Design, Synthesis, Biological Evaluation of Novel Imidazo (2,1-C)(1,2,4)-Triazine Derivatives As Cox-1 And Cox-2 Inhibitors

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Abstract:

In the field of Medicinal chemistry, Triazines are heterocyclic compounds with a wide range of biological activities that have recently received attention because to their adaptable structure (three isoforms) and the enormous number of derivatives that may be generated from them to give functional models. The significance of triazines was highlighted by several literature reports as a source of potential candidates for the treatment of new and emerging diseases. The aim of this research work is to synthesize some new substituted triazines by synthetic methodologies and to evaluate the biological activity. The present study focuses to design new 3,4,7-tri substituted imidazo(2,1-c)(1,2,4) triazines. The structures of the compounds were established by IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies. For all the newly synthesized compounds molecular docking studies were carried out and also evaluated for in vivo anti inflammatory and analgesic activities. All the synthesized novel heterocyclic derivatives were found to have broad spectrum of biological activities.

Keywords: *Imidazo(2,1-c)(1,2,4) triazines, molecular docking, anti-inflammatory activity, and analgesic activity.*

1 Introduction:

Inflammation is a defensive response primarily governed by the immune system, which dispatches white blood cells to the affected sites, resulting in redness, swelling, and other symptoms such as fever. The inflammatory response is an innate system of cellular and humoral responses following injury (such as after exposure to heat or cold, ischemia/reperfusion, blunt trauma, etc.) in which the body attempts to restore the tissue to its pre-injury state [1].

Underlying a vast array of physiological and pathological processes is inflammation. Although the pathological aspects of numerous types of inflammation are well understood, their physiological functions remain largely unknown[2]. Multiple pathophysiological pathways, such as cytokines, interleukins, Nf-kB, protein kinases, protein kinases (Adenosine Monophosphate-activated protein kinase-AMPK), tyrosine kinases, and immunological responses, regulate and mediate the inflammatory process [3-6].

Activating the NF-B/Rel transcription family through nuclear translocation of cytoplasmic complexes plays a central role in inflammation by inducing the transcription of proinflammatory genes[7].

First purified in 1976 and cloned in 1988, cyclooxygenase (COX) is the essential enzyme in synthesizing prostaglandins (PGs) from arachidonic acid. Multiple laboratories identified COX-2 in 1991, the product of a second gene with COX activity.COX is primarily involved in the inflammatory process and is the target of the majority of NSAIDs (non-steroidal anti-inflammatory drugs).COX-1,ubiquitously localized in tissues, constitutive (activated by physiological stimuli), is responsible for homeostatic functions, such as gastric secretions [8]. In contrast, the inducible (by pro inflammatory stimuli) isoform, COX-2, located in inflammatory and neoplastic sites, is involved in the development and progression of numerous pathological conditions, such as inflammation, arthritis, and colon cancer [9].

Previously, 1,2,4-triazine derivatives were reported as inhibitors of various enzymes involved in the inflammatory process [10], allergic reactions (h3 receptor inhibition)[11], and also in the tumor-suppressing process by interacting with DNA, tubulin and thus acting as anticancer agents [12].Moreover, triazine derivatives exhibited remarkable antimicrobial and antifungal properties[13].The design and synthesis of Imidazo(2,1-c)(1,2,4)-triazine derivatives resulted from our desire to discover novel substituted triazine derivatives with anti microbial, anti-inflammatory and analgesic activity.

