Original Article

Synthesis, Characterization, Anti-Inflammatory And Analgesic Activities Of Some Novel Chalcone Derivatives Derived From 3-((4-Formyl-2-Methoxy Phenoxy) Methyl) Benzonitrile Derivative

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Abstract

A series of novel aryl and aroyl-substituted chalcone derivatives were prepared by condensation of vanillin derivative with different acetophenones. Vanillin derivative (3-((4-formyl-2-methoxyphenoxy) methyl) benzonitrile) was prepared by reacting 3-(bromomethyl) benzonitrile with vanillin. All synthesized compounds (**4a-4h**) were evaluated *in vivo* for their anti-inflammatory and analgesic activity. Among all the tested compounds **4b, 4c, 4e**, **4f** and **4g** have shown remarkable anti-inflammatory activity when compared to other compounds. Compound **4h** has shown moderate anti-inflammatory activity and **4a**&**4d** have shown less anti- inflammatory activity. Among the tested compounds **4a,4b, 4g** and **4h** were found to have significant analgesic activity and compound **4c,4d,4e**,and **4f** had moderate analgesic activity. The newly synthesized chalcone derivatives **4a-4h** were characterized by IR, Mass and ^{1}H NMR spectral data.

Key words: Vanillin, Benzonitrile, Chalcone, Synthesis, Anti-inflammatory Activity, Analgesic Activity.

INTRODUCTION

Eicosanoids are a family of lipid mediators derived from the metabolism of arachidonic acid. Eicosanoids such as prostanoids and leukotrienes have a wide range of biological actions including potent effects on inflammation and immunity. Once liberated from the cell membrane, arachidonic acid may become substrate for various metabolic pathways that produce biological mediators [1,2]. The most important of these pathways are the cyclooxygenase and the lipoxygenase [3].

Inflammation is a multifactorial process. It reflects the response of the organism to various stimuli and is related to many disorders such as arthritis, asthma and psoriasis which require prolonged or repeated treatment. Cyclooxygenase (COX) and lipoxygenase (LOX) produce two groups of arachidonic acid metabolites, prostaglandins (COX products) and leukotrienes (LOX products), that play a key role in inflammation [4]. The classical nonsteroidal anti-inflammatory drugs (NSAIDs) act via the inhibition of the COX-1 isoenzyme or the combined inhibition of COX-1 and COX-2 isoenzymes. For example, aspirin is a COX-1 selective inhibitor, whereas indomethacin and naproxen are COX-1/COX-2 inhibitors. Because COX-1 is mainly responsible for mucus formation in the gastrointestinal (GI) tract, COX-1 inhibition is implicated for inducing GI irritation, the main undesired side effect of such agents [5]. Another side effect, mild bleeding diathesis also results from the selective inhibition of the COX-1 catalyzed synthesis of the platelet aggregation factor, thromboxane A2 [6]. COX-2 isoenzyme is evident to be over-expressed during inflammatory conditions and was also found to exhibit a protective role in asthma [7]. Literature reports reveal that COX isoenzymes are the attractive molecular targets for the development of NSAIDs [8]. Thus, inhibition of these enzymes leads to a decreased production of prostaglandins and thromboxanes which in turn accounts for the beneficial effects of NSAIDs (e.g. anti-inflammatory, antipyretic, analgesic and cardiovascular effects) as well as their undesirable side effect profiles (e.g. GI irritation). It has been hypothesized that there might be other forms of the COX enzyme yet to be discovered. The failure of COX-1 and COX-2 selective inhibitors evoked the concept that inflammation be considered as a multifactorial process and all biochemical pathways should be taken into account [9].

It is well known that many natural products and synthetic compounds act by reducing the active site iron thereby uncoupling the catalytic cycle of the enzyme. Thus, phenols like nordihydroguaretic acid, caffeic acid, flavonoids, coumarins, or compounds like phenidone are efficient 5-LO inhibitors *in vitro* and *in vivo*. [10, 11].

Chalcones (1, 3-diaryl-2-propen-1-ones) are a chemical class that has shown promising therapeutic efficacy for the management of several diseases [12]. Many papers have been presented in the literature with references to structural modifications of the chalcone template [13]. They are well known intermediates for synthesizing various organic and heterocyclic compounds. The compounds with the backbone of chalcones have been reported to possess various biological activities such as antimicrobial[14,15], antifungal[16], anti-inflammatory[17], analgesic[18], antiplatelet[19], antimalarial[20], anticancer[21], antiviral[22], antioxidant[23], antitubercular[24],and inhibition of tyrosinase[25] activities. The presence of a reactive α,β-unsatutated keto function in chalcones is found to be responsible for their antimicrobial, antifungal, anti-inflammatory and analgesic activity. They considered as the precursor of flavonoids and isoflavonoids. Chemically they consisted of openchain flavonoid by a three carbon α,β -unsaturated carbonyl system. [26] Recently much attention has been paid on the synthesis of chalcones mainly from acetophenones and aromatic aldehydes by Claisen-Schmidt condensation.

