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In Silico and Docking Studies of Novel Isatin Derivatives for Anti-Inflammatory Activity

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Abstract
In the present work, some new isatin derivatives are planned to synthesise based on the results of *in silico* studies. These compounds are designed from substituted isatins and p-dimethyl amino benzaldehyde. In the first step substituted isatins treated with Hydrazine hydrate to get substituted isatin-3-hydrazones. In the next step isatin-3-hydrazone treated with P-Dimethyl amino benzaldehyde to form new Isatin derivatives. All these isatin derivatives have been investigated for their anti-inflammatory activity using molecular Docking studies. The molecular Docking studies were carried out into the active site COX-2 enzyme (PDB ID: 5F19). Among all the newly synthesised derivatives, 5,6 Dichloro substituted compound (IIIe) showed good docking score and best binding energy. So, this shows the promising anti-inflammatory activity. Among other compounds IIId, IIIh,III,III shows moderate activity. Based on these in silico and docking study results we planned to synthesise the compounds having anti-inflammatory activity.

Keywords
New isatin derivatives, *In silico,* Molecular docking, COX-2 enzyme, Anti-inflammatory activity.

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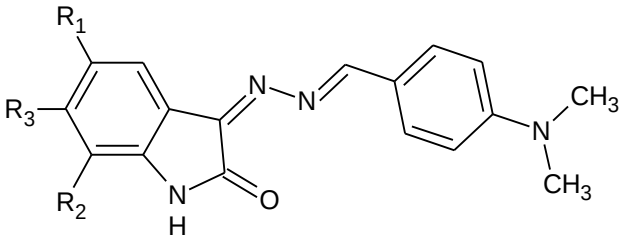
1. INTRODUCTION
Isatin or 1H-indole-2, 3-dione, is an indole derivative containing keto group at position 2 and 3 of the ring. This compound consists of pyrrole ring fused with benzene ring which is first discovered by Erdmann and Laurent in 1841, independently as a product from oxidation of indigo by nitric and chromic acids. In nature, isatin is found in many plants, such as *Isatis tinctoria, Calanthe discolor* and in *couroupita guianensis.* Isatin is a versatile lead molecule for potential bioactive agents and it's derivatives were reported to possess wide variety of important biological activities like antibacterial[2], antifungal[2], anticonvulsant[7], anti HIV[9], antituberculosis[9], antioxidant[5], anti-inflammatory[1] and antidepressant[8] activity etc., It has been reported that the nature of substituents at the 2- or 3- position of the indole nucleus plays an important role in possessing the anti-inflammatory properties. Among other compounds IIId, IIIh shows moderate activity.
Cyclooxygenases (COX) is the key enzyme in the synthesis of prostaglandins which are responsible for inflammation, pain and increased body temperature. Our body produce two main isoforms of COX proteins, that is, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). The COX-1 enzyme is

responsible for formation of prostanoids, prostaglandin, thromboxane and prostacyclin which are involved in causing pain, blood clotting and protecting the stomach, whereas COX-2 is involved in the pain by inflammation and plays a important role in prostaglandin biosynthesis in inflammatory cells. When target is COX-1 inflammation is reduced, but the protection of the lining of the stomach is lost and causing ulceration and bleeding from the stomach and even the intestine. Whereas COX-2 is target there is a less gastric irritation and peptic ulceration. Therefore, selective COX-2 inhibitors are the targets for Anti-inflammatory drugs. In present work, we aim to dock novel isatin derivatives with best binding energy by using COX-2 enzyme as a selective target. Molecular docking is important tool for studying the binding affinities and ligand-target interaction; hence we used them for the study and evaluation of anti-inflammatory activity.

The series of designed compounds were evaluated for *in silico* and docking studies are



S.No	Compound	R_1	R_2	R_3
1.	IIIa	H	H	H
2.	IIIb	H	Cl	H
3.	IIIc	Br	H	H
4.	IIId	Br	NO_2	H
5.	IIIe	Cl	H	Cl
6.	IIIf	F	H	H
7.	IIIg	I	H	H
8.	IIIh	NO_2	H	H
9.	IIIi	NO_2	Cl	H
10.	IIIj	CH_3	H	H

***In silico* studies**

The tools used in the analysis of the compounds were SwissADME and Molinspiration. The molinspiration was used to generate bioactivity scores (like GPCR ligand, ion channel inhibitor, kinase inhibitor, nuclear receptor ligand, protease inhibitor, enzyme inhibitor). SwissADME was used to study various parameters like physicochemical properties, lipophilicity, pharmacokinetic parameters, obeyance of Lipinski rule, bioavailability score and lead likeness.

i) Physicochemical parameters: The parameters of the molecules which effect the nature of the compound. Ex: No: rotatable bonds, H-bond donors or acceptors, molecular weight etc.

ii) Lipophilicity: From the various values of LogP, MlogP value is considered.

iii) Pharmacokinetic parameters: the parameters like gastro-intestinal absorption, blood brain barrier penetrability and P-glycoprotein substrate or inhibitor.

iv) Lipinski rule: Lipinski's rule of five or simply the rule of five (RO5) is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most orally administered drugs are relatively small and moderately lipophilic molecules.

