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SYNTHESIS, 3D QSAR AND DOCKING STUDIES OF NOVEL INDOLYL ISOXAZOLINE DERIVATIVES AS ANTI-INFLAMMATORY AGENT

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ABSTRACT

A series of 3-(2-(4-substitutedphenyl)-1*H*-indol-3-yl)-1-(4-substitutedphenyl)prop-2-en-1-one **3(a-x)** were synthesized from substituted indole aldehydes. Using hydroxylamine hydrochloride the chalcones **3(a-x)** were cyclised to afford a novel series of 2-(4-substitutedphenyl)-3-(3-(4-substitutedphenyl)-4,5-dihydroisoxazol-5-yl)-1*H*-indole **4(a-x).** The structure of all these compounds were established on the basis of spectral (IR, ^{1}H NMR) studies. The compounds **4(a-x)** were evaluated for the anti-inflammatory activity using carrageenan induced rat paw edema method. 3D QSAR studies were performed to understand structural requirement for exploring future potential of synthesized derivatives. QSAR models were generated using k-Nearest Neighbor Molecular Field Analysis (kNN-MFA). The virtual combinatorial library was prepared using the generated model. Docking studies were performed on the series and compared with selective COX-2 inhibitor SC558.

KEYWORDS: Indole, isoxazoline, anti-inflammatory activity, 3D QSAR, kNN-MFA.

INTRODUCTION

Inflammation is the result of concerted participation of a large number of vasoactive, chemotactic and proliferative factors at different stages of infections. The NSAIDs are popular in reducing the acute and chronic inflammation as they have no abuse liability.[1,2] Present NSAIDs have common side effects like gastric and peptic ulceration, acute renal failure, hypersensitivity reaction when administered in large dose or in long term therapy.[3]

The search for newer non-steroidal anti-inflammatory agents is the only way to fortify against these adverse effects. The discovery of indomethacin as a successful agents for clinical treatment of inflammatory disorders has led to the exploration of indole moiety to obtain better anti-inflammatory agents.[4,5] Literature survey revealed that few isoxazoline derivatives possess good anti-inflammatory activity.[6,7] Valdecoxib is an isoxazoline derivative which is widely used in the market as anti-inflammatory drug. This prompted us to synthesize hybrid analogues of two pharmacophores viz. indole and isoxazoline in the hope to achieve safer and better NSAIDs having anti-inflammatory activity with the lower incidences of GI side effects.

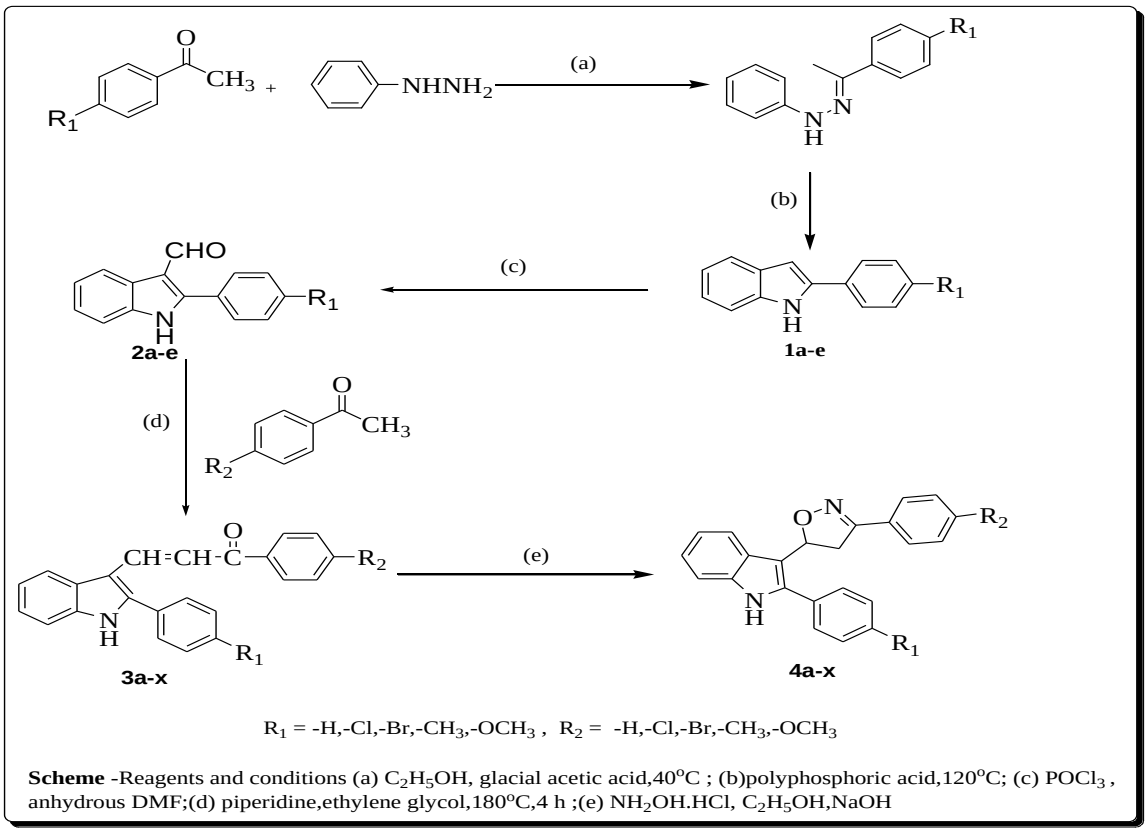
3D QSAR facilitates evaluation of three dimensional molecular fields around molecules and generate relationship of these field values with the activity. k-Nearest Neighbor Molecular Field Analysis (kNN-MFA) is one of the method to generate such relationship which measures the nearness of the molecule by an appropriate distance metric.[8] In this study we report kNN-MFA based QSAR model developed for 24 synthesized Indolylisoxazoline derivatives. The distribution point map derived from kNN-MFA 3D QSAR model permitted an understanding of steric and electrostatic requirements for ligand binding. This model was used for predicting the biological activity of newly designed analogs without synthesizing the compounds. The most and the least active compounds were docked in 1CX2 isoform of cyclooxygenase-2 enzyme. The docking scores compared with the selective COX-2 inhibitor SC558.