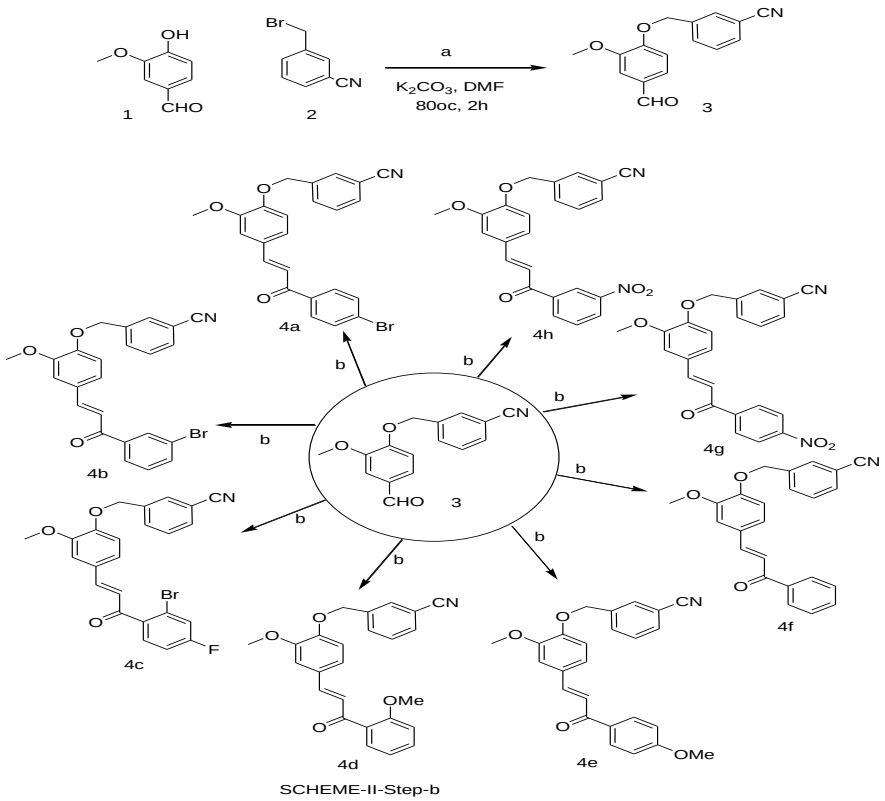
In the present communication we report the reaction of various acetophenone derivatives with different aromatic aldehyde derivatives to form chalcones (4a-4h). Encouraged by the broad range of biological applications of chalcones, the present research work is aspired to describe the synthesis, characterization and anti-inflammatory and analgesic activity of novel chalcone derivatives **4a-4h** derived from vanillin.

The suggested structural variations could affect both efficacy and their tolerability partly due to differences in their physicochemical properties, which determine their distribution in the body and their ability to pass through and to enter in the interior membranes. [27, 28].

2 RESULTS AND DISCUSSIONS

2.1 Chemistry

The newly synthesized chalcone derivatives **4a–4h** described in this paper were prepared according to the synthetic **Scheme 2.** Vanillin derivative (3-((4-formyl-2-methoxyphenoxy) methyl) benzonitrile) **(3)** was prepared by reacting 3-(bromomethyl) benzonitrile **(2)** with vanillin**(1)** in presence of DMF containing K_2CO_3 at 80^{oc}, for 2 hours. Claisen-Schmidt condensation of Vanillin derivative (3-((4-formyl-2-methoxyphenoxy) methyl) benzonitrile) **(3)** with different acetophenones **(a-h)** was carried out in presence of sodium hydroxide in methanol at room temperature for 2h to obtain chalcone derivatives (**4a-4h)** in 98% yield. The structures of the synthesized compounds were confirmed by ^{1}H NMR, IR and Mass data. All the aliphatic and aromatic protons were observed at expected regions. The ^{1}H NMR data for the derivatives **4a – 4h** is in agreement with the assigned structures. The mass spectra of
compounds **4a-4h** showed (M+1) peaks, in agreement with their molecular formula.



SCHEME-II-Step-b

Scheme 1. Synthesis of Novel Chalcone derivatives **4a-4h**

Experimental Conditions: a) Vanillin, 3-(bromomethyl)benzonitrile, K_2CO_3, DMF, rt, 2h b) Benzonitrile derivatives, Acetophenones, NaOH, MeOH, rt, 2h.

2.2 Biological Activity

2.2.1 Anti-inflammatory Activity

The newly prepared chalcone derivatives 4a-4h were screened for anti-inflammatory activity at concentration 10mg/kg. Among all tested compounds, **4a, 4b, 4c, 4e** and **4f** exhibited maximum activity, while compounds 4d and 4g showed moderate activity and the remaining compound **4h** weak activity when comparable with standard anti-inflammatory agent indomethacin. In general, it is observed from **Table 1** that the compounds having 4-bromophenyl, 3-bromophenyl, 2-bromo-4-fluorophenyl, 4-methoxyphenyl and 3-phenyl exhibited excellent anti-inflammatory activity and remaining compounds showed moderate activity. As all the tested compounds emerged as active against inflammation, it indicates that this basic moiety can be a promising scaffold for anti-inflammatory drugs. It may be suggested that the chalcone derivatives with suitable R group may lead to a good anti-inflammatory agent. However, this is a very promising preliminary study and further evaluation is needed to use them for clinical use.

