT OF CANCER

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Keywords:

Cancer, Matrix metalloproteinases-9 (MMP-9), Nonsteroidal anti-inflammatory drugs (NSAIDs), ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity), Molecular Docking

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ABSTRACT: Overexpression of Matrix metalloproteinases (MMPs) in cancers promotes tumor by degrading the barrier of extracellular matrix and angiogenesis. MMP-9 plays a major role in the progression of cancers by enhancing migration, cell survival, angiogenesis, epithelial-to-mesenchymal transition, immune response induction, and tumor microenvironment formation thus, it is considered as a lead drug target to treat cancer. NSAIDs or Nonsteroidal anti-inflammatory drugs have been widely investigated for their effectiveness in different cancer types but promising candidates for clinical use are still halfway. The present study aims to investigate a combination of NSAIDs that can convey high-potential structural inhibition against MMP-9 utilizing a molecular docking-based *in-silico* approach. A thorough literature survey followed by ADMET analysis provided the best NSAID candidates covering diverse chemical space for the analysis. Molecular docking of NSAIDs with MMP-9 individually proved oxaprozin and piroxicam as best candidates for structural inhibition of MMP-9. Combination docking gave a high binding energy of -12.98 kcal/mol for the synergistic inhibitory effect of oxaprozin and piroxicam against MMP-9. Thus, further in-vitro analysis can provide a highly effective NSAID combination to treat the pathologies of cancer in an efficient manner.

INTRODUCTION: Cancer refers unusual division and growth of body cells with the capability to proliferate to distant parts of the body. It is the second major cause of death worldwide. The International Agency for Research on Cancer gave a detailed report on global cancer occurrence and death based on GLOBOCAN (Global Cancer Observatory) 2020 data.

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According to this report, about 19.3 million new cancer cases arose, and about 10.0 million cancer patients died worldwide in the year 2020. Approximately 2.3 million (11.7 %) new breast cancer cases and 2.2 million (11.4 %) new lung cancer cases were identified in the year 2020. Also, an increase to 28.4 million cancer patients by the year 2040 has been proposed [1].

According to the National Cancer Registry Programme Report 2022, India's estimated breast cancer occurrence and prevalence rate was 105.4 per 100000 in females, and the lung cancer rate was 95.6 per 100000 in males. The probability of cancer development is 1 in 9 individuals for all cancers in

both sexes between 0 and 74 years of age. The occurrence of cancer cases in India is known to increase by 12.8% from the year 2022 to 2025. The Global Cancer Observatory (GLOBOCAN) forecasted 2.08 million cancer cases, indicating a rise of 57.5% from the year 2020 to 2040 for India. The increment in cancer cases is associated with lifestyle factors that include tobacco and alcohol consumption, obesity, population aging, and growth. The most common body parts prone to cancer are the digestive system, breast, genitals, oral cavity, and respiratory system. Lung cancer is most prominent in males, while breast cancer is in females. However, in children between 0 to 14 years of age, lymphoid leukemia is the most common cancer [2].

Matrix metalloproteinases (MMPs) are members of the zinc-dependent extracellular matrix (ECM) endopeptidases family, having 23 members in humans. It has two conserved motifs of which one is the prodomain containing cysteine, responsible for partially restricting the catalytic activity, and the second motif is the catalytic domain having histidine, responsible for the endopeptidase activity [3]. They are accountable for the deterioration and modification of the proteins that form the ECM. They have a proteolytic activity that plays an important role in different pathological and physiological processes, including tissue remodeling, organ development, control of inflammatory functions, and cancer progression [4]. The different classes of MMPs perform different functions, such as collagenase mediates the degeneration of triple-helical fibrillar collagen. Gelatinases are important in various physiological and cellular processes like wound healing, cell migration, and angiogenesis [5]. Stromelysins have the potential to degrade laminin, fibronectin, gelatin, and collagen [6]. Matrilysins degrade components of ECM [7]. MT-MMPs are cell surface active enzymes and have collagenolytic and proteolytic activity towards ECM components [8].

Matrix metalloproteinase 9 (MMP-9) is a component of the family of Gelatinase B, and it is capable of degrading gelatin. It is normally present in the cerebellum, hippocampus, and cerebral cortex [9]. The bone marrow is the main site for the synthesis of MMP-9, which is then stored in neutrophils. Further, macrophages are also a dominant originator of MMP-9 [10]. MMP-9 is connected with many physiological processes such as ECM degradation, tissue remodeling, and fractionation of cell surface proteins. Upregulation of MMP-9 has promoted the progression of many diseases, such as emphysema in Smad3-null mice. MMP-9 overexpression also enhances the invasiveness of the LNCaP cell line of prostate tumor. It encourages coronary thrombosis in arteries *in-vivo* [10]. It is a biomarker and a lead therapeutic target of hepatocellular carcinoma, and also increases cardiac inflammation and fibrosis [11]. Thus, MMP-9 overexpression is associated with a diverse range of pathologies of cancers [12]. The restriction of MMP-9 activity is performed by matrix metalloproteinase inhibitors (MMPIs) binding to the zinc ion at the catalytic site [13]. Many of the studies have reported excessive expression of MMP-9 as an important factor for cancer pathogenesis and progression [14]. Gelatinases have an important role in tumorigenesis by controlling the survival of cancer cells, migration, stimulation of immune response, and the generation of cancer microenvironment [13].