MATERIALS AND METHODS

Chemistry

All the chemicals used in the synthesis were of laboratory grade. To monitor the progress of reactions and to establish the identity and purity of reactants and products, Thin Layer Chromatography was performed on aluminium slides coated with silica gel 60, using appropriate solvent systems and the spots were visualized under ultra-violet light. Melting points were determined in open capillary on Veego (model: VMP-D) electronic apparatus and are uncorrected. The IR spectra of the synthesized compounds were recorded on Shimadzu 8400-S FT-IR Spectrophotometer using potassium bromide. The ^{1}H NMR spectra were recorded in $CDCl_3$ using NMR Varian-Mercury 300 MHz spectrometer and chemical shifts are given in units as parts per million, downfield from Tetra Methyl Silane (TMS) as an internal standard.

The steps followed in synthesis are depicted in the scheme



Scheme -Reagents and conditions (a) C_2H_5OH, glacial acetic acid,40°C ; (b)polyphosphoric acid,120°C; (c) $POCl_3$, anhydrous DMF;(d) piperidine,ethylene glycol,180°C,4 h ;(e) $NH_2OH.HCl$, C_2H_5OH,NaOH

Substituted indoles 1(a-e) were synthesized according to Fisher indole synthesis.[9]

Synthesis of indole aldehyde 2(a-e).[10]

Dry dimethylformamide (25 ml) was stirred at 0^0C for 15 min. Phosphonylchloride (2 mol) was added drop wise using dropping funnel for half an hour. Substituted indole (1 mol) was dissolved in 50 ml dry DMF and added drop wise into the formylated solution below 10^0C over the period of two hour. After the completion of addition the reaction mixture was stirred for another half hour. The temperature was brought to 35-40^0C and maintained for 2 h. Then 100 g of crushed ice added slowly. Sodium hydroxide solution (10 mol) was added drop wise. The suspension was heated to boiling to remove diethylamine. The suspension was cooled to room temperature and kept overnight. The reaction mixture was diluted with water and filtered. The crude product obtained was washed with water to remove inorganic material. The crude aldehyde was recrystallized from ethanol.

Synthesis of 3-(2-(4-substitutedphenyl)-1H-indol-3-yl)-1-(4-substitutedphenyl)prop-2-en-1-one 3(a-x)

Equimolar quantity of indole-3-aldehyde and substituted acetophenone was dissolved in 50ml ethylene glycol. Equimolar quantity of piperidine was added and refluxed for 4 h. The reaction mixture was cooled to room temperature. The crude chalcone was filtered and washed with cold ethanol and water. The chalcone was recrystallised from ethanol.

Synthesis of 2-(4-substitutedphenyl)-3-(3-(4-substitutedphenyl)-4,5-dihydroisoxazol -5-yl)-1H-indole 4(a-x).[11]

The target compound was synthesized by reacting the corresponding chalcone (0.01mol) with hydroxylamine hydrochloride (0.02 mol) in presence of NaOH (0.01 mol) using distilled ethanol as solvent. The reaction mixture was refluxed for 14-16 h. The progress of reaction was monitored with TLC. After completion of the reaction, an excess of the solvent was removed by distillation and the resultant mass was poured into ice water with vigorous stirring. The solution was acidified with dilute HCl. It was kept in cool overnight. The crude solid obtained was filtered, washed with sufficient cold water, dried and purified by recrystalization from ethanol. The physical property data of target compounds 4(m-x) is depicted in **Table-1**. The synthesis, characterization and biological activity of compounds 4(a-l) are reported in literature.[12]

Table. 1. Data of physical properties target compounds 4(m-x).

Code	R_1	R_2	Molecular formula	Mol. wt.	M.P. ^{O}C	R_f	% Yield
4m	-Br	-Cl	$C_{23}H_{16}BrClN_2O$	451.5	210-212	0.69	85
4n	-Br	-Br	$C_{23}H_{15}Br_2N_2O$	496	232-234	0.65	79
4o	-Br	$-CH_3$	$C_{24}H_{18}BrN_2O$	431	222-224	0.49	92
4p	$-CH_3$	-H	$C_{24}H_{19}N_2O$	352	187-189	0.48	81
4q	$-CH_3$	-Cl	$C_{24}H_{18}ClN_2O$	386.5	193-195	0.54	86
4r	$-CH_3$	-Br	$C_{23}H_{15}BrClN_2O$	431	190-192	0.59	85
4s	$-CH_3$	$-CH_3$	$C_{25}H_{21}N_2O$	366	189-191	0.76	89
4t	$-CH_3$	$-OCH_3$	$C_{25}H_{21}N_2O_2$	382	199-201	0.71	76
4u	$-OCH_3$	-Cl	$C_{24}H_{18}ClN_2O_2$	402.5	191-193	0.67	82
4v	$-OCH_3$	-Br	$C_{24}H_{18}BrN_2O_2$	447	195-197	0.76	84
4w	$-OCH_3$	$-CH_3$	$C_{24}H_{18}N_2O_2$	382	198-200	0.67	79
4x	$-OCH_3$	$-OCH_3$	$C_{25}H_{21}N_2O_3$	398	189-191	0.56	76

Anti-inflammatory activity[13]

Anti-inflammatory activity was evaluated by carrageenan induced rat paw edema method developed by Winter *et al*. The suspensions of test compounds were prepared in sterile 0.9% w/v NaCl solution. In all cases control received the same quantity of sterile 0.9% w/v NaCl

solution as vehicle. Sprague Dawley rats of either sex weighing between 150-250 g were randomly distributed in control and experimental group of six animals each. At 0 h the target compounds **4(a-x)** (40 mg/kg) and standard (20 mg/kg) doses were administered orally. After 1 h of oral administration of compounds and standard, 0.1 ml of 1% w/v suspension of carrageenan in distilled water was injected into the planter tissue of right paw of rat by using 27 gauge needles. The paw was marked with ink at the level of the tibia-tarsal junction and the initial volume of paw was measured by plathysmometer within 30 second of injection. The relative increase in paw volume was found by remeasuring the paw volume after 3 h of carrageenan injection.

The % inhibition of edema was calculated by following formula and the results are shown in **Table-2**.

$$\% \text{ Inhibition of edema (3h)} = \left[1 - \frac{Vt}{Vc} \right] X\ 100$$

Where, Vt = mean relative change in paw volume in test group.

Vc = mean relative change in paw volume in control group.

Table. 2. Anti-inflammatory activity of target compounds 4(m-x).

