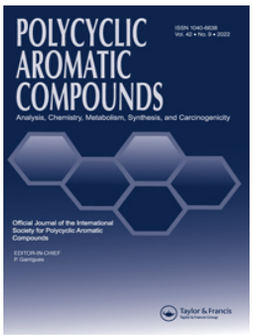

Polycyclic Aromatic Compounds

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ABSTRACT

A new series of 2-(4,5,6,7-tetrahydrobenzo[*c*]isoxazol-3-yl)-4*H*-chromen-4-ones **5a-e** were synthesized from 1-(2-hydroxyphenyl)-3-(4,5,6,7-tetrahydrobenzo[*c*]isoxazol-3-yl)propane-1,3-diones **4a-e** in presence of acetic acid and conc. HCl. Compounds **4a-e** were synthesized by Baker-Venkataraman rearrangement from 2-acetylphenyl 4,5,6,7-tetrahydrobenzo[c]isoxazole-3-carboxylate **3a-e** in presence of pyridine and KOH and compounds **3a-e** were synthesized from 4,5,6,7-tetrahydrobenzo[*c*]isoxazole-3-carboxylic acid **1** and substituted 2-hydroxy acetophenone **2a-e**. All the synthesized compounds were characterized with the help of IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopic techniques. All the compounds were screened for their *in vitro* anti-inflammatory activities. Furthermore, molecular docking study against COX-2 enzyme could provide valuable insight into the binding affinity of these molecules and the type of thermodynamic interactions governing their binding.

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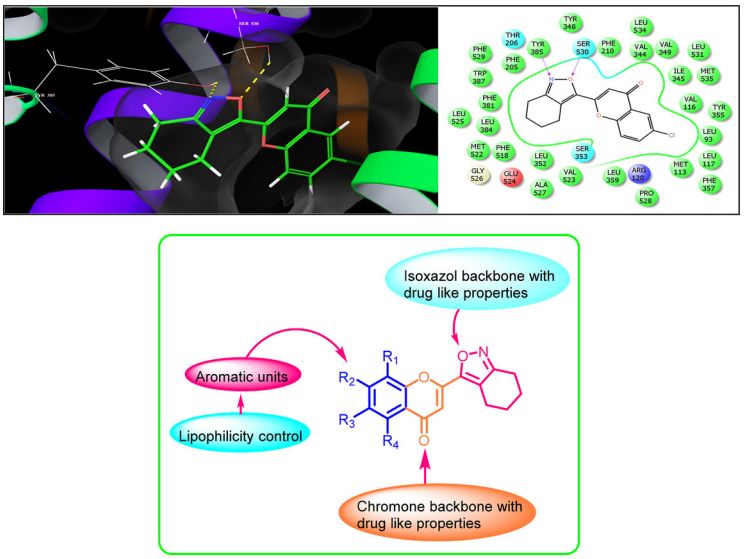


Introduction

Isoxazole is oxygen and nitrogen containing five membered heterocyclic compounds. It contains one Carbon-Nitrogen double bond and one Carbon-Carbon double bond contribute to the unsaturated property of the molecule. The incorporation of isoxazole moiety may contribute to the improved efficacy, less toxicity, and increased pharmacokinetics profiles.[1] Isoxazole exhibits various pharmacological activities such as cytotoxic,[2] anti-diabetic,[3] anti-inflammatory,[4] antibacterial,[5] antifungal,[6] antitumor,[7] antiviral,[8] antitubercular.[9] There are several drugs in market available as drugs containing isoxazole such as oxacillin, sulfamethoxazole, sulfisoxazole, drazoxolon, HWA-486 and NVP-AUY922 (Figure 1).

Chromones are the oxygen containing heterocyclic compounds with benzofused γ- pyrone ring, with the parent compound being chromone (4*H*-chromene-4-one, 4*H*-1- benzopyran-4-one). The derivatives of chromone are the most significant heterocyclic compounds in variety of natural products and medicinal agents. Chromone derivatives have variety of biological activities such as nematicidal,[10] antitumor[11] antioxidant,[12] antibacterial,[13] anticancer,[14] anti-Alzheimer[15] and anti-inflammatory activity[16] etc. There are some marketed drugs having chromone as core heterocyclic ring like khellin act as herbal folk medicine, disodium cromoglycate used in treatment of asthama,[17] flavoxate in smooth-muscle relaxant and apigenin as a skin cancer chemo preventive agent (Figure 2).

1,3-Diketones opens wide prospects for the design of a variety of organic compounds due to its high reactivity, including those structurally related to natural ones. For COX-2 inhibitions the β-diketone scaffold is a very important key intermediate which is a non-steroidal anti-inflammatory agent of the coxib family. The functionalized beta-diketones are clinically important molecules showing antiviral,[18] antibacterial,[18] antitumor,[19] anti-inflammatory,[20] anticancer[20] and antioxidant[21] activities. Gill *et al.*, reported some novel chromones incorporated isoxazole moieties and evaluated for their antimicrobial activity.[22] Wang et. al. reported the synthesis of isoxazole-linked norcantharidin analogues of substituted chromones.[23]

In continuation of our earlier work[24] on synthesis and biological properties of various heterocyclic moieties, herein, a small focused library of 2-heteryl chromones incorporated molecules.

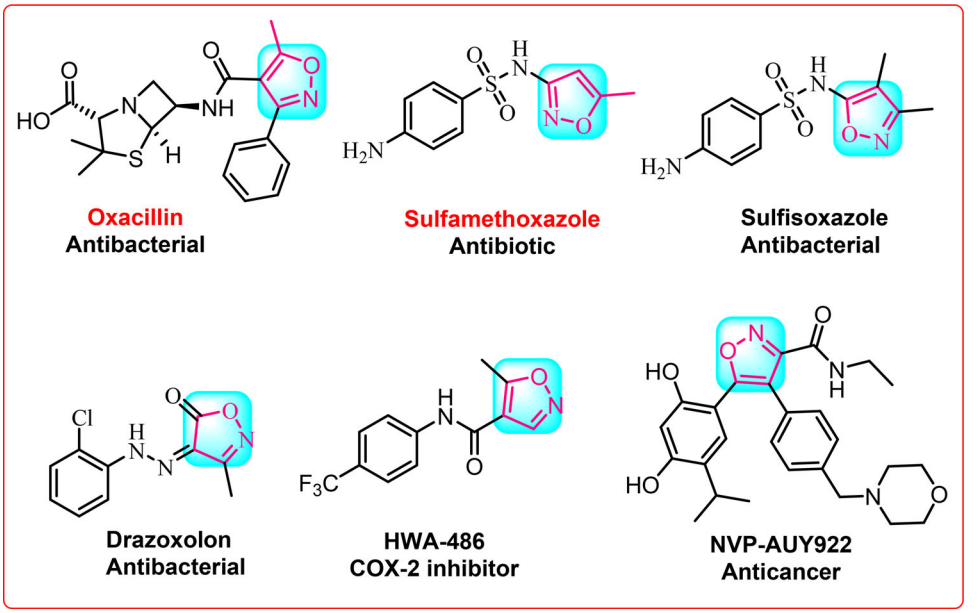


Figure 1. Isoxazole-containing drugs.

