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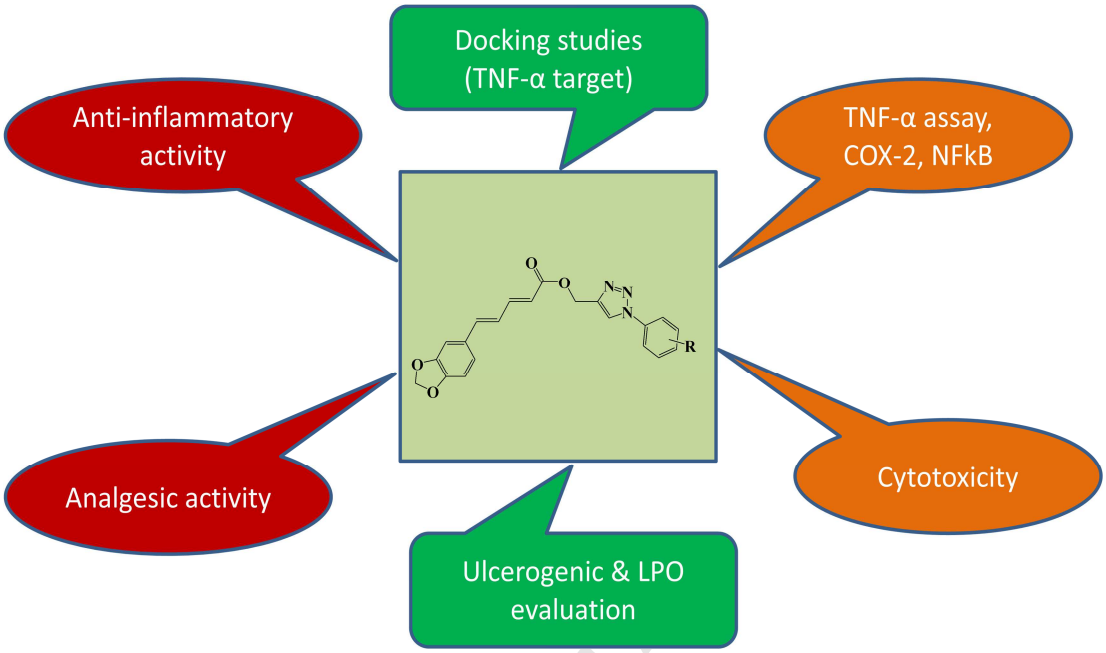

Design, Synthesis and Biological Evaluation of Piperic Acid Triazolyl Derivatives as Potent Anti-inflammatory Agents

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Abstract

Nineteen novel piperine based triazoles have been synthesized using click chemistry approach and were tested for *in vivo* anti-inflammatory activity. The most active compounds were evaluated for *in vitro* TNF-α expression. Compounds **3g** and **3f** were found to show significant *in vivo* inhibition of inflammation**, 80.40 %** and **76.71%,** respectively after 5h in comparison to piperine (**54.72**%) and the standard drug indomethacin (**77.02**%) without causing any damage to the stomach. Compounds **3g** and **3f** suppressed TNF-α level by **73.73%** and **70.64%**, respectively and protein expression of COX-2, NF-κB and TNF-α more than indomethacin. Moreover, the compound **3g** was found to show significant analgesic activity of **54.09%** which was comparable with the indomethacin **(57.43%)**.

Keywords: Piperine, Click chemistry, TNF-α, Cytotoxicity, Immunohistochemistry, Molecular docking.

Abbreviations: Tumour necrosis factor (TNF-α), Cyclooxygenase (COX), Lipid peroxidation (LPO), Non steroidal anti-inflammatory drug (NASIDs)

1. Introduction

Piperine is a major alkaloid constituent of piper species, including *Piper nigrum* Linn and *Piper longum* Linn. It is commonly used in various traditional systems of medicines [1]. Piperine has been reported to exhibit various types of pharmacological activities such as anti-inflammatory [2], anti-oxidant [3], anti-tumor [4], anti-asthmatic [5], hepato-protective [6], anti-thyroid [7], and anti-depressant [8]. Most of non steroidal anti-inflammatory drugs (NSAIDs) used for the treatment of inflammation show adverse effects such as gastric ulcer [9], kidney damage [10] and hepatotoxicity [11]. Therefore, the development of NSAIDs with reduced side effects is still underway all over the world. Plant-derived drugs used in the traditional systems of medicine for the treatment of pain, hay fever and inflammatory ailments have received considerable attention as they are cheap and have no or little side effects [12].