Compound code	180 min (mean vol. ± SEM)	% inhibition (180 mins)
Control (saline)	1.39±0.0081	00 [a,****]
Indomethacin	1.07±0.0036	73.8[a]
4m	1.18±0.0073	47.62[a,****]
4n	1.15±0.0182	59.52 [a,*]
4o	1.14±0.0073	57.14 [a, ns]
4p	1.12±0.0109	64.28 [a, ns]
4q	1.14±0.0146	61.9 [a, ns]
4r	1.14±0.0146	61.9 [a, ns]
4s	1.13±0.0109	64.29 [a, ns]
4t	1.09 ±0.0292	69.05 [a, ns]
4u	1.18±0.0292	50 [a,****]
4v	1.15±0.0223	54.76 [a,*]
4w	1.14±0.0103	57.14 [a, ns]
4x	1.12±0.0073	59.52 [a, ns]

Data were analyzed by one-way ANOVA followed by Bonferroni's multiple comparison test. Values were expressed as Mean ± S.E.M. **** P<0.0001,*** P < 0.001, ** P < 0.01, * P<

0.05, [ns]- non significant as compared with standard group.[a] Statistically significant from the control at $p< 0.05$.

Molecular modeling -3D QSAR[14]

The molecular modeling studies were performed using MDS 3.5, supplied by VLife sciences licensed to PDEA's SGRS college of Pharmacy. The software was installed on Pentium 4 workstation.

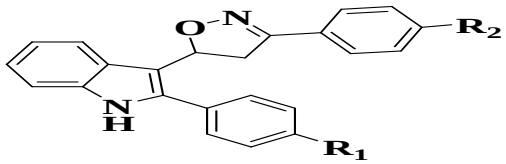
Dataset

The biological data used in this study was percent inhibition of carrageenan induced rat paw edema. The synthesis, characterization and biological activity of compounds 4(a-l) are reported in literature. The synthesis and determination of the activity of compound 4(m-x) were carried out. The biological data for 4(a-x) were converted to logarithmic scale ($-\log_{10}$% inhibition) to reduce skewness of data set and then used for QSAR analysis as dependent variable.

Alignment

All the 24 molecules (**Table 3**) were drawn in ChemDraw software. They were optimized by using Merck Molecular Force Field (MMFF). After this all the 24 molecules were aligned **(Fig.2)** using template based alignment method by choosing a minimum common structure as Template **(Fig.1)** and the most effective one as the Reference Molecule **4(t)**.

Table. 3: Structure with experimental and predicted p% inhibition activities of Indolylisoxazoline derivatives.



Code	R_1	R_2	Experimental Value	Predicted Value	Code	R_1	R_2	Experimental Value	Predicted Value
4a	-H	-H	1.69897	1.71874	4m	-Br	-Cl	1.67778	1.61963
4b	-H	-Cl	1.69897	1.71871	4n	-Br	-Br	1.77466	1.75694
4c	-H	-Br	1.75694	1.71873	4o	-Br	$-CH_3$	1.75694	1.76591
4d	-H	$-CH_3$	1.73846	1.69897	4p	$-CH_3$	-H	1.80807	1.79992
4e	-H	$-OCH_3$	1.80814	1.82471	4q	$-CH_3$	-Cl	1.79169	1.79996
4f	-Cl	-H	1.60724	1.65481	4r	$-CH_3$	-Br	1.79169	1.79991
$4g^t$	-Cl	-Cl	1.58081	1.61966	4s	$-CH_3$	$-CH_3$	1.80814	1.79169
4h	-Cl	-Br	1.63205	1.64228	4t	$-CH_3$	$-OCH_3$	1.83916	1.80811
$4i^t$	-Cl	$-CH_3$	1.65552	1.61964	4u	$-OCH_3$	-Cl	1.69897	1.65493

4j[t]	-Cl	$-OCH_3$	1.67778	1.77433	4v	$-OCH_3$	-Br	1.73846	1.79993
4k[t]	-Cl	$-NH_2$	1.69897	1.68839	4w	$-OCH_3$	$-CH_3$	1.75694	1.76581
4l	-Br	-H	1.71916	1.71872	4x[t]	$-OCH_3$	$-OCH_3$	1.77466	1.77293

[t]Molecules used in test set ,rest of molecules used in training set

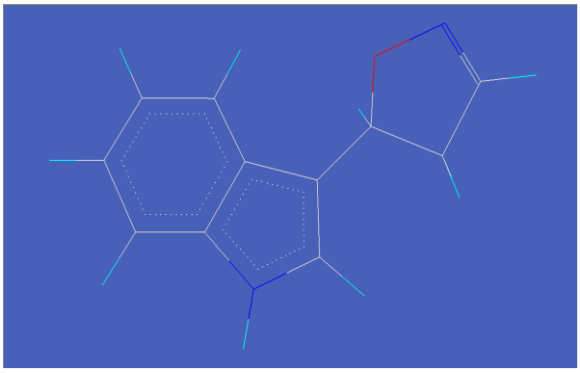


Fig. 1 Template for 3D QSAR.

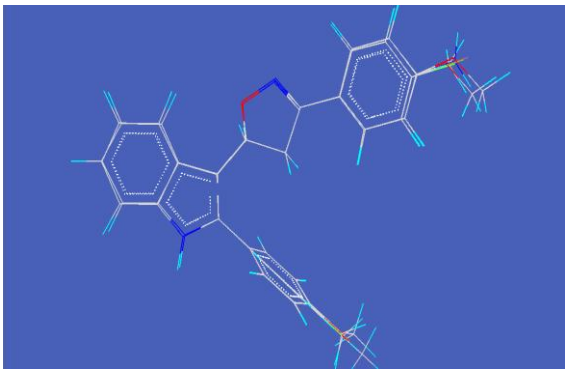


Fig. 2 Aligned molecules.

kNN-MMF methodology: Alignment was followed by generation of common rectangular grid around the molecules. The steric and electrostatic energies were computed at the lattice point of grid using methyl probe of charge +1, these interaction energy values at the grid point were considered for relationship generation using kNN MFA method and utilized as descriptors to decide nearness between molecules. A common rectangular grid around the molecules was generated. The term descriptor was utilized to indicate field values at the lattice points. Total 2080, 3D descriptors (1040 for each electrostatic and steric field) were calculated setting charge types as Gasteiger– Marsili (GM) and dielectric constant value at 1.0.[15] The descriptors with no variation in values were rejected as descriptor with constant value did not contribute to QSAR. From the 24 molecules taken in the study, a training set of 17 molecules and test set of 4 molecules were generated using the manual selection procedures. After the selection of the test and training sets, kNN MFA model was generated using stepwise forward backward variable selection method[16,17] with crosscorrelation limit of 0.5 and Ftest In 4 and Ftest Out 3.99. The relative positions of the local fields around aligned molecules that were important for activity variation in the model observed was shown in the Show points. **(Fig. 3)**.

