Original Article

Synthesis and *in silico* evaluation of indole derivatives as potential anti-inflammatory agents

Bhaskar, Bhupinder Kumar*

Department of Pharmaceutical Chemistry, ISF College of Pharmacy, Moga, Punjab, India

Correspondence:
Bhupinder Kumar, Department of Pharmaceutical Chemistry, ISF College of Pharmacy, Ghal Kalan, G.T Road, Moga - 142 001, Punjab, India. Mobile: +91-9877311037. E-mail: bhupinderkumar25@gmail.com

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ABSTRACT

Indole is one of most promising pharmacologically active molecules with potential anti-inflammatory activity. We clubbed indole with oxazole and oxadiazole to synthesize a potential anti-inflammatory agent. Synthesis of various required intermediates and newly proposed compounds was achieved in satisfactory yield after purification. The title compounds (**5a-g**) were synthesized by condensing Chalcone derivatives with hydroxylamine hydrochloride using glacial acetic acid. All the synthesized compounds were subjected to docking studies against COX-1 enzyme, (PDB ID: 3KK6) and COX-2 enzyme (PDB ID: 4COX) using Genetic Optimization for Ligand Docking program. From the series, it was found that all the synthesized compound fit to well at active site of COX-1 and COX-2 enzymes as in case of standard inhibitor celecoxib and indomethacin. In case of COX-1 compounds **5f** and **5g** was found to have highest docking score with 70.50 and 66.08, respectively, as compared to standard drug indomethacin and celecoxib with docking score of 64.84 and 77.90, respectively. In case of COX-2, compounds **5c**, **5f,** and **5g** were found to have highest docking score with 60.37, 65.13, and 63.18, respectively, among these all compounds as compared to standard drug celecoxib with docking score of 67.90.

Keywords: Anti-inflammatory, chalcone, COX-1, COX-2, indole derivatives

INTRODUCTION

Inflammation, derived from latin word *inflammare*, which is first protective mechanism of body immune system toward damaged tissue or cell by various stimuli such as pathogens, harmful stimuli, and injuries.[1,2] To protect our body from these harmful infections our body protective mechanism initiate and starts healing process, that is, inflammation.[3] In other words, inflammation is a kind of defensive mechanism which is necessary for our body for saving tissue, cell or organ from further damage or for reduction of infection or injury.[4,5] Inflammation involves the production of pro-inflammatory mediators, as influx of innate immune cells and tissue destruction.[6,7] Four major sign of inflammation which cause loss of cell or tissue function are redness, heat, pain, and swelling.[8] During inflammation process various types of microcirculatory events such as white blood cell recruitment, accumulation, and vascular permeability changed in inflammation.[3-9] Inflammation either acute or some time chronic and composed of multiple processes. In case of acute inflammation, our body starts to restore tissue homeostasis and if acute inflammation become worse than it lead to chronic inflammation.[10] Most of the time, inflammation which is normal in condition becomes a chronic and continuous inflammatory disease.

Indole is combination of the word's indigo and oleum. Indole was first isolated by treatment of the indigo dye with oleum. Indole ring also found in a lot of natural products such as fungal metabolites, marine natural products,[11] and in indole alkaloids. Development of indole chemistry began with the study of the indigo dye. In, mid-19th century wide research going on a natural violet-blue dye named indigo leads to the synthesis of indole by zinc distillation of Oxindole in 1866. Indole contains one nitrogen atom in its core structure with molecular formula C_8H_7N. Indole is one of the most promising heterocyclic active compounds having

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wide range of pharmacological activities. At C2 and C3 position pyrrole ring fused to benzene ring. Indole can be modified to variety of indole derivatives with better biological/pharmacological activities which can be further developed as novel drug for the treatment of various diseases such as hypertension, cancer, inflammation, neurodegenerative disease, diabetes, and so on.[12,13] One of the best example of naturally occurring indole derivative present in human body in form of essential amino acid is Tryptophan (2-amino-3-(3′-indolyl)propionic acid). Another example of indole derivative present in the body as neurotransmitter is Serotonin.[14] Melatonin is another type structurally similar hormone which is important to control diurnal rhythm of physiological functions.