MMP-9 (Gelatinase B) has been reported to promote cancers, including breast, lung, gastric, pancreatic, and prostate cancer. In lung cancer, MMP-9 is induced by Skp2, a constituent of the E3 ubiquitin ligase. Skp2 has an important role in the induction of p27 degradation; thus, overexpression of Skp2 may cause an increase in p27 proteolysis and encourage cell and tumor invasion and metastasis [15]. In breast cancer, the overexpression of MMP-9 relates to the expression of transcription factor activator proteins AP-2 and HER2. The overexpression of HER2 and AP-2 is responsible for MMP induction and gelatinase regulation [16]. Overexpression of MMP-9, therefore, has a strong connection with the extensive range of cancers and their progression, so MMP-9 can be considered as a potential target to develop effective therapies against cancer.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are frequently used drugs for the treatment of pain, fever, stiffness, and inflammation. Globally, over 30 million people use NSAIDs every day. Asprin has been used for the last 120 years and is considered the procreator of all NSAIDs. Based on chemical structures, COX inhibitory properties, and

selectivity, the NSAIDs are classified as non-selective and selective NSAIDs. The non-selective NSAIDs include NSAIDs-carboxylic acid (Asprin, Naproxen, Diclofenac, Ibuprofen, Indomethacin, Ketoprofen, and Flurbiprofen), Oxicams (Piroxicam), preferential COX-2 inhibitors-Carboxamides (Meloxicam), Sulphonanilides (Nimesulide), and Naphthalenes (Nabumetone), while the selective COX-2 inhibitors include diaryl-substituted Pyrazoles/Furanones (Celecoxib, Rofecoxib, Valdecoxib) [17]. The major mode of action of NSAIDs is inhibition of cyclooxygenase (COX-1 and COX-2)

Wang et al., 2020, studied that flurbiprofen inhibits inflammatory factor expression, multiplication, invasion, and migration of colorectal cancer cells by suppressing the expression of COX2 and MMP-9. The inflammatory factor inhibition is measured by TNF-α, IL-β, and IL-6 levels through ELISA. These factors are decreased in flurbiprofen-treated cells. Moreover, multiplication, invasion, and migration were measured by transwell and wound healing assay with SW620 cells. The western blotting method showed the inhibited expression of MMP-9 in the samples treated with Flurbiprofen [18]. Prasad *et al.,* 2024 studied the protective effects of NSAIDs (Aspirin and Naproxen) in TMPSS2-ERG fusion-driven prostrate tumorigenesis as inhibitory effects in proliferation and inflammation.

The effect of NSAIDs was concerned with the inhibited expression of M-CSF, IL-33, CCL22, CCL12, and CD93, which are tumor-promoting factors; chemerin, Fit-3 ligand, and IGFBP-5, which are growth signaling molecules, and MMP-9, which are stromal alternation proteins [19]. Syggelos *et al.,* 2007 investigated the inhibitory effects of NSAIDs on both MMP-2 and MMP-9 by gelatin zymography [20]. Fisher & Demel, 2019 discussed NSAIDs as potential therapeutic agents in overcoming inflammation in intracranial aneurysms (IA) progression. They effectively suppress many inflammatory factors, including nuclear factor-kB and MMPs (MMP-9) which are involved in IA. Several other studies have been performed on the MMP-9 inhibition by NSAIDs for treating cancer and other inflammatory responses [21]. Therefore, the effective role of MMP-9 in the development and progression of carcinogenic conditions and the efficient anti-inflammatory properties of the Non-Steroidal Anti-inflammatory drugs (NSAIDs), provide a foundation to the present study for the identification of potential NSAID combinations that display synergistic effects and can inhibit the MMP-9 structurally, using an *in-silico* approach, to provide a high potential treatment against cancers.

METHODOLOGY:

ADMET Analysis and Principal Component Analysis (PCA): Previous studies and anti-inflammatory properties of non-steroidal anti-inflammatory drugs (NSAIDs) led to the selection of Diflunisal, Fenoprofen, Flurbiprofen, Ketoprofen, Ketorolac, Nabumetone, Naproxen, Oxaprozin, Piroxicam, and Celecoxib for identification of their possibilities as MMP-9 inhibitor. The physiochemical properties of these NSAIDs were evaluated using SwissADME [22]. The analysis of toxicity was performed using ProTox II while the bioactivity was analyzed *in-silico* by Molinspiration (https://www.molinspiration.com/) web servers respectively [23]. The Origin 2023b was used for generating a chord diagram for comparison of different NSAID propertie**s.**