2 Materials and Methods:

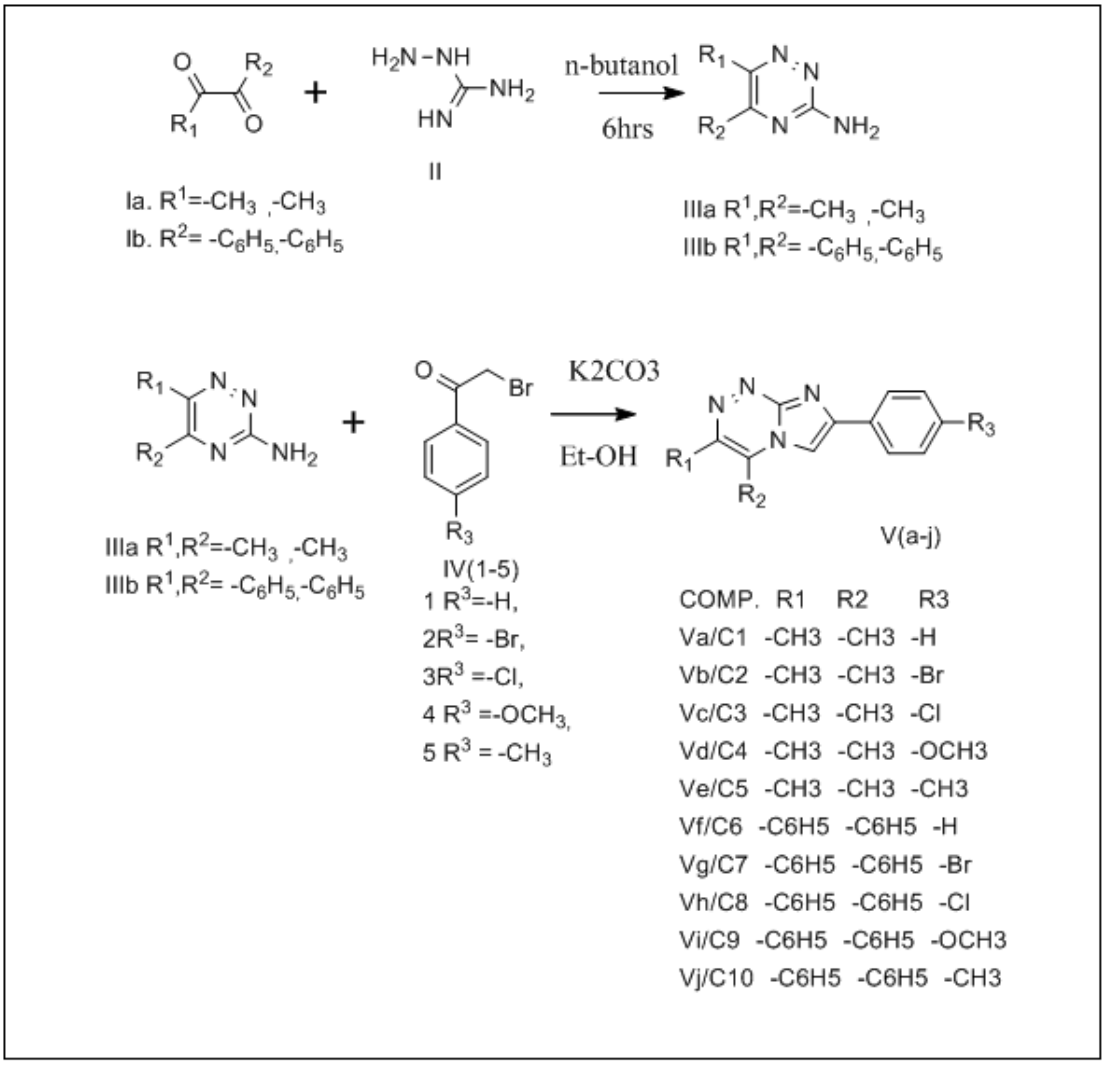
2.1: General

All chemicals and dry solvents were purchased from the local manufacturers and S.D Fine Chem. Ltd, Mumbai, India. All the chemicals used in the synthesis were obtained from standard commercial sources. All the reactions were carried out in dried glassware under an atmosphere of nitrogen and were monitored by thin layer chromatography (TLC) carried out on E. Merck silica gel plates (60 F254) with UV light, iodine as probing agents. Column chromatography separation was performed using Avra Synthesis Pvt. Ltd. Silica gel 60, 0.140-0.25 mm (60-120 mesh) using combination of Ethyl acetate and Hexane. Melting points were determined on an Digital melting point apparatus (Jain Scientific glass works) by open capillary method and are uncorrected. 1H and C NMR were recorded in DMSOd6 or CDCl3 on Varian 400 MHz using TMS as internal standard. The chemical shifts were reported in ppm (δ) and coupling constants (J) values were given in Hertz (Hz). Mass spectra recorded on LCMS 2O1OA, SHIMADZU,

JAPAN. Infrared (IR) spectra were recorded on a BRUKER FTIR or IR Prestige-21, SHIMADZU. The names of all compounds given in the experimental section were taken from Chemdraw Ultra, Version 12.0.

2.2 Chemistry of 3,4,7-tri substituted imidazo(2,1-c)(1,2,4) triazine derivatives :
General procedures for the synthesis of 3,4,7-TRI SUBSTITUTED IMIDAZO(2,1-C)(1,2,4) TRIAZINE DERIVATIVES IV (a-j) /**C1-C10** it invoIves two steps

SCHEME: I



2.2.1 Synthetic procedures for scheme: I

Synthesis of 3,4,7-TRI SUBSTITUTED IMIDAZO(2,1-C)(1,2,4) TRIAZINE DERIVATIVES IV (a-j) /C1-C10 it invoIves two steps

2.2.2 STEP-1: Synthesis of 5,6-di substituted 1,2,4-triazin-3-amine (III):

"Under nitrogen atmosphere, 0.1 moles of di methyl glyoxal (Ia) /di phenyl glyoxal (IIb) and 21.02 g (0.1mol) of amino guanidine hydrogen carbonate were added to a solution of 50 ml of n- butanol. Then the reaction mixture was subjected for intensive stirring, refluxed for 6 hours. Completion of the reaction monitored by TLC (60%Ethyl acetate: Hexane). Then cool the reaction mixture to room temperature. The precipitate was filtered with suction and washed with a mixture of diethyl ether- hexane (1:1).After drying in a vacuum the yield of the product is 97% (24.05gm). Purify the residue by column chromatography to obtain pure 5,6-diphenyl 1,2,4-triazin-3-amine IIIa and IIIb

2.2.3 STEP-II synthesis of 3,4,7-tri substituted Imidazo (2,1-c)(1,2,4) triazine derivatives (Va-Vj) :

"Under nitrogen atmosphere,5,6-di substituted 3-amino-1,2,4-triazine (100 mg, 1,04 mmol) (IIIa and IIIb)and K_2CO_3 (173 mg, 1,25 mmol) were added to a solution of 2-bromo(substituted) acetophenone (1,14 mmol)IV (1-5) in EtOH (2 mL) The resulting solution was heated to reflux for 6 h and then cooled down to r.t.
After concentration under reduced pressure, the residue was diluted with CH_2Cl_2 (10 mL), the CH_2Cl_2 layer was washed with H_2O (2 × 10 mL), and dried ($MgSO_4$).
After filtration and concentration under reduced pressure, the crude was purified by column chromatography on silica gel (EtOAc–PE, 1:9 to 4:6) to obtain pure form of desired titled compounds 3,4,7-trisubstituted Imidazo[2,1-c][1,2,4] triazines (Va-Vj)/C1-C10

2.3: Docking methodology:

Molecular binding of synthesized compounds C1-C10with respect to inhibit the activity of cyclooxygenase-2 protein (PDB ID: 1CX2) and COX-1(PDB ID: 1EQG), we achieved *in silico* docking in the active site of the target protein using Auto Dock Tools 4.2

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 Chem Draw ultra 19.0 software and subsequently converted to 3D structures and saved in .pdb format.

Further, the ligand energies were minimized using MOPAC (semi empirical 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 re docked with the protein.

2.4 Biological Activity:

2.4.1 Acute toxicity studies:

All the test compounds C1-C10 with a dose of 2000 mg/kg delivered no harmful impact on the social reactions of the treated rodents (dosed once) and watched for 14 days. All the animals show neither any poisonous nor deadly impact. The current investigation shows that the administration of test compounds up to 2000 mg/kg did not produce any indication of harmfulness, toxicity or mortality in animals at the time of experimental period.

S. No.	Response	Animals	
		Prior to treatment	Later to treatment
1	Skin color	Normal	Normal
2	Pain response	Normal	Normal
3	Grooming	Absent	Absent
4	Food intake	Normal	Normal
5	Alertness	Normal	Normal
6	Righting reflex	Normal	Normal
7	Corneal reflex	Present	Present
8	Tremors	Absent	Absent
9	Pupils	Normal	Normal
10	Convulsion	Absent	Absent
11	Urination	Normal	Normal
12	Sleep	Normal	Normal
13	Diarrhea	Absent	Absent
14	Torch response	Normal	Normal
15	Lethargy	Absent	Absent
16	Water intake	Normal	Normal
17	Salivation	Normal	Normal
18	Coma	Absent	Absent
19	Gripping	Normal	Normal
20	Mortality	Not applicable	Nil
21	Touch response	Normal	Normal

Table: 1 Effect of test compounds in acute toxicity study.

2.4.2: Anti Inflammatory Activity

In vivo Method for Evaluation of Anti-inflammatory activity:
Anti-inflammatory activity of all synthesized derivatives was determined by the carrageenan-induced rat paw oedema model. Albino rats (100-200 g) were divided into 3 groups as control, test and standard (six animals per group).
Overnight fasted animals were used and during that period only tap water was given.

Generally, Diclofenac was used as standard drug. Both test (20 mg/kg) and standard (20 mg/kg) drugs were suspended in 1% carboxy methyl cellulose (CMC) and administered orally through gastric gavage needle.