Thus, in view of above discussed facts and as need to discover new potentially active anti-inflammatory agents, we have synthesized some new oxazole bridged chalcone derivatives of indole. The synthesized indole derivatives were characterized using nuclear magnetic resonance (NMR), mass and infrared (IR) data and evaluated *in silico* for their binding to COX-1 and COX-2.

EXPERIMENTAL

Chemistry

General procedure for the synthesis of 2-methylindole-3-carboxaldehyde (2) [Scheme 1]

2-Methylindole (3.81 mmol, 1 eq) was added to the Vilsmeier-Haack reagent prepared by the addition of $POCl_3$ (3 eq) in DMF (3.81 mmol, 1 eq) at 0°C. The mixture was stirred at 0°C for 2 h to ensure the complete consumption of starting material reaction monitored by thin layer chromatography (TLC). Then reaction quenched slowly by adding a crystal of ice and neutral the reaction mixture by adding the 20% of NaOH solution. The resulting precipitate was filtered and dried to afford the 2-methylindole-3-carboxaldehyde (**2**) in good yield. Compound (**2**) was used for further reaction. Recrystallize the product if necessary.

General procedure for the synthesis of chalcone derivatives (4) [Scheme 2]

A mixture of 2-metylindole-3-carboxaldehyde (1 g, 4.51 mmol, **2**), acetophenone (0.5 mL, 4.51 mmol, **3**), and piperidine (1.8 mL) was taken in a well dried round bottom flask and heated for 4–6 h to ensure the complete consumption of starting material reaction monitored by TLC. Then ethanol (7 mL), glacial acetic acid and water (1:1) were added to resulting red solution until first appearance cloudiness. The progress of the reaction is monitored by TLC. The resulting product was filtered off and washed with water, dried the desired product (**4**).

General procedure for the synthesis of isoxazole derivatives (5a-e) [Scheme 3]

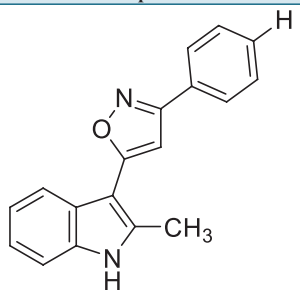
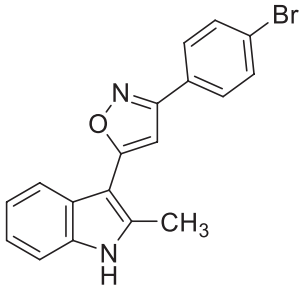
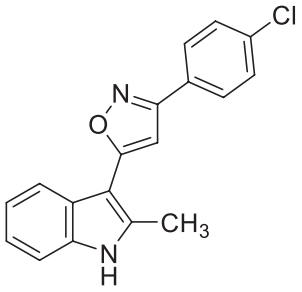
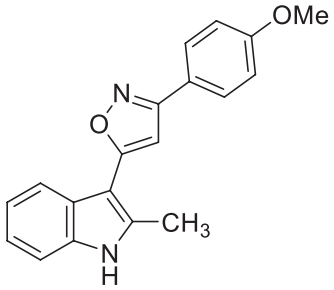
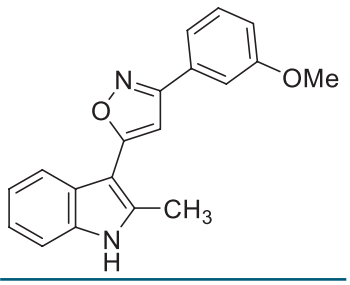
A mixture of Chalcone (500 mg, 1.54 mmol, **4a-e**), Hydroxylamine. HCl (322 mg, 4.638Mmol), and glacial acetic acid (3 mL) were refluxed for 12–14 h continuously. Reaction progress was monitored by TLC continuously using solvent system 30% ethyl acetate and hexane (3:7). On completion of the reaction (TLC monitoring), the resulting solution was cooled and add crushed ice into resulting solution then precipitate was formed, filtered off, washed with cold water, and dried to afford the final product in good yield.

