In-silico pharmacological screening of structurally modified Diclofenac derivatives against peripheral pain.

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ABSTRACT:
Pain is defined as "an unpleasant sensory and emotional experiences associated with actual or potential tissue damage or described in terms of such damage". Intensity and acuity of pain differsliables upon psychological and emotional conditions of pain. NSAIDS, world-widedesired drug for pain deficiencies in synthesising molecules with innovative derivatives. Diclofenac a sketchily used NSAID for wide-ranging disease conditions is OTC drug since years. The present research focuses on structurally altered derivatives of diclofenac against cox-2 for pain. 33 structures are acknowledged and screening is done using AutoDoc software against Cox-2. Binding energies and inhibitory constant assessments obtained operating AutoDoc for 100 runs are recorded and related with the novel Diclofenac result. The binding sites of molecules are visualised using Discovery Studio Visualiser and are list below in figure 2. The molecule with peak binding energy isprogressed for Md simulation using gromax software. The simulation ensued with diclofenac indicative of the molecule is equally efficient in treating pain against cox-2.The molecule may be synthesised and proceeded for preclinical trails in future.

I. INTRODUCTION
Non-Steroidal Anti-inflammatory drugs widely used for Analgesic, Inflammation and Pyretic. A phenyl acetic acid derivative if NSAID i.e., Diclofenac is highly efficient orally.[1]It is widely used in management of pain associated with inflammatory conditions including osteoarthritis, rheumatoid arthritis. It is also approved by FDA for ophthalmic use during cataracts and photophobia. [2]

Diclofenac, synthesized in the year 1973, a widely prescribed OTC drug for fever, gout, migraine, post episiotomy pain, corneal abrasion and biliary colic [3]. Diclofenac gel dosage forms usage for the topical treatment of arthritic pain has been using since 2020. [4]

PAIN:
Pain is defined as "an unpleasant sensory and emotional experiences associated with actual or potential tissue damage or described in terms of such damage". It is widely associated with tissue damage, injuries and diseases [7]. Pain perception is also influenced by psychological and emotional factors. Pain intensity is difficult to measure and the severity of pain depends on psychological and emotional status[8].

Types of pain:
It is of two types depending on the origin of pain impulse. They are:
  i.   Central pain
  ii.   Peripheral pain
  iii. General pain

Central pain
It is a neurological disorder caused by damage or injury or destruction to the sensory pathways of the central nervous system (CNS). Common symptoms include pain (but also pruritus) and loss of sensation, usually in the face, arms and legs.

Peripheral pain
It refers to the condition when the nerves carrying impulses get damaged or destructed or injured. The nerves involved may carry impulse to andfrom brain and spinal cord to various organs located in periphery. As the peripheral nerves are

involved it is considered as peripheral pain. Damage to these nerves may result in impairment of muscle movements causing pain.

General pain
Pain has been classified by anatomic location, body system, duration, severity, frequency and etiology.

Types of pain:
- Acute pain
- Chronic pain
- Neuropathic pain
- Nociceptive pain
- Radicular pain

Acute Pain: Pain lasting for short duration from minutes to 3 months is considered as acute pain. Soft tissue injury and mild broken bones are majorly observed. Pain sensation may persist after treatment of injury also. [14]

Chronic Pain: Pain lasting for longer duration of more than 6 months is categorised as Chronic pain. Based on the severity it may be constant or intermittent. It is majorly observed in case of chronic heart diseases, Burns, Diabetes, Cancer, Asthma and Pulmonary diseases.

Neuropathic Pain: Pain arising due to damage to nerve in the body is considered as Neuropathic pain. This type of pain often results in shooting, stabbing or burning sensation at injury site. The injury site may lose the sensitivity feeling difficult when incident with hot or cold objects. [16]

Nociceptive pain: Pain affecting sensory nerves that may result due to damage or injury to body tissues results in Nociceptive pain. This generally results due to toe stubbing, Dental procedures and during sports injuries.

Radicular pain: Pain affecting spinal nerves that either get compressed or inflamed due to infection or injury considered as Radicular pain. Nerves connecting to back and hip are majorly affected. It is in general considered as Sciatica pain affecting sciatic nerve. [18]

Introduction to NSAIDs:
NSAIDs, one of the most prescribed medication for analgesic, pyretic and inflammation. NSAIDs having frequency of prescription of about 10-50% among all OTC drugs in each year. The prevalence is more in geriatrics with age above 65 years. A survey concluding that 7.3% of elderly patient above 60 years old prescribe NSAIDs. NSAIDs besides having anti-inflammatory property possess analgesic and anti-pyretic effect. The drugs act by inhibiting cyclooxygenase enzyme, that possess crucial role in synthesis of prostaglandins and leukotrienes that are the potent pain mediators. All the NSAIDS act by inhibiting the action of enzyme cyclooxygenase, a rate determining step for synthesis of prostaglandins and leukotrienes.