- No more than 5 hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen–hydrogen bonds)
- No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)
- A molecular mass less than 500 daltons
- An octanol-water partition coefficientlog P not greater than 5

v) Bioactivity score: Biological targets are the most common proteins such as enzymes, ion channels, and receptors. The biological target is also referred to as drug target. The bioactivity scores of the synthesized complexes were calculated for different parameters such as binding to G

protein-coupled receptor (GPCR) ligand and nuclear receptor ligand, ion channel modulation, kinase inhibition, protease inhibition, and enzyme activity inhibition. All the parameters were calculated with the help of online software Molinspiration (www.molinspiration.com), which predicted moderate biological activity for the synthesized complexes. It is known that for metal complexes, if the bioactivity score is more than 0.0, then the complex is active; if it is between −5.0 and 0.0, then the complex is moderately active, and if the bioactivity score is less than −5.0, then it is inactive.

vi) Leadlikeness: A lead compound in drug discovery is a chemical compound that has pharmacological or biological activity likely to be therapeutically useful but may nevertheless have suboptimal structure that requires modification to fit better to the target.

Molecular Docking studies:

Molecular docking studies play an important role in rational drug design. Molecular docking studies are used to find the orientation between the ligand and protein. In the present study Patch dock is a online based tool for studying the binding affinities and amino acid interactions in the active site of the cyclooxygenase 2 enzyme.

Molecular docking studies was performed by using the Patch Dock. All these ten new isatin derivatives were docked into the active site of the enzyme COX-2 (PDB ID: 5F19) which showed better docking scores than the reference compound indomethacin.

PDB ID: 5F19

Preparation of ligand:

In Patch Dock, ligand molecules were built using Chem Draw 12 version and convert in 3D format using Chem Bio 3D and minimize energy after that the structure can be directly save in pdb format and submit it in the Patch Dock.

Software for virtual screening:

The software used for molecular docking was Patch Dock. It is an online free software available for docking. The results are given as best pose binding energy scores. The prepared ligands and target molecules were docked, and the results were recorded.

RESULTS AND DISCUSSION:

SwissADME:

Compound	Physicochemical properties				Lipophilicity	Pharmacokinetics			Lipinski rule	BA score	Leadlikeness
	n-rot bonds	H-acceptor	H-donor	TPSA(Å)	mlogP	GI	BBB	P-gp			
IIIa	3	3	1	57.06	1.73	High	Yes	No	Yes	0.55	Yes
IIIb	3	3	1	57.06	2.24	High	Yes	No	Yes	0.55	Yes
IIIc	3	3	1	57.06	2.35	High	Yes	No	Yes	0.55	No
IIId	4	5	1	102.88	1.36	High	No	No	Yes	0.55	No
IIIe	4	2	2	56.73	2.81	High	Yes	No	Yes	0.55	No
IIIf	3	4	1	57.06	2.12	High	Yes	No	Yes	0.55	Yes
IIIg	4	5	1	102.88	0.74	High	No	No	Yes	0.55	Yes
IIIh	4	2	2	56.73	2.81	High	Yes	No	Yes	0.55	No
IIIi	4	5	1	102.88	1.24	High	No	No	Yes	0.55	No
IIIj	4	5	1	102.88	1.36	High	No	No	Yes	0.55	No

In SwissADME, the compounds showed good lipophilicity, high GI absorption, good bioactivity score. They are BBB permean and were found to be P-glycoprotein substrates it is a P-gp inhibitor. All the compounds obeyed Lipinski rule with no violations and showed leadlikeness.

MOLINSPIRATION:

To calculate molecular properties like miLogp, HBD, HBA, TPSA, MV and MW and to predict the bioactivity of compounds

Properties of compounds

Compound	Mi LogP	TPSA	N of atoms	M.Wt	No ofN	nOHNH	N Violations	No-rot bonds	Volume
IIIa	3.08	60.83	22	292.34	5	1	0	3	268.42
IIIb	3.71	60.83	23	326.79	5	1	0	3	281.96
IIIc	2.25	60.83	23	310.33	5	1	0	3	273.35
IIId	3.86	60.83	23	371.24	5	1	0	3	286.31
IIIe	4.14	60.83	23	418.24	5	1	0	3	292.41
IIIf	3.50	60.83	23	306.37	5	1	0	3	284.98
IIIg	4.34	60.83	24	361.23	5	1	0	3	295.49
IIIh	3.01	106.65	25	337.34	8	1	0	4	291.76
IIIi	3.62	106.65	26	371.78	8	1	0	4	305.29
IIIj	3.75	106.65	26	416.24	8	1	0	5	309.64

In molinspiration, the compounds were found to be active as kinase inhibitors, moderately active as GPCR ligand, protease inhibitor and enzyme inhibitor. They were found to be inactive as ion channel inhibitors and nuclear receptor ligands.