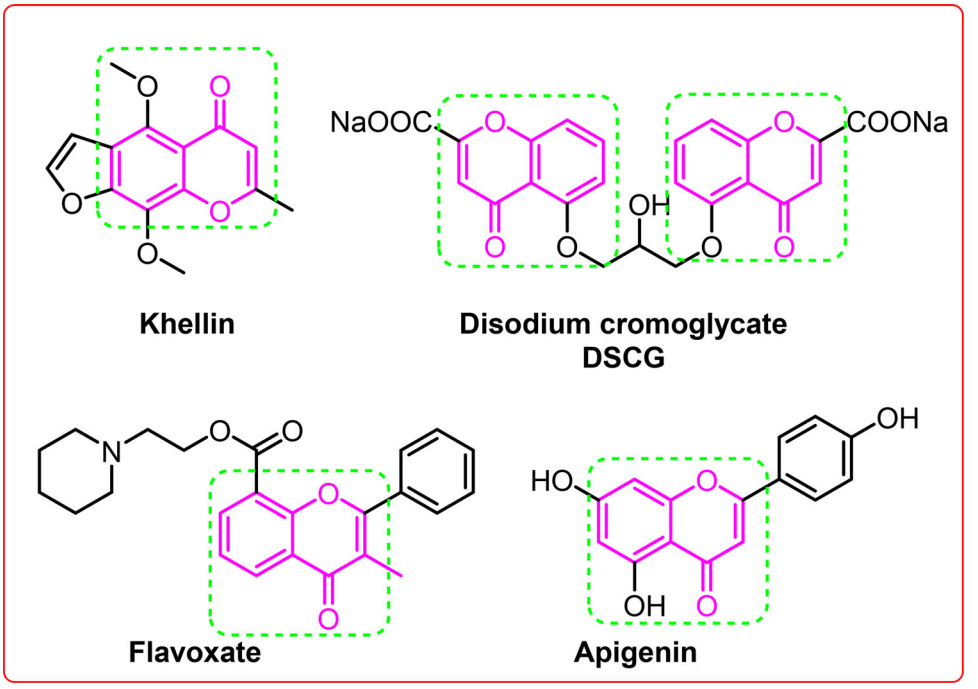


Figure 2. Chromone containing marketed drugs.

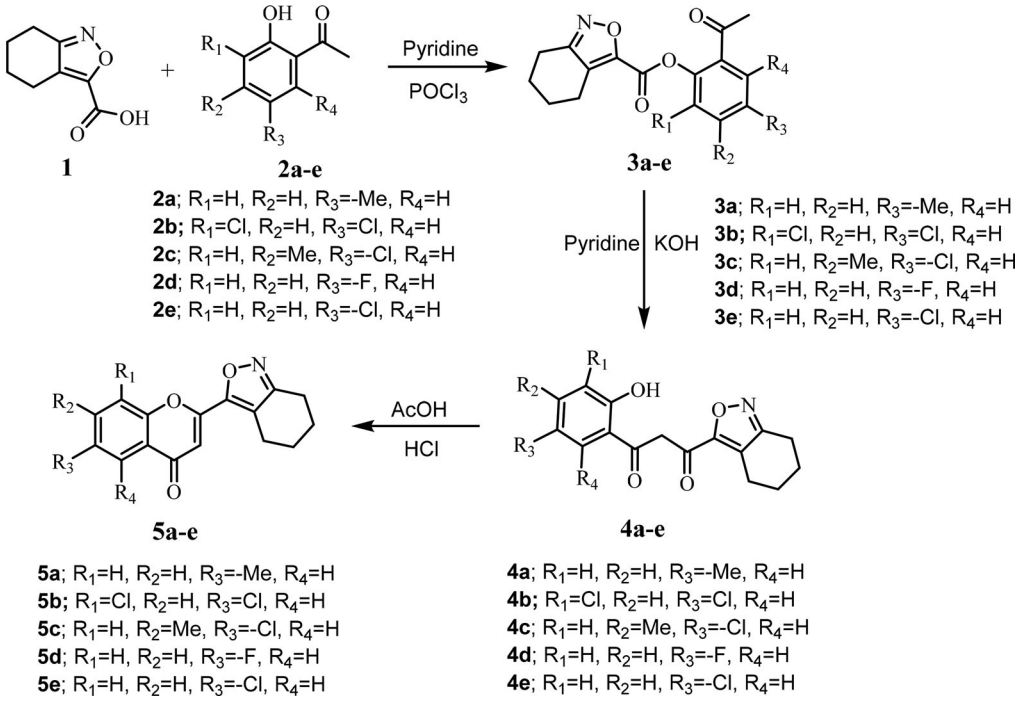
Considering the biological importance of isoxazole, chromones and β-diketones, we construct conjugated isoxazole, chromones and β-diketones in one molecular framework to enhance the anti-inflammatory and antioxidant activity with minimizing the side effects. In addition to this, we have also performed *in silico* molecular docking study against COX-2 enzyme and ADME prediction for the synthesized compounds.

Results and discussion

Chemistry

In the present study, we have described the synthesis of new 2-(4,5,6,7-tetrahydrobenzo[*c*]isoxazol-3-yl)-4*H*-chromen-4-ones (**5a-e**). Initially, a series of 2-acetylphenyl 4,5,6,7-tetrahydrobenzo[*c*]isoxazole-3-carboxylate **3a-e** were synthesized from 4,5,6,7-tetrahydrobenzo[*c*]isoxazole-3-carboxylic acid **1** and substituted 2-hydroxy acetophenone **2a-e** in $POCl_3$ and dry pyridine (Scheme 1). Further, compounds **3a-e** undergoes Baker-Venkataraman transformation to give 1-(2-Hydroxyphenyl)-3-(4,5,6,7-tetrahydrobenzo[*c*]isoxazol-3-yl)propane-1,3-diones (**4a-e**). Finally, the cyclodehydration of compounds **4a-e** in presence of acetic acid and conc. HCl gives corresponding 2-(4,5,6,7-tetrahydrobenzo[*c*]isoxazol-3-yl)-4*H*-chromen-4-ones (**5a-e**) in a good yield. (Scheme 1).

