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Multiple biological activities and molecular docking studies of newly synthesized 3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide chalcone hybrids

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Abstract

A series of fifteen new chemical entities, 3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide chalcones **(6a-o),** were synthesized as new hybrids with enriched biological activities compared to their parent molecules. The compounds were characterized by ^{1}H NMR, ^{13}C NMR, Mass and IR spectral studies. Their antibacterial, anti-inflammatory and antioxidant activities have been evaluated. These compounds showed moderate to good antibacterial, anti-inflammatory and antioxidant activities. The molecular docking analysis was performed with cyclooxygenase enzyme to ascertain the probable binding model.

Keywords*:* pyrazole carboxamide; chalcone; antibacterial; anti-inflammatory; antioxidant.

The overall clinical experiences reveal that the single-targeted drugs will not be effective agents to the biological system even if they have good inhibitory activity against specific target.[1,2] The complex diseases are very difficult to cure with the available drug molecules. The simple drugs and single targeted drugs are not effective to cure complex diseases like cancer. In hyperglycemic patients, there is an excessive production of glucose molecule and the glucose produces AGE (Advanced Glycation End products).[4] This will generate free radical and AGE will denature the protein molecules as well. So, the drug molecule has to be an antioxidant, Anti-AGE and antidenaturant also. Single targeted compound is less effective reaching the market late than expected.[5]

There are several drugs which have been tried and proved for various bioactivities.[6] The best and well-known example is aspirin which is a good pain reliever, anti-arthritic and also used to reduce the platelet aggregation. Curcumin has various proven bioapplications.[7] Minarini *et al* explained the multiple biological activities of poly amines.[8] Kumar *et al* indicated the multiple biological activities of flavone.[9] Curcumin and flavone based natural compounds are nontoxic to humans.[10,11]There are bioactive molecules having potent

activity against complex diseases such as AIDS, arthrosclerosis, cancer and depression.[12,13] The combination of two moieties/hybrids might be covalent or non-covalent bonding.

To identify a new multiple bioactive agent, we have decided to choose two known bioactive units based on literature reports. Pyrazole is an important pharmacophore in many drugs (ex: betazole). 3-Aryl pyrazoles were shown to possess wide-range of biological activities such as antimicrobial,[14] antitubercular,[15] anti-inflammatory,[16] antimalarial,[17] antioxidant,[18] antitumor,[19] antianalgesic[20] and anticancer[21] activities. The pyrazole containing pyridine system has shown very good antiproliferative activity.[22] Thaher *et al* proved the p38 kinase inhibitor nature of pyrazole pyridyl core derivatives.[23] Gellibert *et al* suggested that the inhibitors are of transforming growth factor β type I receptor.[24] 3-Arylpyrazole containing the carboxamide group on 5th position showed potent bioactivities such as anticancer activity,[25] cyclooxygenase inhibitors[26] and antiobesity activity.[27] It can also be used as a cannabinoid receptor which was proved in many literatures.[28,29]

Chalcones are another series of important bioactive molecules found in many natural products.[30] Curcumin contains the bischalcone unit.[31] Chalcones possess antimicrobial,[32] anti-inflammatory,[33] anticancer,[34] antitumor,[35] antioxidant[36] and antitubulin activity.[37] Dominguez *et al* reported the synthetic aryl chalcone with very good antimalarial activity.[38] Recently Bandgar *et al* reported the chalcone containing carboxamide **(Fig 1)** on 3rd position of aryl ring showing excellent anti-inflammatory, antioxidant and anticancer activities.[39]

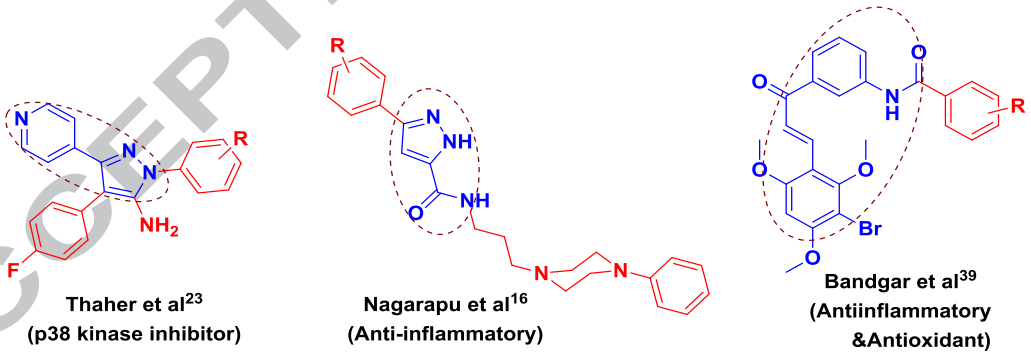
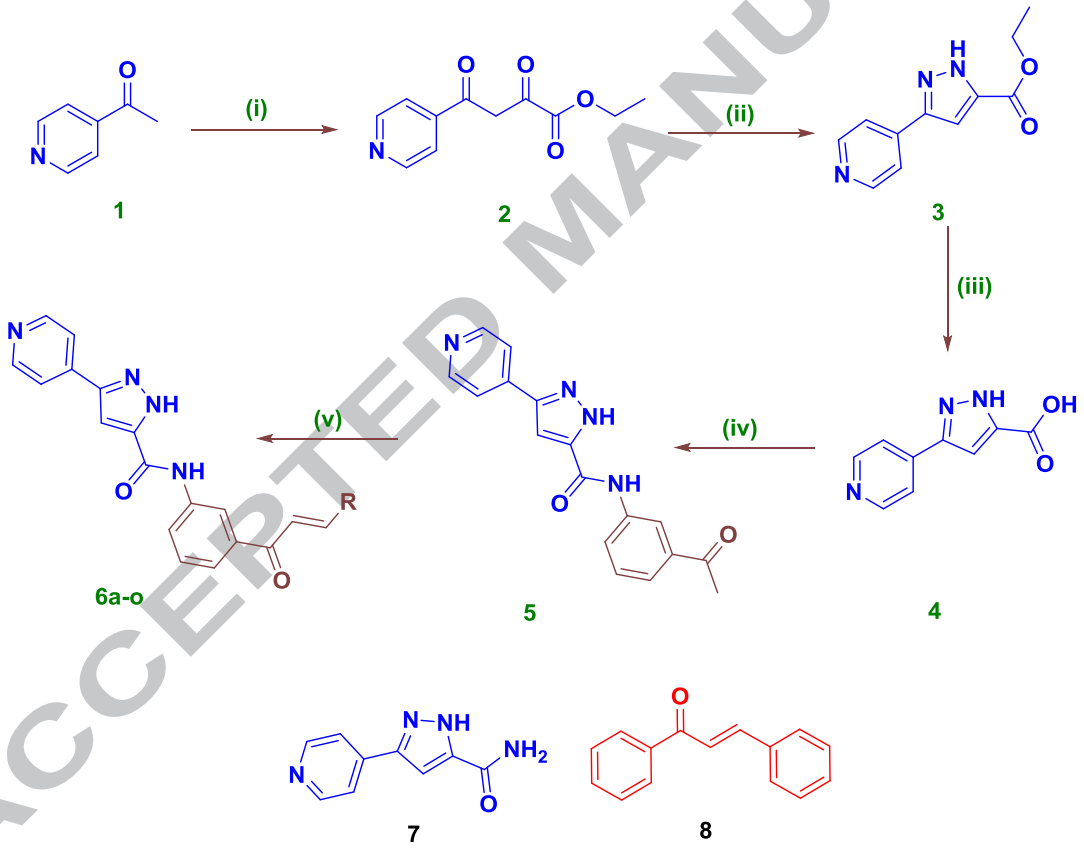


Fig 1: Biologically active pyrazole and chalcone derivatives from literature reports.