Cyclooxygenase, an enzyme plays an important role in the cyclisation of ring. The enzyme exists in two isoforms namely, Cox-1 and Cox-2. Cox-1 playing importance in physiological role in protecting gastric mucosa from action of HCl. Cox-2 being pathological plays an important role in production of Prostaglandins that play a vital role in inflammation and pain mechanism. Majority of NSAIDS inhibit Cox non selectively. NSAIDs playing vital role in treatment of pain and inflammation possess adverse effects that are prominent leading to organ failure.

Diclofenac, an NSAID belongs to the phenyl acetic acid derivative widely used to decrease inflammation. The drug is profoundly anti-inflammatory followed analgesic with least anti-pyretic property. The structure of Diclofenac is as follows:
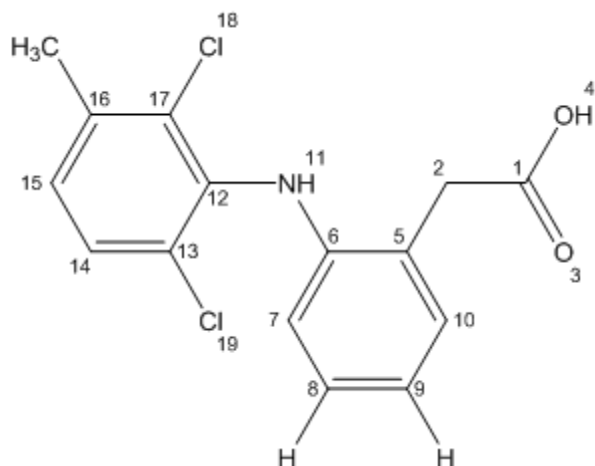
Fig 1: 2-(2-(2,6-dichloro-3-methylphenylamino) Phenyl) acetic acid)

Diclofenac is chemically drawn with IUPAC mentioned above. The drug consisting of two chloro, one phenyl and methyl groups at 18th, 19th and 16th position. Structural modifications are done at 8th, 9th and 16th position with substituents resulting 33 new modified structure as follows:
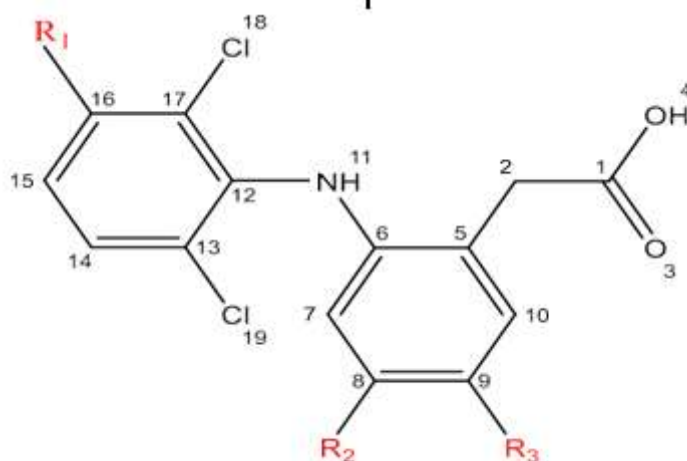
Structural Modifications done at Diclofenac:

Fig 2: structural modifications done at diclofenac

The following are the attached at the R1,R2,R3 of Diclofenac

Table1: Structural modifications of diclofenac

DRUG	R₁	R₂	R₃	CHEMICALFORMULA	IUPACNAMES
DCF	H	H	H	C14H11Cl2NO2	2-(2-(2,6-dichloro-3-methylphenylamino)phenyl)aceticacid