1,2,3-triazoles have been reported to possess a wide range of pharmacological properties including anti-inflammatory, analgesic, anti-microbial, anti-convulsant, anti-neoplastic, anti-malarial, and anti-viral [13-20]. In view of the biological importance of piperine and 1,2,3-triazoles as anti-inflammatory agents we have synthesized piperine based 1,2,3-triazoles as potent anti-inflammatory agents with reduced side effects. We have conjugated these two moieties under one construct using click chemistry approach. The synthesized molecules were evaluated for their *in vivo* anti-inflammatory activity. *In silico* molecular docking study has been done to study the binding interactions of the synthesized compounds with TNF-α protein. The compounds showing significant anti-inflammatory activity and good binding affinity with TNF-α target were further evaluated for their effect on *in vitro* TNF-α level. Furthermore immunohistochemical studies have also been done to explore the effect of the active compounds on expression of COX-2, NF-Kb and TNF-α protein. The synthesized compounds

have also been screened for their anti-nociceptive potential. Lipid peroxidation and ulcerogenic risk evaluation studies have also been carried out.

2. Results and discussion

2.1. Chemistry

For the synthesis of piperine based 1,2,3-triazole library, propargylated ester of piperic acid was prepared by reacting piperic acid with propargyl bromide in the presence of cesium carbonate in dry THF. 1,3-dipolar cycloaddition reaction between the propargylated ester and the aromatic azides resulted in the synthesis of the final compounds. The structural confirmation of target compounds **(3a-s)** was done by ^{1}H NMR (Brucker Avance II 300 NMR Spectrometer) ^{13}C NMR (Brucker Avance II 400 NMR Spectrometer) and mass spectral (LCQ Fleet) data as well as elemental analysis (Elementar GMBH) **(Scheme-1).** In the ^{1}H NMR spectra, the formation of triazole derivatives was confirmed by the presence of proton of triazole ring as a singlet in the range of δ 7.99 to 8.54 ppm along with methylene protons appearing at δ 5.35 to 5.87 ppm. The double doublet of H-3 appeared in the range of δ 7.47 to7.49 ppm and the singlet of H-4 appeared in the range of δ 5.91 to 6.05 ppm (fig A).The remaining two protons H-1and H-2 appeared in aromatic region as expected. ESI-MS of all the compounds showed $[M]^{+}$ and $[M+H]^{+}$ peaks with reasonable intensity.

2.2. Anti-inflammatory activity

All the synthesized compounds have been tested for their *in vivo* anti-inflammatory activity by carrageenan induced rat paw edema model. The results (**table 1**) indicated that compounds **3e-g & 3o-q** possess significant anti-inflammatory activity. Amongst these compounds, the compound **3g** showed better activity **(76.12 %** at 3h and **80.40%** inhibition at 5h) as compared to the standard drug indomethacin **(72.05%** at 3h and **77.02 at 5h)**.

The compounds **3e, 3f, 3o, 3p,** and **3q** also showed significant anti-inflammatory activity (**70.81%, 76.71%, 71.21%, 72.97%,** and **70.27%)** respectively which is comparable to standard drug indomethacin at 5 h of inflammation **(77.02%)** but higher than that of piperine which showed **54.72%** inhibition at 5 h.

2.3. In silico docking studies

It has been reported in earlier studies that piperine can significantly lower the level of TNF-α, which is one among the important pro-inflammatory cytokines. It is expected that the anti-inflammatory effect of piperine might attribute to the inhibition of TNF-α.[21] Therefore, the synthesized piperine derivatives were docked against TNF-α to analyze their binding pattern and energies inside the receptor pocket. Crystallized structure of 2AZ5 was chosen from protein data bank and used as a target for molecular docking studies with the specific ligand indomethacin which inhibits it [22]. The synthesized derivatives were docked individually against the generated grid and were found to show good binding energies ranging from **-34.24** to **-42.41** kcal/ mol. Amongst all the synthesized molecules, the most promising molecules were **3g, 3a, 3f, 3o, 3h, 3e, 3p,** and **3q** which exhibited a glide score of **-5.81, -5.80, -5.78, -5.67, -5.61, -5.59,-5.32** and **-5.13** respectively whereas the glide score of compounds piperine and indomethacin were found to be **-4.42** and **-5.02** respectively**.** Alike indomethacin, the most active compound (3g) were found to align perfectly with the hydrophobic pocket of the TNF-α protein where as piperine was found to form hydrogen bonding with LYS-98 residue. The glide score, binding energies of all the synthesized compounds are shown in **table 2**.