We have synthesized a series of compounds containing the skeletons of 3-pyridylpyrazole 5-carboxamide and diaryl chalcones and the synthesized compounds were studied for their biological activity such as antibacterial, anti-inflammatory and antioxidant activities. The molecular docking studies were also performed for the synthesized hybrids and the results are all presented here.

Synthetic route for the compounds (**6a-o)** is described in **Fig 2**. The reaction of 4-acetylpyridine with diethyl oxalate in the presence of sodium hydride in anhydrous THF at 25 °C gave the pyridyl β-ketopyruvate **2** in 85% yield.[16] Further conversion of intermediate **2** to pyrazole heterocycle as per popular procedure with hydrazine hydrate and acetic acid yielded the pyridyl pyrazole ethyl ester **3** in 88% yield.[16] The major intermediate **5** was obtained by the acid hydrolysis of compound **3** with 6N hydrochloric acid followed by the coupling of 3-aminoacetophenone employing the coupling agent carbonyl diimidazole in 90%.[40] Finally the target compounds **(6a-o)** were synthesized from compound **5** by aldol condensation with various aldehydes.[41] The condensation of benzaldehyde with acetophenone gives compound **8.**[41] The list of synthesized compounds were represented in **Fig 3.**



Reagents and conditions: (i) NaH, Diethyloxalate, THF, 3h, RT; (ii) $NH_2NH_2.H_2O$, CH_3COOH, 15h, RT; (iii) 6N HCl, 3h, reflux; (iv) CDI, 3-aminoacetophenone, 30 min, 0 ^oC-RT; (v) aldehyde, KOH, MeOH, H_2O, 3h, RT.

Fig 2: Synthetic route for pyridine pyrazole carboxamido chalcone

All the synthesized intermediates and targeted compounds were characterized by ^1H NMR, ^{13}C NMR, Mass and IR spectral investigations. The band around 1650 cm^{-1} in the IR spectrum clearly indicates the presence of carboxamide and ketone. The disappearance of acetyl methyl signal at δ 2.65 and the appearance of olefinic peaks around 7.7-7.9 ppm in the ^1H NMR spectrum indicate the formation of chalcones. The parent ion

peak appeared on the positive mode in the mass spectrum confirms the formation of the chalcones. The HMBC correlation of various carbon and protons are represented in supporting information.

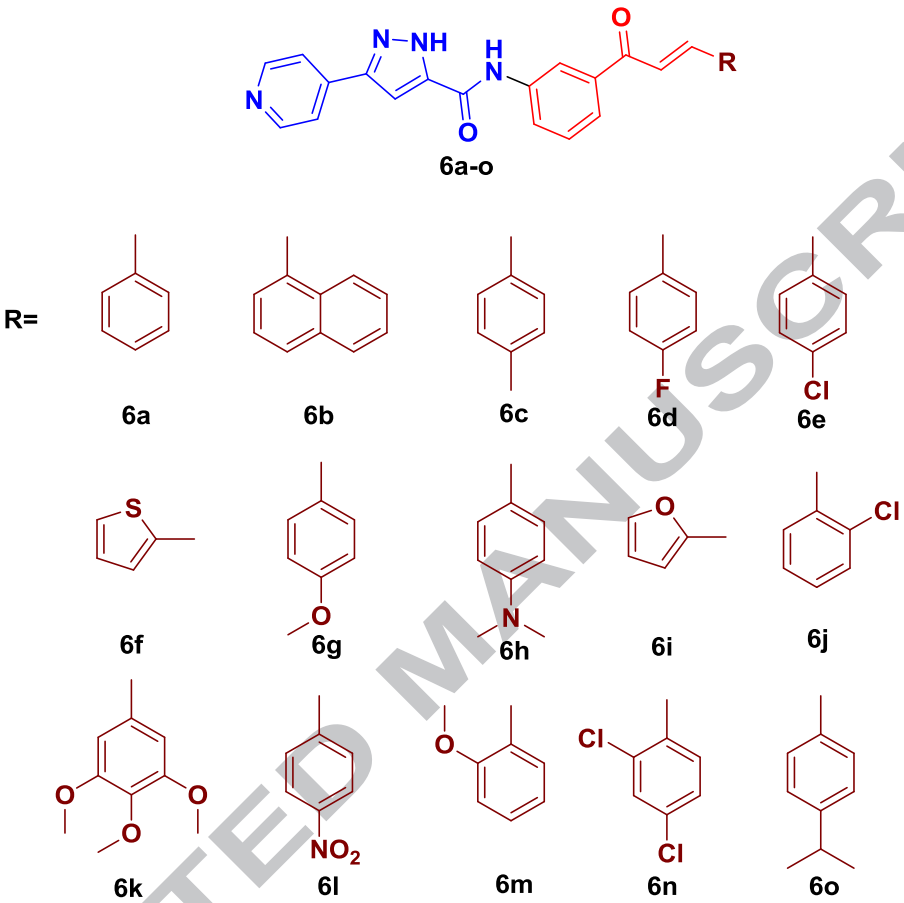


Fig 3: List of synthesized compounds (**6a-o)**

The synthesized compounds were studied for their antibacterial activity. All the compounds were active against *P. aeruginosa* and also majority of the compounds showed enhanced activity than the basic pyrazole and chalcone. Within the series, **6d**, **6g** and **6n** bearing substituents methyl, methoxy and dichloro groups showed highest zone of inhibition than others. The compounds **6f, 6g, 6h, 6i** and **6n** showed potent activity against *K. pneumoniae*. Remaining compounds are inactive against the *K. pneumoniae*. Except compound **6d,** other compounds showed rich activity against *S. pyogenes.* Especially the compound **6l** having the electron withdrawing group on para position has potent zone of inhibition. Similarly except the compound **6j,** remaining compounds showed very good activity against *S. aureus.* The compound **6l** showed highest activity than others. The compounds **6f** and **6i** bearing the heterocyclic unit were active against both gram-positive and gram-negative pathogens. Similarly the compound **6g** and **6i** bearing N,N-dimethylphenyl and furan units also showed activity against both gram-positive and gram-negative pathogens. Over all, the hybrid of

pyridyl pyrazole and chalcone has enhanced antibacterial activity than their parent compounds. The zone of inhibitions for the synthesized compounds and parent molecules were represented in **Table I**.