D1	CH3	H	H	C15H13Cl2NO2	2-(2-(2,6-dichlorophenylamino)-4-methylphenyl)aceticacid
D2	H	CH3	H	C15H13Cl2NO2	2-(2-(2,6-dichlorophenylamino)-4-methylphenyl)aceticacid
D3	H	H	CH3	C15H13Cl2NO2	2-(2-(2,6-dichloro-3-methoxyphenylamino)phenyl)aceticacid
D4	OCH3	H	H	C15H13Cl2NO3	2-(2-(2,6-dichloro-3-methoxyphenylamino)phenyl)aceticacid
D5	H	OCH3	H	C15H13Cl2NO3	2-(2-(2,6-dichlorophenylamino)-4-methoxyphenyl)aceticacid
D6	H	H	OCH3	C15H13Cl2NO3	2-(2-(2,6-dichlorophenylamino)-5-methoxyphenyl)aceticacid
D7	F	H	H	C14H10Cl2FNO2	2-(2-(2,6-dichloro-3-fluorophenylamino)phenyl)aceticacid
D8	H	F	H	C14H10Cl2FNO2	2-(2-(2,6-dichlorophenylamino)-4-fluorophenyl)aceticacid
D9	H	H	F	C14H10Cl2FNO2	2-(2-(2,6-dichlorophenylamino)-5-fluorophenyl)aceticacid
D10	CF3	H	H	C15H10Cl2F3NO2	2-(2-(2,6-dichloro-3-(trifluoromethyl)phenylamino)phenyl)aceticacid
D11	H	CF3	H	C15H10Cl2F3NO2	2-(2-(2,6-dichlorophenylamino)-4-(trifluoromethyl)phenyl)aceticacid
D12	H	H	CF3	C15H10Cl2F3NO2	2-(2-(2,6-dichlorophenylamino)-5-(trifluoromethyl)phenyl)aceticacid
D13	OCF3	H	H	C15H10Cl2F3NO3	2-(2-(2,6-dichloro-3-(trifluoromethoxy)phenylamino)phenyl)aceticacid
D14	H	OCF3	H	C15H10Cl2F3NO3	2-(2-(2,6-dichlorophenylamino)-4-(trifluoromethoxy)phenyl)aceticacid

D15	H	H	OCF3	C15H10Cl2F3NO3	2-(2-(2,6-dichlorophenylamino)-5-(trifluoromethoxy)phenyl)aceticacid
D16	Cl	H	H	C14H10Cl3NO2	2-(2-(2,3,6-trichlorophenylamino)phenyl)aceticacid
D17	H	Cl	H	C14H10Cl3NO2	2-(2-(2,6-dichlorophenylamino)-4-chlorophenyl)aceticacid
D18	H	H	Cl	C14H10Cl3NO2	2-(2-(2,6-dichlorophenylamino)-5-chlorophenyl)aceticacid
D19	OH	H	H	C14H11Cl2NO3	2-(2-(2,6-dichloro-3-hydroxyphenylamino)phenyl)aceticacid
D20	H	OH	H	C14H11Cl2NO3	2-(2-(2,6-dichlorophenylamino)-4-hydroxyphenyl)aceticacid
D21	H	H	OH	C14H11Cl2NO3	2-(2-(2,6-dichlorophenylamino)-5-hydroxyphenyl)aceticacid
D22	COOH	H	H	C15H11Cl2NO4	3-(2-(carboxymethyl)phenylamino)-2,4-dichlorobenzoicacid
D23	H	COOH	H	C15H11Cl2NO4	3-(2,6-dichlorophenylamino)-4-(carboxymethyl)benzoicacid
D24	H	H	COOH	C15H11Cl2NO4	4-(2,6-dichlorophenylamino)-3-(carboxymethyl)benzoic acid
D25	NH2	H	H	C14H12Cl2N2O2	2-(2-(3-amino-2,6-dichlorophenylamino)phenyl)aceticacid
D26	H	NH2	H	C14H12Cl2N2O2	2-(2-(2,6-dichlorophenylamino)-4-aminophenyl)aceticacid
D27	H	H	NH2	C14H12Cl2N2O2	2-(2-(2,6-dichlorophenylamino)-5-aminophenyl)aceticacid

D28	CH2NH2	H	H	C15H14Cl2N2O2	2-(2-(3-(aminomethyl)-2,6-dichlorophenylamino)phenyl)aceticacid
D29	H	CH2NH2	H	C15H14Cl2N2O2	2-(2-(2,6-dichlorophenylamino)-4-(aminomethyl)phenyl)aceticacid
D30	H	H	CHNH2	C15H14Cl2N2O2	2-(2-(2,6-dichlorophenylamino)-5-(aminomethyl)phenyl)aceticacid
D31	CONH2	H	H	C15H12Cl2N2O3	2-(2-(3-carbamoyl-2,6-dichlorophenylamino)phenyl)aceticacid
D32	H	CONH2	H	C15H12Cl2N2O3	2-(2-(2,6-dichlorophenylamino)-4-carbamoylphenyl)aceticacid
D33	H	H	CONH2	C15H12Cl2N2O3	2-(2-(2,6-dichlorophenylamino)-5-carbamoylphenyl)aceticacid

Docking:

Docking, a recent advancement widley preferred in determining the binding interaction of one molecule with the receptor. Docking is used to predit the strength of association or binding affinity between the ligand and the receptor. It is widely used to design binding of structure with protein. The binding interaction and energy for the molecules is predicted by using softwares.Characterization of binding of molecules and their role in drug design aids in drug discovery.