One percent of CMC was administered in control group. After 1 h of administrating the compound, we induced the carrageenan (1%) by the sub plantar surface of the right hind paws of animals.

The initial paw volume and also the paw volume at 1, 2, 3 and 4 h of administrating carrageenan were measured. Percent paw oedema inhibition was calculated.

Percent Paw oedema inhibition (E)%=$V_t - V_0$
Where V_0 is the volume before carrageenan injection (ml),
V_t the volume at t hours after carrageenan injection (ml)
Inhibition rate (I)%= E_c-E_t/ E_c
Where E_c and E_t are the edema rates of control and treated groups respectively

2.4.3 Analgesic Activity

Analgesic activity of tested compounds by Eddy'S Hot Plate Method

The apparatus consists of a hot plate on which the rat was placed for testing. It consists of a 20 cm diameter metal hot plate surface set at 50 ºC, a plexiglass cage that fits the hot metal surface and a timer operated by a foot-switch [13].

For three consecutive days preceding the experiment, rats were adapted on the hot plate by placing them on a plate maintained at room temperature for 15 min each day.
All groups were given vehicle and/or the different compounds (10 mg/kg) and the last group received indomethacin (10 mg/Kg) 60 min prior to testing.

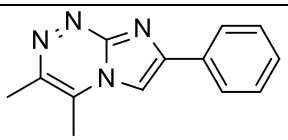
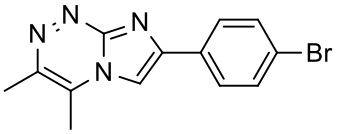
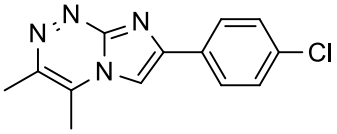
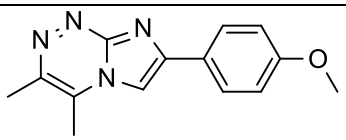
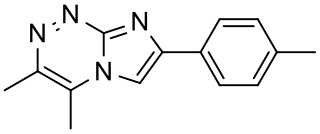
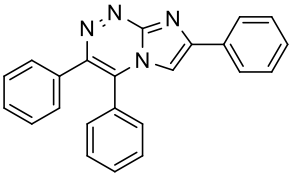
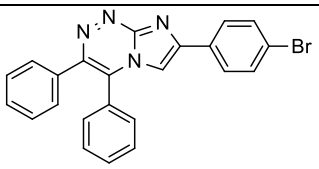
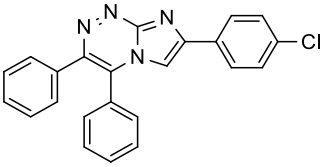
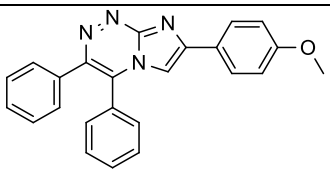
Each animal was then placed gently onto a 50 _C hot plate to perform the test. Latency to exhibit nociceptive responses, such as licking paws or jumping off the hot plate was determined 30, 60, and 90 min after administration of test substances or the saline [14].

Statistical analysis

The results obtained were expressed as mean ± SEM (Standard error of mean) of six animals. Statistical analysis was done using Graph Pad prism software. One way analysis of variance (ANOVA) was also used followed by post-Dunnett's test. Values were considered to be significant at the $P < 0.05$ level.

3 Results and discussion:

3.1: Physical data of Synthesized compounds (C1-C10):

S.No	Compound no.	Molecular Structure	Molecular formula	Mp ^{0} c	TLC r_f value (ethyl acetate:hexane,1:9)	Yield %
1	C-1		**$C_{13}H_{12}N_4$**	**280-283**	**0.7**	**78**
2	C-2		**$C_{13}H_{11}BrN_4$**	**288-289**	**0.9**	**75**
3	C-3		**$C_{13}H_{11}ClN_4$**	**285-287**	**0.7**	**77**
4	C-4		**$C_{14}H_{14}N_4O$**	**283-284**	**0.8**	**78**
5	C-5		**$C_{14}H_{14}N_4$**	**281-283**	**0.6**	**80**
6	C-6		**$C_{23}H_{16}N_4$**	**291-293**	**0.8**	**77**
7	C-7		**$C_{23}H_{15}BrN_4$**	**292-293**	**0.7**	**75**
8	C-8		**$C_{23}H_{15}ClN_4$**	**291-292**	**0.8**	**82**
9	C-9		**$C_{24}H_{18}N_4O$**	**295-297**	**0.5**	**85**

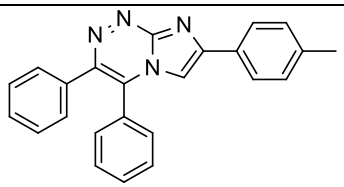
10	C-10		**$C_{24}H_{18}N_4$**	**290-201**	**0.8**	**86**

Table no.2 Physical data of Synthesized compounds (C1-C10)

3.2 Spectral data of synthesized compounds:

3.2.1: 3,4-dimethyl-7-phenylimidazo[2,1-c][1,2,4]triazine

- Yield 78%
- Light brown crystals, m.p. 280–2830°C
- IR (KBr): max in cm-1 (3010 for Ar-H Str, 2949 for Al-CH_2, 1600 for Ar C=C, 1606.5 for N=NStr, 1607.5 for C=N, and 718.7 for Ar C-HBen.
- The following values for the **^{1} H NMR** spectrum (400 MHz, in DMSO-d_6) are given in ppm: 2.6 (6H,S,2*-CH3), 7.6-7.8 (10H,m, C6H5), and 8.60 (1H,s,C5-H of imidazole).
- **^{13}C NMR** SPECTRUM (400MHZ, in CdCl3): ppm: 21.2, 24.3, 127.2, 129.6, 129.9,131.0,133.2,134.5,139.2, 144.5, 158.3, and 168.7
- m/z 225.5 (M+1), $C_{13}H_{12}N_4$ in MS (ESI)

3.2.2: 7-(4-bromophenyl)-3,4-dimethylimidazo[2,1-c][1,2,4]triazine

- Yield 75%
- Crystals, m.p.288-2890C, dark brown
- IR (KBr): max in cm-1 (3010 for Ar-H Str, 2949 for Al-CH2, 1600 for Ar C=C, 1606.5 for N=NStr, 1607.5 for C=N, 718.7 for Ar C-HBen, and 596.0 for C-BrStr.
- SPECTRUM OF **^{1} H NMR** (400 MHz, in DMSO-d_6) The following values are given in parts per million (ppm): 2.3 (6H,S,2*-CH3); 7.6 (2H,d, C ′3 H, C ′5H 4-bromophenyl protons); 7.7 (d,2H,J=5.0 Hz,C ′2 H, C ′6H,4-bromo phenyl protons); and 8.80 (1H,s,C5-H of imidazole).
- $C_{13}H_{11}BrN_4$, 304 (M+1), MS (ESI)m/z:

3.2.3: 7-(4-chlorophenyl)-3,4-dimethylimidazo[2,1-c][1,2,4]triazine

- Yield 77%
- m.p.285-287^{0}C, brown crystals
- IR (KBr): max in cm-1 (3010 for Ar-H Str, 2949 for Al-CH2, 1600 for Ar C=C, 1606.5 for N=NStr, 1607.5 for C=N, 718.7 for Ar C-HBen, and 696.3 for C-Cl Str.
- **^{1} H NMR** SPECTRUM (400 MHz, in DMSO-d_6): in ppm, the following values were found: 2.6 (6H,S,2*-CH3), 7.5 (2H,d, C ′3 H, C ′5H 4-chloro phenyl protons), 7.9 (d,2H,J= 5.0 Hz,C ′2 H, C ′6H,4- chloro phenyl protons), and 8.80 (1H,s,C5-H of imidazole).

- **^{13}C NMR** SPECTRUM (400MHZ, in CdCl3): 21.2, 24.4, 127.2, 129.6, 129.9,131.0,133.2,134.5,139.2, 144.5, 158.3, and 168.7 ppm
- 260 (M+2) (ESI) m/z, $C_{13}H_{11}ClN_4$

3.2.4: 7-(4-methoxyphenyl)-3,4-dimethylimidazo[2,1-c][1,2,4]triazine

- Yield 78%
- Crystals of Light Brown, m.p. 283-284^{0}C
- IR (KBr): max in cm-1 (3010 for Ar-H Str, 2949 for Al-CH2, 1600 for Ar C=C, 1606.5 for N=NStr, 1607.5 for C=N, 1118.3 for C-O Str OCH3, and 718.7 for Ar C-HBen).
- **^{1} H NMR** SPECTRUM (400MHZ, in DMSO-d_6) in ppm: 2.6 (6H,S,2*-CH3), 3.8 (3H,s,-OCH3), 7.0 (2H,d, C ′3 H, C ′5H 4-methoxy phenyl protons), 7.9 (d,2H,J=5.0 Hz,C ′2 H, C ′6H,4- 4-methoxy
- $C_{14}H_{14}N_4O$, MS (ESI)m/z: 255 (M+1).

3.2.5: 3,4-dimethyl-7-(p-tolyl)imidazo[2,1-c][1,2,4]triazine

- Yield 80%
- Crystals of Light Brown, m.p. 281-283^{0}C
- IR (KBr): max in cm-1 (3010 for Ar-H Str, 2949 for Al-CH2, 1600 for Ar C=C, 1606.5 for N=NStr, 1607.5 for C=N, 853.8 for Ar-CH3, and 718.7 for Ar C-HBen.
- **^{1}H NMR** SPECTRUM (400 MHz, in DMSO-d_6): ppm: 2.6 (9H,s,3*-CH3); 7.2 (2H,d, C ′3 H, C ′5H 4-meyhyl protons); 7.6 (d,2H,J= 5.0 Hz,C ′2 H, C ′6H, 4-meyhyl protons); and 8.80 (1H,s,C5-H
- **^{13}C NMR** SPECTRUM (400 MHz, in CdCl3): 18.9, 21.2, 24.4, and 57.7 ppm 127.2,129.6,129.9,131.0,133.2,134.5,139.2,144.5,158.3,168.7
- $C_{14}H_{14}N_4$, MS (ESI)m/z: 239.0(M+1H).

3.2.6: 3,4,7-triphenylimidazo[2,1-c][1,2,4]triazine

- Yield 77%
- Crystals with a dark brown color, m.p. 291-293^{0}C
- IR (KBr) maximum values are as follows: 3010 (Ar-H Str), 1600.18 (Ar C=C), 1606.5 (N=NStr), 1607.5 (C=N), and 853.8 (Ar-CH3) in cm-1.
- **^{1}H NMR** SPECTRUM (400 MHZ, in DMSO-d_6): ppm: 7.2–7.6 (10H,m, C6H5), 7.6–7.8 (5H,m,2*3,4-di phenyl C6H5), and 8.80 (1H,s,C5-H of imidazole).
- **^{13}C NMR** SPECTRUM (400MHZ, in CdCl3): 127.2, 129.6, 129.9, 131.0, 133.2, 134.5, 139.2, 144.5, 158.3, and 168.7 ppm
- m/z 349(M+1H), $C_{23}H_{16}N_4$ MS (ESI)

3.2.7: 7-(4-bromophenyl)-3,4-diphenylimidazo[2,1-c][1,2,4]triazine

- Yield 82%
- m.p.291-292^{0C}, brown crystals

- IR (KBr): max in cm-1 (3010 for Ar-H Str, 1600.18 for Ar C=C, 1606.5 for N=N Str, 1607.5 for C=N, 853.8 for Ar-CH3, and 696.3 for C-Cl Str).
- **^{1}H NMR** SPECTRUM (400MHZ, in DMSO-d_6): 7.6 (2H,d, C ′3 H, C ′5H 4-bromo phenyl protons), 7.6-7.8 (10H,m, 2*C6H5), 7.9 (d,2H,J=5.0 Hz,C ′2 H, C ′6H, 4-bromo phenyl protons), and 8.80 (1H,s,C
- **^{13}C NMR** SPECTRUM (400MHZ, in CdCl3): 127.2, 129.6, 129.9, 131.0, 133.2, 134.5, 19.2, 144.5, 158.3, and 168.7 ppm
- ESI MS (M+2H) m/z 384.9, $C_{23}H_{15}ClN_4$

3.2.8: 7-(4-chlorophenyl)-3,4-diphenylimidazo[2,1-c][1,2,4]triazine

- Yield 85%
- Crystals of Light Brown, m.p. 295-297^{0C}
- IR (KBr) maximum values in cm-1 are as follows: 3010 (Ar-H Str), 2949.6 (Al-CH2), 1600.18 (Ar C=C), 1606 (N=NStr), 1607 (C=N), 1118.3 (-C-O Str OCH3), and 853.8 (Ar-CH3).
- **^{1}H NMR** SPECTRUM (400MHZ, in DMSO-d_6):3.8 (3H,s,-OCH3), 7.0 (2H,d, C ′3 H, C ′5H 4-methoxy phenyl protons), 7.4-7.7 (10H,m, 2*C6H5), 7.8 (d,2H,J=5.0 Hz,C ′2 H, C ′6H, methoxy phenyl protons), and 8.80 (1H,s,C5-H of imidazo
- **^{13}C NMR** SPECTRUM (400 MHz, in CdCl3): ppm: 57.7, 127.2, 129.6, 129.9, 131.0, 133.2, 134.5, 139.2, 144.5, 158.3, and 168.
- m/z 379(M+1), $C_{24}H_{18}N_4O$, MS (ESI)