The formation of 2-acetylphenyl 4,5,6,7-tetrahydrobenzo[*c*]isoxazole-3-carboxylate **3a-e** were confirmed by ^{1}H NMR, ^{13}C NMR and mass spectra. In the ^{1}H NMR spectrum of compound **3a**, the two singlets observed at δ 2.41 and 2.52 ppm for the -CH_3 group attached to the phenyl and carbonyl carbon respectively. The ^{13}C NMR spectrum of compound **3a** revealed that the peak appears at δ 197.89 ppm is due to the presence of carbonyl carbon. Structure of compound **3a** also confirmed by molecular ion peak at m/z 300.11 $(M+H)^+$. Similarly, the synthesis of 1-(2-hydroxyphenyl)-3-(4,5,6,7-tetrahydrobenzo[*c*]isoxazol-3-yl)propane-1,3-diones **4a-e** were also confirmed by the spectral techniques. In the ^{1}H NMR spectrum of compound **4a**, the singlet observed at δ 16.12 ppm confirms the presence of enolic-OH proton. The ^{13}C NMR spectrum of compound **4a** showed the peak at δ 196.80 ppm confirms the presence of carbonyl carbon. Further, the structure of compound **4a** also confirmed by molecular ion peak at *m/z* 300.16 $(M+H)^+$.



Scheme 1. Synthesis of 2-(4,5,6,7-tetrahydrobenzo[*c*]isoxazol-3-yl)-4*H*-chromen-4-ones (**5a-e**)

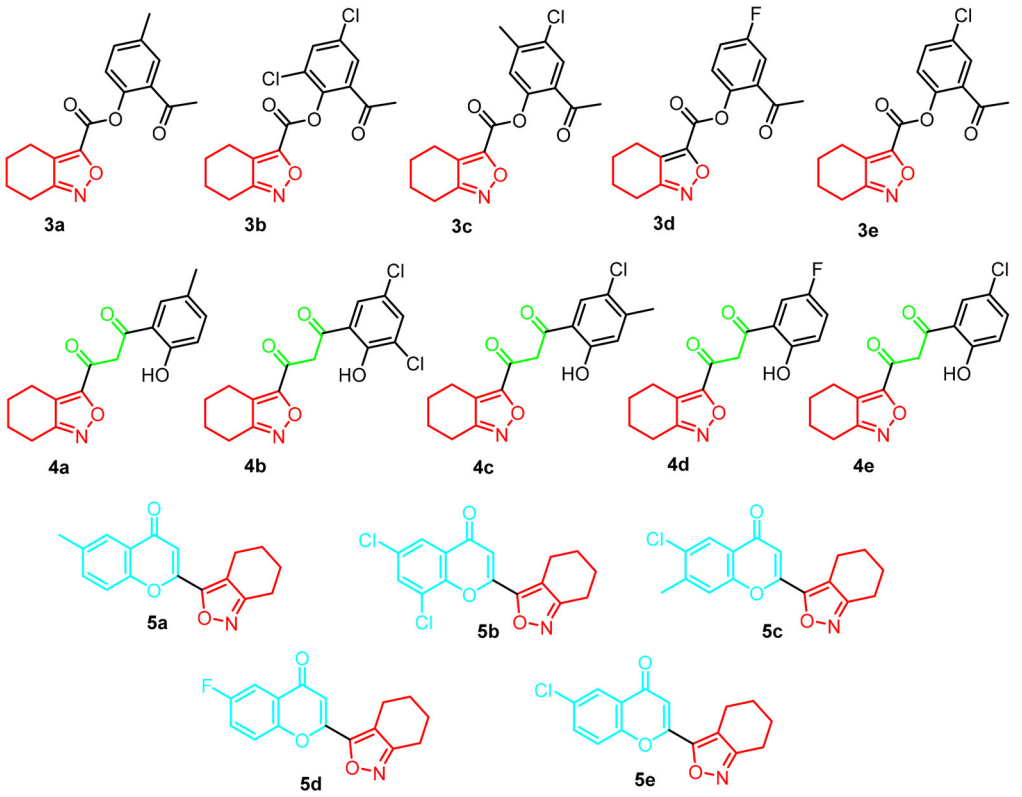


Figure 3. Structures of all the synthesized derivatives.

Finally, the formation of 2-(4,5,6,7-tetrahydrobenzo[*c*]isoxazol-3-yl)-4*H*-chromen-4-ones (**5a-e**) were confirmed by the various spectral techniques. In the ^{1}H NMR spectrum, the formation of chromone ring in compound **5a** confirmed by the singlet peak observed at δ 6.58 ppm for the proton present on chromone ring. The ^{13}C NMR spectrum of compound **5a** showed that the signal appears at δ 176.67 ppm for carbonyl carbon of chromone ring. Structure of compound **5a** also confirmed by mass spectra, molecular ion peak observed at *m/z* 282.15 $(M+H)^{+}$. Similarly, all the synthesized compounds were characterized by the spectral analysis. Structures of all the synthesized derivatives shown in Figure 3.

Biological evaluation

Anti-inflammatory activity

All the synthesized compounds were screened for their *in vitro* anti-inflammatory activities in comparison to standard drug Diclofenac sodium. Inflammation is related to pain and inflammation of surrounding tissues that involves elevated protein denaturation, vascular permeability and membrane alterations.[28] Non-steroidal anti-inflammatory drugs (NSAIDs) including diclofenac sodium are common choice of the drugs. Role of inhibition of protein denaturation carried out by such drug is reported [28] and ability of reversal of enhanced plasma protein coagulation is also seen in drug-treated animals. Previous literature[28] on *in vitro* anti-inflammatory activity demonstrated the application of bovine serum albumin model in para-medical research.

Table 1. *In vitro* anti-inflammatory (percent of inhibition), anti-oxidant activity (MIC in μg/mL) and molecular docking score.

Cpd	Anti-inflammatory	Anti-oxidant	Glide Score	Glide energy (Kcal/mol)	H-bonding (Å)
3a	14.53	153.507	−7.881	−34.912	Ser530(2.599)
3b	11.50	309.5597	−7.189	−31.726	Ser530(2.731)
3c	18.03	141.9384	−8.001	−36.449	Ser530(2.538)
3d	21.88	142.1429	−8.107	−37.199	Ser530(2.051), Tyr385(2.653)
3e	NT	NT	−6.741	−28.606	Ser530(2.579)
4a	25.93	214.75	−8.178	−36.065	Ser530(1.701)
4b	29.58	269.3371	−8.294	−38.031	Ser530(1.864)
4c	NT	NT	−6.649	−27.864	Ser530(2.307, 2.639), Tyr385(2.575)
4d	32.89	239.507	−8.668	−39.907	Ser530(2.561)
4e	33.33	157.8333	−8.726	−40.076	Ser530(2.567)
5a	34.21	471.598	−9.121	−42.596	Ser530(2.181), Tyr385(2.596)
5b	33.33	882.875	−9.031	−41.868	Ser530(2.232), Tyr385(2.614)
5c	41.18	650.475	−9.177	−44.257	Ser530(2.466), Tyr385(2.369)
5d	48.72	628.325	−9.577	−47.365	Ser530(2.013), Tyr385(2.729)
5e	49.75	NT	−9.638	−48.518	Ser530(2.215), Tyr385(2.628)
DFS	77.78	–	–	–	–
AA	–	41.69611	–	–	–

Cpd: Compounds; DFS: Diclofenac sodium; AA: Ascorbic acid.