2.4. TNF-α assay

Compounds showing significant *in vivo* anti-inflammatory activity were further evaluated for *in vitro* TNF–α level in LPS induced RAW 264.7 cell lines. The results are shown in **fig 2**. It was found that compound **3g** and **3f** showed better suppression of **73.73%** & **70.64%** in TNF-α concentration as compared to the standard drug indomethacin as well as piperine which decrease TNF-α level by **67.99%** and **49.67%** respectively. The compounds **3p (63.58%)** and **3o (60.71%)** also showed significant decrease in TNF-α concentration. From the above results, it is clear that compounds **3g** and **3f** showing significant *in vivo* anti-inflammatory activity and glide scores also significantly decreased the level of TNF-α in LPS induced RAW 264.7 cell line.

2.5. Cytotoxicity profile

Cytotoxicity effect of active compounds in RAW 264.7 macrophages was carried out by MTT assay. The results of cytotoxicity assay are shown in **Fig 3**. The results clearly indicates that the cell viability of compounds **3f, 3g, 3o** and **3p** was more than 90% i.e. these compounds did not cause any abnormal cell death as compared to standard drug indomethacin which has cell viability of **55**% only.

2.6. Antinociceptive activity

Compounds showing significant anti-inflammatory activity were also further tested for their *in vivo* analgesic activity by writhing test method. The results of analgesic activity are shown in **table 3**. Amongst all the active compounds, compound **3g** showed significant analgesic activity **(54.09%)** in comparison to standard drug indomethacin **(57.43%)** Compounds **3f**, **3o** and **3p** also showed good analgesic activity.

2.7. Ulcerogenic studies

The compounds **3f, 3g, 3o** and **3p** showing most potent *in vivo* anti-inflammatory activity were further evaluated for ulcerogenic risk. They were not found to cause any damage to the stomach of the animal, whereas standard group (indomethacin) treated animals caused mucosal and epithelium tissue damage. Piperine treated animals also did not show any damage to the stomach of animals (**fig4**)

2.8. Lipid peroxidation assay

The result of LPO is shown in **fig 5** and it is measured as nmol of MDA per 100 mg of gastric mucosa tissue. It has been observed that carrageenan increased the level of LPO in animals. However the level of LPO was found to decrease significantly in the presence of the standard drug and the test compounds **3f, 3g, 3o** and **3p.**

2.9. Immunohistochemistry

The modulation of cellular signalling network involved in induction and activity of pro-inflammatory cytokines like TNF-α, COX-2 and NF-κB has been considered a paradigm for preventing inflammation [21]. Therefore in the present study the potential of the active compounds in suppressing these cytokines was studied. NF-κB plays an important role in maintaining immune response by regulating the production of cytokines responsible for inflammation [22]. NF-κB stimulates the expression of enzymes such as nitric oxide synthase (iNOS) which is involved in the pathogenesis of inflammatory process. Therefore, the activation of NF-κB acts as a key mediator in controlling inflammatory responses. Cyclooxygenase enzyme mediates the formation of several biological precursors [23]. Three isoenzyme of COX mainly are COX-1, COX-2 and COX-3. COX-2 is inducible and gets activated at the site of inflammation. The effects of active compounds **3f** and **3g** on paw tissue expression of COX-2 and NF-κB are shown in fig **7a** and **7b**. Group II i.e. only carrageenan treated group showed

more intense brown color which clearly indicated increase in the number of cells with COX-2 and NF-κB expression as compared to that of normal control group (I) which showed no expression of COX-2, NF-κB and TNF-α. The treatment of animals with indomethacin and active compound **3f** and **3g** reduced the number of cells showing expression of these proteins. However compounds **3f** and **3g** showed more reduction in the expression of these proteins as compared to standard drug.

3. Structure activity relationship

The structure activity relationship of the synthesized compounds has been analyzed as follows.