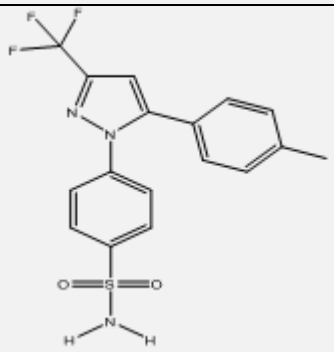
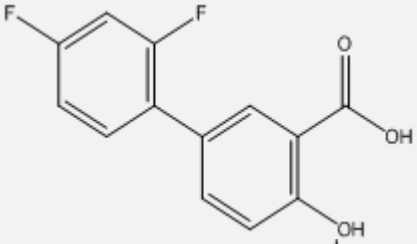
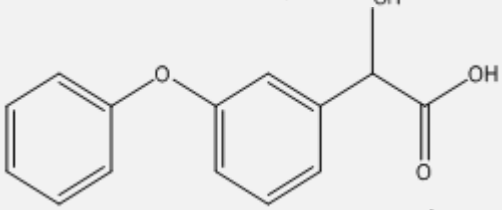
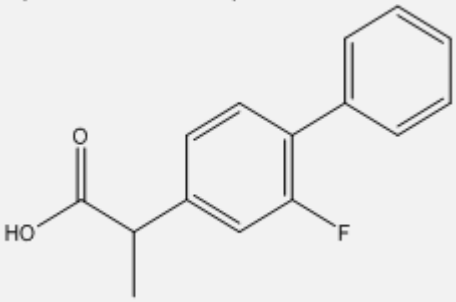
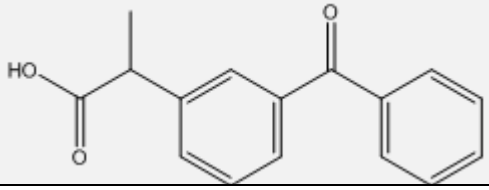
The Minitab trial version 2021 was utilized for conducting the Principal Component Analysis (PCA) which evaluates the correlation between the bioactivity, physiochemical properties, and toxicity of the selected NSAIDs.

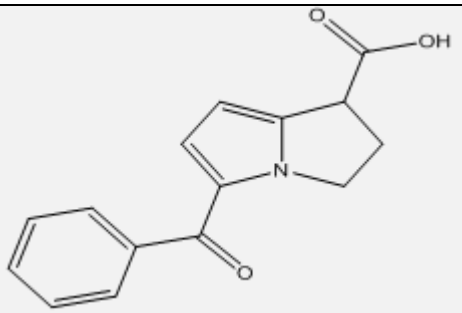
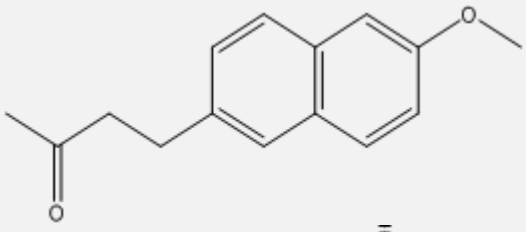
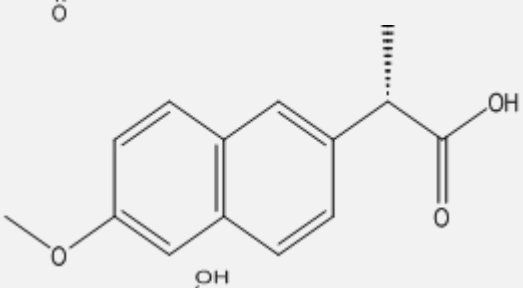
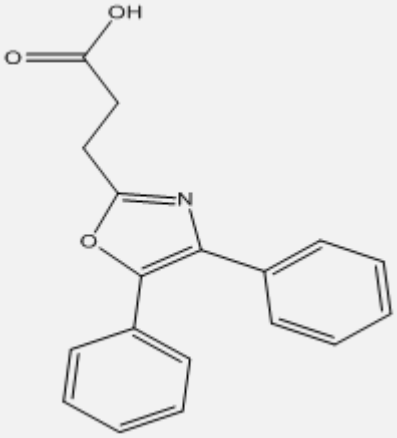
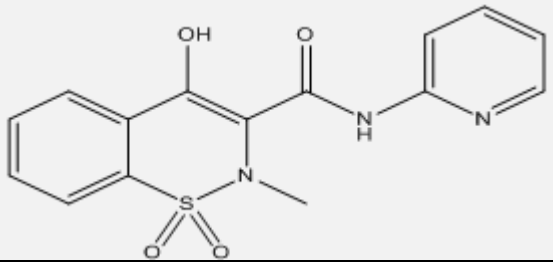
Molecular Docking: The crystal structure of MMP-9 was downloaded from RCSB-PDB, which had PDB ID 6ESM. The structures of selected non-steroidal anti-inflammatory drugs (NSAIDs) in 3D-conformations were generated by online smile translator tool (https://cactus.nci.nih.gov/translate/). Molecular docking was performed by AutoDockTools 1.5.6 (https://autodock.scripps.edu/), individually and in combinations at the active site having coordination of 3 histidine (His 226, His 230, His 236) and Zn. For combination docking, two NSAIDs were considered together in pdbqt format to perform docking. The Kollman charges of -75.265 atomic units were added to the MMP-9. The grid size X = 12.165, Y= 15.184, Z= 18.128, grid center: X = 1.582, Y = 50.36, Z = 19.54; and grid spacing of 0.33 Å was used for docking. The population size =150, the number of evaluations =25,00000, and

the number of generations =27,000 were used in the Lamarckian Genetic Algorithm in docking. The crossover and gene mutation rates were 0.8 and 0.02 respectively. The docking binding energies and docking interactions of both individual and combination dockings were analyzed to study the individual and synergistic effect of molecules and the images of best conformations were generated using PyMol (https://www.pymol.org/).