SPECTRAL DATA OF REPRESENTATIVE COMPOUND

5-(2-methyl-1H-indol-3-yl)-3-phenylisoxazole 13a

Pale brown; 65.35%; ^{1}H-NMR (500 MHz, $CDCl_3$): 11.07 (s, 1H, NH), 7.77 (t, 1H, Ar-CH, J_1 = 8 Hz, J_2 = 4Hz), 7.50 (t, 1H, Ar-H,

Table 1: Structure of various Indole containing Isoxazole derivatives (5a-e)

S. No.	Compound Name	R	Final product
1.	**5a**	H	
2.	**5b**	Br	
3.	**5c**	Cl	
4.	**5f**	O-Me	
5.	**5g**	O-Me	

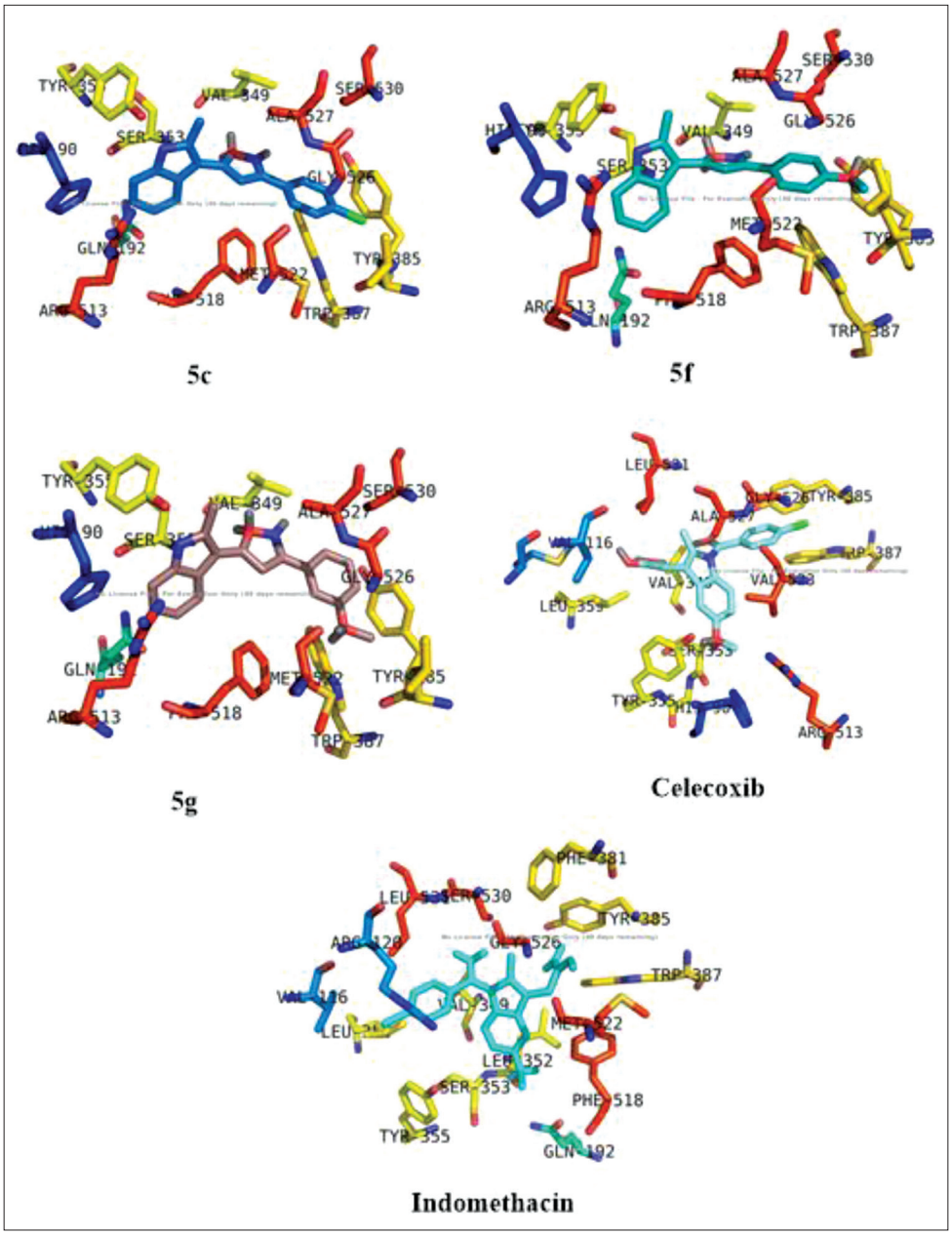
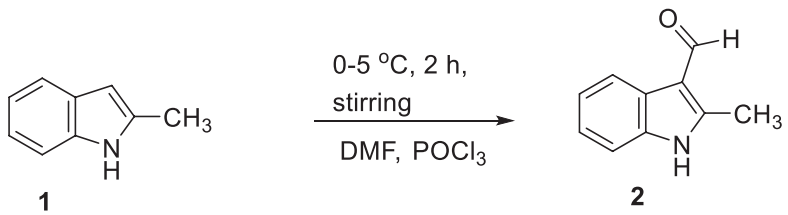