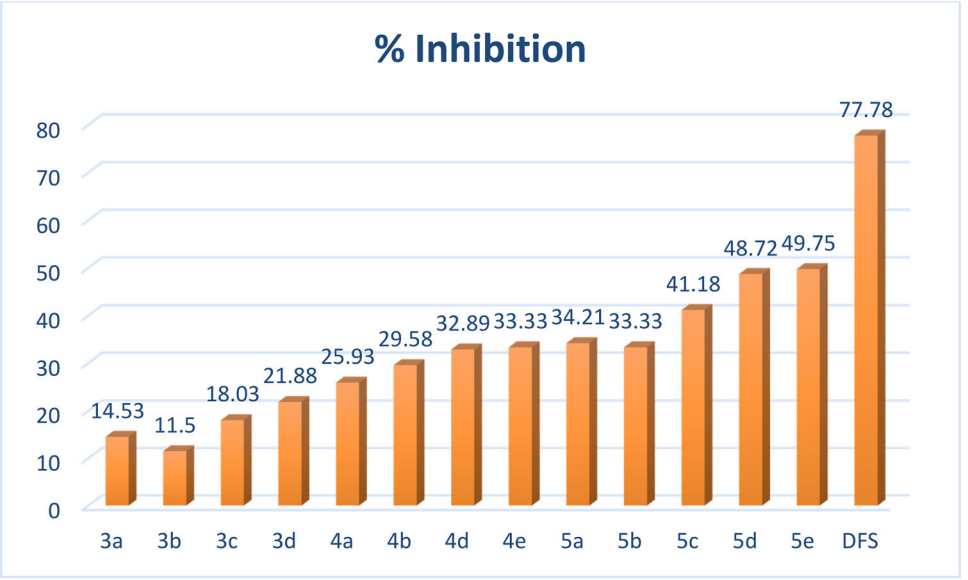


Figure 4. The percent inhibition of compounds *in vitro* anti-inflammatory model.

Among all the synthesized compounds, 2-(4,5,6,7-tetrahydrobenzo[*c*]isoxazol-3-yl)-4*H*-chromen-4-ones (**5a-e**) compound **5c**, **5d** and **5e** all other compounds exhibited moderate anti-inflammatory activity compare to the standard drug diclofenac sodium. (Table 1).

The percent inhibition of compounds *in vitro* anti-inflammatory model shown in Figure 4.

Anti-oxidant activity by DPPH

According to the DPPH assay, all the synthesized compound displays comparable antioxidant activity than standard drug Ascorbic acid (Table 1). The percent inhibition of compounds *in vitro* antioxidant model shown in Figure 5.

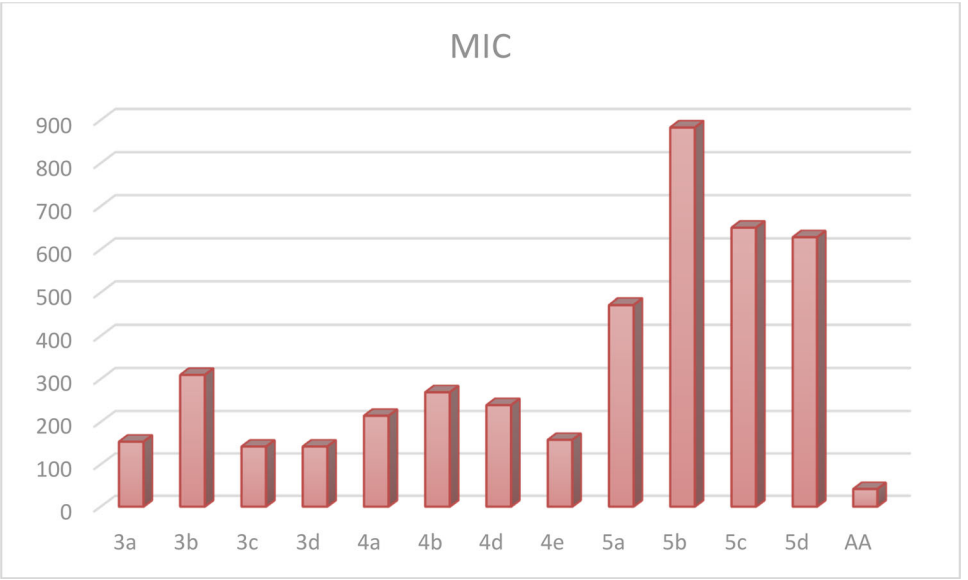


Figure 5. The percent inhibition of compounds *in vitro* antioxidant model.

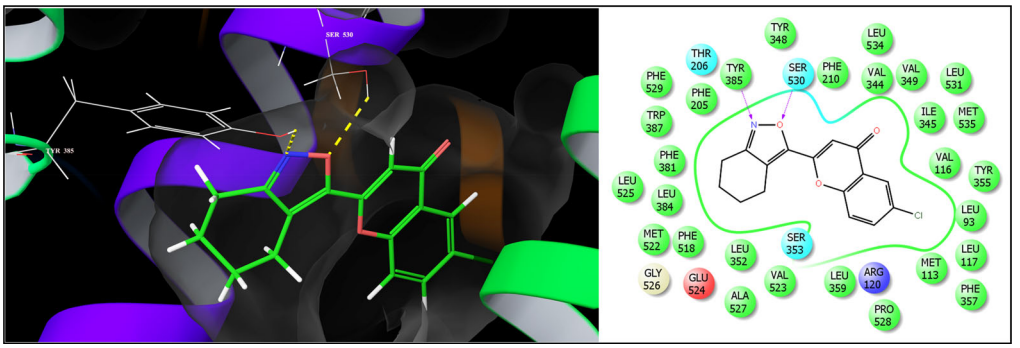


Figure 6. Binding mode of **5e** at the active site of COX-2 (on right side: the pink lines represent the hydrogen bonding interactions).

Computational study

Molecular docking

A perusal of the docked complexes of chromones derivatives (**3a-3e**, **4a-4e**, **5a-5e**) revealed that they could snuggly fit into the active site of COX-2 engaging a series of bonded and non-bonded interactions. Their binding affinity was found to be in agreement with the observed anti-inflammatory activity with active compounds showing relatively higher binding compared to compounds with moderate activity (Table 1). Quantitative analysis of the per-residue interactions between one of the most active molecule **5e** (Figure 6) and the residues lining the active site of the enzyme was carried out to identify the most significantly interacting residues and the nature of thermodynamic interactions governing their affinity to COX-2.