RESULTS: MMP-9 has been established as an effective cancer target due to its overexpression in different types of cancers. In this study, different NSAIDs have been selected based on their chemical and drug-like properties and have further been analyzed for their potential to inhibit MMP-9 using molecular docking. The 10 selected NSAIDs effective candidates are Celecoxib, Diflunisal, Fenoprofen, Flurbiprofen, Ketoprofen, Ketorolac, Nabumetone, Naproxen, Oxaprozin, and Piroxicam **Table 1** and the PDB ID of 6ESM was used for 3D structure of MMP-9.

TABLE 1: CHEMICAL STRUCTURE AND PUBCHEM CID OF CONSIDERED COMPOUNDS

S. no.	Compound Name	PubChem CID	Chemical Structure
1	CELECOXIB	2662	
2	DIFLUNISAL	3059	
3	FENOPROFEN	3342	
4	FLURBIPROFEN	3394	
5	KETOPROFEN	3825	

6	KETOROLAC	3826	
7	NABUMETONE	4409	
8	NAPROXEN	156391	
9	OXAPROZIN	4614	
10	PIROXICAM	54676228	

ADMET and PCA Analysis: The ADMET analysis was done by SwissADME for all the selected NSAIDs. The bioavailability score for most of the NSAIDs was 0.85, indicating a high predicted probability that at least ten percent of the NSAIDs will be orally bioavailable in rats **Table 2**. The predicted LD_{50} values present the lethal median dose of substance required to kill 50% of the test animals. These values ranged from 49 mg/kg to 3880 mg/kg for the considered NSAIDs, and showed the diverse nature of the selected NSAIDs **Table 2** and **Fig. 1**. Lipinski's rule of 5 is considered to assess the drug-likeness of molecules, and molecules having molecular weight ≤ 500 Daltons, LogP ≤ 5, hydrogen bond donors < 5, and hydrogen bond acceptors < 10 are considered to have effective drug-like properties. The analysis based on Lipinski's rule of 5 to evaluate drug-likeness proved that all the compounds follow these rules with no violation,

proving that all NSAIDs have high drug-like properties **Table 2** and **Fig. 1.** Bioactivity scores showed negative score values for the ability of the considered NSAIDs to convey inhibition of common off-targets or toxic targets **Table 3.**

TABLE 2: ADMET PROPERTIES OF CONSIDERED COMPOUNDS WERE ANALYZED BY USING SWISS ADMET AND PROTOX II(MMP-9)

Molecule	MW	Heavy atoms	Aromatic heavy atoms	Rotatable bonds	H-bond acceptors	H-bond donors	MR	TPSA	Log P	Lipinski violations	Ghose violations	Veber violations	Bio availability Score	Lead likeness violations	LD50 (mg/Kg)
Celecoxib	381.37	26	17	4	7	1	89.96	86.36	3.4	0	1	0	0.55	1	1400
Diflunisal	250.2	18	12	2	5	2	60.78	57.53	3.27	0	0	0	0.85	1	392
Fenoprofen	242.27	18	12	4	3	1	69.31	46.53	3	0	0	0	0.85	1	800
Flurbiprofen	244.26	18	12	3	3	1	68.19	37.3	3.59	0	0	0	0.85	2	117
Ketoprofen	254.28	19	12	4	3	1	72.67	54.37	2.84	0	0	0	0.85	0	49
Ketorolac	255.27	19	11	3	3	1	69.81	59.3	2.05	0	0	0	0.85	0	189
Nabumetone	228.29	17	10	4	2	0	70.03	26.3	3.23	0	0	0	0.55	1	3880
Naproxen	230.26	17	10	3	3	1	66.79	46.53	2.76	0	0	0	0.85	1	248
Oxaprozin	293.32	22	17	5	4	1	83.73	63.33	3.4	0	0	0	0.85	1	1210
Piroxicam	331.35	23	12	3	5	2	87.52	107.98	1.38	0	0	0	0.56	0	216

TABLE 3: BIOACTIVITY SCORES OF THE CONSIDERED COMPOUNDS ESTIMATED BY THE MOLINSPIRATION ONLINE SERVER

S. no.	Molecule	GPCR ligand	Ion channel modulator	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
1	CELECOXIB	-0.06	-0.27	0.01	-0.28	-0.06	0.17
2	DIFLUNISAL	0.01	0.15	0.05	0.26	-0.14	0.22
3	FENOPROFEN	-0.02	0.02	-0.26	0.29	-0.27	0.20
4	FLURBIPROFEN	0.09	0.20	-0.12	0.30	-0.03	0.28
5	KETOPROFEN	0.09	0.07	-0.15	0.39	-0.09	0.27
6	KETOROLAC	0.29	-0.04	-0.09	-0.03	-0.29	0.62
7	NABUMETONE	-0.26	-0.09	-0.70	-0.25	-0.33	0.08
8	NAPROXEN	-0.11	-0.06	-0.38	0.14	-0.26	0.15
9	OXAPROZIN	0.27	0.05	0.06	0.40	-0.16	0.32
10	PIROXICAM	-0.42	-0.57	-0.50	-0.73	-0.04	0.18