Table 1. Antibacterial activity of the synthesized compounds (Zone of Inhibition in mm)

S.No	Compound	**Gram-negative**		**Gram-positive**	
		P. aeruginosa	*K. pneumoniae*	*S. pyogenes*	*S. aureus*
1	6a	9.5±0.2	-	13.2±0.3	7.5±0.2
2	6b	6.3±0.4	-	15.4±0.2	12.3±0.3
3	6c	7.8±0.3	-	7.9±0.4	5.9±0.3
4	6d	12.1±0.4	-	-	5.4±0.5
5	6e	10.2±0.2	-	15.3±0.4	12.3±0.3
6	6f	8.6±0.4	7.5±0.2	13.5±0.3	15.4±0.2
7	6g	12.5±0.3	5.2±0.5	10.6±0.3	4.5±0.2
8	6h	10.5±0.2	5.9±0.4	12.4±0.3	7.8±0.2
9	6i	5.7±0.2	5.8±0.4	13.2±0.3	10.7±0.2
10	6j	8.3±0.2	-	7.4±0.3	-
11	6k	5.2±0.4	-	11.6±0.3	12.6±0.2
12	6l	10.4±0.4	-	19.3±0.2	20.2±0.3
13	6m	8.8±0.3	-	9.7±0.2	6.8±0.5
14	6n	12.2±0.4	6.8±0.4	15.5±0.2	17.5±0.4
15	6o	9.6±0.5	-	9.4±0.4	6.8±0.3
16	7	4.0±0.3	5.0±0.4	6.0±0.3	5.0±0.2
17	8	7.0±0.2	5.0±0.4	6.5±0.3	6.0±0.3
18	Control	-	-	-	-

Control: DMSO

The synthesized compounds **(6a-o)** are tested for radical scavenging activity against DPPH free radical. All the synthesized compounds showed potent radical scavenging activity than the parent pyrazole and chalcone. Compounds **6f** and **6j** containing thiophene and o-chlorophenyl units have highest activity than the others, their IC_{50} values being 27.59 and 27.71µM respectively. The compound **6g** and **6n** bearing methoxy and chloro substituents on phenyl ring of chalcone unit also showed potent activity. Similarly the compound **6m** and **6i** bearing *p*-N,N dimethylamino and o-methoxy substituents on phenyl ring of chalcone showed very good activity. The IC_{50}values of the synthesized compounds and basic cores are represented in **Table2**.

The synthesized compounds **(6a-o)** were tested for H_2O_2 scavenging activity. All the compounds showed better activity than the parent compounds (**7** and **8**). Within this series the compounds **6g**, **6k** and **6m** bearing p-methoxy, 3,4,5-trimethoxy and o-methoxy groups showed better scavenging activity than others.

Similarly the compounds having the methyl and isopropyl units on para position of the cinnamyl, namely **6b** and **6o,** showed good scavenging activity. Nitro and N,N dimethylamino groups on para position of phenyl ring slightly reduces the scavenging activity. The compounds **6e**, **6j** and **6n** having chloro in the ortho or para or both showed good activity. The IC_{50} values of the synthesized compounds and parents were listed in **Table 2**.

Table 2: Antioxidant activity of synthesized compounds

S. No	Compound	DPPH (IC_{50})(µM)	H_2O_2 (IC_{50})(µM)
1.	6a	67.09	71.52
2.	6b	64.47	72.06
3.	6c	69.42	64.56
4.	6d	56.02	58.70
5.	6e	63.92	78.90
6.	6f	27.59	70.02
7.	6g	38.44	54.55
8.	6h	41.89	60.92
9.	6i	41.76	72.34
10.	6j	27.71	79.76
11.	6k	54.47	52.90
12.	6l	52.79	64.56
13.	6m	41.67	56.70
14.	6n	35.02	80.94
15.	6o	52.27	60.24
16.	7	82.10	89.12
17.	8	70.90	98.09

The synthesized compounds **(6a-o)** were studied for their anti-inflammatory activity against Bovine serum albumin (BSA) (protein denaturation technique). Most of the compounds have shown better activity than pyrazole and chalcone. Within this series, the compounds bearing electron donating groups such as phenyl, methoxy, N,N-dimethyl and isopropyl units (**6b, 6e, 6h, 6k** and **6o)** showed better activity. The compound with electron withdrawing groups like nitro showed almost equal activity to parent compounds. Compounds **6f** and **6i** bearing heterocyclic units such as thiophene and furan have moderate antidenaturation activity. The compound **6e**, **6j** and **6n** bearing chloro substitution showed little enhanced activity than parent compound. The percentage inhibition of the synthesized compounds and parent compounds were represented in **Fig 4**.

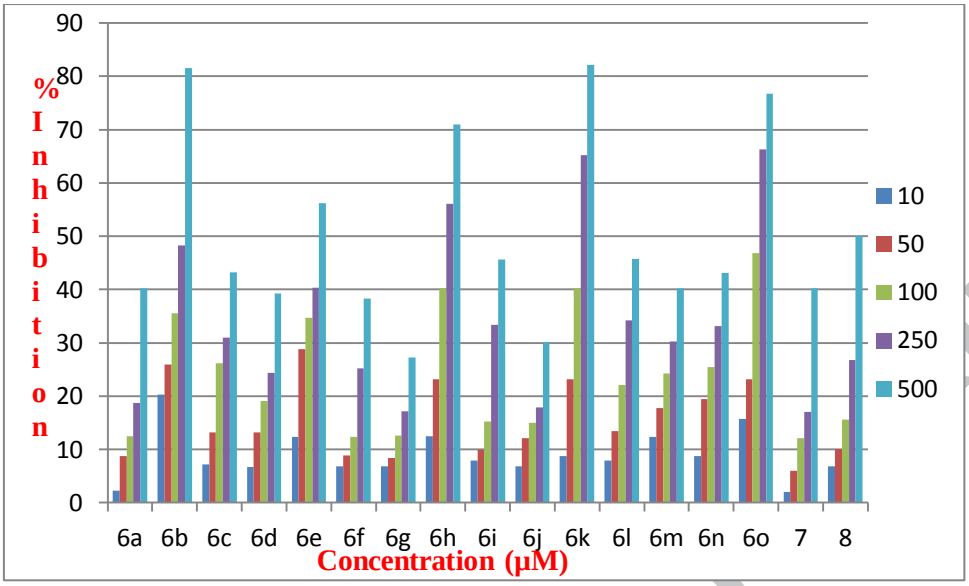


Fig 4: Anti-inflammatory activity (BSA) of synthesized compounds

The synthesized compounds **(6a-o)** were studied for anti-inflammatory activity using Human red blood cells (membrane stabilization method). The entire compounds showed very good anti-inflammatory activity than the parent compounds **(7** and **8)**. Within this series the compounds **6c** bearing methyl group on the para position of the cinnamyl unit showed highest activity than others. Similarly the compounds **6m**, **6e** and **6n** bearing o-methoxy, p-chloro and 2,4-dichloro units showed higher activity than others. The compound **6l** having the nitro group at para position of cinnamyl unit also showed good inhibition. The compounds **6f** and **6i** bearing heterocyclic rings such as thiophene and furan showed moderate activity. Similarly the compounds having the units like phenyl, naphthalene, p-fluorophenyl and p-N,N-dimethylaminophenyl also showed moderate activity. The percentage inhibitions for the synthesized compounds at various concentrations were represented in **Fig 5**.