The lowest energy docked complex of **5e** showed that molecule could aptly bind to the target protein with high binding affinity (Glide docking score of −9.638/intermolecular binding energy of −48.518 kcal/mol) interactions at co-ordinates close to the co-crystallized ligand. The major driving force for mechanical interlocking of this molecule into the active site was observed to be

favorable van der Waals interactions observed with Ser530(-2.359 kcal/mol), Ala527(-4.548 kcal/mol), Gly526(-2.363 kcal/mol), Val523(-2.387 kcal/mol), Met522(-1.969 kcal/mol), Phe518(-1.035 kcal/mol), Trp387(-1.482 kcal/mol), Tyr385(-2.183 kcal/mol), Leu384(-1.135 kcal/mol), Phe381(-1.213 kcal/mol) and Leu352(-1.088 kcal/mol) residues through the 4,5,6,7-tetrahydrobenzo[c]isoxazol-3-yl component while the 6-Chloro-4H-chromen-4-one component of the molecule engaged in similar type of interactions with Leu534(-1.018 kcal/mol), Leu531(-3.071 kcal/mol), Tyr355(-1.296 kcal/mol), Ser353(-1.794 kcal/mol) and Val349(-3.014 kcal/mol). The enhanced binding affinity is also attributed to a favorable electrostatic interaction with Ser530(-1.729 kcal/mol), Glu524(-1.479 kcal/mol), Glu510(-1.045 kcal/mol) and Tyr385(-1.833 kcal/mol). Along with these non-bonded interactions, **5e** also portrayed two very close hydrogen bonding interactions through the isoxazole ring, firstly with Ser530(2.215 Å) and second with Tyr385(2.628 Å) residues. Such hydrogen bonding interactions ″anchor″ the ligand guiding its orientation into the 3 D space of the active site and further facilitates the non-bonded interactions. A similar mode and type of bonded and non-bonded interactions were involved in stabilizing the complexes of other compounds with COX-2 speculating an identical mechanism of action (Figure 1S-14S). This *in silico* binding affinity data suggest that structural modifications in the isoxazole clubbed chromen-4-one scaffold directed toward improving the steric and electrostatic interactions along with other bonded interactions *viz*. Hydrogen bonding can lead to compounds with improved binding affinity toward COX-2.

In silico ADME prediction

Important task for the lead compounds is early prediction of drug likeness properties, as it resolves the cost and time of drug development and discovery. Due to the inadequate drug likeness properties of many active agents with a significant biological activity have failed in clinical trials.[26] On the basis of Lipinski's rule of five, the drug likeness properties were analyzed by ADME parameters using Molinspiration online property calculation toolkit[27] and data are summarized in Table 2.

All the compounds exhibited noteworthy values for the various parameters analyzed and showed good drug-like characteristics based on Lipinski's rule of five and its variants that characterized these agents to be likely orally active. For all the synthesized compound, the data obtained

Table 2. Pharmacokinetic parameters of (**4a-e**, **5a-e** & **6a-e**) compounds.

Entry	% ABS	TPSA (A^2)	n-ROTB	MV	MW	miLog *P*	n-ON	n-OHNH	Lipinski violation	Drug-likeness model score
Rule	–	–	–	–	< 500	≤ 5	< 10	< 5	≤ 1	–
3a	85.05	69.41	4	269.30	299.33	3.64	5	0	0	0.34
3b	85.05	69.41	4	279.81	354.19	4.48	5	0	0	0.16
3c	85.05	69.41	4	282.84	333.77	4.25	5	0	0	0.32
3d	85.05	69.41	4	257.67	303.29	3.36	5	0	0	0.31
3e	85.05	69.41	4	266.28	319.74	3.87	5	0	0	0.37
4a	81.26	80.40	4	268.58	299.33	3.14	5	1	0	0.42
4b	81.26	80.40	4	279.09	354.19	3.77	5	1	0	0.31
4c	81.26	80.40	4	282.11	333.77	3.75	5	1	0	0.24
4d	81.26	80.40	4	256.95	303.29	2.86	5	1	0	0.50
4e	81.26	80.40	4	265.55	319.74	3.37	5	1	0	0.60
5a	89.59	56.24	1	250.33	281.31	3.94	4	0	0	0.43
5b	89.59	56.24	1	260.84	336.17	4.78	4	0	0	−0.07
5c	89.59	56.24	1	263.87	315.76	4.55	4	0	0	0.37
5d	89.59	56.24	1	238.70	285.27	2.69	4	0	0	0.57
5e	89.59	56.24	1	247.31	301.73	4.17	4	0	0	0.65

% ABS: Percentage absorption, TPSA: Topological polar surface area, n-ROTB: Number of rotatable bonds, MV: Molecular volume, MW: Molecular weight, milogP: Logarithm of partition coefficient of compound between n-octanol and water, n-ON Acceptors: Number of hydrogen bond acceptors, n-OHNH donors: Number of hydrogen bonds donors.

were within the range of accepted values. None of the synthesized compounds violated the Lipinski's rule of five. The parameters like the number of rotatable bonds and total polar surface area are linked with the intestinal absorption; results showed that all synthesized compounds had good absorption. The larger the value of the drug likeness model score, the higher is also probability that the particular molecule will be active. The *in-silico* assessment of all the synthetic compounds has shown that they have very good pharmacokinetic properties, which is reflected in their physicochemical values, thus ultimately enhancing pharmacological properties of these molecules.

Conclusion

A new series of 2-(4,5,6,7-tetrahydrobenzo[c]isoxazol-3-yl)-4H-chromen-4-ones **5a-e** were synthesized from 1-(2-hydroxyphenyl)-3-(4,5,6,7-tetrahydrobenzo[c]isoxazol-3-yl)propane-1,3-diones **4a-e** in presence of acetic acid and conc. HCl. Compounds **4a-e** were synthesized by Baker-Venkataraman rearrangement from 2-acetylphenyl 4,5,6,7-tetrahydrobenzo[c]isoxazole-3-carboxylate **3a-e** in presence of pyridine and KOH and compounds **3a-e** were synthesized from 4,5,6,7-tetrahydrobenzo[c]isoxazole-3-carboxylic acid **1** and substituted 2-hydroxy acetophenone **2a-e**. All the synthesized compounds were characterized with the help of IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopic techniques. All the compounds were screened for their in vitro anti-inflammatory activities. Furthermore, *in-silico* molecular docking study against COX-2 enzyme and ADME properties of the synthesized compounds also carried out. The results of *in silico* binding affinity study were found to be in agreement with the in vitro data suggesting that these molecules could serve as a pertinent starting point for structure-based optimization of this novel scaffold and therefore efforts are under way to optimize scaffold for arriving at potent molecules with improved anti-inflammatory and selectivity.

Experimental

All organic solvents were acquired from commercial sources and used as received. The melting points were measured on a DBK melting point apparatus and are uncorrected. IR spectra were recorded on Shimadzu IR Affinity 1S (ATR) FTIR spectrophotometer. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on Bruker Advance neo 500 spectrophotometers using TMS as an internal standard and DMSO-d_6 as solvent and chemical shifts were expressed as δ ppm units. Mass spectra were obtained on Waters, Q-TOF micromass (ESI-MS) mass spectrometer.