Principal Component Analysis (PCA) is a multivariate method that integrates information from a large number of observed variables related to a subject into a smaller number of variables. It reduces a large dataset of variables to extract essential features known as principal components. These components are linear combinations of variables that explain the maximal variance of the variables. Variance indicates the amount of variability of the variables [24]. PCA was performed for the compounds to study the variance and the association among the compounds based on their ADMET properties. The first two components generated by the analysis define the 80.8% variance of the data. The contribution of the top ten principal components in defining explained variance is presented in the Scree Plot **Fig. 1.** Close association among naproxen, flurbiprofen, ketorolac, and ketoprofen was observed on the basis of ADMET properties in the score plot, where the scores of these molecules lie in the same quadrant and are closely linked to each other **Fig. 1.** The loading plot displays the association among the variables or the ADMET properties selected for analysis **Fig. 1**. Among the physicochemical properties of the selected NSAIDs, molecular refractivity, aromatic heavy atoms, heavy atoms, molecular weight, hydrogen bond acceptors, and total polar surface area (TPSA) were found to be associated with the first principal component, while the number of rotatable bonds was associated with the second component. Further, the number of hydrogen bond donors was negatively associated with the second component, while it was positively associated with the first component. The bioavailability score was negatively associated with both components, but

the LD_{50} values were positively associated with the second component. The association was observed between the number of rotatable bonds and LD_{50} values. The biplot compiles both score and loading plots and defines the association of compounds with different properties as well as with first and second components **Fig. 1.** The compounds fenoprofen, ketoprofen, ketorolac, flurbiprofen, and naproxen were observed to have similar bioavailability scores and thus were closely arranged in the biplot. Nabumetone, with the least TPSA, significant bioavailability, and extremely high LD_{50} of 3880 mg/kg was observed to be different among all the selected NSAIDs. Oxaprozin and piroxicam were distantly associated with each other based on bioavailability. Still, they were found to have a positive association with the first component due to mild similarity in physicochemical properties. This analysis proves that a diverse variety of NSAIDs have been selected to explore large chemical space for identifying potential compounds.

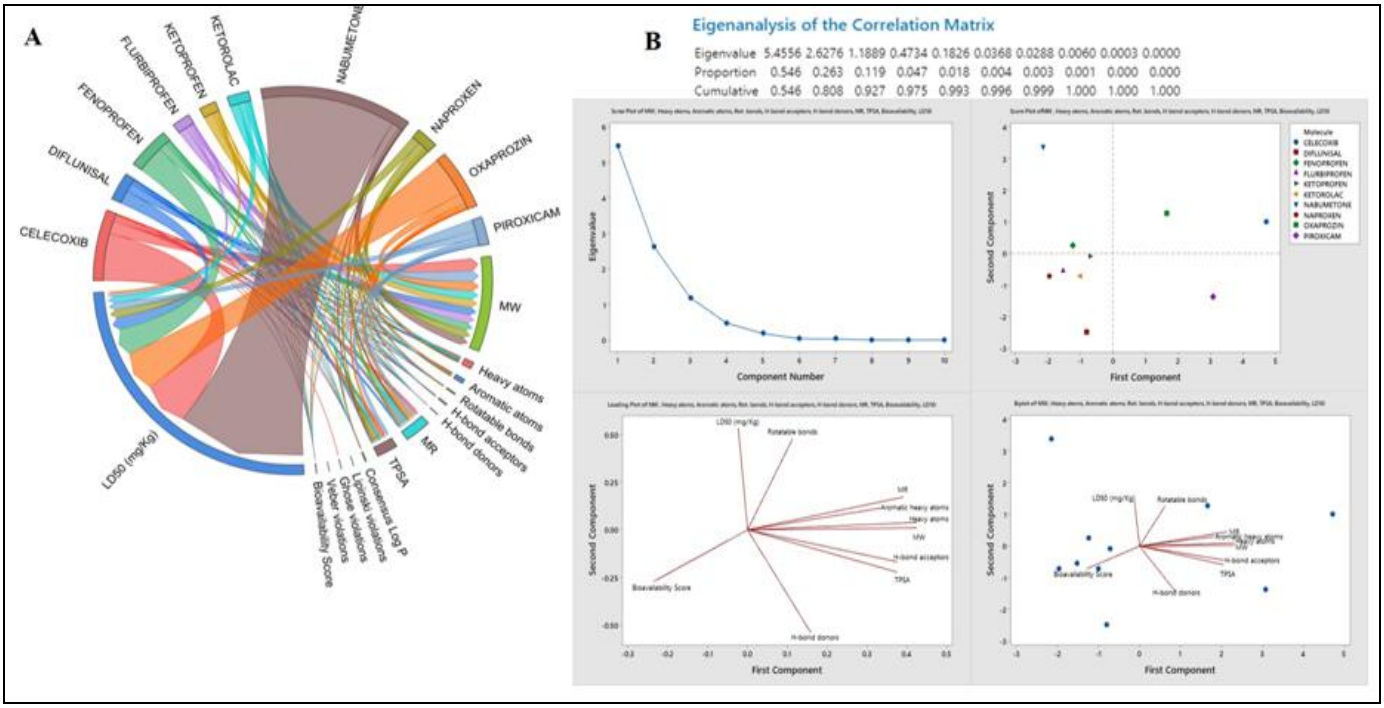


FIG. 1: A). CHORD DIAGRAM SHOWING ADMET PROPERTIES OF THE SELECTED 10 NSAIDS B). PRINCIPLE COMPONENT ANALYSIS EIGEN VALUE CORRELATION MATRIX, SCREE PLOTS, SCORE PLOTS, LOADING PLOTS, AND BIPLOTS

Molecular Docking of Individual NSAIDs: Molecular docking of MMP-9 with the selected NSAIDs was performed to identify the high-affinity inhibitor. The highest negative binding energies of -12.98 and -12.98 kcal/mol were obtained for Oxaprozin and Piroxicam with MMP-9. The binding energies of other NSAIDs were significant ranging from -12.42 to -11.31 kcal/mol **Table 4**.