Table 1: Anti-inflammatory activity data of the chalcone derivatives (4a-4h) on carrageenan-induced paw edema in rats.

Groups	Dose	1 hr	2 hr	3 hr	4 hr	5 hr
Disease control	Vehicle	0.43±0.021	0.56±0.021	0.7±0.036	0.76±0.021	0.8±0.000
Standard	10mg/kg	0.38±0.016 ns (11.6%)	0.4±0.000*** (28%)	0.36±0.021*** (47%)	0.26±0.033*** (65%)	0.2±0.000*** (75%)
4a	10mg/kg	0.41±0.016 ns (4.6%)	0.48±0.04 ns (14%)	0.4±0.036*** (42.8%)	0.31±0.03*** (58.6%)	0.26±0.021*** (66.7%)
4b	10mg/kg	0.38±0.016 ns (11.6%)	0.41±0.016*** (26.7%)	0.38±0.016*** (45%)	0.28±0.040*** (63%)	0.23±0.021*** (70.8%)
4c	10mg/kg	0.4±0.025 ns (6.9%)	0.46±0.021 ns (17.8%)	0.41±0.016*** (40%)	0.3±0.036*** (60.8%)	0.25±0.022*** (68.7%)
4d	10mg/kg	0.41±0.016 ns (4.6%)	0.45±0.022 ns (10.7%)	0.43±0.021*** (38%)	0.33±0.021*** (56.5%)	0.3±0.025*** (62.5%)
4e	10mg/kg	0.4±0.000 ns (6.9%)	0.43±0.021** (23%)	0.41±0.016*** (40%)	0.3±0.025*** (60.8%)	0.25±0.022*** (68.5%)
4f	10mg/kg	0.38±0.016 ns (11.6%)	0.43±0.021** (23%)	0.4±0.000*** (42.8%)	0.3±0.025*** (60.8%)	0.26±0.021*** (66.7%)
4g	10mg/kg	0.41±0.03 ns (4.6%)	0.46±0.021ns (17.6%)	0.43±0.021*** (38%)	0.33±0.021** (56.5%)	0.28±0.016*** (64.5%)
4h	10mg/kg	0.4±0.000 ns (6.9%)	0.48±0.03ns (14%)	0.43±0.021*** (38%)	0.35±0.022*** (54.3%)	0.31±0.016*** (60.4%)

All the values were expressed as mean ±sem, n=6. A significant difference with respect to disease control groups was evaluated by ANOVA, Tukey's test.

* p <0.05, **p <0.01, ***p <0.001, ns p>0.05 when compared with Disease control. Statistically significant when compared to disease control.

2.2. Analgesic Activity

The newly prepared chalcone derivatives 4a-4h were screened for analgesic activity by using hot plate method. Among all tested compounds, 4a, 4b, 4g and 4h showed maximum activity, while compounds 4c,4d,4e and 4f showed moderate activity when comparable with disease control. In general, it is observed from **Table 2** that the compounds having 4-bromophenyl, 3-bromophenyl, 4-nitrophenyl, and 3-nitrophenyl exhibited excellent analgesic activity and remaining compounds showed moderate activity. As all the tested compounds emerged as active against analgesia, it indicates that this basic moiety can be a promising scaffold for analgesic drugs. It may be suggested that the chalcone derivatives with suitable R group may lead to a good analgesic agent. However, this is a very promising preliminary study and further evaluation is needed to use them for clinical use.

Table-2: Analgesic activity of the chalcone derivatives (**4a-4h**) on hot plate method in albino mice

S.no	0min	30min	60min	90min	120 min	180min
Disease control	4.66±0.33	**4.66**±0.21	**5.33**±0.33	5.33±0.49	**8.5**±0.22	**5**±0.51
03-101	8.3± .21***	**18.1**±0.30***	18.6±0.20***	46.83±0.36***	71.8±0.30***	17.5±0.22***
03-102	**8.5**±0.22***	**18.5**±0.22***	**18.6**±0.21***	**46.83**±0.40***	**73**±0.00***	**16.83**±0.30***
03-103	**6.16**±0.16***	**10.5**±0.34***	**17.83**±0.30***	**23.16**±0.54***	**23.5**±0.34***	**44.66**±0.33***
03-104	7.5±0.22***	11.0±0.36***	38±0.36***	51.5±0.42***	23.1±0.47***	36.5±0.22***
03-105	**7.5**±0.22***	**11.3**±0.33***	**38.16**±0.30***	**51.16**±0.54***	**23.66**±0.42***	**36.3**±0.21***
03-106	**14.83**±0.16***	**10.3**±0.33***	**63.33**±0.42***	**80.66**±0.49***	**23.33**±0.42***	**91.5**±0.88***
03-108	**32.0**±0.44***	**53.3**±0.33***	**53.5**±0.50***	**55.33**±0.42***	**69.33**±0.21***	**62.16**±1.04***
03-110	31.33±0.49***	**52.8**±0.30***	53.5±0.22***	54.66±0.21***	69±0.36***	62.5±0.95***