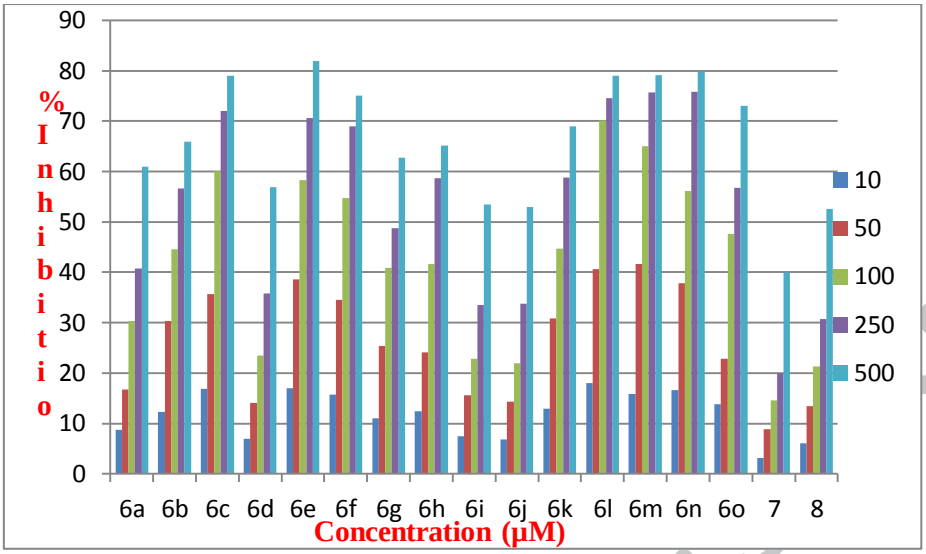


Fig 5. Anti-inflammatory of activity (HRBC) of synthesized compounds

In the biological system the cyclooxygenase is the enzyme responsible for inflammation. So, the inhibition of cyclooxygenase (COX-1 & COX-2) will reduce the pain and inflammation. The synthesized compounds were docked with COX-1 and COX-2 enzymes using molecular docking tools and the results were presented here.

All the synthesized compounds were examined for the interaction with COX-1 enzyme (1PGG.pdb). In most of the molecules, the pyridyl, pyrazole and phenyl rings showed pi-pi interactions or pi-alkyl interactions with amino acid residues. Along with that, the functional groups (amide and ketone) are making the hydrogen bond with amino acid residues. Most of the compounds showed hydrogen bonding with various amino acid. Especially the compound **6c** containing methyl substituent showed hydrogen bonding with three amino acids (ASN515, GLY96, HIS90). The compounds **6e**, **6f**, and **6i** containing the substitutions p-chlorophenyl, thiophene and furan showed the hydrogen bonding with TRP98. Other amino acids ARG120, TYR355, PHE150 and GLN358 showed hydrogen bonding with some other derivatives. The protein ligand binding energies also better. All the compounds showed better binding energy than parent compound **7**. Majority of compounds showed superior binding energy than their parents **7** and **8**. In addition most of the compounds showed superior inhibition constant than the parent compounds. Especially within this series compound **6a**, **6b** and **6h** have the highest binding energy and inhibition constant. Their binding energies are -9.13, -9.04 and -8.44 kcal/mol and their inhibition constants are 0.20, 0.23 and 0.65 µM correspondingly. Overall the synthesized hybrid molecule

enhances/retain the binding energy and inhibition constant than the parent compounds. The molecular docking results of COX-1 with ligand are represented in **Table 3.**

Table3: Molecular docking interaction of synthesized compounds against COX-1.

S. No	Compound Name	COX-1 (1PGG)			
		Binding energy (kcal/mol)	Inhibition constant (μM)	No. of hydrogen bonding	Hydrogen bonded amino acid residue
1.	6a	-9.13	0.20	-	-
2.	6b	-9.04	0.23	1	ARG120
3.	6c	-7.6	1.71	3	ASN515, GLY96, HIS90
4.	6d	-7.88	1.67	-	-
5.	6e	-6.26	6.91	1	TRP98
6.	6f	-4.85	278	1	TRP98
7.	6g	-5.51	92.2	-	-
8.	6h	-8.44	0.65	1	TYR355
9.	6i	-4.54	466	1	TRP98
10.	6j	-7.45	3.44	-	-
11.	6k	-5.71	64.7	-	-
12.	6l	-4.08	1010	-	-
13.	6m	-6.8	10.4	1	PHE580
14.	6n	-4.82	298	1	GLN358
15.	6o	-7.26	4.73	-	-
16.	7	-4.01	1150	1	HIS95
17.	8	-5.46	99	-	-

Similarly, all the synthesized and their parent compounds were proceeded to examine the interaction with COX-2 enzyme (4COX). Similar to COX-1 the pyridyl, pyrazole and phenyl rings showed pi-pi interactions or pi-alkyl interactions with amino acid. Along with that, the functional groups (amide and ketone) and pyrazole nitrogen are making the hydrogen bond with amino acid residues. Especially, compound **6l** containing p-nitrophenyl group showed two hydrogen bonding with the amino acid SER471 and LYS473. Other amino acids ARG456, GLU254, TYR355, ARG120, GLU520 and TYR122 were in hydrogen bonding with synthesized derivatives. In COX-2 all the compounds showed better binding energy and inhibition constant than the parent compounds **7** and **8**. Compounds **6e**, **6l**, **6m** and **6n** having p-chlorophenyl, p-nitrophenyl, 2,4-dichlorophenyl and p-isopropylphenyl groups showed highest binding energy such as -8.49, -9.43, -8.71, -8.71 and -8.37 kcal/mol and inhibition constants are 0.60, 0.12, 0.41, 0.41 and 0.73 μM respectively. Overall the

synthesized hybrid molecule completely enhances the binding energy and inhibition constant than parent compound with COX-2 enzyme. The molecular docking results of COX-2 with ligand are represented in **Table 4.** The docked complex image of ligand **6b** and COX-1, **6o** and COX-2 is represented in **Figs 6 & 7.**

Table 4: Molecular docking interaction of synthesized compounds against COX-2.

S. No	Compound Name	COX-2 (4COX)			
		Binding energy (kcal/mol)	Inhibition constant (μM)	No. of hydrogen bonding	Hydrogen bonded amino acid residue
1.	6a	-7.72	2.2	-	-
2.	6b	-6.52	16.7	1	ARG456
3.	6c	-7.64	2.52	-	-
4.	6d	-7.45	3.42	1	GLU254
5.	6e	-8.49	0.60	1	TYR355
6.	6f	-6.36	21.8	1	LYS83
7.	6g	-6.72	11.8	-	-
8.	6h	-7.24	4.9	-	-
9.	6i	-7.33	4.2	1	ARG120
10.	6j	-6.42	19.61	1	GLU520
11.	6k	-7.2	5.24	-	-
12.	6l	-9.43	0.12	2	SER471, LYS473
13.	6m	-8.71	0.41	-	-
14.	6n	-8.71	0.41	-	-
15.	6o	-8.37	0.73	1	TYR122
16.	7	-5.76	59.6	1	MET522
17.	8	-6.23	27.2	2	GLN42, ASN43