The interaction analysis showed that all the NSAIDs formed significant number of hydrogen bonds with MMP-9 at the active site of the enzyme, containing the three histidine-Zn coordination complex, which mediates the catalysis **Fig. 2**. Flurbiprofen having a binding energy of -12.56 kcal/mol, was observed to form a hydrogen bond with His226 of this coordination complex **Fig. 2D.** The sulfonamide group of celecoxib formed four hydrogen bonds at the active site of MMP-9 with backbone atoms of Val223, Leu243, Tyr248 and Leu222. The diflunisal formed 6 hydrogen bonds with the Met247, Pro246, Tyr245, Leu243, Ala242, and Arg249.

Extensive hydrogen bonding was also observed for the fenoprofen-MMP-9 docked conformation. Oxaprozin and Piroxicam with the highest binding affinities formed 3 and 5 hydrogen bonds with Gln227, Ala191, and His190 and, Met247, Tyr248, Pro246, His190, and Leu187, respectively.

TABLE 4: MOLECULAR DOCKING BINDING ENERGIES AND KI VALUES OF SELECTED COMPOUNDS WITH MMP-9

S. no.	Ligands	Binding energies (kcal/mol)	Ki (µM)
1	CELECOXIB	-12.42	0.0008
2	DIFLUNISAL	-12.42	0.0008
3	FENOPROFEN	-11.31	0.0051
4	FLURBIPROFEN	-12.56	0.0006
5	KETOPROFEN	-12.56	0.0006
6	KETOROLAC	-12.12	0.0013
7	NABUMETONE	-11.54	0.0035
8	NAPROXEN	-11.6	0.0031
9	OXAPROZIN	-12.98	0.0003
10	PIROXICAM	-12.98	0.0003