3.2.9: 7-(4-methoxyphenyl)-3,4-diphenylimidazo[2,1-c][1,2,4]triazine

- Yield 85%
- Crystals of Light Brown, m.p. 295-297^{0C}
- IR (KBr) maximum values in cm-1 are as follows: 3010 (Ar-H Str), 2949.6 (Al-CH2), 1600.18 (Ar C=C), 1606 (N=NStr), 1607 (C=N), 1118.3 (-C-O Str OCH3), and 853.8 (Ar-CH3).
- **^{1}H NMR** SPECTRUM (400MHZ, in DMSO-d_6):3.8 (3H,s,-OCH3), 7.0 (2H,d, C ′3 H, C ′5H 4-methoxy phenyl protons), 7.4-7.7 (10H,m, 2*C6H5), 7.8 (d,2H,J=5.0 Hz,C ′2 H, C ′6H, methoxy phenyl protons), and 8.80 (1H,s,C5-H of imid
- **^{13}C NMR** SPECTRUM (400 MHz, in CdCl3): ppm: 57.7, 127.2, 129.6, 129.9, 131.0, 133.2, 134.5, 139.2, 144.5, 158.3, and 168.
- m/z 379(M+1), $C_{24}H_{18}N_4O$, MS (ESI)

3.2.10: 7-(4-methylphenyl)-3,4-diphenylimidazo[2,1-c][1,2,4]triazine

- Yield 86%
- Crystals, m.p.290-291^{0}C, dark brown
- Maximum values in cm-1 for the IR (KBr) include 3010 (Ar-H Str), 1600.18 (Ar C=C), 1606.5 (N=NStr), 1607.5 (C=N), 853.8 (Ar-CH3), and 718.7 (Ar C-HBen).

- **^{1}H NMR** SPECTRUM (400 MHZ, in DMSO-d_6): in ppm: 2.3 (3H,s,-CH3), 7.2 (2H,d, C ′3 H, C ′5H 4-methyl phenyl protons), 7.3-7.6 (10H,m, 2*C6H5), 7.8 (d,2H,J= 5.0 Hz,C ′2 H, C ′6H, methyl
- **^{13}C NMR** SPECTRUM (400MHZ, in CdCl3): 21.2, 127.2, 129.6, 129.9, 131.0, 133.2, 134.5, 139.2, 144.5, 158.3, and 168.7 ppm
- $C_{24}H_{18}N_4$, MS (ESI)m/z: 364.9(M+2)

3.3. *In silico* binding studies:

Docking with COX-2

***In silico* binding studies:** In order to evaluate the anti inflammatory and analgesic activity of the synthesized C1-C10 compounds, were subjected to molecular docking studies using Auto dock 4.2 to know their binding affinity between the ligands and their receptors cyclooxygenase-I (COX-I) and cyclooxygenase-II (COX-II) which forms the basis of pharmacological effects of the synthesized compounds.

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

	Binding energy (kcal/mol)	**H-bond**			**Hydrophobic**	
Compound		**Residue No**	**Amino acid**	**Distance**	**Residue No**	**Amino acid**
Co-Crystal Ligand	9.69	90A	HIS	3.08	349A	VAL
		355A	TYR	2.42	352A	LEU
		513A	ARG	2.53	352A	LEU
		517A	ILE	2.85	518A	PHE
		518A	PHE	2.57	523A	VAL
		518A	PHE	2.15	523A	VAL
C1	-7.39	522A	MET	2.01	531A	LEUA
		526	VAL	1.78	526A	ALA
					352A	LEU
C2	-8.03				527A	ALA
					349A	VAL
					359	LEU
C3	-7.85	355A	TYR	2.71	527A	ALA
					527A	ALA
					349A	VAL
C4	-7.25	523A	VAL	3.05	349A	VAL
		120A	ARG	4.74	116A	VAL

		527A	ALA	3.82	522A	MET
C5	-7.35	120A	ARG	3.99	527A	ALA
		355A	TYR	2.62	526A	GLY
					349A	VAL
C6	-9.56	523A	VAL	2.93	359A	LEU
		113A	MET	2.14	523A	VAL
C7	-3.56	120A	ARG	3.3	113A	MET
		518A	PHE	2.8	349A	VAL
		355A	TYR	2.84	523A	VAL
C8	-5.82	355A	TYR	2.85	526A	GLY
		522A	MET	4.01	522A	MET
					385A	TYR
C9	-8.88	384A	LEU	2.9	349A	VAL
		120A	ARG	3.62	523A	VAL
		355A	TYR	3.17	527A	ALA
C10	-7.32				359A	LEU
					527A	ALA
					113A	MET

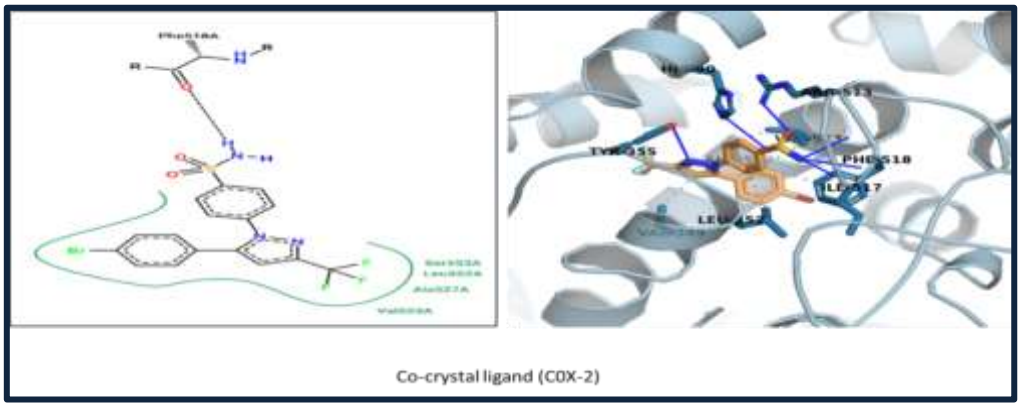
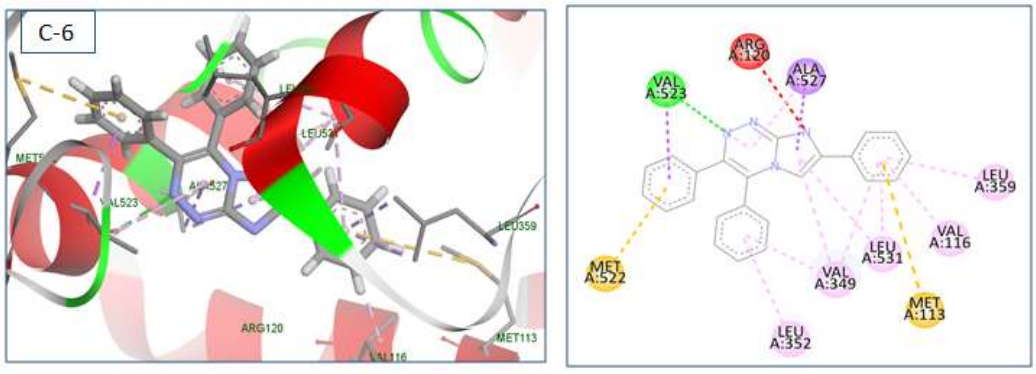


Figure no.1: In silico binding interactions of the active ligands towards the active site of COX-2 protein (PDB ID: 1CX2). The best docked pose for active compounds was represented in the image. The co-crystal ligand was used for the results validation.