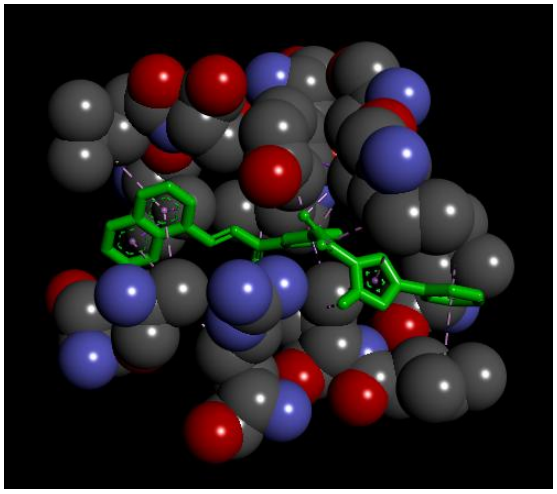


Fig 6: Binding of **6b** into the active site of COX-1

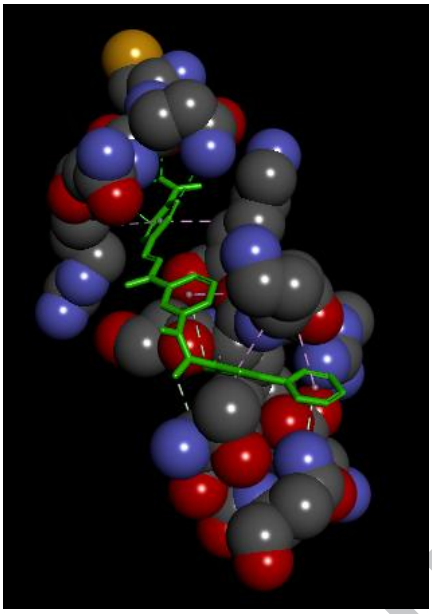


Fig 7: Binding of **6o** into the active site of COX-2

The pyrazole - chalcone hybrids were synthesized, characterized and studied for their *in vitro* biological evaluation such as antibacterial, anti-inflammatory and antioxidant activities. As expected, these compounds have enhanced biological activity than the basic pyrazole and chalcones. Some of the compounds showed potent activity. Also the molecular docking studies also proved that hybrid molecule enhanced binding energy and inhibition constant with COX-1 and COX-2 enzyme. These synthesized compounds are currently under study for the biological evaluation of other therapeutic targets.

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Synthetic procedure for ethyl2,4-dioxo-4-(pyridin-4-yl)butanoate **(2):** To sodium hydride (60 % in paraffin oil) (1.98 g, 0.049mol) tetrahydrofuran (30 mL) was added drop wise in ice cold condition under nitrogen atmosphere. Then 4-acetyl pyridine (3.0 g, 0.024 g) was added and stirred for 30 min at room temperature. Finally diethyl oxalate (4.03 mL, 0.029 mol) was added and the reaction mixture was again stirred for 3h at room temperature. The completion of the reaction was monitored by TLC and quenched with crushed ice. Then the reaction mixture was extracted with ethyl acetate (2 x 100 mL), washed with water (150 mL) and brine solution (150mL). The organic layer was separated, dried over anhydrous sodium sulphate and evaporation of solvent afforded the expected product **(2)**. White solid. Yield 75%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.78 (d, *J* = 5.5 Hz, 2H), 7.86 (d, *J* = 5.5 Hz, 2H), 4.42 (q, *J* = 14.0, 7.1 Hz, 2H), 1.42 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.22, 173.23, 165.26, 150.73, 137.86, 122.95, 61.91, 29.81, 14.31. ESI-LC/MS calculated m/z 221.07, found 220.05 (M^--1). IR : 3124, 1710, 1621, 1567, 821, 720. Anal.Calcd for: $C_{11}H_{11}NO_4$: C, 59.73; H, 5.01; N, 6.33%. Found: C, 59.77; H, 5.00; N, 6.30%.

Synthetic procedure for ethyl 3-(pyridin-4-yl)-1H-pyrazole-5-carboxylate **(3):** To acetic acid (10 mL) solution of intermediate **2** (2.0g, 9.04 mmol), hydrazine hydrate (2.26 mL) was added drop wise at

0°C. The reaction mixture was stirred for 15h at room temperature quenched with crushed ice, basified with sodium bicarbonate solution. Then extracted with ethyl acetate (2 x 100mL) washed with water (100 mL) and brine solution (100mL). Then the organic layer was separated, dried over anhydrous sodium sulphate and evaporated under reduced pressure afforded product. White solid. Yield 87%. ^{1}H NMR (300 MHz, DMSO-D_6) δ 14.40 (s, 1H), 8.68 (d, *J* = 5.7 Hz, 2H), 7.90 (d, *J* = 5.7 Hz, 2H), 7.56 (s, 1H), 4.39 (q, *J* = 14.0, 7.0 Hz, 2H), 1.38 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 163.75, 150.81, 135.90, 127.70, 120.08, 107.35, 61.32, 14.68. ESI-LC/MS calculated m/z 217.09, found 218.08 (M^{+}+1). IR: 3124, 2978, 1725, 1608, 1571, 835, 765. Anal.Calcd for: $C_{11}H_{11}N_3O_2$: C, 60.82; H, 5.10; N, 19.34%. Found: C, 60.85; H, 5.11; N, 19.30%.

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Synthetic procedure for 4-(5-carboxy-1H-pyrazol-3-yl)pyridin-1-iumchloride **(4):** The intermediate **3** (1.5 g, 6.9 mmol) was dissolved in 6N hydrochloric acid (7.5 mL) and heated to reflux for 3h. The completion of the reaction was monitored by TLC. Then the reaction mixture was stirred with tetrahydrofuran (20mL) and filtered, washed with tetrahydrofuran (20 mL). The filtered product **4** was taken for the next step. White solid. Yield 95%. ^{1}H NMR (300 MHz, DMSO-D_6) δ 8.97 (bs, 2H), 8.51 (d, J = 5.3 Hz, 2H), 7.85 (s, 1H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 160.59, 148.01, 142.48, 122.53, 109.74. ESI-LC/MS calculated m/z 189.05, found 190.11 (M^++1). IR: 3046, 2865, 1694, 1631, 1589, 830, 773. Anal.Calcd for: $C_9H_8ClN_3O_2$: C, 47.91; H, 3.57; N, 18.62%. Found: C, 47.87; H, 3.59; N, 18.64%.

Synthetic procedure for N-(3-acetylphenyl)-3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide**(5):** The intermediate **4** (1.0g, 5.29 mmol) was dissolved in Dimethylformamide (5 mL) and

carbonyldiimidazole (1.02 g, 6.3 mmol) was added at 0° C. The reaction mixture was stirred for 30 min at room temperature. To the reaction mixture 3-amino acetophenone (0.714 g, 5.29mmol) was added at 0° C and again stirred for 24 h at ambient temperature. Then the reaction mixture was quenched with crushed ice. The white precipitate **5** obtained was filtered, dried in vacuum and used for further reactions. White solid. Yield 92%. ^{1}H NMR (300 MHz, DMSO-D_6) δ 14.25 (s, 1H), 10.15 (s, 1H), 8.64 (d, *J* = 4.5 Hz, 2H), 8.40 (s, 1H), 8.12 (d, *J* = 7.8 Hz, 1H), 7.76 (bs, 2H), 7.70 (d, *J* = 7.4 Hz, 1H), 7.50–7.45 (m, 2H), 2.63 (s, 3H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 196.25, 158.71, 148.91, 150.91, 137.73, 136.13, 127.71, 123.50, 122.32, 118.60, 118.39, 103.13, 25.44. ESI-LC/MS calculated m/z 306.11, found 307.31 (M^++1). IR: 3309, 3123, 1681, 1645, 1607, 1554, 818, 771. Anal.Calcd for: $C_{17}H_{14}N_4O_2$: C, 66.66; H, 4.61; N, 18.29%. Found: C, 66.62; H, 4.68; N, 18.28%