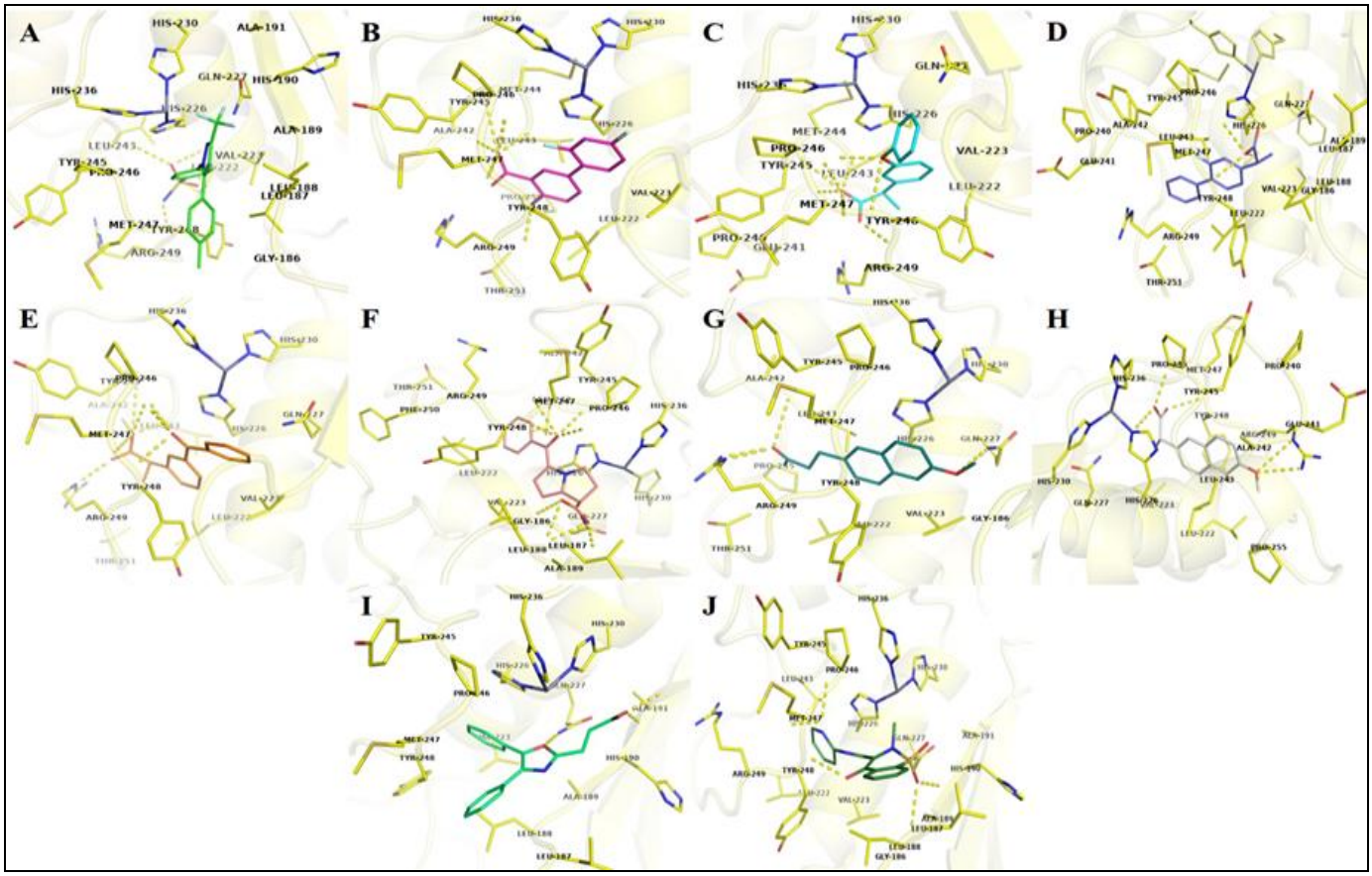


FIG. 2: DOCKING INTERACTIONS OF MMP-9 WITH THE 10 SELECTED NSAIDS A). CELECOXIB B). DIFLUNISAL C). FENOPROFEN D). FLURBIPROFEN E). KETOPROFEN F). KETOROLAC G). NABUMETONE H). NAPROXEN I). OXAPROZIN J). PIROXICAM

Molecular Docking of Combination of NSAIDs: The synergistic effect of these compounds for inhibition of MMP-9 was observed by combination docking of the selected compounds into the active site of the MMP-9. The two compounds that exhibited high negative binding affinities in individual molecular docking were considered for evaluation of the synergistic inhibition effect. The binding energy of the combination docking was the same as that of the dockings performed individually. The Oxaprozin Piroxicam combination gave the binding energy of -12.98 kcal/mol with MMP-9 showing consistency of interactions of the two compounds (Figure 3). The combination of ligands formed 2 hydrogen bonds of which one was with Ala191 and the other with His226 which participates in the coordination complex with the Zn ion at the active site. Thus, our analysis of NSAIDs suggested that the identified combination of NSAIDs can convey highly effective synergistic structural inhibition of MMP-9 by binding at the active site of the enzyme.

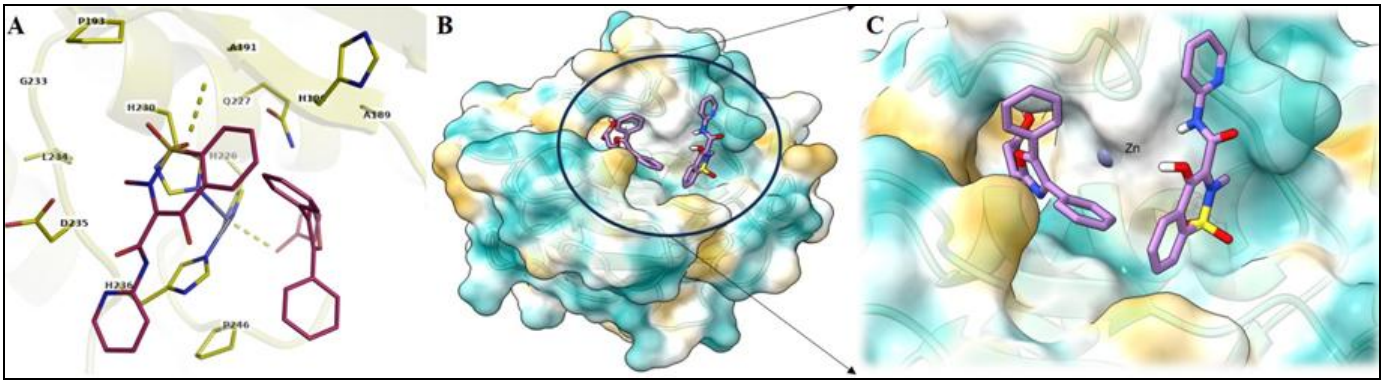


FIG. 3: DOCKED INTERACTION OF MMP-9 WITH THE BEST NSAIDS COMBINATION AS A). 3D CARTOON AND STICK REPRESENTATION B) 3D SURFACE REPRESENTATION AND C). CLOSE-UP OF 3D SURFACE REPRESENTATION AT THE ACTIVE SITE

DISCUSSION: NSAIDs have been investigated as drugs against cancer, but their combinations have been scarcely evaluated or exploited. Cancer the lead death cause is associated with uncontrolled cell proliferation in different parts of the body. Many drug targets that can be targeted to decrease the harshness of the disease have been identified. MMPs have been reported to be overexpressed in a wide range of cancers. MMP-9 promotes several cancer pathologies like differentiation, invasion, and metastasis of cancerous cells.