Docking with COX-1

Figure no. 2: In silico binding interactions of the active ligands towards the active site of COX-1 protein (PDB ID: 1EQG). The best docked pose for active compounds was represented in the image. The co-crystal ligand was used for the results validation.

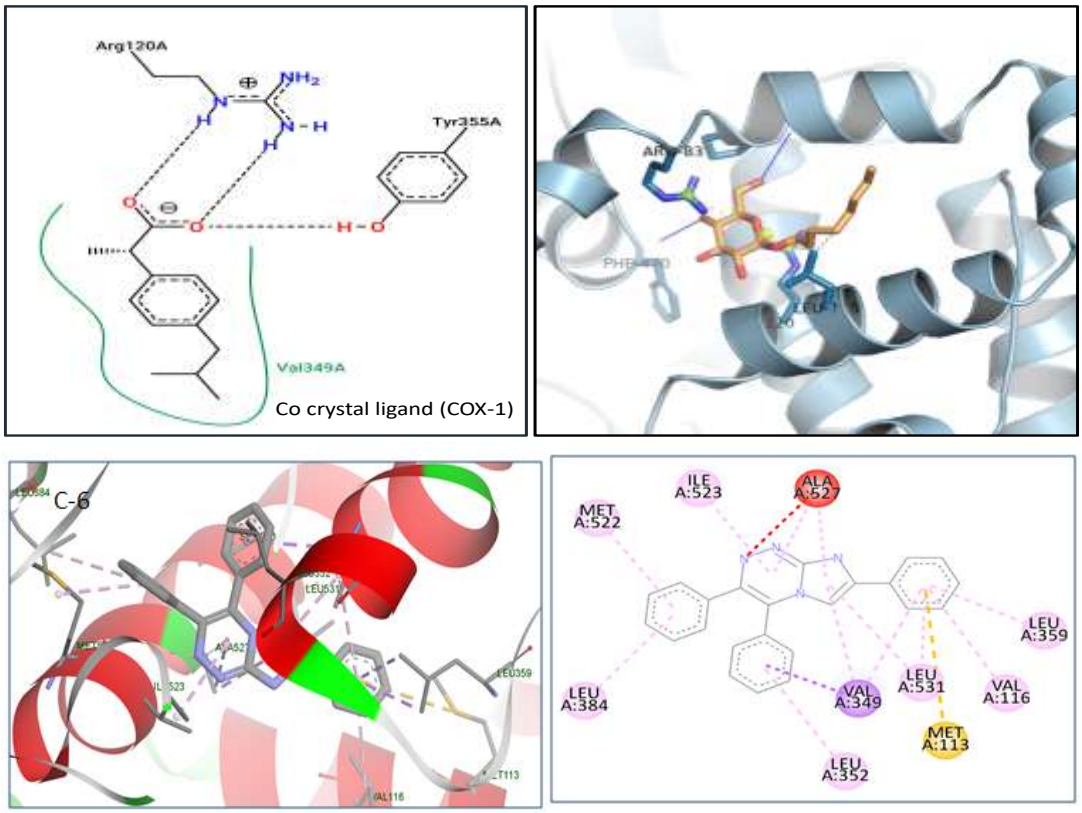


Table 4: Binding energies and interacted amino acid residues of the target protein COX-1 protein (PDB ID: 1EQG) with docked ligands.

		Binding energy (kcal/mol)	**H-bond**			**Hydrophobic**	
	Compound		**Residue No**	**Amino acid**	**Distance**	**Residue No**	**Amino acid**
	Co-Crystal Ligand	-9.17	86A	PRO	3.3	115	LEU
			120A	ARG	3.25		
			470A	PHE	3.11		
	C1	-8.14				349A	VAL
						527A	ALA
						526	GLY
	C2	-7.95	523A	ILE	3.69	527A	ALA
						522A	MET
						385A	TYR
	C3	-7.78	120A	ARG	2.21	527A	ALA
						116A	VAL

						359A	LEU
	C4	-7.36	120A	ARG	3.16	385A	TYR
			527A	ALA	3.3	355A	TYR
						526A	GLY
	C5	-7.3				527A	ALA
						385A	TYR
						526A	GLY
	C6	-9.42	530A	SER	3.39	349A	VAL
			113A	MET	2.89	527A	ALA
						359A	LEU
	C7	-7.55	120A	ARG	3.54	89A	ILE
			355A	TYR	4.06	93A	LEU
						116A	VAL
	C8	-7.55	113A	MET	3.27	116A	VAL
						349A	VAL
						359A	LEU
	C9	-7	120A	ARG	2.74	89A	ILE
						385A	TYR
						349A	VAL
	C10	-6.24	120A	ARG	2.82	116A	VAL
						359A	LEU
						527A	ALA

From the overall docking and interaction analysis, the best confirmation among C-series, found to be C-6 exhibited highest binding energies -9.56 kcal/mol (COX-2) & -9.42 kcal/mol (COX-I) and good hydrogen bond interaction with the active site of (COX-II:ICX2) and (COX-I:1EQZ). With respect to cyclooxygenase –II, hydrogen bond formed between amino acid valine 523 with the H (O-CH_3) of compound **C-6** with a hydrogen bond distance of 2.93 A˚. Other hydrogen bond also formed between amino acid methionine 522 O(oxygen) with the H(hydrogen) of the compound **C-6** with hydrogen bond distance of 2.14 A˚. With respect to cyclooxygenase –I, hydrogen bond formed between amino acid valine 349 H(Hydrogen) with the N of compound **C-6** with a hydrogen bond distance of 3.39 A˚. Other hydrogen bond also formed between amino acid methionine 113 O(oxygen) with the H(hydrogen) of the compound **C-6** with hydrogen bond distance of 2.89 A˚.