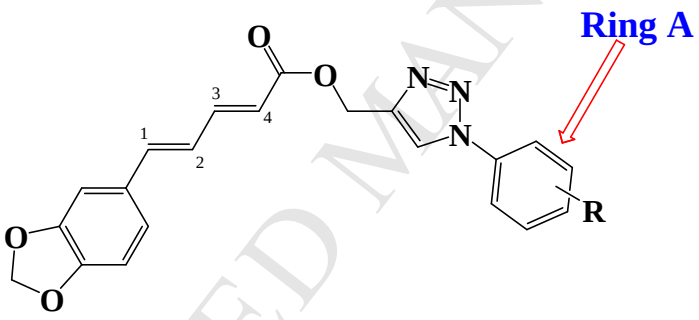

Fig A

- Presence of electron withdrawing groups on the aryl moiety decreased the *in vivo* anti-inflammatory activity as compared to the presence of electron donating groups.
- Better *in vivo* anti-inflammatory activity was observed for compounds having halogen at ortho position of the aromatic **ring A** as compared to halogen attached at para position. Increasing order of activity found was F< Cl<Br.
- Compound with methoxy group at ortho position of aromatic **ring A (3m**) showed better activity (*in vivo*) as compared to compound with methoxy at para position **(3l)**.

- Compound with methyl group at para position showed less activity **(3i)** as compared to when methyl group is at ortho position **(3j)**.
- Compound **(3q)** with di-subsitituted aromatic ring (methyl group) showed better activity as compared to when mono substituted methyl group is present **(3i, 3j)**.
- Position of chlorine groups at aromatic ring **(3o and 3p)** did not affect the activity.
- Ethoxy group substituted compound **(3n)** showed less activity as compared to compound having ethyl substituent **(3k)**.

4. Conclusion

It may be concluded that the compound **3g** showed better anti-inflammatory and significant analgesic activity *in vivo* as compared to piperine and the standard drug indomethacin. The anti-inflammatory activity of the compound **3g** could be attributed to its inhibition of TNF-α in addition to the reduction in protein expression of COX-2, NF-Kb and TNFα. Furthermore, no ulcerogenic and cytotoxic effect was observed for compound **3g**. Therefore, the compound**3g** may be considered as a promising candidate for development of new and safer anti-inflammatory agents.

5. Experimental protolcol

5.1. *Isolation of piperine*

The coarsely powdered fruits of *Piper nigrum* (3kg) were extracted three times with chloroform by cold maceration for 24 h. The extract was filtered and concentrated to dryness at 60^{o}C on a water bath. The residue was dissolved in minimum quantity of chloroform and addition of diethyl ether to this solution resulted in immediate separation of crude piperine. The needle-shaped crystals of compound were obtained by repeated crystallization with diethyl ether.

5.2. *Preparation of piperic acid (1)*

Piperine (2g) was dissolved in 200 ml of methanol containing 20% KOH in a 500 ml reaction flask. The reaction mixture was refluxed with stirring for 48 h. After completion of hydrolysis and removal of methanol under reduced pressure, a yellow colored oily solid was obtained which was then dissolved in water (100 mL) and acidified with HCl yielding yellowish precipitate of piperic acid. Crystallization with methanol gave yellow needle shaped crystals.

5.3. Synthesis of compound (2)

To a solution of **(1)** in THF, cesium carbonate and propargyl bromide were added and the reaction mixture was refluxed with stirring for 12h. After completion of the reaction, the reaction mixture was poured into crushed ice and extracted with ethyl acetate. The ethyl acetate layer was then dried over sodium sulfate and concentrated to obtain the crude product. The crude product **(2)** was then crystallized from methanol.

5.4. General procedure for the synthesis of compounds (3a-s)

To a solution of compound **(2)** (100 mg, 0.03 mol) in *t*-BuOH: H_2O (2:1, 2 ml), sodium ascorbate (1.2 mg, 0.06 mol) and $CuSO_4$ (1.2 mg, 0.045 mol) were added at room temperature. To this solution, aromatic azide (0.06 mol) was added and the reaction mixture was stirred for 24 h. After completion of the reaction, the reaction mixture was poured into crushed ice and extracted with ethyl acetate. The ethyl acetate layer was then dried over sodium sulfate, filtered and concentrated. The final product was then crystallized with methanol.