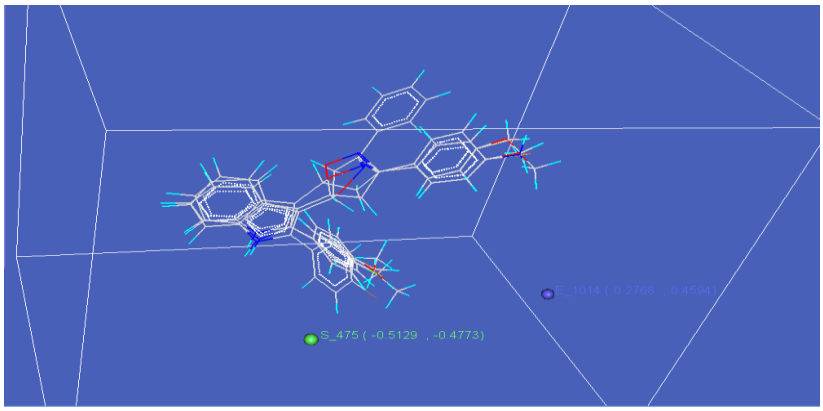


Fig. 3: Show points for the molecules.

Evaluation of QSAR model

The QSAR models were evaluated using following statistical parameters number of observations i.e. molecules in dataset (n= 19), number of nearest neighbors (k = 2), number of descriptors (vn =2), cross validated r^2 (q^2= 0.7496), predicted r^2 for the external test set (pred_r^2 = 0.6991), Standard error (SE=0.0553).

Combinatorial library

Combinatorial library of molecules was generated using template and various substituent atoms and groups employing the LeadGrow module of VLifeMDS. The template molecule with two substitution sites X27 and X28 was shown in **(Fig. 4)**. The X27 site was subjected to 20 choices of atoms/ groups. The X28 site was also subjected to 20 choices of atoms/ groups. The permutation and combination generated the virtual library of 400 compounds. The activity of compounds from library was predicted by the use of previously generated 3D QSAR equation. The compounds showing better activity than the most active synthesized compound 4(t) were listed in **Table 4**.

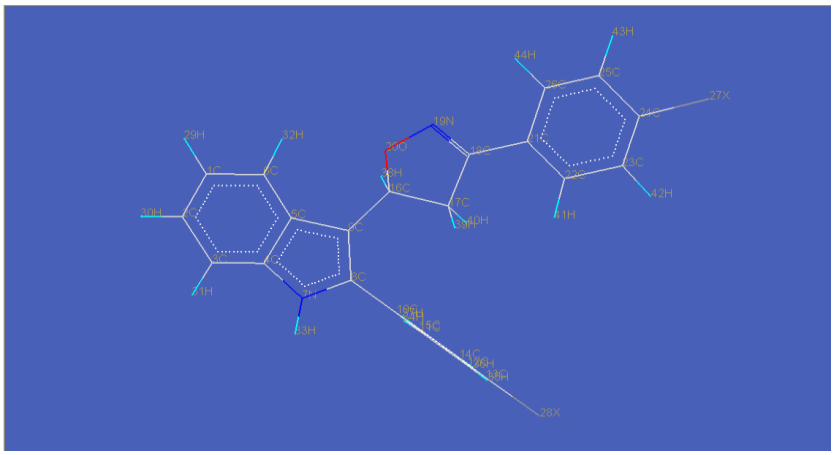


Fig. 4. Template for combinatorial library.

Table 4 Model summary

kNN Method
Training Set Size = 19
Test Set Size = 5
Selected Descriptors:
E_1014
S_475
Statistics:
K Nearest Neighbour = 2
n = 19
Degree of freedom = 16
q2 = 0.7496
q2_se = 0.0311
Predr2 = 0.6991
Pred_r2se = 0.0553
Descriptor Range:
E_1014 0.2768 0.4594
S_475 -0.5129 -0.4773

Docking Study[18]

Optimization of Protein

1CX2 isoform of human cyclooxygenase was downloaded from Protein Databank website. The tetramer is converted into monomer. Water molecules, Cofactors and Heme were deleted from protein. The SC558 **(Fig. 5)** reference molecule was extracted. The bond order, bond angle, peptide bond check performed using the local geometry check option from the analyze menu of the BioPredicta module. Hydrogens were added in molecule and energy minimized using Merk Molecular Force Field.

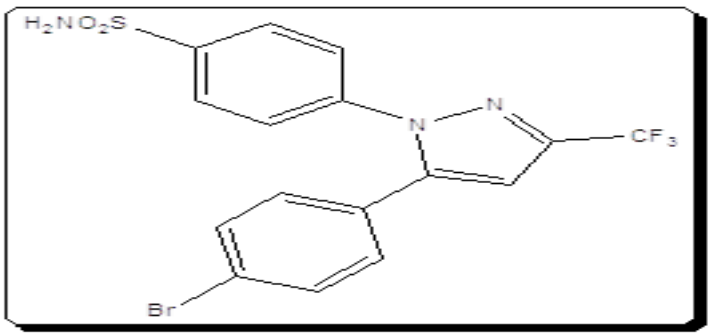


Fig. 5 Reference molecule SC558.

Optimization of ligand

The structure of SC558, 4(t) and 4(g) compounds were energy minimized using Merk molecular Force Field (MMFF) until the root mean square gradient values became smaller than 0.0001 kcal/mol A^o.

Docking

Cavity 2 in 1CX2 (protein and co crystallized ligand) was the cavity where the co crystallized ligand was found to be located. Grid based docking performed in cavity number 2 with grid size 1^0A, number of bump 4 and rotation angle 100^0. Fitness Function selected as Dock Score. The final minimum score (dock score interaction of receptor- ligand) for the best ligand pose was considered. Batch docking performed to find docking scores of all molecules **(Table 5)**. These docking scores recorded were compared for reference, the most active and the least active compound. After merging and energy minimization the amino acid interactions were also observed **(Fig. 6, 7, 8).**

Table. 5: Docking scores of all synthesized molecules.