P value *p<0.05,p<0.01,***p<0.001 followed by Dunnet's test Compare with disease control**

3 Conclusion:

In conclusion, the present paper describes the synthesis, spectroscopical characterization and anti-inflammatory and analgesic activities of eight novel chalcones derivatives (prepared from commercially available vanillin). The newly synthesized novel chalcone derivatives (**4a-4h**) were screened for in vivo anti-inflammatory and analgesic activities. Within the chalcone derivatives **4a-4h**, The compounds incorporated with the substituents such as with4-bromophenyl,3-bromophenyl, 2-bromo-4-fluorophenyl, 4-methoxyphenyl, and 3-phenyl, were found to have good anti-inflammatory activity while the remaining compounds exhibited equipotent (**4d & 4g**) or moderate (**4h**) anti-inflammatory activity, which is on a par with indomethacin. Within the chalcone derivatives **4a-4h**, The compounds incorporated with the substituents such as with 4-bromophenyl, 3-bromophenyl, 4-nitrophenyl and 2-nitrophenyl exhibited excellent analgesic activity, while the remaining compounds displayed equipotent (**4c, 4d, 4e, and 4f)** analgesic activity.

In silico binding studies:

Docking with COX-2

The structural relation of synthesized chalcone derivatives **4a–4h** with the binding of cyclooxygenase-2 (PDB ID: 1CX2) protein was identified using molecular docking. From the preliminary anti-inflammatory results, the synthesized compounds were examined for docking with target protein co-crystalized with 1CX2. The ligands exhibited good binding affinities towards target protein. Both hydrogen and hydrophobic interactions were played crucial role and were influenced the docking results. Nearly ten different docking conformations per each ligand was generated and the best conformation was showed in the Figure 1. The docking results revealed that the all docked ligands were occupied the same binding site of the co-crystalized ligand (1CX2) binding site. Further, the ligands **4b** and **4c** exhibited -10.22, and -7.88 Kcal/mol binding energies, respectively.. The co-crystal ligand 1CX2 exhibited -9.74 Kcal/mol binding energy. The amino acid residue Trp355 in the active site plays a major role in the interaction with all the tested ligands. The amino acid residues in the active site of the target protein interacted with the docked ligands were presented in the Table 3.