Jiang & Li, 2021 conducted a thorough review of breast cancer cases to investigate the connection between MMP-9 overexpression and cancer. The MMP-9 overexpression was found to be associated with lymph node metastasis with large tumor size. Thus, overexpression of MMP-9 is the potential biomarker for prognosis in breast cancer cases. The overexpression of MMPs including MMP-9 is controlled and regulated by several cellular signaling pathways such as the E3 ubiquitin ligase pathway [25]. Liu *et al.,* 2022 discussed molecular mechanisms that target E3 ubiquitin ligases for the treatment of several cancers [26]. Another study suggested that the expression of MMPs has a significant role in cancer-associated fibroblasts and skin cutaneous melanoma (SKCM). The enhanced expression of MMP-9, and Her2/neu in gastric cancer was studied by Jafari *et al.,* 2021 and revealed that 50% cancers showed higher expression of MMP-9 [27]. Li *et al.,* 2009 studied the overexpression of MMP-9 in esophageal squamous cell carcinoma (ESCC). Their analysis concluded increased expression of MMP-9 in ESCC by 60.3% compared to the corresponding esophageal and paired normal epithelium (8.9 %) [28].

Hence, proved that MMP-9 significantly participates in ESCC carcinogenesis. All the above studies highlighted MMP-9 as a promising target for cancer treatment development.

Shah & Patel, 2019 conducted an *in-silico* approach to design a hybrid compound of NSAIDs with benzothiazole and thiadiazole ring, to explore multi-targeted anticancer therapy. Their analysis revealed that salicylic acid has the highest negative binding energy (-6.54, -7.0 & -8.84 kcal/mol) with TNF-α, COX-II, and MPS1 receptors, respectively. Thus, salicylic acid hybrid NSAIDs may reduce inflammation and potentially target cancer [29]. Zeeshan *et al.,* 2023 performed an *in-silico* molecular docking study on ten Ketoprofens (NSAIDs) compounds to investigate their anticancer potential against COX-2 (PDBID: 3Q7D) and found that 2-(3-benzoyl phenyl)-N-cyclohexyl propenamide has the highest negative binding affinity of -9.932 kcal/mol against COX-2 [30]. Zia *et al.,* 2020 identified NSAID derivatives as TNF-α inhibitors. The high negative binding energies, showing high binding affinity, of -8.4 kcal/mol & -7.4 kcal/mol were given by the benzophenone derivative (ketoprofen) and Fluorobenzene derivative (Flurbiprofen) [31]. Ashraf *et al.,* 2019 performed an *in-silico* study to evaluate the Dexibuprofen amide derivative of Ibuprofen as an anticancer agent. The molecular docking of BRCA1 with N-(2,5-chlorophenyl)-2- (4-isobutyl phenyl) propionamide and N-(2-chlorophenyl)-2-(4-isobutyl phenyl) propionamide derivative revealed significant binding energy of -6.39 and -6.34 kcal/mol [32]. Gamarra-Sánchez *et al.,* 2024 evaluated forty-two ketorolac derivatives for structural inhibition of COX-2 and found the most

potent ketorolac derivate (Molecule 34) against COX-2 which gave the highest binding energy of -7.7 kcal/mol with the cavity [33]. Ianni *et al.,* 2019 conducted an *in-silico* study using molecular docking to find the ability of oxaprozin to regulate the expression of MMP-9 [34]. Celecoxib and aspirin have been studied for their anticancer synergistic effect in NSCLC [35]. Bharathi *et al.,* 2020 conducted an *in-silico* interaction study of epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGFR2), and lipoxygenase receptors (LOX) with naproxen. In-vitro analysis showed suppressed expression of these receptors in cancerous cells in the presence of the compound, thus providing scope for naproxen as an anticancer drug [36].

The present study evaluated the physiochemical and biological properties of NSAIDs by PCA analysis. Further, effective interactions of 10 NSAIDs with MMP-9, a well-established cancer target were studied. The docking binding energies in the present study were highly significant compared to previous studies and thus showed the immense potential of the selected NSAIDs to inhibit MMP-9. The combination analysis of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) for inhibiting MMP-9 revealed that the combination of Oxaprozin-Piroxicam with MMP-9 was the most effective one. Oxaprozin and Piroxicam revealed high affinity in both individual and combination dockings and the docking conformations were Consistent, blocking the active site of the MMP-9 thus inhibiting its activity. Therefore, further *in-vitro* studies are highly demanded to provide effective synergistic NSAIDs combinations for inhibition of MMP-9 to treat a wide range of cancers.

CONCLUSION: Nonsteroidal anti-inflammatory drugs (NSAIDs), due to their anti-inflammatory and anti-cancer properties have been investigated in numerous studies, yet an effective treatment has left been identified. MMP-9, a lead member of the matrix metalloproteinase family, is responsible for ECM remodeling and has been reported to be over-express in a diverse range of cancers, proving it an effective target for cancer treatment. This study presents an *in-silico* analysis of well-known NSAIDs for their potential to inhibit MMP-9 both individually and in combination using a molecular docking approach. The best combination of NSAIDs identified was Oxaprozin and Piroxicam, which gave a high negative binding energy of -12.98 kcal/mol with effective interaction with the active site histidine residues. This work provides a potent combination of NSAIDs, which can be evaluated by *in-vitro* and *in-vivo* analysis, which is out of the scope of the present study. Many similar *in-silico* studies have been validated by *in-vitro* analysis and have provided promising outcomes. Therefore, to develop a high-potential treatment of cancer by mediating effective structural inhibition of MMP-9, the identified combination of NSAIDs seems to be highly beneficial.

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