Sr. No.	Compound code	Docking Score	Sr. No.	Compound code	Docking Score
1	SC558	-4.811594	13	4l	2.837429
2	4a	-3.384984	14	4m	-3.768558
3	4b	4.505558	15	4n	-2.750497
4	4c	-3.603202	16	4o	-2.598937
5	4d	-3.278354	17	4p	-2.325447
6	4e	-5.145282	18	4q	-3.618280
7	4f	-5.435064	19	4r	-3.549439
8	4g	-3.933550	20	4s	-3.329397
9	4h	1.706499	21	4t	-2.261698
10	4i	-3.545179	22	4u	-3.307991
11	4j	-2.284846	23	4v	-4.364930
12	4k	-2.813328	24	4w	-4.159828

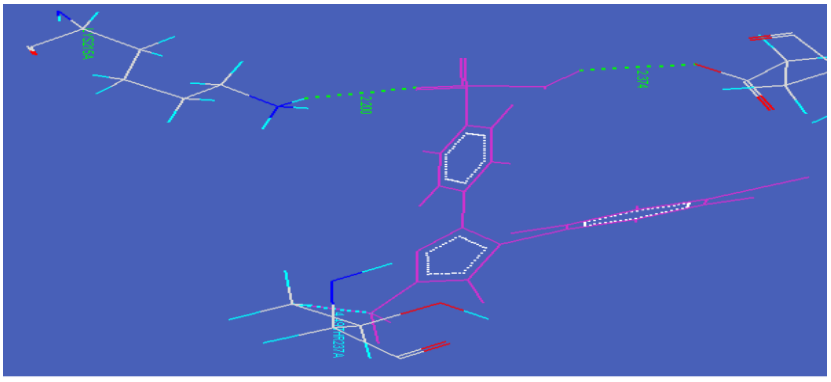


Fig. 6. Docking of reference compound in 1CX2 isoform of cyclooxygenase-2.

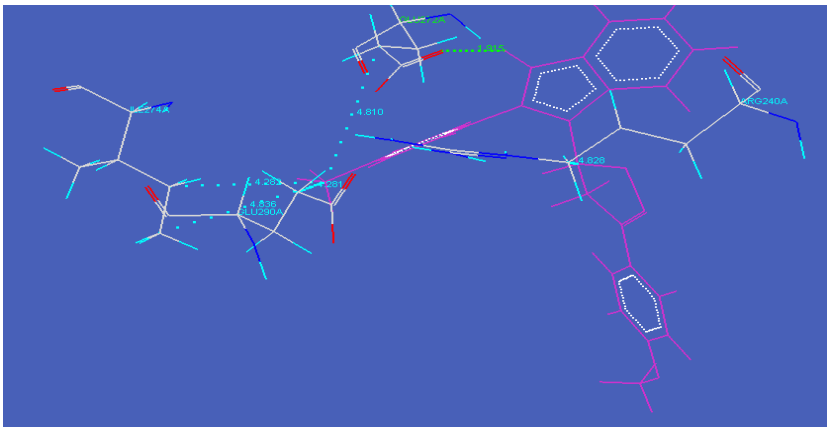


Fig. 7. Docking of the most active compound 4(t) in 1CX2 isoform of cyclooxygenase-2.

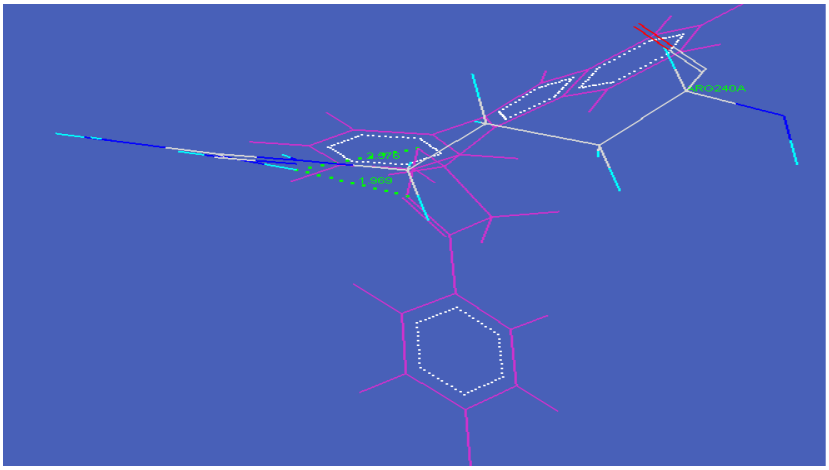


Fig. 8. Docking of the least active compound 4(g) in 1CX2 isoform of cyclooxygenase-2.

RESULTS AND DISCUSSION

Substituted indoles **1(a-e)** were synthesized by using substituted acetophenone and phenylhydrazine in presence of polyphosphoric acid. Substituted phenylindole reacted with phosphorous oxychloride and anhydrous DMF which afforded aldehyde **2(a-e).** The compounds **3(a-x)** were synthesized by reaction of substituted indole aldehydes **2(a-e)** with substituted acetophenone using ethylene glycol as solvent in presence of piperidine. The **4(a-x)** compounds were synthesized by refluxing corresponding chalcone and hydroxylamine hydrochloride in presence of sodium hydroxide using ethanol as solvent. The spectral characteristics and physical properties of the synthesized compounds were identified with the help of FT-IR, ^{1}H NMR spectroscopy and melting point.

Spectral data of target compounds 4(m-x)

2-(4-bromophenyl)-3-(3-(4-chlorophenyl)-4,5-dihydroisoxazol-5-yl)-1*H*-indole 4(m)

IR(KBr,ν_{max})cm^{-1}:3226.05(N-H), 3059.20 (Ar-C-H), 1626.00(Ar- C=C), 1588.54(C=N), 1305.91(C-O-N),831.12(C-Cl),754.98(C-Br).**^{1}H NMR ($CDCl_3$, δ ppm):** 3.531-3.626(dd,1H,-CH_2),3.689-3.778(dd,1H,-CH_2),5.988-6.060(t,1H,-CH),7.076-7.150 (m,4H,Ar-H), 7.232-7.265 (m,2H,Ar-H), 7.388-7.462 (m,2H,Ar-H), 7.529-7.552 (m,2H,Ar-H), 7.614-7.698(m,2H,Ar-H), 8.321(s,1H,-NH).

2-(4-bromophenyl)-3-(3-(4-bromophenyl)-4,5-dihydroisoxazol-5-yl)-1*H*-indole 4(n)

IR(KBr,ν_{max})cm^{-1}:3436.30(N-H), 3060.17 (Ar-C-H), 1596.15(Ar- C=C), 1559.89(C=N), 1322.67(C-O-N),746.33(C-Br).**^{1}H NMR ($CDCl_3$, δ ppm):** 3.537,3.587 (dd,1H,-CH_2), 3.632,3.696 (dd, 1H,-CH_2), 5.995-6.058 (t,1H,-CH), 7.105,7.149 (m, 4H,Ar-H), 7.264 (m,4H, Ar-H), 7.410,7.445 (m,2H,Ar-H), 7.674,7.73 (d,2H,Ar-H), 8.221 (s,1H,-NH).