Protein used in Docking:

NSAIDs, acts on the enzyme cyclooxygenase that exists in two isoforms. The target selected is cyclooxygenase-2. The terget i.e. protein is downloaded from protein data bank and is purified by removing the unwanted ligand structures by using Discovery studio visualiser. The purified structure of protein is saved in the form of .pdb and is preferred for furthur docking.

The protein consisting of 4 chains that are homologus in nature. Hence docking can be done using any one of the chain. The downloaded and modified form of protein is given below in fig.1.

Fig.3: Figure showing downloaded and purified chain of cyclooxyhenase 2 used for docking.

DOCKING PROCEDURE:
The steps involved in docking are:
1.   Preperation of coordinate files
2.   Docking of molecules using AutoDoc.
3.   Analysis of results using Discovery studio Visualiser.

Preparation of coordinate files:
        The molecules in docking that are in .pdb format are needed to be converted to PDBQT by uploading into Auto Doc. The protein and drug molecules are PDBQT files that include information on the torsional degrees of freedom.
        Protein uploaded initially are added with kollman charges, polar hydrogen groups and made ready for docking by saving into docking parametric file in PDBQT format. Drug molecule added in .pdb format is enabled to detect the root and saved in PDBQT format.
The molecules are converted into docking format and initialized the process of autogrid prior before docking.

Docking of molecules using AutoDoc:
        The molecules are initially gridded by including molecule in grid box at x,y and z

coordinates at 126. The setting of molecule into grid box with coordinates can be more selective based on exact binding site at receptor. The grid coordinate files formed are used for further.
        The docking process is done after grid of molecules. The number of times i.e. runs the molecules performs will be decided accordingly and placed in genetic algorithm. Docking is initiated by saving the coordinate files.
The result of docking is obtained in a .dlg file containing cluster rankings and docking binding energies, inhibitory constant and coordinates of docking.

Analysis of results using Discovery studio Visualiser
        AutoDoc software lags in visualising of result of docking. The interaction between the ligand and protein which are a result of autogrid and AutoDoc are visualised with Discovery studio visualiser. Discovery studio visualiser works in identifying the binding sites of docking in 2D and 3D.The resultant images can be saved as jpg format.
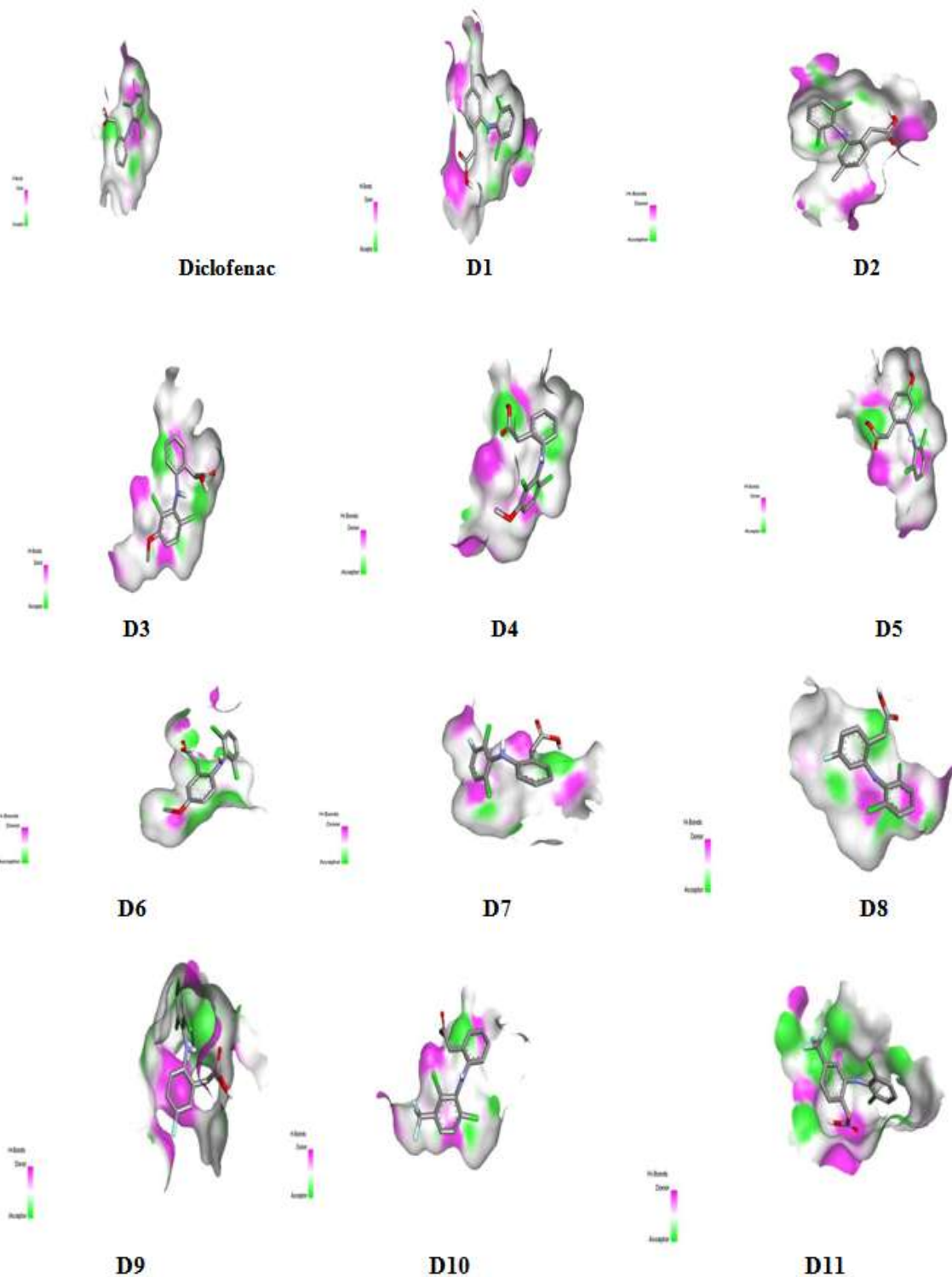
DOCKING RESULTS:

Table 2: The binding energies and inhibitory constants obtained as a result of AutoDoc are listed below
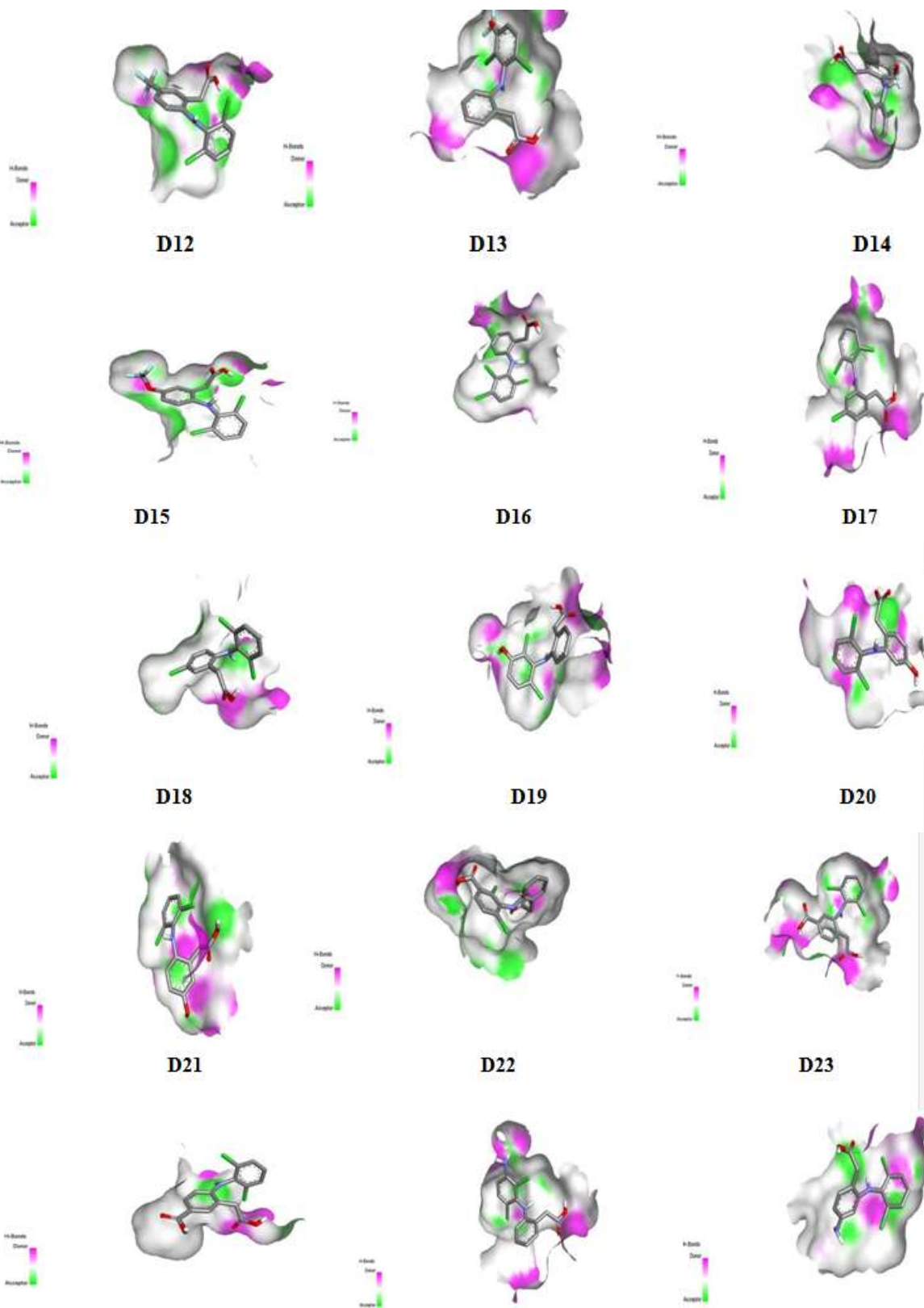
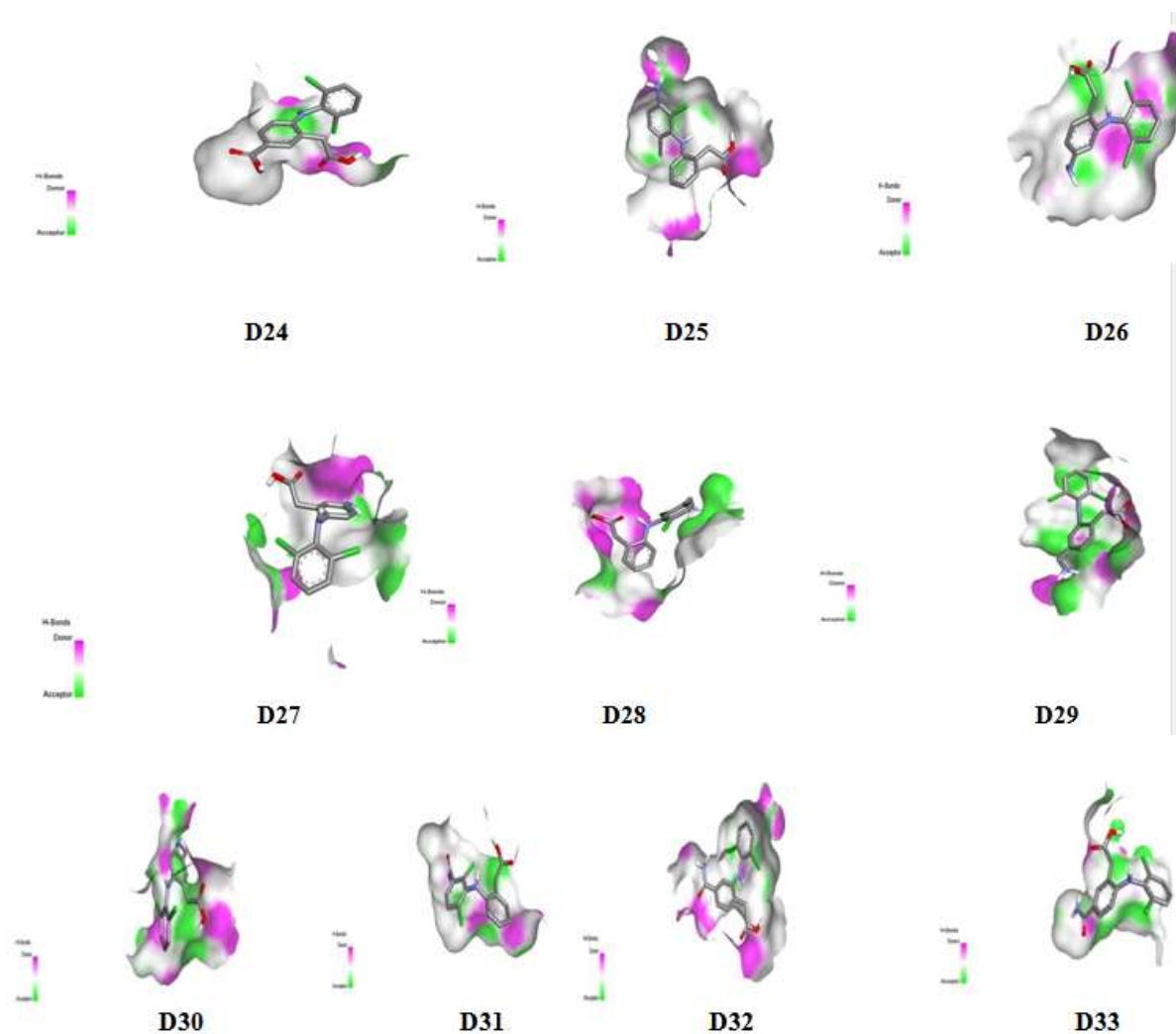| Molecule	BindingEnergy	InhibitoryConstant
Diclofenac	-9.28kcal/mol	157.88nM
D1	-8.30kcal/mol	817.51nM
D2	-8.32kcal/mol	793.97nM
D3	-7.70kcal/mol	2.27uM
D4	-8.04kcal/mol	1.28uM
D5	-8.45kcal/mol	643.38nM
D6	-8.72kcal/mol	407.14nM
D7	-7.99kcal/mol	1.39uM
D8	-7.82kcal/mol	1.85uM
D9	-7.76kcal/mol	2.04uM
D10	-8.67kcal/mol	443.04nM
D11	-7.56kcal/mol	2.85uM
D12	-8.16kcal/mol	1.04uM
D13	-8.08kcal/mol	1.20uM
D14	-8.56kcal/mol	534.33nM
D15	-8.16kcal/mol	1.05uM
D16	-8.43kcal/mol	658.50nM
D17	-8.54kcal/mol	546.61nM
D18	-8.64kcal/mol	460.98nM
D19	-8.14kcal/mol	1.09uM
D20	-8.41kcal/mol	679.01nM
D21	-8.37kcal/mol	727.59nM
D22	-7.93kcal/mol	1.53uM
D23	-8.25kcal/mol	899.82nM
D24	-8.42kcal/mol	671.89nM
D25	-8.25kcal/mol	900.46nM
D26	-8.96kcal/mol	270.04nM
D27	-8.35kcal/mol	758.64nM
D28	-8.35kcal/mol	758.64nM
D29	-8.96kcal/mol	272.31nM
D30	-8.66kcal/mol	452.38nM
D31	-8.47kcal/mol	617.37nM
D32	-8.75kcal/mol	383.41nM
D33	-8.86kcal/mol	321.35M