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N-(3-cinnamoylphenyl)-3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide **(6a):** White solid.Yield 90%.Mp 183-185 ^{o}C. ^{1}H NMR (300 MHz,) δ 13.96 (s, 1H), 9.62 (s, 1H), 8.65 (d, *J* = 3.0 Hz, 2H), 8.45 (s, 1H), 7.81 (d, *J = 8.1*, 1H), 7.77-7.68 (m, 3H), 7.70–7.69 (m, 3H), 7.68–7.64 (m, 2H), 7.53–7.48 (m, 2H), 7.45–7.38 (m, 2H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 189.82, 158.00, 150.28, 144.67, 138.77, 138.64, 134.72, 130.51, 129.16, 128.87, 128.40, 124.30, 123.84, 122.07, 119.89, 119.69, 104.50. ESI-LC/MS calculated m/z 394.14, found 395.08 (M^++1). IR : 3249, 2978, 1663, 1548, 817, 753. Anal.Calcd for: $C_{24}H_{18}N_4O_2$: C, 73.08; H, 4.60; N, 14.20%. Found: C, 73.09; H, 4.62; N, 14.14%.

(E)-N-(3-(3-(naphthalen-1-yl)acryloyl)phenyl)-3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide**(6b):**

White solid. Yield 85%.Mp 194-197 ^{o}C. ^{1}H NMR (300 MHz, DMSO-D_6) δ 10.12 (s,1H), 8.66–8.58 (d, *J = 4.2 Hz*, 2H), 8.52 (s, 1H), 8.25 (d, *J* = 8.1 Hz, 1H), 8.16 (d, *J* = 7.6 Hz, 1H), 8.00 (d, *J* = 6.8 Hz, 1H), 7.96–7.92 (m, 2H), 7.82 (d, *J* = 7.6 Hz, 1H), 7.73–7.69 (m, , 4H), 7.60–7.49 (m, 4H), 7.44 (s, 1H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 189.48, 159.37, 150.79, 145.30, 144.18, 143.03, 139.52, 138.44, 138.17, 135.58, 134.03, 130.85, 129.62, 129.39, 125.24, 124.45, 123.41, 120.51, 119.93, 105.00. ESI-

LC/MS calculated m/z 444.16, found 445.51 (M^++1). IR : 3058, 1660, 1596, 1543, 778, 689. Anal.Calcd for: $C_{28}H_{20}N_4O_2$ C, 75.66; H, 4.54; N, 12.60%. Found: C, 75.68; H, 4.55; N, 12.55%

(E)-3-(pyridin-4-yl)-N-(3-(3-(p-tolyl)acryloyl)phenyl)-1H-pyrazole-5-carboxamide**(6c):** White solid. Yield 92%. Mp 228-231 ^oC. ^1H NMR (300 MHz, DMSO-D_6) δ 9.84 (s, 1H), 8.65 (d, *J* = 5.3 Hz, 2H), 8.41 (s, 1H), 8.15 (d, *J* = 7.8 Hz, 1H), 7.80 (d, *J* = 7.8 Hz, 1H), 7.77 (bs, 1H), 7.73 (d, *J* = 9.7 Hz, 2H), 7.61-7.59 (m, 3H), 7.55–7.49 (m, 2H), 7.25 (d, *J* = 7.9 Hz, 2H), 2.40 (s, 3H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 187.83, 157.50, 148.76, 142.85, 139.29, 137.66, 130.55, 128.12, 127.53, 127.10, 122.95, 122.21, 119.70, 118.68, 118.14, 104.49, 19.81. ESI-LC/MS calculated m/z 408.16, found 409.05 (M^++1). IR : 3224, 3023, 1661, 1544, 864, 795. Anal.Calcd for: $C_{25}H_{20}N_4O_2$: C, 73.51; H, 4.94; N, 13.72%. Found: C, 73.56; H, 4.92; N, 13.70%

(E)-N-(3-(3-(4-fluorophenyl)acryloyl)phenyl)-3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide**(6d):** Yellowish White solid.Yield 89%.Mp 247 -250 ^oC. ^1H NMR (300 MHz, DMSO-D_6) δ 8.69 (d, *J* = 6.0 Hz, 2H), 8.54 (s, 1H), 8.23 (d, *J* = 7.9 Hz, 1H), 8.05–7.95 (m, 4H), 7.93–7.78 (m, 3H), 7.65–7.58 (m, 2H), 7.34 (t, *J* = 8.8 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 189.37, 165.45, 162.14, 159.37, 150.59, 145.42, 144.08, 143.14, 139.48, 138.49, 131.62, 131.38, 131.27, 129.40, 124.91, 124.13, 122.48, 120.37, 119.80, 116.37, 116.08, 104.79. ESI-LC/MS calculated m/z 412.13, found 413.10 (M^++1). IR : 3361, 3229 , 1662, 1538, 801, 737. Anal.Calcd for: $C_{24}H_{17}FN_4O_2$: C, 69.89; H, 4.15; N, 13.59%. Found C, 69.91; H, 4.17; N, 13.55%

(E)-N-(3-(3-(4-chlorophenyl)acryloyl)phenyl)-3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide**(6e):** White solid. Yield 82%. Mp 275-278 ^oC. ^1H NMR (300 MHz, DMSO-D_6) δ 10.54 (s, 1H), 8.73 (d, *J* = 5.6 Hz, 2H), 8.55 (s, 1H), 8.22 (d, *J* = 9.3 Hz, 1H), 8.01–7.95 (m, 4H), 7.89–7.79 (m, 3H), 7.67–7.59 (m, 4H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 189.48, 159.37, 150.79, 143.03, 139.52, 138.44, 135.58, 134.03, 130.85, 129.62, 129.39, 125.24, 124.45, 123.41, 120.51, 119.93, 105.00, 79.54. ESI-LC/MS calculated m/z 428.10, found 429.07 (M^++1). IR: 3240, 1665, 1599, 1560, 809, 788. Anal.Calcd for: $C_{24}H_{17}ClN_4O_2$: C, 67.21; H, 4.00; N, 13.06%. Found: C, 67.22; H, 4.03; N, 13.00%

(E)-3-(pyridin-4-yl)-N-(3-(3-(thiophen-2-yl)acryloyl)phenyl)-1H-pyrazole-5-carboxamide**(6f):** Brown solid. Yield 88%. Mp 224–226 ^oC. ^1H NMR (300 MHz, DMSO-D_6) δ 10.59 (s, 1H), 8.72 (d, *J* = 5.0 Hz, 2H), 8.53 (s, 1H), 8.22 (d, *J* = 7.9 Hz, 1H), 8.02 (d, *J* = 15.4 Hz, 1H), 7.92–7.86 (m, 4H), 7.76 (d, *J* = 3.3 Hz, 1H), 7.67–7.53 (m, 3H), 7.31–7.24 (m, 1H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 188.97,

162.72, 159.44, 150.74, 145.40, 144.18, 140.08, 139.54, 138.48, 138.33, 137.29, 133.29, 130.74, 129.60, 129.13, 124.95, 124.03, 120.87, 120.26, 119.88, 104.97. ESI-LC/MS calculated m/z 400.10, found 401.04 (M^++1). IR: 2924, 2853, 1655, 1542, 815, 758. Anal.Calcd for: $C_{22}H_{16}N_4O_2S$: C, 65.99; H, 4.03; N, 13.99%. Found: C, 66.01; H, 4.05; N, 13.94%.