2-(4-bromophenyl)-3-(4,5-dihydro-3-*p*-tolylisoxazol-5-yl)-1*H*-indole 4(o)

IR(KBr,ν_{max})cm^{-1}: 3281.02(N-H), 3048.59 (Ar-C-H), 2914.29(Aliphatic C-H) , 1596.57 (Ar-C=C), 1556.30(C=N), 1304.22(C-O-N),741.65(C-Br).**^{1}H NMR ($CDCl_3$, δ ppm):** 2.416(s,3H,-CH_3), 3.503-3.598(dd,1H,-CH_2), 3.679-3.769(dd,1H,-CH_2),6.040-6.112 (t,1H,-CH), 7.048-7.102(m,2H,Ar-H), 7.176-7.227 (m, 4H,Ar-H), 7.230-7.299(m,3H,Ar-H), 7.379-7.512 (m,2H,Ar-H), 7.660-7.688(m,2H,Ar-H),8.238(s,1H,-NH).

3-(4,5-dihydro-3-phenylisoxazol-5-yl)-2-*p*-tolyl-1*H*-indole 4(p)

IR(KBr,ν_{max})cm^{-1}:3283.92(N-H), 3055.35 (Ar-C-H), 2912.12(Aliphatic C-H), 1615.25 (Ar-C=C), 1563.36(C=N), 1305.54(C-O-N).**^{1}H NMR ($CDCl_3$, δ ppm):** 2.422(s,3H,-CH_3), 3.552-3.647(dd,1H,-CH_2), 3.735-3.825 (dd,1H,-CH_2),6.043-6.115(t,1H,-CH), 7.054-7.104(m,2H,Ar-H), 7.181-7.232 (m,4H,Ar-H), 7.258-7.309(m,3H,Ar-H), 7.385-7.488 (m,2H,Ar-H), 7.537-7.564 (m,2H,Ar-H),8.225(s,1H,-NH).

3-(3-(4-chlorophenyl)-4,5-dihydroisoxazol-5-yl)-2-*p*-tolyl-1*H*-indole 4(q)

IR(KBr,ν_{max})cm^{-1}:3380.06(N-H), 3101.66 (Ar-C-H),2923.22(Aliphatic-C-H),1596.15 (Ar-C=C), 1567.93(C=N), 1313.57(C-O-N),816.88(C-Cl).**^{1}H NMR (DMSO, δ ppm):** 3.357(s,3H,-CH_3),3.579-3.670(dd,1H,-CH_2),3.811-3.906(dd,1H,-CH_2),5.956-6.025 (t,1H,-CH), 6.961-6.986(d,2H,Ar-H), 7.097-7.147 (m,4H,Ar-H), 7.296-7.433(m,2H,Ar-H), 7.490-7.572 (m,2H,Ar-H), 7.768-7.792(d,2H,Ar-H), 11.533(s,1H,-NH).

3-(3-(4-bromophenyl)-4,5-dihydroisoxazol-5-yl)-2-*p*-tolyl-1*H*-indole 4(r)

IR(KBr,ν_{max})cm^{-1}:3216.41(N-H), 3052.45 (Ar-C-H),2963.55(Aliphatic C-H), 1639.55 (Ar-C=C), 1587.47(C=N), 1279.81(C-O-N),815.92(C-Br).**^{1}H NMR (CDCl$_3$, δ ppm):** 2.426(s,3H,-CH_3), 3.504-3.599(dd,1H,-CH_2), 3.683-3.773(dd,1H,-CH_2),6.050-6.123 (t,1H,-CH), 7.059-7.110 (m,2H,Ar-H), 7.184-7.301 (m, 4H,Ar-H), 7.380-7.407(m,3H,Ar-H), 7.443-7.517 (m,2H,Ar-H), 7.549-7.630(m,2H,Ar-H),8.288(s,1H,-NH).

3-(4,5-dihydro-3-*p*-tolylisoxazol-5-yl)-2-*p*-tolyl-1*H*-indole 4(s)

IR(KBr,ν_{max})cm^{-1}:3266.56(N-H), 3029.31 (Ar-C-H),2925.87(Aliphatic C-H), 1614.47 (Ar-C=C), 1555.04(C=N), 1300.79(C-O-N). **^{1}H NMR (CDCl$_3$, δ ppm):** 2.403-2.415(s,6H,-CH_3), 3.528-3.622(dd,1H,-CH_2), 3.709-3.799(dd,1H,-CH_2),6.015-6.088 (t,1H,-CH), 7.168-7.325 (m,2H,Ar-H), 7.353-7.397 (m, 2H,Ar-H), 7.434-7.478(m,4H,Ar-H), 7.533-7.571 (m,2H,Ar-H), 7.630-7.658 (m, 2H,Ar-H),8.228(s,1H,-NH).

3-(4,5-dihydro-3-(4-methoxyphenyl)isoxazol-5-yl)-2-*p*-tolyl-1*H*-indole 4(t)

IR(KBr,ν_{max})cm^{-1}:3244.38(N-H), 3056.31 (Ar-C-H), 2963.12(Alkyl-C-H),2840.16(-OCH_3) ,1606.76(Ar- C=C), 1551.84(C=N), 1257.63(C-O-N).**^{1}H NMR (CDCl$_3$, δ ppm):** 2.421(s,3H,-CH_3), 3.526-3.620(dd,1H,-CH_2), 3.707-3.796(dd,1H,-CH_2),3.860(s,3H,-OCH_3), 6.006-6.077 (t,1H,-CH), 6.942-6.971 (d,2H,Ar-H), 7.178-7.229 (m,2H,Ar-H), 7.382-7.409(d,4H,Ar-H),7.463-7.490(m,2H,Ar-H),7.544-7.571(d,2H,Ar-H),8.190 (s,1H,-NH).

3-(4,5-dihydro-3-*p*-tolylisoxazol-5-yl)-2-*p*-tolyl-1*H*-indole 4(u)

IR(KBr,ν_{max})cm^{-1}:3431.38(N-H), 3055.36 (Ar-C-H), 1614.47(Ar- C=C), 1544.07(C=N), 1243.99(C-O-N),843.07(C-Cl).**^{1}H NMR (CDCl$_3$, δ ppm):** 3.531-3.626(dd,1H,-CH_2), 3.689-3.778(dd,1H,-CH_2),3.858(s,3H,-OCH_3),5.988-6.060(t,1H,-CH),6.967-7.032 (m,2H,Ar-H),7.082-7.193(m,4H,Ar-H),7.370-7.470(m,2H,Ar-H), 7.579-7.609 (m,2H,Ar-H), 7.579-7.609 (m,2H,Ar-H),8.251(s,1H,-NH).