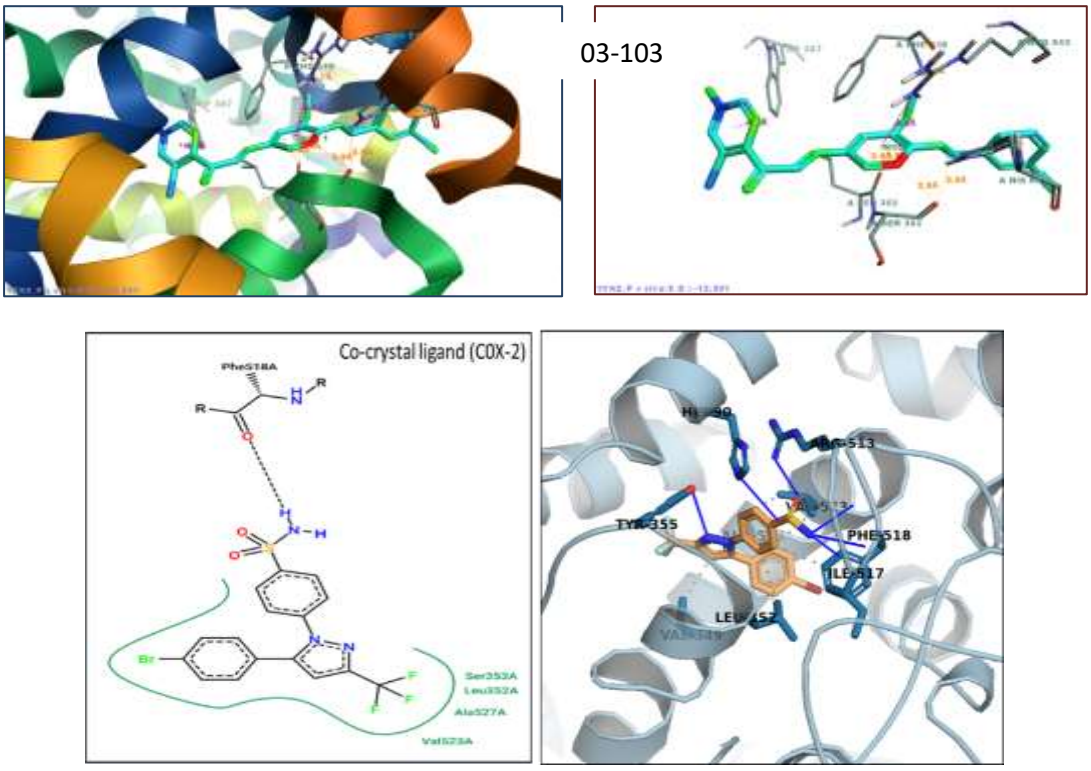


Figure 1: In silico binding interactions of the active ligands towards the active site of COX-2 protein (PDB ID: 1CX2). The best docked pose for active compounds was represented in the image. The co-crystal ligand was used for the results validation.

Table 3: Binding energies of the target protein COX-2 protein (PDB ID: 1CX2) with docked ligands.

Compound Code	LF Rank Score	LF dG	LF Vscore	LF LE
03-101	-5.98	-8.61	-10.84	-0.29
03-102	-5.98	-10.22	-12.42	-0.35
03-103	-12.33	-7.88	-11.02	-0.26
03-104	-6.52	-8.08	-10.58	-0.26
03-105	-5.48	-7.24	-10.25	-0.24
03-106	-5.98	-7.7	-9.94	-0.27
03-108	-5.41	-7.05	-9.01	-0.22
03-110	-6.8	-8.58	-10.84	-0.27
Co-crystal ligand	-10.25	-11.02	-9.25	-0.09

4. Experimental

4.1 General

The solvents were purified according to standard procedures prior to use, and all commercial chemicals were used as received. For thin-layer chromatography (TLC) analysis, Merck pre-coated Plates (silica gel 60 F254) were used and spots were visualized with UV light. Merck silica gel 60 (230-400 mesh) was used for flash column chromatography and the eluting solvents are indicated in the procedures. ^{1}H NMR spectra were recorded in Varian MR-400 MHz instrument. Chemical shifts are reported in δ parts per million (ppm) downfield from tetramethylsilane (TMS) with reference to internal standard and the signals were reported as s (singlet), d (doublet), dd (doblet of doblet), t (triplet), q (quartet), m (multiplet) and coupling constants in Hz. ^{13}CNMR .The mass spectra were recorded on Agilent ion trap MS. Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR spectrometer.

4.2 Synthesis

4.2.1 Preparation of 3-((4-formyl-2-methoxyphenoxy) methyl) benzonitrile (3) from Vanillin (1) with 3-(bromomethyl) benzonitrile (2).

Vanillin was used as a starting material for the preparation of chalcones. To the DMF solution containing Vanillin 1(0.05g mg, 0.32 mmol), K_2CO_3 (0.045g, 3.25 mmol) was added followed by 3-(bromomethyl) benzonitrile (2) (0.071g, 0.35 mmol) and the reaction mixture was stirred at 80^{o}c, for 2hours. After the reaction reached completion, the reaction mixture is cooled to room temperature and filtered the precipitated solids and washed with pet-ether, to obtain the pure compounds (3). The yield of the product was to be 95%. The the 3-((4-formyl-2-methoxyphenoxy) methyl) benzonitrile (3) has been synthesized and the quantified data along with the spectral data is presented.

White solid; Yield:95%; m.p:88^{o}c; IR (KBr): υ_{max} 3448, 3348, 2973, 2824, 2739, 2229, 1680, 1590, 1517, 1451, 1425, 1386, 1341, 1318, 1275, 1244, 1177, 1125, 1029, 887, 870, 816, 796, 785, 683, 639, 619, 551cm^{-1}; ^{1}H NMR(400MHz, DMSO-d_6): δ 9.85(s,1H), 7.94(s,1H), 7.84(d, *J*=7.6Hz,1H), 7.80(d, *J*=8.0Hz,1H), 7.64(t, *J*=8.0Hz,1H),7.55(dd, *J*=2.0,8.4Hz,1H), 7.44(d, *J*=1.6Hz,1H), 7.26(d, *J*=8.0Hz,1H); MS: m/z, 267.8 (M+1).

4.2.2 General Experimental procedure for the Synthesis of Chalcone derivatives (4a-4h)

Acetophenone was added to the solution mixture of methanol and sodium hydroxide, containing 3-((4-formyl-2-methoxy phenoxy) methyl) benzonitrile (3) (0.2 mmol) and the contents were stirred at room temperature for 2hours. After the reaction reached completion, the reaction mixture is cooled to room temperature and filtered to get the precipitated solids and washed with pet-ether, to obtain the pure Chalcone compounds **(4a-4h)**. The yield of the product varied from 90 – 96%.