*5.5.1. 5-Benzo[1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(phenyl)-1H-[1,2,3]trizol-4-ylmethyl ester **(3a)*:** White crystals; yield: 64%; M.p: 145-146^{o}C; R_f : 0.51; FT-IR (cm^{-1} KBr); 1661, 1594, 1509, 1403. ^{1}H-NMR (300MHz, $CDCl_3$, δ_{ppm}): 5.40 (s, 2H, CH_2), 5.97 (s, 2H, CH_2), 6.00 (s, 1H,

CH=CH-CH=CH), 6.80-7.21 (m,7H, Ar-H), 7.48 (dd,1H, CH=CH-CH=CH, J=10.8, J=14.4), 7.59-7.70 (m, 3H, Ar-H,), 8.01 (s, 1H, triazole ring proton). ^{13}C-NMR (120MHz, $CDCl_3$): 57.75 (OCH_2), 101.68 (CH_2 piperine), 117.90-149.18 (Ar-C), 166.66 (C=O). ESI-MS m/z: 376 $[M+H]^+$ Anal. Calcd. for $C_{21}H_{17}N_3O_4$: C, 67.19; H, 4.56; N, 11.19; found C, 67.45; H, 4.65; N, 11.34.

5.4.2. 5-Benzo[1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(4-fluoro-phenyl)-1H-[1,2,3]trizol-4-ylmethyl ester ***(3b)*****:** White crystals; yield: 71%; M.p: 121-122^{o}C; R_f : 0.53; FT-IR (cm^{-1} KBr); 1668, 1599, 1520, 1421; ^{1}H-NMR (300 MHz, CDCl3, δ_{ppm}): 5.41 (s, 2H, CH_2), 5.95 (s, 2H, CH_2), 6.00 (s, 1H, CH=CH-CH=CH), 6.66-7.00 (m, 5H, Ar-H), 7.23 (d, 2H, Ar-H, J= 8.4), 7.47 (dd, 1H, CH=CH-CH=CH, J=11.1, J=15), 7.73 (dd, 2H, Ar-H, J= 4.5, J=8.4), 8.07 (s, 1H, triazole ring proton). ^{13}C-NMR (120MHz, $CDCl_3$): 57.40 (OCH_2), 101.44 (CH_2 piperine), 116.66-148.73 (Ar-C), 166.96 (C=O). ESI-MS m/z: 416 $[M+Na]^+$. Anal. Calcd. for $C_{21}H_{16}FNO_4$: C, 64.12; H, 4.10; N, 10.68; found C, 64.38; H, 4.39; N, 10.84.

5.4.3. 5-Benzo[1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(4-chloro-phenyl)-1H-[1,2,3]trizol-4-ylmethyl ester ***(3c)*****:** Brown crystals; yield: 65%; M.p: 176-177^{o}C; R_f : 0.51; FT-IR (cm^{-1} KBr); 1658, 1590, 1515, 1414. ^{1}H-NMR (300 MHz, CDCl3, δ_{ppm}): 5.32 (s, 2H, CH_2), 5.86 (s, 2H, CH_2), 5.91 (s, 1H, CH=CH-CH=CH), 6.58-6.92 (m, 5H, Ar-H), 7.34-7.45 (m, 3H, Ar-H), 7.62 (d, 2H, Ar-H, J=8.4), 7.99 (s, 1H, triazole ring proton). ^{13}C-NMR (120MHz, $CDCl_3$): 57.40 (OCH_2), 102.34 (CH_2 piperine), 118.16-148.73 (Ar-C), 166.66 (C=O). ESI-MS m/z: 410 $[M+H]^+$. Anal. Calcd. for $C_{21}H_{16}N_3O_4$: C, 61.54; H, 3.94; N, 10.25; found C,61.10; H, 3.26; N, 10.91.

5.4.4. 5-Benz [1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(4-bromo-phenyl)-1H-[1,2,3]trizol-4-ylmethyl ester ***(3d)*****:** Brown crystals; yield: 67%; M.p: 188-191^{o}C; R_f : 0.51; FT-IR (cm^{-1} KBr);

1668, 1585, 1520, 1410. ^{1}H-NMR (300 MHz, $CDCl_3$, δ_{ppm}): 5.43 (s,2H, CH_2), 5.99 (s, 2H, CH_2), 6.05 (s, 1H, CH=CH-CH=CH), 6.71-7.10 (m, 5H, Ar-H), 7.49 (dd, 1H, CH=CH-CH=CH, J= 10.4, J= 14.9), 7.67 (broad, s, 4H, Ar-H), 8.00 (s, 1H, triazole ring proton). ^{13}C-NMR (120MHz, $CDCl_3$): 57.89 (OCH_2), 102.49 (CH_2 piperine), 120.93-149.64(Ar-C), 166.10 (C=O). ESI-MS m/z: 456 $[M+2]^+$ Anal. Calcd. for $C_{21}H_{16}BrF_3NO_4$: C, 55.52; H, 3.35; N, 9.25; found C,55.81; H, 3.51; N, 9.53.