3-(3-(4-bromophenyl)-4,5-dihydroisoxazol-5-yl)-2-(4-methoxyphenyl)-1*H*-indole 4(v)

IR(KBr,ν_{max})cm^{-1}:3333.10(N-H), 3049.56 (Ar-C-H), 2957.68(-OCH_3),1610.29(Ar- C=C), 1567.69(C=N), 1237.38(C-O-N),749.37(C-Br).**^{1}H NMR (CDCl$_3$, δ ppm):** 3.392-3.570(dd,1H,-CH_2), 3.591-3.785(dd,1H,-CH_2), 3.898(s,3H,-OCH_3)6.066-6.101(t,1H,-CH), 6.984(m,2H,Ar-H), 7.034-7.064 (m,4H,Ar-H), 7.273(m,2H,Ar-H), 7.392-7.415 (m,2H,Ar-H), 7.486-7.514 (m,2H,Ar-H),8.428 (s,1H,-NH).

3-(4,5-dihydro-3-*p*-tolylisoxazol-5-yl)-2-(4-methoxyphenyl)-1*H*-indole 4(w)

IR(KBr,ν_{max})cm^{-1}:3420.87(N-H),3051.49(Ar-C-H),2943.23(Alkyl-C-H),2851.32 (-OCH_3) ,1620.10(Ar- C=C), 1535.39(C=N), 1299.10(C-O-N).**^{1}H NMR ($CDCl_3$, δ ppm):**2.210(s,3H,-CH_3),3.412-3.597(dd,1H,-CH_2),3.601-3.798(dd,1H,-CH_2),3.898 (s,3H, -OCH_3),6.012-6.110(t,1H,-CH), 6.993(m,2H,Ar-H), 7.034-7.064 (m,2H,Ar-H), 7.250-7.314(m,4H,Ar-H),7.393-7.417 (d,2H,Ar-H), 7.486-7.515(d,2H,Ar-H), 8.428 (s,1H,-NH).

3-(4,5-dihydro-3-*p*-tolylisoxazol-5-yl)-2-*p*-tolyl-1*H*-indole 4(x)

IR(KBr,ν_{max})cm^{-1}:3326.35(N-H), 2938.66 (Ar-C-H),2836.17(-OCH_3) 1606.76(Ar- C=C), 1550.33(C=N), 1254.74(C-O-N).**^{1}H NMR ($CDCl_3$, δ ppm):** 3.516-3.610(dd,1H,-CH_2), 3.705-3.760(dd,1H,-CH_2), 3.860 (s,3H,-OCH_3),5.981-6.053(t,1H,-CH), 7.374-7.400 (d,2H,Ar-H), 7.493-7.538 (m,4H,Ar-H), 7.565-7.620(m,2H,Ar-H), 7.679-7.709(d,2H,Ar-H), 8.177(s,1H,-NH).

The compounds synthesized were evaluated for anti-inflammatory activity by employing carrageenan induced rat paw edema method using indomethacin as reference standard. The pharmacological screening results of the synthesized compounds indicate that the compounds 3-(3-(4-bromophenyl)-4,5-dihydroisoxazol-5-yl)-2-phenyl-1H-indole **4(c)**,3-(4,5-dihydro-3-(4-methoxyphenyl)isoxazol-5-yl)-2-phenyl-1H-indole **4(e)**, 2-(4-bromo phenyl)-3-(4,5-dihydro-3-p-tolylisoxazol-5-yl)-1H-indole **4(o)**, 3-(4,5-dihydro-3-phenyl isoxazol-5-yl)-2-p-tolyl-1H-indole **4(p)**, 3-(3-(4-chlorophenyl)-4,5-dihydroisoxazol-5-yl)-2-p-tolyl-1H-indole **4(q)**, 3-(3-(4-bromophenyl)-4,5-dihydroisoxazol-5-yl)-2-p-tolyl-1H-indole **4(r)**, 3-(4,5-dihydro-3-p-tolylisoxazol-5-yl)-2-p-tolyl-1H-indole **4(s)**, 3-(4,5-dihydro -3-(4-methoxy phenyl) isoxazol-5-yl)-2-p-tolyl-1H-indole **4(t)**, 3-(4,5-dihydro-3-p-tolylisoxazol-5-yl)-2-(4-methoxyphenyl)-1H-indole **4(w)**, 3-(4,5-dihydro-3-p-tolyl isoxazol-5-yl)-2-p-tolyl-1H-indole **4(x)** had exhibited significant anti-inflammatory activity. The compound **4(t)** was found to be the most active among the series. The compound **4(g)** was found to be the least active among the series.

The 3D QSAR models had been established using kNN- MFA method in conjunction with Stepwise Forward-Backward selection method for the selected members of training and test set. From these several models, one model was selected having the best internal and external predictivity. This model was only reported herein. For this model training and test sets were selected using manual selection method. The test set finalized was having the compounds **4(g), 4(i), 4(j), 4(k), 4(x)**. The kNN-MFA method allowed choosing probe, grid size and grid

interval for the generation of descriptors. Structural features of the compounds and the dependent variable values (experimental and predicted) for the optimal training and test data are depicted in **Table 3**. The summary of the selected model **(Table 4)** can be given as: k = 2; q^2=0.7496; pred_r^2= 0.6991; Descriptor range: E_1014 0.2768 0.4594; S_475 -0.5129 -0.4773. Descriptor range **(Fig. 3)** for the selected model of the series elaborates that (i) Indole and isoxazoline rings are essential for the activity. (ii) Negative range of steric field indicates that less bulky substituent would be favorable for the activity. (iii) Positive range of electronic field indicates that less electronegative would be favorable for the anti-inflammatory activity. The deactivating electron withdrawing, higher electronegative and bulky groups like –Cl, -Br showed significant decrease in anti-inflammatory activity as compared to electron donating, less electronegative and less bulky groups like $–CH_3$,$-OCH_3$,-H,$-NH_2$ exhibit increases anti-inflammatory activity. The synthesized compound **4(g)** with 2 chlorine atom substitution exhibited the least activity. The synthesized compound **4(t)** with no chlorine atoms but with $–CH_3$ and $–OCH_3$ substitution proved to be the most active compound. The activity of compounds from virtual combinatorial library was predicted using previously generated 3D QSAR equation. The compounds **(Table 6)** showing better activity than the most active compound can be synthesized in future and can be tested for better anti-inflammatory activity. All synthesized compounds were docked in the same cavity (cavity no.2) as of reference molecule SC558 which is the selective cyclooxygenase-2 inhibitor. Few of the synthesized compounds found to possess comparable docking scores **(Table 5)**.