4.2.2.1. (E)-3-((4-(3-(4-bromophenyl)-3-oxoprop-1-enyl)-2-methoxyphenoxy)methyl) benzonitrile (4a). Light Yellow solid; Yield:94%; m.p:178^{o}c; IR (KBr):υ_{max} 3432,3063, 2227, 1658,1592, 1513, 1421, 1386,1310,1268,1214,1168, 1137,1070,1035, 1008,824, 789, 683,554, 464 cm^{-1}; ^{1}H NMR (400MHz, DMSO-d_6): δ 8.09 (d, J=8.4Hz,2H),7.92(s,1H),7.85-7.60(m,5H) ,7.65-7.60 (m,3H), 7.40(d, J=7.6Hz, 1H), 7.13(d,J=8.4Hz,1H),5.23(s,2H),3.89(s,3H) ; ^{13}CNMR (DMSO-d_6); 188.158, 149.958, 149.316, 144.969, 138.498, 136.786,132.661, 131.821, 131.253, 130.529, 129.846, 128.067,127.137, 125.853, 123.935, 119.563, 118.699, 113.265, 112.763, 111.454, 111.256, 109.840, 68.710, 55.883, 55.644; MS: m/z,448.40(M+1).

4.2.2.2. (E)-3-((4-(3-(3-bromophenyl)-3-oxoprop-1-enyl)-2-methoxyphenoxy) methyl) benzonitrile (4b). Light Yellow solid; Yield:96%; m.p:179^{o}c; IR (KBr):υ_{max} 3442,3061, 2923, 2227, 1655,1589, 1578, 1561, 1510,1459,1426,1390, 1312,1268,1234, 1210,1171, 1146,1031, 979,851 ,793,686, 631,555 ,449 cm^{-1}; ^{1}H NMR(400MHz, DMSO-d_6): δ 8.27(s,1H),8.15 (d,J=7.6Hz,1H), 7.92 (s,1H), 7.88-7.80 (m,4H), 7.76(d,J=12.0Hz,1H),7.65-7.53(m,3H) , 7.43 (d,J=8.0Hz,1H), 7.14 (d,J=8.0,1H), 5.28(s,J-17.6Hz, 2H), 3.90(d,J=14.0Hz,3H) ; ^{13}CNMR (DMSO-d_6);187.746, 150.024, 149.299, 145.290, 139.857, 138.490, 135.625, 132.661, 131.821, 131.253, 131.015, 130.875, 130.002, 129.837, 128.026, 127.516, 123.984, 122.321, 119.489, 118.690,113.265, 111.503, 111.446, 68.710, 55.924; MS: m/z, 448.40(M+1).

4.2.2.3. (E)-3-((4-(3-(2-bromo-4-fluorophenyl)-3-oxoprop-1-enyl)-2-methoxyphenoxy) methyl) benzonitrile (4c). Light Yellow solid; Yield:93%; m.p:175^{o}c; IR (KBr):υ_{max} 3444, 3066, 2922,2229, 1680, 1655,1591,1512,1425,1388,1312,1268,1200,1172,1147, 1030, 801, 684,672,614,555,447 cm^{-1}; ^{1}H NMR(400MHz, DMSO-d_6): δ 8.48(d, J=4.0Hz,1H), 8.24 (d,J=4.0Hz,1H),7.93-7.80 (m,4H), 7.76 (d,J=15.6Hz,1H),7.65-7.55(m,3H), 7.44(d,J=7.2Hz,1H), 7.14(d,J=8.0Hz,1H), 5.24 (d,J=17.2Hz, 2H),3.89(d,J=13.6Hz,3H) ; MS: m/z, 466.40(M+1).

4.2.2.4. (E)-3-((2-methoxy-4-(3-(2-methoxyphenyl)-3-oxoprop-1-enyl)phenoxy)methyl) benzonitrile (4d). Pale Yellow solid; Yield:94%; m.p:95^{o}c; IR (KBr):υ_{max} 3443,3071,2942, 2231, 1680,1649, 1594, 1509,1465,1422,1382, 1316,1265,1243,1200,1148,1112,1028, 863, 810,784, 753, 681,629,540,447 cm^{-1}; ^{1}H NMR(400MHz, DMSO-d_6): δ 7.91 (d,J=8.0 Hz, 1H), 7.85-7.78 (m,2H),7.66-7.61 (m, 1H),7.56-7.50(m,1H), 7.43 (d,d,J=1.6, 4.0Hz, 1H), 7.39 (t, J=3 2Hz, 2H),7.31 7.25(m,2H), 7.19 (d,J=8.0Hz,1H), 7.09-7.03(m,2H),5.21(d,J=28Hz,2H), 3.84 (t,J=3.6 Hz, 6H) ; ^{13}CNMR (DMSO-d_6); 192.562, 191.426, 157.433, 152.732, 149.604, 149.291, 143.265, 138.515, 138.070, 132.554, 131.764,

131.155, 129.788, 129.228, 128.026, 125.342, 122.741, 120.485, 118.666, 113.397, 112.738, 112.269, 111.429, 111.190, 109.840, 68.685, 55.751, 55.710; MS: m/z, 400.48(M+1).