(E)-N-(3-(3-(4-methoxyphenyl)acryloyl)phenyl)-3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide**(6g):** Pale yellow solid. Yield 94%. Mp 245-248 ^oC. ^1H NMR (300 MHz, DMSO-D_6) δ 10.54 (s, 1H), 8.73 (d, *J* = 3.3 Hz, 2H), 8.53 (s, 1H), 8.21 (d, *J* = 7.8 Hz, 1H), 7.98 (d, *J* = 7.6 Hz, 1H), 7.92-7.81 (m, 6H), 7.70–7.59 (m, 2H), 7.09 (d, *J* = 8.4 Hz, 2H), 3.88 (s, 3H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 189.44, 161.89, 159.35, 154.96, 152.19, 150.80, 144.56, 139.47, 138.88, 138.22, 131.08, 129.57, 127.71, 124.94, 124.28, 120.44, 120.16, 119.95, 114.91, 105.03, 55.80. ESI-LC/MS calculated m/z 424.15, found 425.10 (M^++1). IR: 3368, 3210, 1650, 1589, 1553, 821, 795. Anal.Calcd for: $C_{25}H_{20}N_4O_3$: C, 70.74; H, 4.75; N, 13.20%. Found: C, 70.75; H, 4.76; N, 13.22%.

(E)-N-(3-(3-(4-(dimethylamino)phenyl)acryloyl)phenyl)-3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide**(6h):** Orange solid. Yield 87%. Mp 253-256 ^oC. ^1H NMR (300 MHz, DMSO-D_6) δ 10.69 (s, 1H), 8.80 (d, *J* = 2.5 Hz, 2H), 8.57 (s, 1H), 8.26 (d, *J* = 7.3 Hz, 1H), 8.04-7.94 (m, 4H), 7.93–7.80 (m, 3H), 7.73–7.65 (m, 2H), 6.89 (d, *J* = 8.2 Hz, 2H), 3.14 (s, 6H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 189.02, 159.41, 152.55, 150.89, 145.89, 139.50, 138.36, 131.21, 129.59, 124.86, 124.60, 124.14, 122.40, 120.34, 120.01, 116.56, 112.26, 105.13, 40.15. ESI-LC/MS calculated m/z 437.19, found 438.10 (M^++1). IR: 3196, 1644, 1596, 1565, 800, 734. Anal.Calcd for: $C_{26}H_{23}N_5O_2$: C, 71.38; H, 5.30; N, 16.01%. Found: C, 71.35; H, 5.31; N, 16.04%

(E)-N-(3-(3-(furan-2-yl)acryloyl)phenyl)-3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide**(6i):** White solid. Yield 88%. Mp 254-256 ^oC . ^1H NMR (300 MHz, DMSO-D_6) δ 10.59 (s, 1H), 8.73 (d, *J* = 5.0 Hz, 2H), 8.56 (s, 1H), 8.22 (d, *J* = 7.9 Hz, 1H), 8.00 (d, *J* = 15.4 Hz, 1H), 7.92–7.86 (m, 3H), 7.70–7.54 (m, 4H), 7.18 (bs, 1H), 6.77 (bs,1H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 188.90, 159.48, 151.61, 150.95, 146.81, 139.71, 138.53, 131.16, 129.86, 125.08, 124.12, 120.43, 120.07, 119.05, 117.96, 113.73, 105.20. ESI-LC/MS calculated m/z 384.12, found 385.06(M^++1). IR : 3209, 1725, 1663, 1597, 1551, 819, 742. Anal.Calcd for: $C_{22}H_{16}N_4O_3$: C, 68.74; H, 4.20; N, 14.58%. Found: C, 68.75; H, 4.22; N, 14.55%

(E)-N-(3-(3-(2-chlorophenyl)acryloyl)phenyl)-3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide**(6j):** Yellowish white solid. Yield 80%. Mp 210-211 ^{o}C. ^{1}H NMR (300 MHz, DMSO-D_6) δ 10.56 (s, 1H), 8.71 (d, *J* = 4.5 Hz, 2H), 8.57 (s, 1H), 8.27–8.23 (m, 2H), 8.15-8.09 (m, 1H), 8.06–7.98 (m, 2H), 7.88 (d, *J* = 4.5 Hz, 2H), 7.67–7.62 (m, 3H), 7.57–7.50 (m, 2H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 189.35, 159.71, 150.86, 145.57, 144.42, 139.79, 139.17, 138.55, 138.26, 134.90, 132.78, 132.55, 130.58, 129.79, 129.01, 128.23, 125.46, 125.33, 124.59, 120.57, 119.98, 105.13. ESI-LC/MS calculated m/z 428.10, found 429.06 (M^++1). IR: 3176, 1663, 1540, 810, 748. Anal.Calcd for: $C_{24}H_{17}ClN_4O_2$: C, 67.21; H, 4.00; N, 13.06%. Found: C, 67.25; H, 4.02; N, 13.04%

(E)-3-(pyridin-4-yl)-N-(3-(3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)-1H-pyrazole-5-carboxamide**(6k):**Pale yellow solid. Yield 84%. Mp 287-290 ^{o}C. ^{1}H NMR (300 MHz, DMSO-D_6) δ 8.67 (d, *J* = 5.9 Hz, 2H), 8.51 (s, 1H), 8.22 (d, *J* = 7.9 Hz, 1H), 8.00 (d, *J* = 7.8 Hz, 1H), 7.91–7.80 (m, 4H), 7.64 (t, *J* = 7.9 Hz, 1H), 7.55 (s, 1H), 7.30 (s, 2H), 3.93 (s, 6H), 3.78 (s, 3H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 189.81, 160.13, 153.69, 150.80, 146.12, 145.27, 144.81, 140.39, 139.85, 139.34, 138.84, 130.78, 129.71, 125.11, 124.46, 122.03, 120.34, 119.97, 107.10, 104.99, 79.70, 60.73, 56.71. ESI-LC/MS calculated m/z 484.17, found 485.07 (M^++1). IR: 3210, 1720, 1618, 1584, 835, 799. Anal.Calcd for: $C_{27}H_{24}N_4O_5$: C, 66.93; H, 4.99; N, 11.56%. Found: C, 66.95; H, 4.97; N, 11.55%

(E)-N-(3-(3-(4-nitrophenyl)acryloyl)phenyl)-3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide**(6l):** Pale yellow solid. Yield 81%. Mp 296-298 ^{o}C. ^{1}H NMR (300 MHz, DMSO-D_6) δ 8.71 (d, *J* = 5.8 Hz, 2H), 8.57 (s, 1H), 8.35 (d, *J* = 8.7 Hz, 2H), 8.23-8.16 (m, 3H), 8.14 (d, *J* = 15.7 Hz, 1H), 8.04 (d, *J* = 7.7 Hz, 1H), 7.92–7.86 (m, 3H), 7.68-7.62 (m, 2H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 189.43, 159.55, 150.74, 148.55, 145.48, 144.30, 141.57, 139.65, 138.42, 138.13, 130.14, 129.67, 126.64, 125.46, 124.54, 124.32, 120.56, 119.93, 105.01. ESI-LC/MS calculated m/z 439.13, found 440.20 (M^++1). 3221, 1689, 1643, 1557, 1521, 851, 756. Anal.Calcd for: $C_{24}H_{17}N_5O_4$: C, 65.60; H, 3.90; N, 15.94%. Found: C, 65.62; H, 3.92; N, 15.90%