Figure 1: Docking pose of compounds for COX-2



1 2

Scheme 1: Synthesis of 2-Methylindole-3-carboxaldehyde

J_1 = 4 Hz), 7.30-7.20 (d, 1H, Ar-H, J_1 = 4 Hz), 7.02-6.96 (3H, m, Ar-H), 6.02-5.97 (1H, m, Ar-H), 2.50 (s, 1H, CH_3), ^{13}C-NMR (100 MHz, $CDCl_3$): 156.17, 135.44, 134.09, 129.78, 129.68, 128.77, 126.36, 125.68, 120.42, 118.70, 117.80, 110.78, 110.78, 108.99, 76.56, 108.17, 39.70, 11.20.

Molecular docking studies

Molecular docking was carried out to investigate the potential binding patterns of the most potent anti-inflammatory agent with pocket residues involved in the binding of the molecule at the active site. Genetic

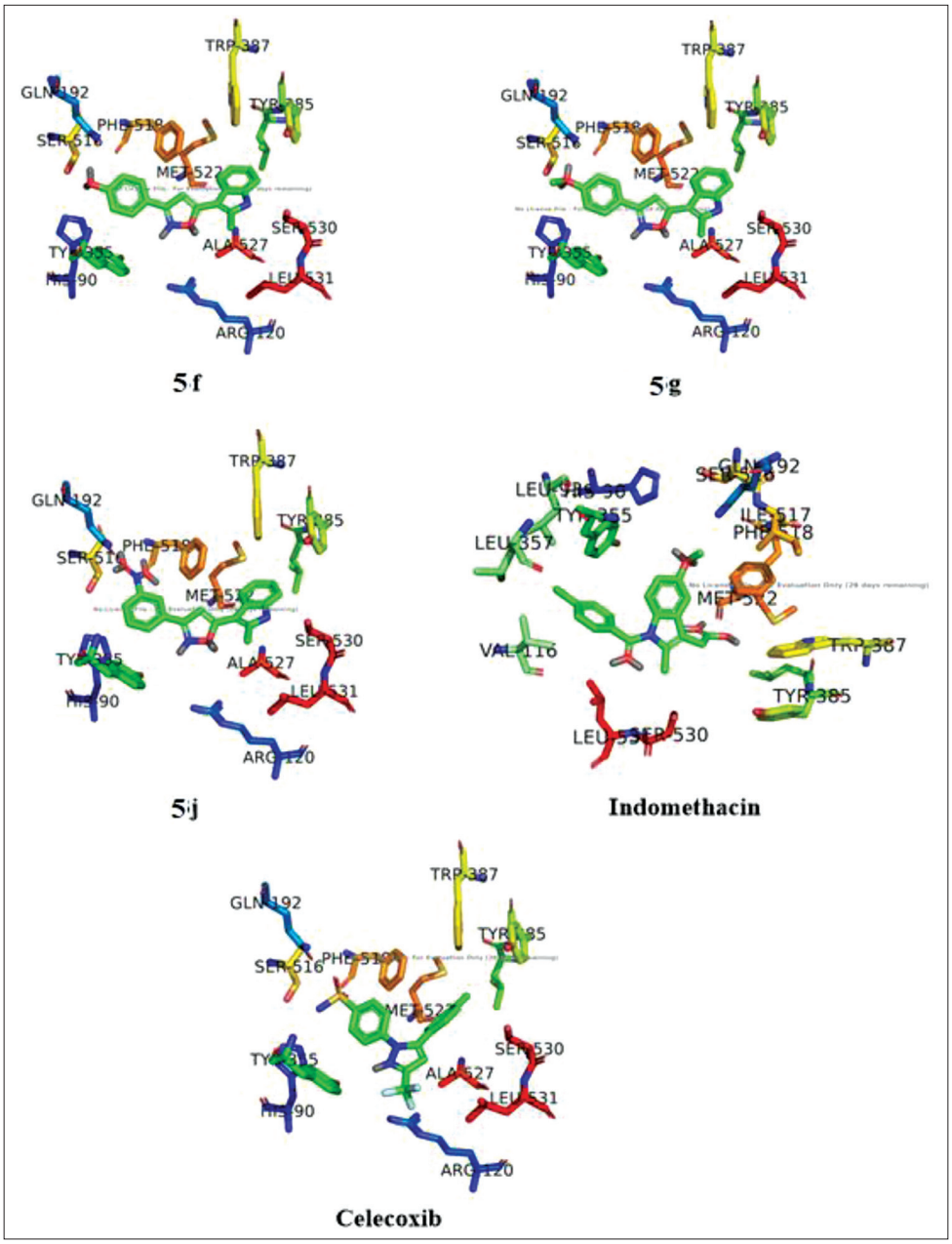
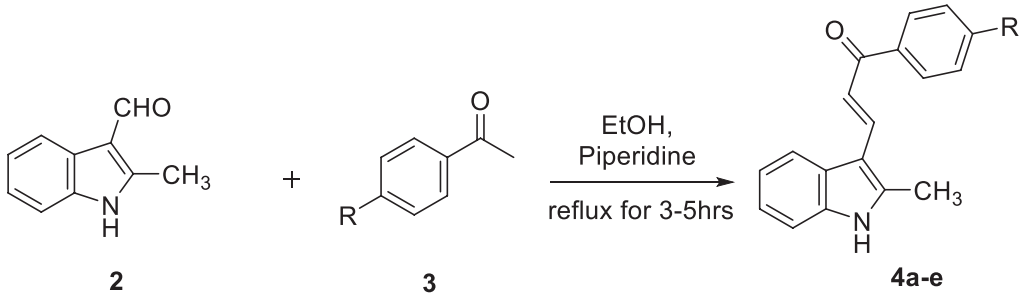
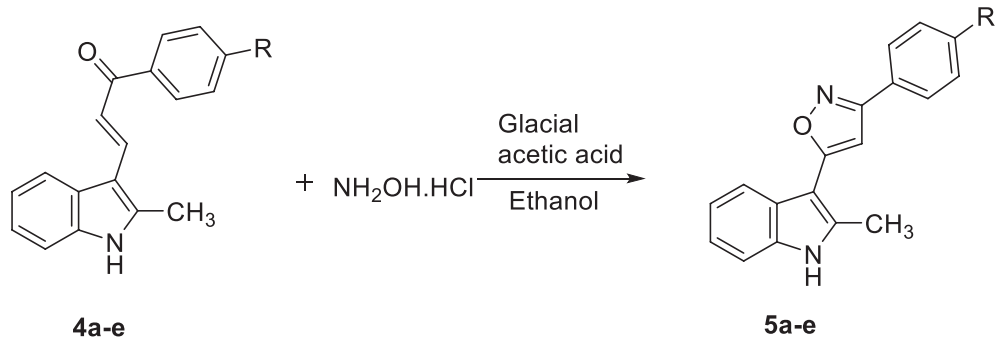