General procedure for the synthesis of 2-acetylphenyl 4,5,6,7-tetrahydrobenzo[c]isoxazole-3-carboxylate (3a-e)

A mixture of 4,5,6,7-tetrahydrobenzo[*c*]isoxazole-3-carboxylic acid **1** (0.001 mol) and substituted 2-hydroxy acetophenone **2a-e** (0.001 mol) were taken in dry round bottom flask and dissolved in 10 mL dry pyridine. The reaction mixture was then cooled to 0 °C. To this solution, $POCl_3$ (0.01 mol) was added drop-wise maintaining temperature below 5 °C. Then the reaction mixture was kept overnight at RT under stirring. It was then poured over crushed ice with vigorous stirring. The crude solid product was separated by filtration and washed with ice-cold water followed by ice cold solution of dil. NaOH and finally again washed with ice cold water. The crude solid product purified by crystallization from ethyl alcohol to get pure compounds **3a-e**.

General procedure for the synthesis of benzoxazole β diketones (4a-e)

Compound **3a-e** (0.05 mol) was taken in 15 mL dry pyridine, and to this reaction mixture an excess of powdered KOH (0.1 mol) was added with constant stirring and the reaction mixture

was stirred at RT for 3 hr. Thereafter the contents were poured over crushed ice and acidified with dil. HCl. The resulting product was separated by filtration and purified by crystallization from ethanol to afforded **4a-e**.

General procedure for the 6-methyl-2-(4, 5, 6, 7-tetrahydrobenzo[c]isoxazol-3-yl)-4H-chromen-4-one (5a-e)

Compound **4a-e** (1 mmol) taken in acetic acid (5 mL) and to this conc. HCl (1 mL) was added. Reaction mixture was heated under reflux for 2 hr. After completion of heating, the reaction mixture was cooled and poured over crushed ice. The resulting product was separated by filtration and purified by crystallization from ethanol to give **5a-e**.

2-Acetyl-4-methylphenyl 4,5,6,7-tetrahydrobenzo[c]isoxazole-3-carboxylate (3a)

Yield: 61%; White solid; mp: 172-174 °C; ^{1}H NMR (500 MHz, DMSO-D6): $\delta = 1.71$-1.79 (m, 4H), 2.41 (s, 3H, Ar-CH_3), 2.52 (s, 3H,-CH_3), 2.76-2.81 (m, 4H), 7.27-7.29 (d, 1H, $J = 10$ Hz, Ar-H), 7.50-7.52 (d, 1H, $J = 10$ Hz, Ar-H), 7.84-7.86 (d, 1H, $J = 10$ Hz, Ar-H); ^{13}C NMR (100 MHz, DMSO- D6): $\delta = 197.9$, 162.6, 156.0, 153.0, 145.6, 137.0, 134.7, 131.7, 130.0, 124.1, 123.3, 29.6, 21.7, 21.7, 21.4, 20.8, 20.6; MS (ESI-MS): m/z 300.11 $(M + H)^+$.

2-Acetyl-4, 6-dichlorophenyl 4,5,6,7-tetrahydrobenzo[c]isoxazole-3-carboxylate (3b)

Yield: 61%; White solid; mp: 120-122 °C; ^{1}H NMR (500 MHz, DMSO-D6): $\delta = 2.36$ (s, 1H, -CH_3), 2.70-2.68 (dd, 4H, Ar-H), 2.76-2.73 (dd, 4H, Ar-H), 7.88 (d, 2H, $J = 2.5$ Hz, Ar-H), 7.99 (d, 2H, $J = 2.5$ Hz, Ar-H); ^{13}C NMR (100 MHz, DMSO-D6): δ 162.8, 162.4, 154.9, 154.0, 152.1, 151.2, 147.2, 141.3, 132.6, 131.1, 131.0, 129.0, 128.8, 124.8, 123.4, 111.6, 21.5, 21.5, 21.4, 21.4, 20.5, 20.4.

2-Acetyl-4-chloro-5-methylphenyl 4,5,6,7-tetrahydrobenzo[c]isoxazole-3-carboxylate (3c)

Yield: 61%; White solid; mp: 122-124 °C; ^{1}H NMR (500 MHz, DMSO-D6): $\delta = 1.79$-1.70 (m, 4H), 2.41 (s, 3H, Ar-CH_3), 2.62 (s, 3H, -CH_3), 2.76-2.81 (m, 4H), 7.47 (s, 1H, Ar-H), 8.07 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-D6): $\delta = 196.5$, 162.7, 155.7, 152.7, 146.3, 142.8, 131.8, 131.5, 129.3, 126.9, 123.6, 29.6, 21.7, 21.6, 21.4, 20.6, 20.2; MS (ESI-MS): m/z 334.11 $(M + H)^+$.

2-Acetyl-4-fluorophenyl 4, 5, 6, 7-tetrahydrobenzo[c]isoxazole-3-carboxylate (3d)

Yield: 61%; White solid; mp: 68-70 °C; ^{1}H NMR (500 MHz, DMSO-D6): $\delta = 1.70$-1.81 (m, 4H, Ar-H), 2.51 (s, 1H,-CH_3), 2.77-2.81 (m, 4H, Ar-H), 7.50 (dd, 1H, $J = 3$ Hz & 9 Hz, Ar-H), 7.57-7.61 (m, 1H, Ar-H), 7.88-7.91 (dd, 1H, $J = 3$ Hz & 9 Hz, Ar-H); ^{13}C NMR (100 MHz, DMSO-D6): $\delta = 197.0$, 162.7, 161.1, 159.1, 155.9, 152., 143.8, 143.7, 131.9, 131.9, 126.5, 126.5, 123.5, 121.1, 120.9, 118.0, 117.6, 29.7, 21.7, 21.6, 21.4, 20.6; MS (ESI-MS): m/z 304.12 $(M + H)^+$.

2-Acetyl-4-chlorophenyl 4,5,6,7-tetrahydrobenzo[c]isoxazole-3-carboxylate (3e)

Yield: 61%; White solid; mp: 108-110 °C; ^{1}H NMR (500 MHz, DMSO-D6): $\delta = 1.71$-1.80 (m, 4H), 2.56 (s, 3H,-CH_3), 2.77-2.81 (m, 4H), 7.48-7.50 (d, 1H, $J = 10$ Hz, Ar-H), 7.78-7.80 (dd, 1H, $J = 10$ Hz, Ar-H), 8.08-8.09 (d, 1H, $J = 5$ Hz, Ar-H); ^{13}C NMR (100 MHz, DMSO-D6): $\delta = 197.0$, 162.7, 155.6, 152.7, 146.4, 134.0, 132.0, 131.7, 130.9, 126.5, 123.6, 29.8, 21.7, 21.6, 21.4, 20.6; MS (ESI-MS): m/z 320.11 $(M + H)^+$.