The most active compound **4(t)**, the least active compound **4(g)** and selective COX-2 inhibitor (SC558) had docking score of -4.261698, -3.933550, -4.811594 respectively. The most active compound **4(t)** had better docking score than the least active compound **4(g)** and both are comparable with reference molecule signify the better positioning of **4(t)** in 1CX2 cyclooxygenase than **4(g)**. Thus, the synthesized indolylisoxazoline derivatives hold potential for development as anti-inflammatory agents after further structural optimization based on QSAR and Docking study.

Table. 6: Virtual Combinatorial Library.

Sr. No.	27X	28X	E_1014	S_475	Predicted value
1	pyrrole	isoButyl	0.380861	-0.460208	1.808111
2	pyridine	pyrrole	0.378246	-0.465775	1.808111
3	tertiaryButyl	phenyl	0.403411	-0.478361	1.823224
4	$-OC_2H_5$	imidazole	0.388566	-0.468047	1.823224
5	isoPropyl	phenyl	0.412178	-0.478552	1.823224

6	pyrrole	phenyl	0.429945	-0.477101	1.823224
7	butyl	phenyl	0.401167	-0.480972	1.823224
8	phenyl	phenyl	0.458578	-0.476913	1.823224
9	phenyl	butyl	0.401115	-0.452097	1.823224
10	$-OCH_3$	amine	0.442434	-0.441952	1.823224
11	phenyl	isoPropyl	0.384129	-0.491992	1.823224
12	phenyl	ethyl	0.393365	-0.444245	1.823224
13	imidazole	pyrrole	0.438047	-0.465219	1.823224
14	-H	phenyl	0.394921	-0.480259	1.823224
15	phenyl	tertiaryButyl	0.395954	-0.525743	1.823224
16	methyl	phenyl	0.400577	-0.476441	1.823224
17	imidazole	amine	0.384257	-0.442057	1.823224
18	ethyl	phenyl	0.402909	-0.480784	1.823224
19	isoButyl	phenyl	0.401393	-0.480643	1.823224
20	phenyl	isoButyl	0.409701	-0.460362	1.823224
21	phenyl	methyl	0.389166	-0.442644	1.823224
22	pyridine	amide	0.448537	-0.474674	1.823224
23	pyridine	isoPropyl	0.456842	-0.492834	1.823224
24	pyridine	-H	0.420874	-0.418683	1.823224
25	pyridine	ethyl	0.460272	-0.447314	1.823252
26	pyridine	methyl	0.460489	-0.442612	1.823258
27	pyridine	tertiaryButyl	0.468212	-0.526492	1.823489
28	pyridine	butyl	0.472585	-0.452145	1.823619
29	imidazole	-H	0.481236	-0.418377	1.823876
30	pyridine	isoButyl	0.481816	-0.459982	1.823895
31	amine	imidazole	0.486012	-0.466407	1.824024
32	$-OCH_3$	pyrrole	0.493955	-0.465374	1.824075

Table. 6: Virtual Combinatorial Library contd…

Sr. No.	27X	28X	E_1014	S_475	Predicted value
33	phenyl	imidazole	0.6603	-0.466983	1.8241
34	Br	imidazole	0.5703	-0.470678	1.8241
35	pyridine	imidazole	0.7315	-0.466968	1.8241
36	$-OCH_3$	isoPropyl	0.5714	-0.493967	1.8241
37	-F	imidazole	0.5435	-0.469713	1.8241
38	$-OCH_3$	amide	0.5542	-0.471902	1.8241
39	ethyl	imidazole	0.6019	-0.467343	1.8241
40	butyl	imidazole	0.6033	-0.466915	1.8241
41	$-OCH_3$	butyl	0.5886	-0.451659	1.8241
42	isoButyl	imidazole	0.6052	-0.468316	1.8241
43	$-OCH_3$	$-OCH_3$	0.5279	-0.482762	1.8241
44	$-NO_2$	imidazole	0.5013	-0.466712	1.8241
45	$-OCH_3$	isoButyl	0.5598	-0.449202	1.8241
46	thiophene	imidazole	0.5734	-0.466869	1.8241
47	imidazole	ethyl	0.5213	-0.446754	1.8241
48	imidazole	isoPropyl	0.5133	-0.491446	1.8241

49	imidazole	butyl	0.5323	-0.451808	1.8241
50	imidazole	isoButyl	0.5471	-0.454124	1.8241
51	imidazole	tertiaryButyl	0.5329	-0.527803	1.8241
52	imidazole	methyl	0.5231	-0.442476	1.8241
53	imidazole	phenyl	0.5903	-0.476746	1.8241
54	$-OCH_3$	imidazole	0.8482	-0.466778	1.8241
55	tertiaryButyl	imidazole	0.6038	-0.468631	1.8241
56	$-OCH_3$	-H	0.5380	-0.418503	1.8241
57	$-OCH_3$	tertiaryButyl	0.5815	-0.525911	1.8241
58	imidazole	imidazole	0.7917	-0.466772	1.8241
59	$-OCH_3$	phenyl	0.6444	-0.479126	1.8241
60	$-OCH_3$	methyl	0.5715	-0.443921	1.8241
61	-Cl	imidazole	0.5667	-0.466658	1.8241
62	imidazole	amide	0.4998	-0.472208	1.8241
63	methyl	imidazole	0.6022	-0.466786	1.8241
64	pyrrole	imidazole	0.6328	-0.469134	1.8241

CONCLUSION

This paper describes the process for the synthesis of novel Indolylisoxazoline derivatives. The method of biological evaluation as anti-inflammatory had also been discussed. Most of the synthesized compounds exhibited anti-inflammatory activity compared with standards. The 3D-QSAR study had shown that less electronegative and less bulky substituents would be favorable for the activity. Few compounds exhibited comparable docking score with selective COX-2 inhibitor. Thus, the synthesized indolylisoxazoline derivatives with less bulky and less electronegative substituents at *para* position of phenyl rings have potential for development as safer and better novel anti-inflammatory agents in future taking help of 3D QSAR, Docking and combinatorial library aspects of molecular modeling.

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