(E)-N-(3-(3-(2-methoxyphenyl)acryloyl)phenyl)-3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide**(6m):** Yellowish white solid. Yield 82%. Mp 116-119 ^{o}C. ^{1}H NMR (300 MHz, DMSO-D_6) δ 8.69 (d, *J* = 5.8 Hz, 2H), 8.56 (s, 1H), 8.21 (d, *J* = 7.8 Hz, 1H), 8.12 (d, *J* = 15.7 Hz, 1H), 8.00 (d, *J* = 7.6 Hz, 1H), 7.96–7.84 (m, 4H), 7.63 (t, *J* = 7.9 Hz, 1H), 7.57–7.49 (m, 2H), 7.19 (d, *J* = 8.3 Hz, 1H), 7.11 (t, *J* = 7.4 Hz, 1H), 3.97 (s, 3H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 189.92, 160.11, 158.89, 150.81, 146.05,

144.79, 139.86, 139.41, 138.91, 132.97, 129.75, 129.18, 125.02, 124.20, 123.60, 123.45, 122.77, 122.50, 121.32, 120.41, 120.14, 120.00, 112.43, 105.00, 56.29. ESI-LC/MS calculated m/z 424.15, found 425.11 (M^++1). IR: 3126, 2980, 1690, 1608, 1551, 830, 775. Anal.Calcd for: $C_{25}H_{20}N_4O_3$: C, 70.74; H, 4.75; N, 13.20%. Found: C, 70.75; H, 4.75; N, 13.23%

(E)-N-(3-(3-(2,4-dichlorophenyl)acryloyl)phenyl)-3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide**(6n):**White solid. Yield 80%. Mp 194-197 ^oC. ^1H NMR (300 MHz, DMSO-D_6) δ 8.66 (d, *J* = 5.8 Hz, 2H), 8.58 (s, 1H), 8.31 (d, *J* = 8.6 Hz, 1H), 8.25 (d, *J* = 7.8 Hz, 1H), 8.05-8.00 (m, 3H), 7.87–7.83 (m, 3H), 7.68–7.59 (m, 2H), 7.53 (s, 1H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 189.19, 160.23, 150.55, 146.16, 144.92, 139.88, 139.52, 138.10, 137.74, 136.02, 135.50, 131.83, 130.15, 129.90, 129.59, 128.34, 125.94, 125.16, 124.12, 120.28, 119.72, 104.67. ESI-LC/MS calculated m/z 462.07, found 463.09 (M^++1). IR : 3127, 2954, 1700, 1590, 1572, 820, 734. Anal.Calcd for: $C_{24}H_{16}Cl_2N_4O_2$: C, 62.22; H, 3.48; N, 12.09%. Found: C, 62.23; H, 3.49; N, 12.05%

(E)-N-(3-(3-(4-isopropylphenyl)acryloyl)phenyl)-3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide**(6o):**
White solid. Yield 92%. Mp 198-200 ^oC. ^1H NMR (300 MHz, DMSO-D_6) δ 14.24(s, 1H), 10.57 (s, 1H), 8.73 (bs, 2H), 8.55 (s, 1H), 8.23 (d, *J* = 8.0 Hz, 1H), 8.00 (d, *J* = 7.7 Hz, 1H), 7.92–7.81 (m, 5H), 7.69–7.60 (m, 2H), 7.41 (d, *J* = 7.9 Hz, 2H), 3.06–2.56 (m, 1H), 1.28 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 189.54, 167.10, 159.28, 151.90, 150.77, 144.58, 139.48, 138.66, 132.73, 129.54, 129.28, 127.29, 125.02, 124.27, 121.74, 120.46, 119.93, 105.02, 33.78, 23.91. ESI-LC/MS calculated m/z 436.19, found 437.20 (M^++1). IR: 3102, 2879, 1705, 1601, 1589, 815, 746. Anal.Calcd for: $C_{27}H_{24}N_4O_2$: C, 74.29; H, 5.54; N, 12.84%. Found C, 74.31; H, 5.56; N, 12.85%

42. Tyagarajan, S.; Chakravarty, P.K.; Zhou, B.; Taylor, B.; Fisher, M.H.; Wyvratt, M.J. et al., *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5480-5483. 3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide **(7):**
White solid. Yield 75%. ^1H NMR (300 MHz, DMSO-D_6) δ 8.94 (bs, 2H), 8.49 (d, *J* = 5.1 Hz, 2H), 8.32 (s, 1H), 7.84-7.82 (m, 2H) ^{13}C NMR (75 MHz, DMSO-D_6) δ 163.50, 149.00, 142.47, 122.89, 109.98. ESI-LC/MS calculated m/z 188.07, found 189.12 (M^++1). IR : 3042, 2789, 1690, 1620, 1595, 845, 760. Anal.Calcd for: $C_9H_8N_4O$ C, 57.44; H, 4.29; N, 29.77%. Found C, 57.38; H, 4.33; N, 29.79%

43. Sribalan, R.; Padmini, V.; Lavanya, A.; Ponnuvel, K. *Saudi Pharm. J.* **2015,** http://dx.doi.org/10.1016/j.jsps.2015.05.003.The bacterial strains used for the examinations were *P. aeruginosa (ATCC 10145), K. Pneumoniae (ATCC 10031), S. pyogenes(ATCC 12358)* and *S.*

aureus(ATCC 11632) obtained from either American type culture collection or purchased from Himedia, Mumbai. The experiments were repeated twice. The experimental technique used is as followed by Sribalan *et al*

44. Lavanya, A.; Sribalan, R.; Padmini, V. *J. Saudi Chem. Soc*. 2015. http://dx.doi.org/10.1016/j.jscs.2015.06008.The synthesized compounds were tested for anti-inflammatory activity (bovine serum albumin denaturation technique and HRBC membrane stabilization method). The experimental technique used is as followed by Lavanya *et al*
45. Sribalan, R.; Kirubavathi, M.; Banuppriya, G.; Padmini, V. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 4282-4286.The DPPH radical and H_2O_2 scavenging activity studied for the synthesized compounds. The experimental technique used is as followed by Sribalan *et al.*
46. Rizvi, S.M.D.; Shakil, S.; Haneef, M. *EXCLI J.* **2013,** *12*, 831-857. Molecular docking of compounds into the COX-1 and COX-2 enzymes was carried out using the Auto-Dock software (version 4.2). Accelrys discovery studio client 4.1 visualizer was used for the visualizing protein-ligand complex. Three dimensional structures of synthesized derivatives were constructed using ChemBio 3D ultra 13.0 software, then they were energetically minimized by using MMFF94 with 5000 iterations and minimum RMS gradient of 0.10. The crystal structure of COX-1 and COX-2 (1PGG.pdb, 4COX.pdb) co-crystallized with indomethacin were taken from Protein Data bank. All bound water and ligand were eliminated from the protein and polar hydrogen was added. Moreover all docking a grid box size of 60x60x60 points in X, Y and Z direction. A grid spacing of 0.375 Å and ten runs were generated by using Lamarckian genetic algorithm searches.

Multiple biological activities and Molecular docking studies of newly synthesized 3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide chalcone hybrids.

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Graphical abstract