1-(2-Hydroxy-5-methylphenyl)-3-(4,5,6,7-tetrahydrobenzo[c]isoxazol-3-yl) propane-1,3-dione (4a)

Yield: 61%; Yellow solid; mp: 90-92 °C; ^{1}H NMR (500 MHz, DMSO-D6): $\delta = 1.64$-1.76 (m, 8H), 2.26 (s, 3H), 2.62-2.82 (m, 8H), 2.97 (d, $J = 16.5$ Hz, 1H), 3.34 (d, $J = 16.5$ Hz, 1H), 7.0 (d, 1H, $J = 8.5$ Hz, Ar-H), 7.44 (dd, 1H, $J = 8$ & 2 Hz, Ar-H), 7.71 (d, 1H, $J = 2$ Hz, 10.89 (s, 1H, -O-H), 15.85 (broad singlet, 1H, enolic-OH); MS (ESI-MS): *m/z* 300.16 $(M + H)^{+}$.

1-(3,5-Dichloro-2-hydroxyphenyl)-3-(4,5,6,7-tetrahydrobenzo[c]isoxazol-3-yl)propane-1,3-dione (4b)

Yield: 61%; White solid; mp: 220-222 °C; ^{1}H NMR (500 MHz, DMSO-D6): $\delta = 1.68$-1.76 (m, 8H, Ar-H), 2.69-2.82 (m, 8H, Ar-H), 3.10 (d, $J = 16$ Hz, 1H), 3.50 (d, $J = 16$ Hz, 1H), 7.18 (S, 1H, enol-olefinic proton), 7.71 (d, 1H, $J = 2$ Hz), 7.85 (s, 1H), 7.97 (s, 1H, enolic -OH), 8.00 (d, 1H, $J = 2$ Hz, 1H), 8.64 (s, 1H), 11.92 (keto-OH); ^{13}C NMR (100 MHz, DMSO- D6): $\delta = 188.7$, 162.6, 162.3, 161.5, 152.2, 135.8, 134.4, 128.2, 126.4, 124.4, 124.2, 123.0, 112.6, 100.2, 46.9, 22.3, 21.9, 21.8, 21.7, 21.6, 21.5, 20.8, 19.9; MS (ESI-MS): m/z 354.10 $(M + H)^{+}$.

1-(5-Chloro-2-hydroxy-4-methylphenyl)-3-(4,5,6,7-tetrahydrobenzo[c]isoxazol-3-yl)propane-1,3-dione(4c)

Yield: 61%; White solid; mp: 150-152 °C; ^{1}H NMR (500 MHz, DMSO-D6): $\delta = 1.68$-1.76 (m, 4H, Ar-H), 2.69-2.82 (m, 4H, Ar-H), 3.51(d, 2H, -CH_2), 4.83(S, 1H, -OH),7.96 (d, 1H, $J = 5$ Hz, Ar-H), 8.003(d, 1H, $J = 2$ Hz, Ar-H), 15.97 (broad singlet, 1H, enolic-OH); ^{13}C NMR (100 MHz, DMSO-D6): $\delta = 189.1$, 183.3, 174.3, 162.9, 162.5, 161.4, 157.9, 157.8, 156.2, 145.2, 143.2, 129.1, 127.2, 125.5, 124.4, 122.2, 121.4, 120.3, 120.1, 119.5, 112.3, 99.3, 99.2, 47.1, 22.3, 22.0, 21.9, 21.7, 21.6, 21.5, 20.9, 20.6, 20.5, 19.9; MS (ESI-MS): m/z 334.13 $(M + H)^{+}$.

1-(5-Fluoro-2-hydroxyphenyl)-3-(4,5,6,7-tetrahydrobenzo[c]isoxazol-3-yl)propane-1,3-dione(4d)

Yield: 61%; White solid; mp: 172-174 °C; ^{1}H NMR (500 MHz, DMSO-D6): $\delta = 4.64$ (S, 1H, olefinic proton), 11.13 (S, 1H, enolic -OH proton); ^{13}C NMR (100 MHz, DMSO-D6): $\delta = 189.7$, 183.2, 174.7, 162.9, 162.5, 161.4, 157.9, 156.7, 156.3, 155.5, 154.7, 154.0, 124.2, 124.0, 122.3, 122.1, 121.4, 121.0, 120.9, 120.8, 120.7, 120.5, 119.4, 119.3, 114.9, 114.7, 112.3, 111.3, 111.1, 99.5, 99.2, 47.1, 22.3, 22.0, 21.9, 21.7, 21.6, 21.5, 20.9, 20.6, 19.8; MS (ESI-MS): m/z 304.15 $(M + H)^{+}$.

1-(5-Chloro-2-hydroxyphenyl)-3-(4, 5, 6, 7-tetrahydrobenzo[c]isoxazol-3-yl) propane-1, 3-dione (4e)

Yield: 61%; White solid; mp: 150-152 °C ^{1}H NMR (500 MHz, DMSO-D6): $\delta = 4.63$ (S, 1H, olefinic proton), 11.39 & 11.25 (bs 2H, phenolic -OH & keto-enol tautomeric proton); ^{13}C NMR (100 MHz, DMSO-D6): $\delta = 189.4$, 182.8, 174.9, 162.7, 162.5, 161.4, 157.9, 157.8, 156.3, 136.4, 134.5, 128.7, 126.5, 125.2, 123.7, 121.9, 121.7, 121.2, 120.5, 119.9, 112.4, 99.6, 99.3, 47.1, 22.3, 21.9, 21.8, 21.7, 21.6, 21.5, 20.9, 20.6, 19.8; MS (ESI-MS): m/z 320.13 $(M + H)^{+}$.

6-Methyl-2-(4,5,6,7-tetrahydrobenzo[c]isoxazol-3-yl)-4H-chromen-4-one (5a)

Yield: 61%; White solid; ^{1}H NMR (500 MHz, DMSO-D6): δ ppm 1.75-1.78 (m, 6H), 2.43 (s, 3H,-CH_3), 2.43-2.46 (m, 3H), 6.67(s, 1H, chromone), 3.02 (s, 2H), 7.63-7.68 (m, 2H, Ar-H), 7.84-7.86 (d, 1H, $J = 10$ Hz, Ar-H); ^{13}C (100 MHz, DMSO-D6): δ ppm = 176.67, 162.78, 162.53, 154.76, 154.05, 153.50, 137.07, 136.27, 136.18, 124.68, 124.61, 123.75, 121.98, 120.86, 119.06, 118.79, 118.32, 108.81, 21.98, 21.73, 21.61, 21.49, 20.91, 20.49; MS (ESI-MS): m/z 282.15 $(M + H)^{+}$.