3.4: Biological activity:

3.4.1: Studies on oral acute toxicity

The social behaviors of the treated mice (dosed once) were unaffected by any of the test substances C1-C10 when they were all given at a dose of 2,000 mg/kg and examined for 14 days. None of the creatures show a poisonous or fatal effect. According to the current study,

the administration of test chemicals up to 2,000 mg/kg over the experimental period did not cause any signs of harm, toxicity, or animal death.

S. No.	Response	Animals	
		Prior to treatment	**Later to treatment**
1	Skin color	Normal	Normal
2	Pain response	Normal	Normal
3	Grooming	Absent	Absent
4	Food intake	Normal	Normal
5	Alertness	Normal	Normal
6	Righting reflex	Normal	Normal
7	Corneal reflex	Present	Present
8	Tremors	Absent	Absent
9	Pupils	Normal	Normal
10	Convulsion	Absent	Absent
11	Urination	Normal	Normal
12	Sleep	Normal	Normal
13	Diarrhea	Absent	Absent
14	Torch response	Normal	Normal
15	Lethargy	Absent	Absent
16	Water intake	Normal	Normal
17	Salivation	Normal	Normal
18	Coma	Absent	Absent
19	Gripping	Normal	Normal
20	Mortality	Not applicable	Nil
21	Touch response	Normal	Normal

Table 5 Effect of test compounds in acute toxicity study

3.4.2: Anti-inflammatory activity

Compound	Percentage inhibition at different time intervals (inhibition ± SEM)			
	1 hour	**2 hours**	**3 hours**	**4 hours**
Diclofenac	19.32 ± 7.1	19.24 ± 1.2	49.63 ± 7.9	45.88 ± 4.6
C1	20.05 ± 1.2	43.09 ± 8.9	31.21 ± 4.6	50.16 ± 7.0*
C2	42.04 ± 4.8	51.67 ± 3.8	36.32 ± 4.2	55.25 ± 2.4*
C3	21.53 ± 1.5	46.31 ± 10.3	34.36 ± 3.5	52.93 ± 6.2*
C4	24.93 ± 6.3	45.73 ± 7.8	31.54 ± 1.9	43.82 ± 2.4

C5	20.56 ± 4.1	41.38 ± 5.1	35.26 ± 2.2	49.28 ± 2.7
C6	42.03 ± 9.8	26.19 ± 4.9	49.38 ± 2.6	62.84 ± 5.6*
C7	18.40 ± 8.4	39.76 ± 2.3	29.23 ± 2.4	38.52 ± 2.8
C8	19.65 ± 3.8	41.83 ± 4.9	30.62 ± 2.5	40.82 ± 7.2
C9	40.30 ± 4.8	45.67 ± 3.5	35.32 ± 4.2	58.25 ± 2.5*
C10	21.02 ± 9.8	28.19 ± 4.9	37.38 ± 1.2	45.84 ± 6.5

Table no.6 Anti-inflammatory activity of tested compounds at a dose molecularly equivalent to 20 mg/Kg Diclofenac

Data was analyzed by one way ANOVA followed by Dunnett's test (n=5). Values at *P<0.05 were considered significant.

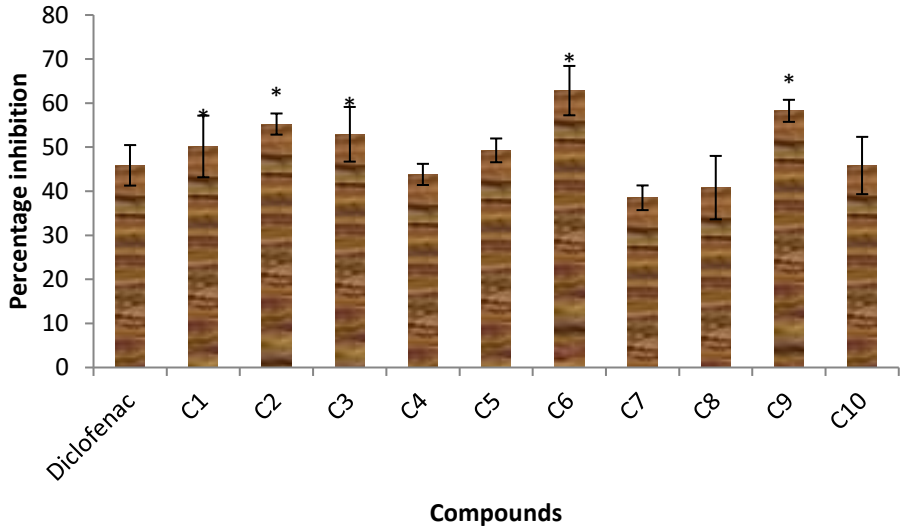


Figure no.3: Anti-inflammatory activity of tested compounds by carrageenan model

The maximum edema inhibition was seen at 4 hours, when the 3,4,7-tri substituted imidazo(2,1-c)(1,2,4) triazines(C1-C10) compound C6 showed a 62.48 percent inhibition. Among the C-series compounds, compound C6 having a phenyl substituent at the Imidazo(2,1-c)(1,2,4) triazines ring at position 7 may have the highest percentage of inhibition. Diclofenac sodium was outperformed in terms of anti-inflammatory efficacy by compounds C9, C2, C3, and C1. Other substances, however, showed lower potency than the common reference medication.

3.4.3: Analgesic activity

Group	Reaction time (min)			
	0 min	**30 min**	**60 min**	**90 min**
Control	4.04 ± 0.68	4.89 ± 0.52	5.87 ± 0.41	5.65 ± 0.46
Indomethacin	5.65 ± 0.32	8.40 ± 0.38*	10.68 ± 0.75*	9.68 ± 1.02*
C1	3.32 ± 0.44	6.13 ± 0.45	6.85 ± 0.75	10.16 ± 0.75*
C2	3.84 ± 0.32	4.47 ± 0.53	5.83 ± 0.35	10.24 ± 0.73*
C3	3.63 ± 0.36	5.41 ± 0.62	6.82 ± 0.75	10.20 ± 0.63*
C4	2.49 ± 0.34	4.21 ± 0.62	6.29 ± 6.40	7.49 ± 0.65
C5	3.06 ± 0.81	5.08 ± 0.34	6.75 ± 0.83	9.75 ± 0.57*
C6	3.84 ± 0.13	5.29 ± 0.57	7.85 ± 0.86	10.87 ± 1.04*
C7	2.83 ± 0.34	3.68 ± 0.26	7.08 ± 0.35	6.29 ± 0.71
C8	3.74 ± 0.64	5.60 ± 0.16	5.69 ± 0.96	6.89 ± 0.87
C9	3.83 ± 0.40	5.02 ± 0.84	6.63 ± 0.44	10.45 ± 0.81*
C10	3.25 ± 0.41	5.43 ± 0.62	6.21 ± 0.16	7.38 ± 0.94

Table no.7 Analgesic activity of tested compounds by hot plate method.