5.4.5. 5-Benzo[1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(2-fluoro-phenyl)-1H-[1,2,3]trizol-4-ylmethyl ester ***(3e)*****:** Light yellow crystals; yield: 62%; M.p: 134-135^{o}C; R_f : 0.52; FT-IR (cm^{-1} KBr); 1670, 1590, 1525, 1418. ^{1}H-NMR (300 MHz, $CDCl_3$, δ_{ppm}): 5.43 (s, 2H, CH_2), 5.96 (s, 2H, CH_2), 6.00 (s, 1H, CH=CH-CH=CH), 6.67-7.01(m, 5H, Ar-H), 7.32-7.52 (m, 4H, Ar-H), 7.97 (dd, 1H, Ar-H, J=7.8 J= 13.8), 8.19 (s, 1H, triazole ring proton). ^{13}C-NMR (120MHz, $CDCl_3$): 57.48 (OCH_2), 101.56 (CH_2 piperine), 114.67-145.53 (Ar-C), 165.19 (C=O). ESI-MS m/z: 416 $[M+Na]^+$. Anal. Calcd. for $C_{21}H_{16}FNO_4$: C, 64.12; H, 4.10; N, 10.68; found C,64.98; H, 4.61; N, 10.56.

5.4.6. 5-Benzo[1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(2-chloro-phenyl)-1H-[1,2,3]trizol-4-ylmethyl ester ***(3f)*****:** White powder; yield: 60%; M.p: 142-143^{o}C; R_f : 0.50; FT-IR (cm^{-1} KBr); 1660, 1580, 1520, 1410. ^{1}H-NMR (300 MHz, $CDCl_3$, δ_{ppm}): 5.44 (s, 2H, CH_2), 5.89 (s, 2H, CH_2), 6.00 (s, 1H, CH=CH-CH=CH), 6.72-7.00 (m, 5H, Ar-H), 7.37 (dd, IH, Ar-H, J=7.8, J=18), 7.47-7.82 (m, 4H, Ar-H), 8.11 (s, 1H, triazole ring proton). ^{13}C-NMR (120MHz, $CDCl_3$): 57.36 (OCH_2), 101.44 (CH_2 piperine), 119.33-148.70 (Ar-C), 166.95 (C=O). ESI-MS m/z: 410 $[M+H]^+$. Anal. Calcd. for $C_{21}H_{16}N_3O_4$: C, 61.54; H, 3.94; N, 10.25; found C,61.13; H, 3.34; N, 10.67.

5.4.7. 5-Benzo[1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(2-bromo-phenyl)-1H-[1,2,3]trizol-4-ylmethyl ester ***(3g)*****:** Brown crystals; yield: 67%; M.p: 180-181^{o}C; R_f : 0.50; FT-IR (cm^{-1} KBr); 1665, 1598, 1515, 1425. ^{1}H-NMR (300MHz, $CDCl_3$, δ_{ppm}): 5.41(s, 2H, CH_2), 5.95 (s, 2H, CH_2), 6.00 (s, 1H, CH=CH-CH=CH), 6.66-7.00 (m, 5H, Ar-H), 7.47 (dd, 1H, CH=CH-CH=CH, J= 10.5, J= 14.7), 7.66 (broad, s, 4H, Ar-H), 8.00 (s, 1H, triazole ring proton). ^{13}C-NMR (120MHz, $CDCl_3$): 57.35 (OCH_2), 101.44 (CH_2 piperine), 119.33-148.74(Ar-C), 166.95 (C=O). ESI-MS m/z: 456 $[M+2]^+$. Anal. Calcd. for $C_{21}H_{16}BrF_3NO_4$: C, 55.52; H, 3.35; N, 9.25; found C,55.81; H, 3.51; N, 9.53.