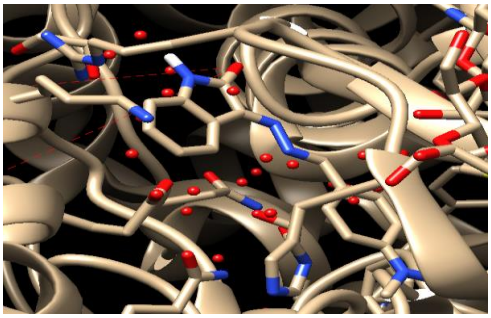
Prediction of bioactivity:

Compound	MI bioactivity score	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
IIIa	2018.03	-0.60	-0.76	-0.17	-0.92	-1.00	-0.32
IIIb	2018.03	-0.57	-0.81	-0.14	-0.91	-0.98	-0.30
IIIc	2018.03	-0.53	-0.74	-0.12	-0.82	-0.96	-0.31
IIId	2018.03	-0.70	-0.85	-0.2	-1.03	-1.08	-0.41
IIIe	2018.03	-0.59	-0.71	-0.11	-0.84	-1.02	-0.37
IIIf	2018.03	-0.60	-0.82	-0.20	-0.89	-0.99	-0.36
IIIg	2018.03	-0.51	-0.66	-0.16	-0.83	-0.89	-0.30
IIIh	2018.03	-0.64	-0.71	-0.26	-0.87	-0.96	-0.37
IIIi	2018.03	-0.64	-0.75	-0.23	-0.88	-0.99	-0.37
IIIj	2018.03	-0.68	-0.81	-0.27	-1.00	-0.97	-0.37

Molecular Docking Results:

The docking results of the compounds were recorded. In series-1 compounds all the compounds showed moderate to good docking score.

S.No	Compound	Score	Bond length (Å)	ACE
1.	IIIa	4912	46.33	- 328.0
2.	IIIb	5272	53.32	- 286.7
3.	IIIc	4620	39.86	- 303.08
4.	IIId	4832	53.32	- 339.6
5.	IIIe	4958	48.00	- 348.48
6.	IIIf	4950	33.07	- 290.88
7.	IIIg	5018	47.7	- 298.3
8.	IIIh	5522	48.08	- 336.7
9.	IIIi	5056	48.7	- 327.1
10.	IIIj	4786	48.7	- 307.6



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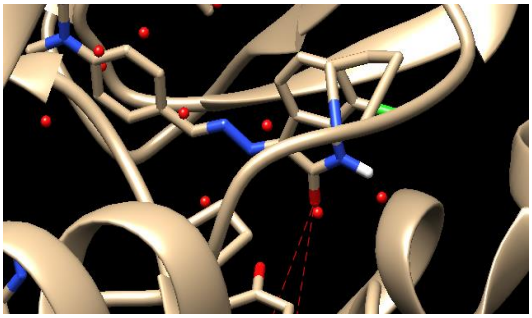

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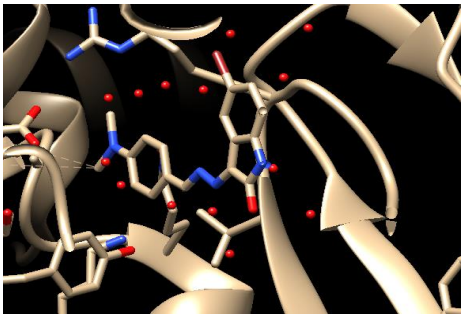

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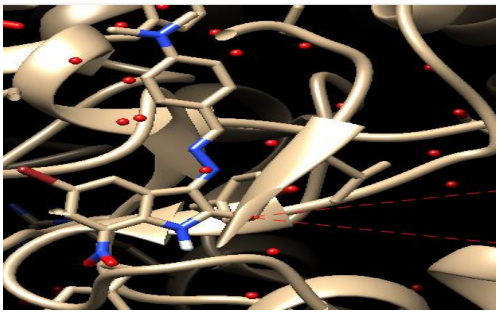

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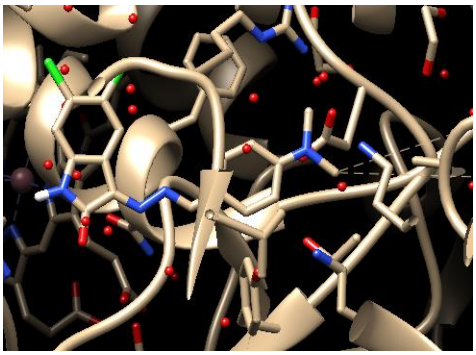