4.2.2.5.(E)-3-((2-methoxy-4-(3-(4-methoxyphenyl)-3-oxoprop-1-enyl)phenoxy)methyl) benzonitrile(4e). White Solid; Yield:96%; m.p:110ºc; IR (KBr):υ_{max} 3444,3076,3010, 2835, 2229, 1658, 1606,1596,1457,1421,1385,1356,1309,1260,1217,1164,1031,972,825, 798,684, 604,552, 449 cm^{-1}; ^{1}H NMR(400MHz, DMSO-d_6): δ 8.16(d, J=8.0Hz,2H),7.92(s,1H),7.86-7.80(m,3H),7.69-7.57 (m,3H),7.37 (d, J=8.0Hz,1H),7.12-7.02(m,3H),5.23(s,2H), 3.87 (t, J=8.0 Hz,7H) ; ^{13}CNMR (DMSO-d_6); 187.260, 163.106, 149.621, 149.291, 143.487, 138.548, 132.645, 131.805, 131.270, 131.188, 130.883, 130.644, 129.813, 128.314, 123.506, 119.933, 118.699, 113.981, 113.290, 111.437, 111.100, 68.693, 55.850, 55.562; MS: m/z, 400.48(M+1).

4.2.2.6. (E)-3-((2-methoxy-4-(3-oxo-3-phenylprop-1-enyl)phenoxy)methyl)benzonitrile(4f) ; White Solid. Yield:91%; m.p:160ºc; IR (KBr):υ_{max} 3443,3062,2835,2229,1659, 1590,1573, 1510, 1422,1388,1310, 1268,1215,1168,1138,1035,1019,850,771, 685,633, 599,554,447 cm^{-1}; ^{1}H NMR (400MHz, DMSO-d_6):δ 8.14(d,J=4. 0Hz,2H), 7.92-7.80(m,4H),7.73-7.62(m,3H),7.59-7.56 (m,3H),7.39(d,J=8.0Hz,1H), 7.13 (d,J=8.0Hz, 1H),5.23(s,2H),3.89(s,3H) ; ^{13}CNMR (DMSO-d_6); 189.055, 149.810, 149.316, 144.385, 138.515, 137.798, 132.982, 132.620, 131.797, 131.212, 129.813, 128.742, 128.463, 128.158, 123.720, 119.958, 118.674, 113.281, 111.437, 111.190, 68.701, 55.858; MS: m/z, 370.47(M+1).

4.2.2.7. (E)-3-((2-methoxy-4-(3-(4-nitrophenyl)-3-oxoprop-1-enyl)phenoxy)methyl) benzonitrile (4g); Dark Brown Solid. Yield:95%; m.p:175ºc; IR (KBr):υ_{max}3444,3110,2954, 2227,1693, 1656,1582, 1513, 1423,1348,1318,1268,1212, 1156,1034,986, 844,801, 705,684, 556,446 cm^{-1}; ^{1}H NMR (400MHz, DMSO-d_6): δ 8.40-8,34(m,4H),7.92-7.74(m,5H),7.65-7.60 (m,2H), 7.43(d,J=4.0Hz,1H), 7. 15 (d,J=8.0Hz,1H), 5.24 (s,2H),3.89(s,3H) ; ^{13}CNMR (DMSO-d_6); 188.199, 150.213, 149.719, 149.308, 146.015, 142.672, 138.432, 132.636, 131.813, 131.237, 129.813, 129.788, 127.870, 124.190, 123.828, 119.719, 118.666, 113.240, 111.437, 111.380, 68.701, 55.867; MS: m/z, 415.47(M+1).

4.2.2.8.(E)-3-((2-methoxy-4-(3-(2-nitrophenyl)-3-oxoprop-1-enyl)phenoxy)methyl)benzonitr ile (4h). Pale Green Solid; Yield:93%; m.p:210ºc; IR (KBr): υ_{max} 3444,3080,2922, 2229,1681,1658, 1591, 1512, 1424,1349,1315,1270,1214,1156,1084,1032,986,800,691,554,450 cm^{-1}; ^{1}H NMR (400MHz, DMSO-d_6): δ 8.79(s,1H),8.61(d,J=8.0Hz,1H), 8.49(d, d, J=1.6,8.0 Hz, 1H), 7.947.78(m,6H),7.62(t, J=8.0Hz,2H), 7.46(d, J=4.0Hz,1H),7.15(d, J=8.0Hz,1H), 5.24 (s,2H), 3.89(s,3H) ; ^{13}CNMR (DMSO-d_6); 191.476, 152.749, 149.406, 146.023, 139.074, 138.465, 134.703, 132.744, 131.953, 130.595, 129.887, 127.186, 125.845, 124.157, 122.741, 112.771, 111.487, 68.850, 55.932, 55.644, 12.307; MS: m/z, 415.47(M+1).