5.4.8. 5-Benzo[1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(3-chloro-phenyl)-1H-[1,2,3]trizol-4-ylmethyl ester ***(3h)*****:** White powder; yield: 63%; M.p: 161-162^{o}C; R_f : 0.50; FT-IR (cm^{-1} KBr); 1675, 1610, 1530, 1405. ^{1}H-NMR (300MHz, $CDCl_3$, δ_{ppm}): 5.33 (s, 2H, CH_2), 5.87 (s, 2H, CH_2), 5.91 (s, 1H, CH=CH-CH=CH), 6.57-6.91 (m, 7H, Ar-H), 7.38 (dd, 1H, Ar-H, J=7.8, J=18), 7.58 (d, 1H,Ar-H, J=7.2), 7.73(s, 1H, Ar-H), 8.11 (s, 1H, triazole ring proton). ^{13}C-NMR (120MHz, $CDCl_3$): 58.91 (OCH_2), 102.18 (CH_2 piperine), 120.60-150.23 (Ar-C), 166.59 (C=O). ESI-MS m/z: 410$[M+H]^+$. Anal. Calcd. for $C_{21}H_{16}N_3O_4$: C, 61.54; H, 3.94; N, 10.25; found C,61.45; H, 3.44; N, 10.80.

5.4.9. 5-Benzo[1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(4-methyl-phenyl)-1H-[1,2,3] trizol-4-ylmethyl ester ***(3i)*****:** White crystal; yield: 60%; M.p: 151-152^{o}C; R_f : 0.55; FT-IR (cm^{-1} KBr); 1689, 1620, 1513, 1409. ^{1}H-NMR (300MHz, $CDCl_3$, δ_{ppm}): 2.36 (s, 3H, CH_3), 5.45 (s, 2H, CH_2), 5.93 (s, 2H, CH_2), 6.03 (s, 1H, CH=CH-CH=CH), 6.63-6.98 (m, 5H, Ar-H), 7.32-7.52 (m, 4H, Ar-H), 7.97 (dd, 1H, Ar-H, J=15.6 J= 18), 8.19 (s, 1H, triazole ring proton). ^{13}C-NMR (120MHz,

$CDCl_3$): 22.12 (CH_3), 58.91 (OCH_2), 102.59 (CH_2 piperine) 121.68-149.53 (Ar-C), 166.59 (C=O). ESI-MS m/z: 390 $[M+H]^+$. Anal. Calcd. for $C_{22}H_{19}N_3O_4$: C, 67.86; H 4.92; N, 10.79; found C,68.05; H, 4.67; N, 10.57.

5.4.10. 5-Benzo[1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(2-methyl-phenyl)-1H-[1,2,3]trizol-4-ylmethyl ester ***(3j)*****:** White powder; yield: 58%; M.p: 146-147^oC; R_f : 0.55; FT-IR (cm^{-1} KBr); 1666, 1618, 1518, 1420. ^{1}H-NMR (300MHz, CDCl3): δ 2.39 (s, 3H, CH_3), 5.47 (s, 2H, CH_2), 5.95 (s, 2H, CH_2), 6.01 (s, 1H, CH=CH-CH=CH), 6.70-6.96 (m, 5H, Ar-H), 7.35-7.56 (m, 4H, Ar-H), 7.97 (dd, 1H, Ar-H, J=7.8, J=13.8), 8.16 (s, 1H, triazole ring proton). ^{13}C-NMR (120MHz, $CDCl_3$) 22.89 (CH_3), 57.81 (OCH_2), 101.19 (CH_2 piperine), 118.18-147.23 (Ar-C), 166.18 (C=O). ESI-MS m/z: 390 $[M+H]^+$. Anal. Calcd. for $C_{22}H_{19}N_3O_4$: C, 67.86; H, 4.92; N, 10.79; found C,67.45; H, 4.10; N, 10.50.