The images obtained as a result of interaction with Discovery Studio Visualiser are pasted below:
Figure showing surface ligand interaction of diclofenac structures with protein COX-2

D12   D13   D14

D15   D16   D17

D18   D19   D20

D21   D22   D23

D24   D25   D26

D27   D28   D29

D30   D31   D32   D33

Molecular dynamic simulation

Molecular dynamic simulation (Md) is a computer simulationmethod for analyzing the physical movements atoms and molecules. The atoms and the molecules are not allowed to interact for a fixed period of time, giving a view of the dynamic" evolution of the system in the most common version ,the trajectories of atoms and the molecules are determined by numerically solving Newtons equation of motion for a system of interacting particles, where forces between the particles and their potential energies are often calculated using inter atomic potentials are molecular mechanic force fields the method is applied mostly in chemical physics, material science and biophysics. Molecular dynamic simulation is often used study biophysical systems depicted here is a hundred of ps simulation of water.

Because of molecular system typically consist of a vast number of particles, it is impossible to determine by using the properties of such complex system analytically MD simulation circumvents this problem by using numerical methods. However long MD simulations are mathematically ill-conditioned, generating cumulative errors in numerical interrogation that can be minimized with proper selection of algorithms and parameters, but not eliminated entirely. For a system that obeys the ergodic hypothesis the evaluation of molecular dynamic simulation may be used to determine the macroscopic thermodynamic properties of the system the time averages on ergodic system corresponded to microanonical assembles the averages. By numbers and lapceplacs vision of newtons mechanism o predating the future by animating nature forces and allowing insight into