Figure 2: Docking pose of compounds for COX-1



Scheme 2: Synthesis of chalcone derivatives

Optimization for Ligand Docking (GOLD) docking program version 2.0 used to evaluate the binding study of synthesized derivatives. GOLD program based on the genetic algorithm to investigate the full range of rotational flexibility and ligand flexibility of selected receptor hydrogen's. From protein data bank, the 3-D structure of COX-1 (PDB ID: 3KK6) AND COX-2 (PDB ID: 4COX) was downloaded.



Scheme 3: Synthesis of Indole containing isoxazole derivatives (5**a-e**)

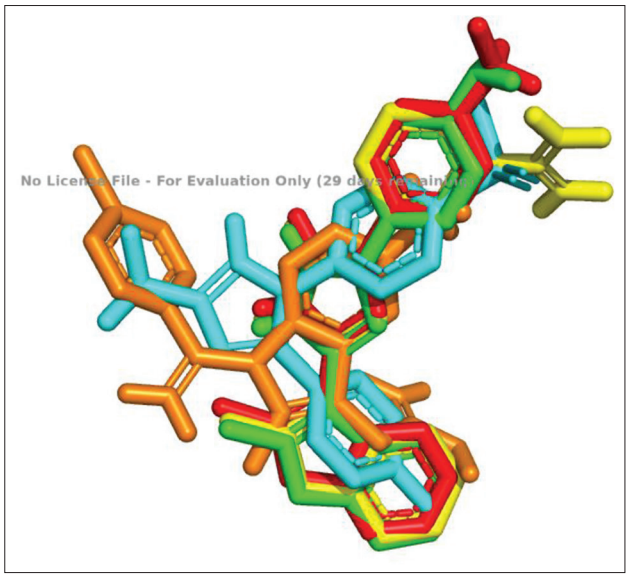


Figure 3: Overlay pose of compounds with standard

RESULTS AND DISCUSSION

Chemistry

The intermediate chalcones (**4a-c**) were synthesized through Claisen-Schmidt condensation using various substituted acetophenones with 2-methylindole-3-carboxaldehyde as described in synthetic scheme. Further, these intermediate chalcones were reacted with hydroxylamine hydrochloride in the presence of glacial acetic acid using solvent ethanol under refluxing conditions to give target oxazole derivatives (5**a-c**). The final products were purified using column chromatography or recrystallization and were obtained in moderate to good yields. The characterization of target compounds was done using spectral techniques that include FTIR, ^{1}H NMR, and mass spectroscopy which fully supported their structural identity.

Molecular docking studies

All the synthesized derivatives were evaluated *in silico* to identify their hypothetical binding mode using the X-ray crystal structure of COX-1 (PDB ID: 3KK6) and COX-2 (PDB ID: 4COX), respectively, and also to rationalize their structure activity relationship. Indomethacin and celecoxib used referenced drug to investigate the molecular docking. It was docked back into its binding site of the crystal structure of the COX-2 using GOLD docking program (Srivastava *et al.*, 2019). Among the synthesized series of compounds derivatives **5c, 5f,** and **5g** showed most promising binding and were docked within the active sites of COX-2 enzyme (PDB ID: 4COX). Analysis of the docking pose of compounds **5c**, **5f,** and **5g** highlighted highest docking score with 60.37, 65.13, and 63.18, respectively, as compared to referenced drug celecoxib and indomethacin with docking score of 67.90 and 49.40, respectively. Among the synthesized compounds in case of COX-1 compounds **5f**, **5g,** and **5j** showed most promising binding compared to standard drug indomethacin and celecoxib. Compounds **5f**, **5g,** and **5j** displayed highest docking score with 70.50, 66.08, and 68.34, respectively, as compared to referenced drug indomethacin and celecoxib with docking score of 64.84 and 77.90, respectively.