6,8-Dichloro-2-(4,5,6,7-tetrahydrobenzo[c]isoxazol-3-yl)-4H-chromen-4-one(5b)

Yield: 61%; White solid; ^{1}H NMR (500 MHz, DMSO-D6): δ ppm 1.78-1.79 (m, 5H), 2.77-2.78 (m, 2H), 2.98-3.00 (m, 2H), 6.84 (s, 1H, chromone), 7.95-7.96 (d, $J = 5$ Hz, 1H, Ar-H), 8.25(s, 1H,

Ar-H); ^{13}C (100 MHz, DMSO-D6): δ ppm = 162.73, 134.66, 126.21, 124.44, 123.66, 119.16, 108.86, 21.96, 21.70, 21.49, 20.77; MS (ESI-MS): m/z 336.08 $(M+H)^+$.

6-Chloro-7-methyl-2-(4,5,6,7-tetrahydrobenzo[c]isoxazol-3-yl)-4H-chromen-4-one (5c)

Yield: 61%; White solid; ^{1}H NMR (500 MHz, DMSO-D6): δ ppm 1.78-1.79 (m, 6H), 2.77 (s, 3H, -CH_3), 2.95 (m, 2H), 6.73 (s, 1H, chromone), 7.87 (s, 1H, Ar-H), 7.98 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-D6): δ ppm = 144.24, 124.64, 118.49, 116.24, 109.00, 107.00, 21.96, 21.74, 21.48, 20.54; MS (ESI-MS): m/z 316.12$(M+H)^+$.

6-Fluoro-2-(4,5,6,7-tetrahydrobenzo[c]isoxazol-3-yl)-4H-chromen-4-one (5d)

Yield: 61%; White solid; ^{1}H NMR (500 MHz, DMSO-D6): δ ppm 1.78-1.79(m, 4H), 2.77-2.79 (d, 2H, J = 10Hz), 2.93-2.96 (d, 2H, J = 15Hz), 6.73(s, 1H, chromone), 7.73-7.75 (m, 1H, Ar-H), 7.77-7.79 (d, J = 4Hz, 1H, Ar-H), 7.87-7.89 (d, J = 10 Hz, 1H, Ar-H); ^{13}C (100 MHz, DMSO-D6): δ ppm = 176.14, 162.60, 158.75, 154.56, 153.90, 152.90, 125.30, 125.24, 123.43, 123.22, 121.94, 121.87, 118.72, 110.23, 110.04, 108.24, 21.95, 21.73, 21.48, 20.49; MS (ESI-MS): m/z 286.13$(M+H)^+$.

6-Chloro-2-(4,5,6,7-tetrahydrobenzo[c]isoxazol-3-yl)-4H-chromen-4-one (5e)

Yield: 61%; White solid; ^{1}H NMR (500 MHz, DMSO-D6): δ ppm 1.77-1.80 (m, 4H), 2.76-2.79 (m, 2H), 2.92-2.95 (m, 2H), 6.74 (s, 1H, chromone), 7.82-7.84 (d, J = 10 Hz, 1H, Ar-H), 7.90-7.92 (m, 1H, Ar-H), 7.98-7.99 (d, J = 5 Hz, 1H, ArH); (100 MHz, DMSO-D6): δ ppm = 175.68, 162.60, 154.47, 154.38, 153.91, 135.12, 130.94, 125.17, 124.40, 121.57, 118.81, 108.85, 21.94, 21.72, 21.48, 20.50; MS (ESI-MS): m/z 302.12 $(M+H)^+$.

Anti-inflammatory activity

All the synthesized compounds were screened for their in vitro anti-inflammatory activities according to the literature procedure[28] with slight modification in the procedure using bovine serum albumin (BSA) as protein and the standard drug diclofenac sodium. A volume of 1 ml of diclofenac sodium at concentrations 50, 100, 200, 400, 800, and 1,000 μg/mL and synthetic compounds was allowed to homogenized separately with 1 mL of aqueous solution of BSA (5%) and incubated for 15 minutes at 27oC. The mixture of distilled water and BSA was used as the control. Denaturation of the proteins was caused by placing the mixture in a water bath for 10 minutes at 70oC. The denaturation of all samples was carried out. The mixture was cooled within the ambient room temperature, and the activity of each mixture was measured at 660 nm by measuring extent of turbidity in terms of percent inhibition in each sample tube. Each test was conducted thrice and mean of the readings were recorded. The following formula was used to calculated inhibition percentage:

$$\%\text{inhibition} = (\text{absorbanceofcontrol} - \text{absorbanceofsample})/\quad(\text{absorbanceofcontrol}) \times 100$$

Antioxidant activity

Various concentrations (50, 100, 150 µg/mL) were prepared by dilution method. The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 255 nm. by using spectrophotometer (UV-VIS Shimadzu). Reference standard compounds being used was ascorbic acid and experiment was done in triplicate. The IC_{50} value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher

free radical activity. The percent DPPH scavenging effect was calculated by using following equation:

DPPH scavenging effect (%) or Percent inhibition = A0 - A 1/A0 × 100.

Where A0 was the Absorbance of control reaction and A1 was the Absorbance in presence of test or standard sample.

Molecular docking

A very promising level of anti-inflammatory activity demonstrated by the title compounds in the *in vitro* assay paved the way to gain an insight into the plausible mechanism of action. The *in-silico* techniques of molecular docking are now a well-established approach for evaluation of binding affinity of a bioactive molecule toward the target protein and predict the type of thermodynamic interactions between the compounds and the active site amino acids to rationalize the obtained biological results. With this objective, Cyclo-oxygenase 2 (COX-2) was chosen as the model protein to perform the molecular docking study for the title compounds. Critical in the inflammation pathway, Cyclo-oxygenase 2 (COX-2) is essential for the formation of proteinoids including thromboxane and prostaglandins which mediate the inflammation and pain. Molecular docking study was performed using the standard protocol implemented in the GLIDE (Grid-based Ligand Docking with Energetics) module of the Schrödinger Molecular modeling package.[25] The three-dimensional X-ray crystal structure of cyclooxygenase-2 (COX-2) enzyme complexed with its inhibitor Diclofenac was retrieved from the Protein Data Bank (www.rcsb.org/1PXX) and subjected to docking against the title compounds.

In silico ADME

In the present study, we have calculated molecular volume (MV), molecular weight (MW), logarithm of partition coefficient (miLog *P*), number of hydrogen bond acceptors (n-ON), number of hydrogen bonds donors (n-OHNH), topological polar surface area (TPSA), number of rotatable bonds (n-ROTB) and Lipinski's rule of five[29] using Molinspiration online property calculation toolkit.[27] Absorption (% ABS) was calculated by: % ABS = 109-(0.345 × TPSA).[30] Drug-likeness model score (a collective property of physic-chemical properties, pharmacokinetics and pharmacodynamics of a compound is represented by a numerical value) was computed by MolSoft software.[31]

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Disclosure statement

No potential conflict of interest was reported by the authors.

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