4.3. Biological experiments

4.3.1. Anti-inflammatory activity: by carrageenan-induced rat paw edema assay

In vivo anti-inflammatory [29,30] screening for the synthesized compounds was performed by using the functional model of carrageenan-induced rat paw edema and is presented as the percentage inhibition of edema at the right hind paw in comparison to the disease control (Table 1). Carrageenan-induced edema is a nonspecific inflammation but is highly sensitive to NSAIDs. Indomethacin, a potent NSAID was used as a reference standard.

Wistar albino rats (Either sex) weighing between 160 and 220 g, were used in the present study. Animals were kept in wire-mesh cages and maintained under constant environmental conditions [23± 2˚C, 12 h, light]. All animals had free access to feed and water (ad libitum), in a constant light dark cycle. During the course of experiment, the general behavior of animals was normal. All the experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC) and experiments were conducted according to the standard guidelines in Albino research & Training Institute, Bachupally, Hyderabad, Telangana State, India. Carrageenan and indomethacin were procured from Himedia Laboratories Pvt Ltd, Mumbai and Oxford Laboratory, Mumbai. For statistical analysis we have used Graph Pad Prism 3.0 version [31].

The synthesized compounds were tested for their anti-inflammatory activity against carrageenan-induced paw edema at dose of 10 mg/kg. The percentage inhibition of edema was calculated using the formula given below:

$$\frac{Vc \_ Vt \times 100}{Vc}$$

where Vc is the increase in paw volume of control (in the absence of test compound) and Vt is the increase in paw volume after administration of the test compound.

The given data is of six animals (Either sex wistar albino rats) per group and divided into eleven groups. Group-1treated as Normal control(animal received normal saline(0.9%w/v NaCl)] and Group-2 referred as disease control [(animals received 0.1ml carrageenan along with the vehicle [1% carboxymethylcellulose (CMC)]) and Group-3 received standard reference (indomethacin) along with the vehicle prior to the administration of carrageenan. Group-4, 5, 6, 7,8,9,10 and 11 received the test compounds (4a-4h) respectively half an hour h prior to the administration of carrageenan. All the test

compounds were suspended in 1% of CMC and administered orally (10 mg/kg) half an hour h prior to the injection of 0.1 ml of freshly prepared carrageenan (1%) in physiological normal saline solution (0.9%w/v NaCl) into the sub-planter tissue of hind paw of each rat.

The equivalent volume of carrageenan (1%) in physiological normal saline solution (0.9%w/v NaCl) was injected into hind paw of the disease control. The inflammation of the paw was measured for all the animals using plethysmograph before the administration of the carrageenan and after the administration of the carrageenan at 60,120,180,240 and 300min. The increase in volume of the paw was adopted as a measure of edema [32]. The percentage protection of the compounds was calculated and presented in Table I.

The antiedematous effects of the compounds were estimated as percentage inhibition of the induced inflammation in comparison with disease control. Statistical analysis was carried out using a one-way analysis of variance (ANOVA). In all cases, post-hoc comparisons of the means of individual groups were performed using Tukey's test. A significance level of $p< 0.001$ denoted significance in all cases.

4.3.2. Analgesic activity:

Hot Plate reaction Time in Mice

The animals were placed individual in Hot plate regulated at temperature (55±0.5ºC) before the Treatment & its reaction time was determined. After noting the initial reaction time, the treatment should be given to each mice. Then the each animal was placed on the Eddy's hot plate regulated temp. to obtain animal response licking of the forepaws or jump of the Hot plate surface was recorded as the hot-plate latency. Mice with baseline latencies of <5s or >30s were eliminated from the study. The reaction time is noted by stop-watch and then the reaction time was re-determined after 0, 30, 60, 90, 120 & 180 mints. after oral administration of standard and test drug [33].

Experimental Animal:

Male albino mice weighing between 20-25 gm were selected for the analgesic activity was housed under the uniform laboratory condition fed with commercial diet & provided with waterad-libitum, during the experiment. The animals were procured from Gwalior & permitted for the study under the Institutional Animal Ethical Committee (IAEC).

All protocols of the study were approved by the Institutional Animal Ethical Committee with reference number ARTI/2014. The IAEC is approved by committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Molecular Docking

Molecular binding of synthesized compounds with respect to inhibit the activity of cyclooxygenase-2 protein (PDB ID: 1CX2), we achieved *in silico* docking in the active site of the target protein using AutoDockTools 4.2 [Ghanbari et al., 2019]. The 3D crystal structure of target protein (PDB ID: 1CX2) was downloaded from RCSB Protein Data Bank and used as the model for docking. The water molecules and co-crystalized hetero molecules were removed from the target protein. The ligand structures were built using ChemDraw ultra 19.0 software and subsequently converted to 3D structures and saved in .pdb format. Further, the ligand energies were minimized using MOPAC (semiempirical quantum mechanics) with AM1 MOZYME geometry acceleration with 100 iterations, and RMS gradient of 0.10. For each docked ligand ten confirmations were generated. Structure with relative lower binding free energy (Kcal/mol) was selected as the best conformation among all the poses. In order to validate the results, the co-crystal ligand was redocked with the protein

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