From docking studies, it was found that synthesized compounds were bind at same site as standard inhibitor indomethacin and celecoxib. For COX-2 enzyme compounds **5c, 5f,** and **5g** displayed finest docking score. Compound **5c** displayed amino acid residue interactions with SER-353, GLN-192, TRP-387, TYP-385, GLY-526, VAL-349, SER-530, MET-522, and ARG-513. Compounds **5f** and **5g** displayed same amino acid residue interactions with SER-353 ARG-513, MET-522, TRP-387, GLY-526, ALA-527, SER-530, VAL-349, TYP-355, HIS-90, and GLN-192 [Table 1 and Figure 1].

S(hb_ext) = Hydrogen bond energy external (between ligand and protein); S(vdw_ext) = van der Waals energy external (between ligand and protein); S(int): Sum of internal torsional and internal Van der Waals energies; intcor = internal corrections [Table 2 and Figure 2].

For COX-1 enzyme, compounds **5f, 5g,** and **5g** displayed best docking score. Compounds **5f, 5g,** and **5g** displayed same amino acid residue interactions with MET-522, SER-516, GLN-192, TYR-355, HIS-90, ARG-120, ALA-527, LEU-531, SER-530, TYR-385, TRP-387, and PHE-518, respectively [Table 3].

S(hb_ext) = Hydrogen bond energy external (between ligand and protein); S(vdw_ext) = van der Waals energy external (between ligand and protein); S(int): Sum of internal torsional and internal Van der Waals energies; intcor = internal corrections.

Table 2: Gold dock score of synthesized compounds with COX-2 (PDB ID: 4COX)

S. No.	Compound name	Gold score (Fitness score)	S(hb_ext)	S(vdw_ext)	S(int)	intcor
1.	5a	59.01	2.13	41.48	−0.15	−0.58
2.	5b	60.02	2.69	41.80	−0.13	−0.58
3.	5c	60.37	0.47	45.98	−3.33	−0.56
4.	5d	57.51	2.82	39.87	−0.13	−0.61
5.	5e	58.44	0.53	45.13	−4.15	−2.16
6.	5f	65.13	0.34	49.60	−3.41	−2.00
7.	5g	63.18	0.16	46.81	−1.35	−2.02
8.	5h	56.88	3.78	39.06	−0.60	−0.67
9.	5i	60.22	0.19	45.83	−2.98	−1.36
10.	5j	58.28	1.60	43.41	−3.00	−1.58
11.	Indomethacin	49.40	0.00	37.98	−2.83	−4.68
12.	Celecoxib	67.90	0.17	49.51	−0.35	−3.28

Table 3: Gold dock score of synthesized compounds with COX-1 (PDB ID: 3KK6)

S. No.	Compound name	Gold score (Fitness score)	S(hb_ext)	S(vdw_ext)	S(int)	intcor
1.	5a	63.76	0.46	46.32	−0.39	−0.58
2.	5b	65.65	2.00	47.10	−1.12	−0.58
3.	5c	65.85	1.05	47.66	−0.75	−0.58
4.	5d	65.83	1.80	47.43	−1.20	−0.57
5.	5f	66.20	3.59	45.79	−0.36	−2.18
6.	5g	70.50	2.18	49.95	−0.38	−1.98
7.	5h	66.08	2.02	47.88	−1.77	−1.98
8.	5h	60.80	0.01	45.49	−1.76	−0.67
9.	5i	65.42	1.36	46.79	−0.28	−1.39
10.	5j	68.34	3.86	48.77	−2.57	−1.49
11.	Indomethacin	64.84	0.51	47.96	−2.27	−4.07
12.	Celecoxib	77.90	4.93	54.72	−2.27	−4.07

From the result of docking study, it was found that all the synthesized compounds were fit to well at same active site of the inhibitors such as celecoxib and indomethacin [Figure 3].