Values represent the mean ± SE of six animals for each group
P<0.05: * Statistically significant from control using one way ANOVA followed by Dunnett's test

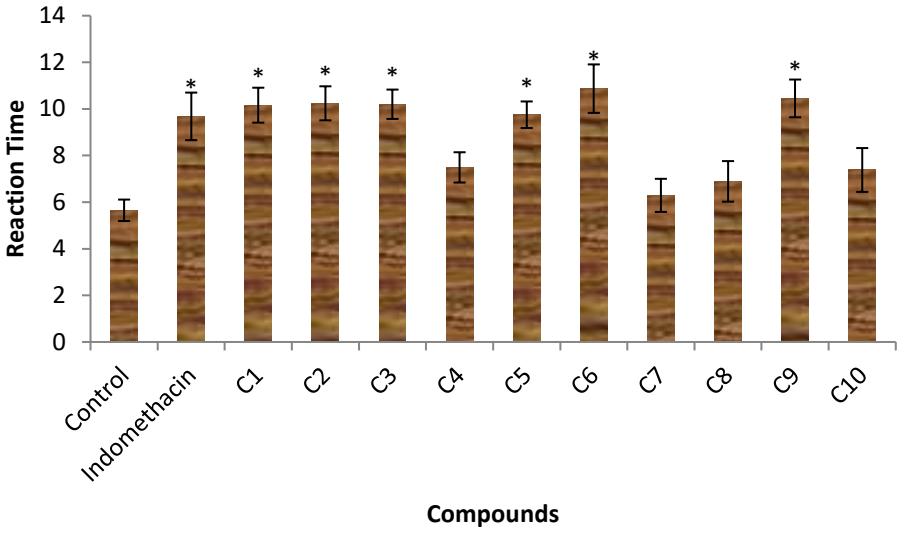


Figure no.4: Analgesic activity of tested compounds by Eddy's Hot Plate Method

All of the 3,4, 7-tri substituted Imidazo(2,1-c)(1, 2, 4) triazines (C1-C10) investigated substances displayed outstanding analgesic effects, according to the data (tables) that were acquired increased activity leading to longer reaction times. Standard Indomethacin and all produced compounds (C1-C10) were assessed at a dose of 10 mg/kg p.o.

Out of all the synthetic C-series compounds comprising trisubstituted imidazo(2,1-c)(1,2,4) triazines (C1-C10), compound C6 had the best analgesic activity. The most potent analgesic effect was seen in compound C6, which had a phenyl substituent at the Imidazo(2,1-c)(1,2,4) triazines ring's 7th position. It was discovered that compounds C9, C2, C3, C1, and C5 have more analgesic effectiveness than Indomethacin. Other substances, however, showed less action than the common reference medication.

4.Conclusion:

The present research work includes design, synthesis and biological evaluation of novel 3,4,7-tri substituted Imidazo(2,1-c)(1,2,4) triazines (C-series,C1-C10) compounds.

All the molecules (C1-C10) were synthesized using straightforward and efficient techniques, and the compounds were characterized using IR, ^{1}H, and ^{13}C NMR, as well as MASS. All new compounds were evaluated for anti-inflammatory, analgesic, and antibacterial properties using molecular docking techniques.

The docking studies showed that novel 3,4,7-tri substituted Imidazo(2,1-c)(1,2,4) triazines (C-series,C1-C10) derivatives were found to have good binding affinities with the cyclooxygenase-I (COX-I PDB ID:1EQZ) and cyclooxygenase-II (COX-II PDB ID:1CX2).
The carrageenan-induced rat paw edema method and the Eddy's heated plate method were used to measure anti-inflammatory and analgesic activity, respectively.

The binding mechanisms and affinities of synthesized compounds with cyclooxygenase-I (COX-I PDB ID:1EQZ) and cyclooxygenase-II (COX-II PDB ID:1CX2) were investigated for all molecules C1-C10 screened for molecular docking using auto dock tools. C6 "3,4,7-triphenylimidazo[2,1-c][1,2,4]triazine" C-6" exhibited the highest binding energies with (COX-2:1CX2) and -9.42 kcal/mol with (COX-I:1EQZ) due to the presence of a phenyl group required for binding with amino acid residues in the active binding region.

With inhibition rates of 62.84 percent, C6 demonstrated the greatest anti-inflammatory and analgesic effect after 90 minutes. This research established the groundwork for the continued development of compounds that functioned as effective pharmacophores and has enormous potential for the discovery of future anti-inflammatory and analgesic medications.

9. Conflicts of Interest.

None

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List of abbreviations

IR - Infrared Spectroscopy

^{1}H NMR -proton nuclear magnetic resonance spectroscopy

^{13}C NMR- carbon nuclear magnetic resonance spectroscopy

COX-I -Cyclo Oxygenase-I

COX-II- Cyclo Oxygenase-II

DMSO - Dimethylsulfoxide

$CDCl_3$ - Deuterated chloroform

TMS- Tetramethylsilane

MHz - Mega Herts

Ethics approval and consent to participate

Materials and Methods :

Animal procurement :

Swiss albino rats (approx 200-250 g) were procured from Albino research, Hyderabad. Present studies were carried out in CPCSEA approved animal house of Sri Venkateshwara College Of Pharmacy, Hyderabad, India.

(Reg. No.1791/PO/Re/S/14/CPCSEA).

Animal housing :

The animals were housed in poly acrylic cages with not more than six animals per cage, with 12 hr - light/12 hr - dark cycle. They have free access to standard diet and drinking water ad libitum.

The animals were allowed to acclimatize the laboratory environment for a week before the start of the experiment.

The care and maintenance of the animals were carried out as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

Chemicals:

Carrageenan, carboxymethyl cellulose (CMC), and all the chemicals used were of analytical grade.

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Lr.No:SES/SVCP/IAN/2023-41

Date: 25.01.2023

To

Sposrthi Pohar
Assistant Professor
Department of Pharmaceutical Chemistry

Dear Mrs. Sposrthi Pohar

This is to inform you that the IAEC has approved your project entitled "Design, Docking, Synthesis and biological evaluation of novel triazine derivatives as anti-inflammatory activity." in the meeting held on 29-12-2022, the protocol No is IAEC/SVCP/2022/09.

Date: 29-12-2022
Place: Hyderabad

IAEC- Chairman

[Dr. M. Bhagavan Raju]

IAEC- Member Secretary

[Dr. G. Anantha Lakshmi]

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Principal

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Lr.No:SES/SVCP/IAN/2023-42

Date: 25.01.2023

To

To

Sposrthi Pohar
Assistant Professor
Department of Pharmaceutical Chemistry

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