molecular motion on an atomic scale

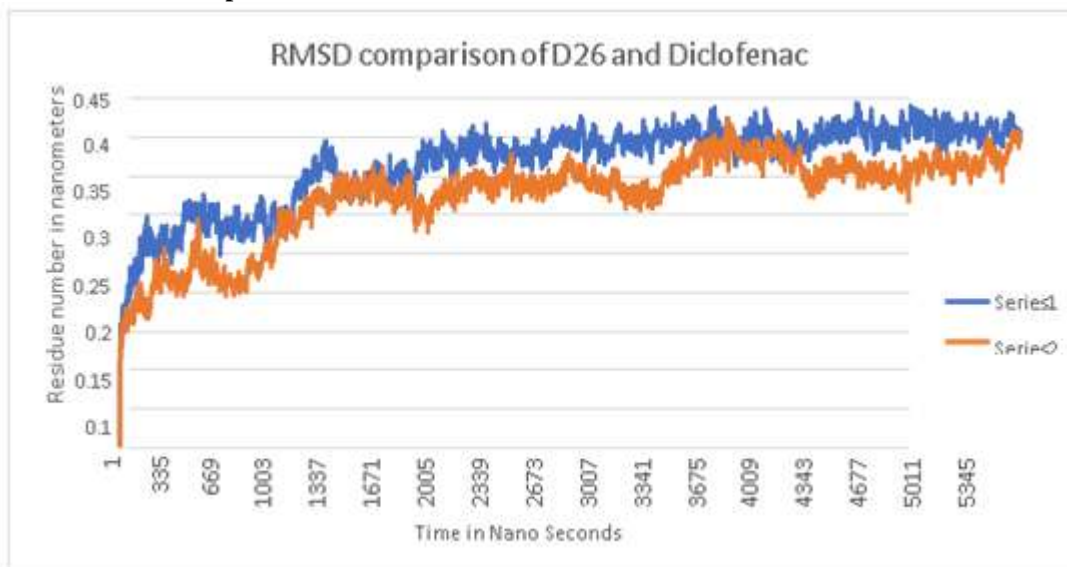**Mdsimulati on result comparison ofRMSDvaluesforD26and Diclofenac**

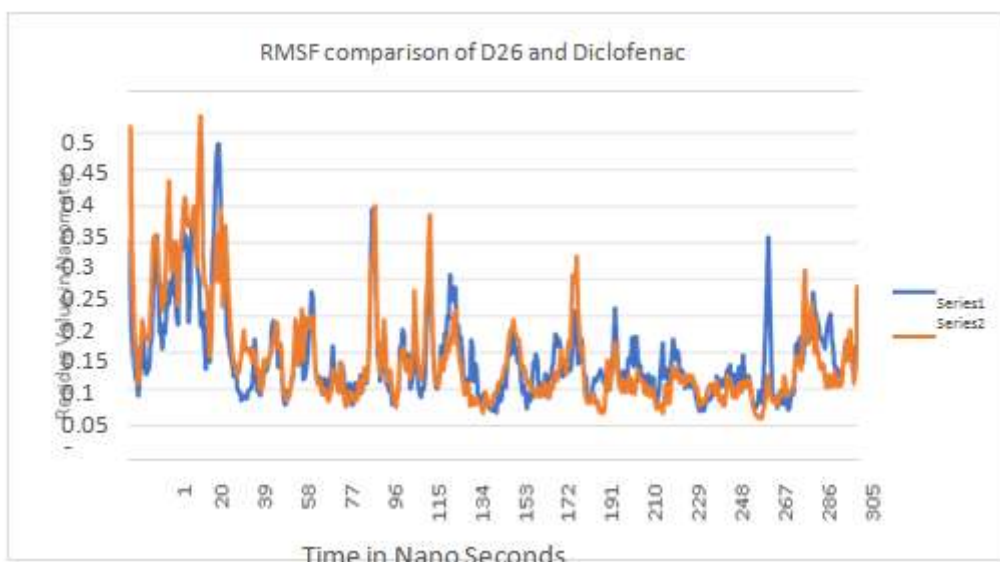Fig 4: RMSD comparison of D26 and Diclofenac

Fig 5: RMSF Comparison of D26and diclofenac

II. CONCLUSION

Pain, an unpleasant sensory and emotional experience associated with actual or potential tissuedamage,describedin termsofsuchdamage

Two types of pain namely peripheral and neuropathy pain are observed. NSAIDs,Non-steroidal anti-inflammatorydrugswidelyusedfor the treatment to analgesics,anti-inflammatoryandanti-pyretic. Thepotencyfor varies amongthe drugs.

Diclofenac, a well-known and widely used drug for analgesic is selected. Structural

alterationsare made by changing the substituent. A total of 33 structures are drawn by changing thesubstituents.

Docking is a method which predicts the preferred orientation of one molecule to a secondwhen a ligand and a target are bound to each other to form a stable complex Knowledge of thepreferred orientation in turn may be used to predict the strength of association or bindingaffinitybetween two molecules.

Docking of all the modified structures is

performed with cyclooxygenase-2 enzyme Insilicousing auto dock software for 100 runs a comparison among the binding energies and inhibitoryconstants for all molecules is done. The molecule with good binding energy and inhibitoryconstant that is (2, 6-dichlorophenylamino)-4 –amino phenyl) acetic acid molecule along withstandard diclofenacis forwardedformolecular dynamicsimulation for100 runs.

Molecular dynamic simulation resulted that the structurally modified derivative of diclofenac i.e.D26 is showing functional similarity with diclofenac.The newly derived structure may perform in similar way with diclofenac.

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