In silico drug-likeness predications

Drug likeness is the complex balance between various molecular and structural features such as stability, oral availability, good pharmacokinetic properties, lack of toxicity, and minimum addictive potential. The ADMET prediction for central nervous system (CNS) active agents is an essential tool to determine the safety and target reaching ability of designed compounds. Many of these properties depend on the inherent biological and physicochemical parameters of the molecule; however, the complex structure of the whole drug molecule makes correlating attempts difficult.

In silico drug likeness prediction of various designed and synthesized derivatives was carried out using a Swiss ADME predictor tool and PreADME. The results obtained from prediction data [Table 4] revealed that the synthesized compounds follow the Lipinski's rule of drug like molecules. Most of the synthesized compounds showed LogP value less than or around 5. The molecular weight is <500 and presence of hydrogen bond donors and hydrogen bond acceptors atoms is also favorable. The compounds showed promising % oral absorbance (80–100%). Further, all the compounds were able to cross the blood brain barrier which is essential to reach target site in the brain and to exhibit CNS activity. With no violation to Lipinski's rule, it can be concluded that the synthesized compounds have optimum chemical skeleton that can be developed as potential drug molecule.

Table 4: Physiochemical properties and toxicological profile of designed derivatives

Compounds	TPSA (Å^2)	MW (g/mol)	RoB	HBD	HBA	ILogP[a]	LogS[b]	% ABS
5a	41.82	274.32	2	1	2	3.92	−4.77	95.7
5b	41.82	353.21	2	1	2	4.54	−5.66	100
5c	41.82	308.76	2	1	2	4.46	−5.35	100
5d	41.82	292.31	2	1	3	4.24	−4.91	100
5e	62.05	290.32	2	2	3	3.51	−4.61	96.1
5f	51.05	304.34	3	1	3	3.92	−4.81	94.6
5g	51.05	304.34	3	1	3	3.93	−4.81	100
5h	67.84	289.33	2	2	2	3.37	−4.40	100
5i	41.82	308.76	2	1	2	4.43	−5.35	90
5j	63.95	310.31	3	1	4	3.16	−4.79	90

ABS: Percentage absorption, HBD: Hydrogen bond donors, HBA: Hydrogen bond acceptors, MW: Molecular weight, RoB: Number of rotatable bonds, TPSA: Topological polar surface area. aLogarithm of compound partition coefficient between n-octanol and water, bLogarithm of water solubility

CONCLUSION

The present project work involved design, synthesis, characterization of indole appendant isoxazole derivatives as anti-inflammatory, and antioxidant potentials. The title compounds **5a-g** were synthesized by condensing Chalcone derivative with hydroxylamine hydrochloride using glacial acetic acid. The synthesized compounds were structurally characterized by physico-chemical studies (such as TLC and melting point) and spectral study (such as FT-IR, ^{1}H-NMR, and ^{13}C-NMR) which confirms their structural identity. All the synthesized compounds **5a-g** were subjected for docking studies COX-1 enzyme (PDB ID: 3KK6) and COX-2 enzyme (PDB ID: 4COX) using GOLD software. From the series of synthesized derivatives, it was found that all synthesized compound fit to well at active site of COX-1 and COX-2 enzymes as in case of referenced inhibitors indomethacin and celecoxib. In case of COX-1 compounds **5f, 5g,** and **5j** were found to be have highest docking score with 66.20, 70.50, and 68.34, respectively, as compared to references drug indomethacin and celecoxib with docking score of 64.84 and 77.90, respectively. For COX-2 enzyme, compounds **5c, 5f,** and **5g** were found to be have best docking score with 60.37, 65.13, and 63.18, respectively, as compared to referenced celecoxib and indomethacin with docking score of 67.90 and 49.40, respectively.

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