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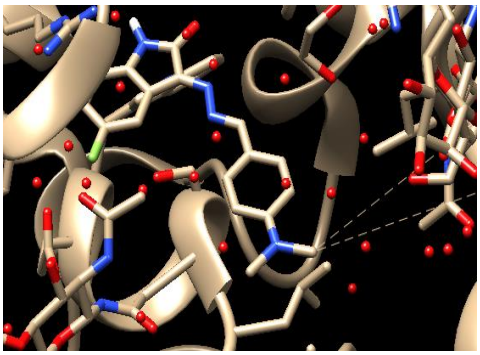

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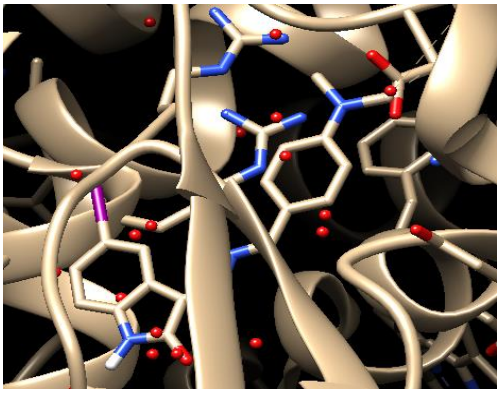
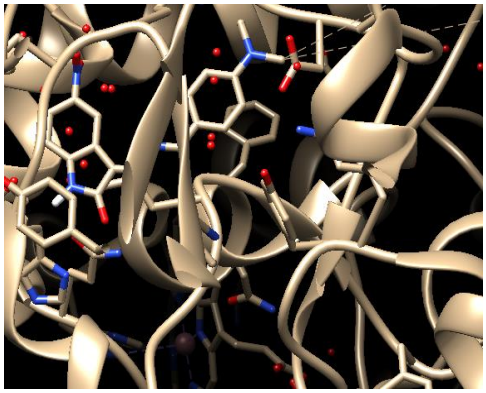
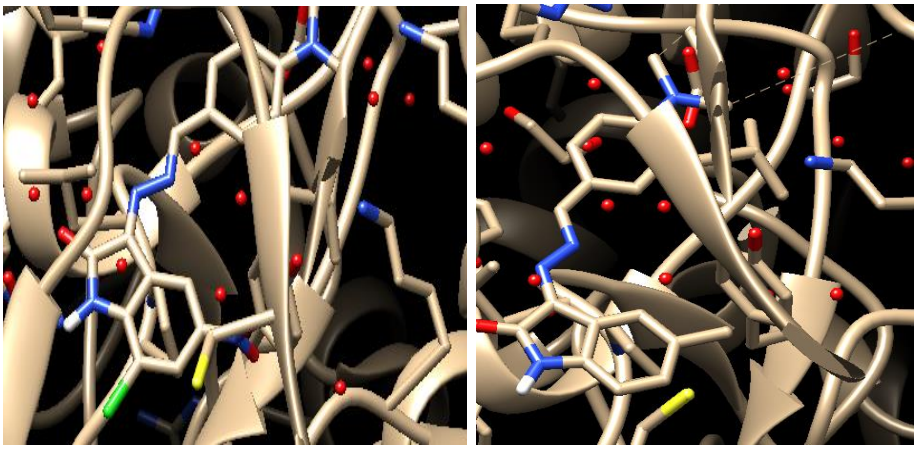


IIIg



IIIh



IIIi **IIIj**

3D binding modes and interactions of the synthesised compounds with COX-2 enzyme**.**

CONCLUSION:

All the synthesised compunds were evaluated using Molecular Docking and in silico studies (Molinspiration, SwissADME) . All the compounds according to SwissADME study, the compounds showed good lipophilicity, high GI absorption and good bioavailability score. They are BBB permeant and were found to be P-glycoprotein substrates. The compounds obeyed Lipinski rule with no violations which shows leadlikeness. According to Molinspiration studies the compounds were found to be active as kinase inhibitors, moderately active as GPCR ligand, protease inhibitor and enzyme inhibitor. The molecular docking study by using one target showed good to moderate activity. Based on the *in-silico* results designate that all the compounds could be used as potential lead for designing more potent anti-inflammatory agents. The 5,6 dichloro substituted compound (IIIe) showed the best binding energy and virtual screening docking scores. Among other compounds IIIa, IIId, IIIh and IIIi shows moderate activity. Based on these results these five comounds are going to plan for synthesis which could be a good candidate for anti-inflammatory drug of higher activity, C0X-2 enzyme selectivity, and lower gastrointestinal side effects.

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