5.4.11. 5-Benzo[1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(4-ethyl-phenyl)-1H-[1,2,3]trizol-4-ylmethyl ester ***(3k)*****:** White powder; yield: 61%; M.p: 155-156^oC; R_f : 0.54; FT-IR (cm^{-1} KBr); 1680, 1582, 1510, 1412. ^{1}H-NMR (300MHz, $CDCl_3$, δ_{ppm}): 1.46 (t, 3H, CH_3 J= 6.0), 1.76 (q, 2H, OCH_2 J=6.8), 5.42 (s, 2H, CH_2), 5.99, (s, 2H, CH_2), 6.01 (s, 1H, CH=CH-CH=CH), 6.75-7.15 (m, 7H, Ar-H), 7.48 (dd, 1H, CH=CH-CH=CH, J= 9.9, J=14.4), 7.60-7.88 (m, 2H, Ar-H,), 8.04 (s, 1H, triazole ring proton). ^{13}C-NMR (120MHz, $CDCl_3$): 18.18 (CH_3), 30.12, (CH_2), 58.99 (OCH_2), 101.90 (CH_2 piperine) 119.13-150.24 (Ar-C), 166.18 (C=O). ESI-MS m/z: 404 $[M+H]^+$. Anal. Calcd. for $C_{23}H_{21}N_3O_4$: C, 68.47; H, 5.25; N, 10.42; found C,68.21; H, 5.45; N, 10.71.

5.4.12. 5-Benzo[1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(4-methoxy-phenyl)-1H-[1,2,3]trizol-4-ylmethyl ester ***(3l)*****:** White powder; yield: 63%; M.p: 130-131^oC; R_f : 0.53; FT-IR (cm^{-1} KBr); 1680, 1582, 1510, 1418. ^{1}H-NMR (300MHz, $CDCl_3$, δ_{ppm}): 3.88 (s, 3H, OCH_3), 5.41(s, 2H,

CH_2), 5.95 (s, 2H, CH_2), 6.00 (s, 1H, CH=CH-CH=CH), 6.66-7.05 (m, 7H, Ar-H), 7.47(dd, 1H, CH=CH-CH=CH, J=10.8, J=15.3), 7.64 (d, 2H, Ar-H, J= 9.0), 8.01 (s,1H, triazole ring proton). ^{13}C-NMR (120MHz, $CDCl_3$): 55.69 (OCH_3), 57.50 (OCH_2), 101.43 (CH_2 piperine), 114.29-148.32 (Ar-C), 166.97 (C=O), ESI-MS m/z: 406 $[M+H]^+$. Anal. Calcd. for $C_{22}H_{19}N_3O_5$: C, 64.12; H, 4.10; N, 10.68; found C,64.98; H, 4.61; N, 10.56.

5.4.13. 5-Benzo[1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(2-methoxy-phenyl)-1H-[1,2,3]trizol-4-ylmethyl ester ***(3m)*****:** White cystral; yield%: 59; M.p: 141-142^{o}C; R_f : 0.53; FT-IR (cm^{-1} KBr); 1668, 1570, 1529, 1429. ^{1}H-NMR (300MHz, $CDCl_3$, δ_{ppm}): 3.86 (s, 3H, OCH_3), 5.43(s, 2H, CH_2), 5.98 (s, 2H, CH_2), 6.03 (s, 1H, CH=CH-CH=CH), 6.70-7.10 (m, 9H, Ar-H), 7.47 (dd, 1H,CH=CH-CH=CH , J=10.5, J=15.3), 8.03 (s,1H, triazole ring proton). ^{13}C-NMR (120MHz, $CDCl_3$): 55.18 (OCH_3), 57.90 (OCH_2), 102.43 (CH_2 piperine) 115.19-149.42 (Ar-C), 166.11 (C=O). ESI-MS m/z: 406 $[M+H]^+$. Anal. Calcd. for $C_{22}H_{19}N_3O_5$: C, 64.12; H, 4.10; N, 10.68; found C,65.08; H, 4.89; N, 10.91.

5.4.14. 5-Benzo[1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(4-ethoxy-phenyl)-1H-[1,2,3]trizol-4-ylmethyl ester ***(3n)*****:** Light brown; yield: 65%; M.p: 162-163^{o}C; R_f : 0.54; FT-IR (cm^{-1} KBr); 1670, 1575, 1520, 1408. ^{1}H-NMR (300MHz, $CDCl_3$, δ_{ppm}): 1.46 (t, 3H, CH_3 J= 6.9), 4.08 (q, 2H, OCH_2 J=6.9), 5.40 (s, 2H, CH_2), 5.95, (s, 2H, CH_2), 6.00 (s, 1H, CH=CH-CH=CH), 6.75-7.03 (m, 7H, Ar-H), 7.47 (dd, 1H, CH=CH-CH=CH , J= 10.5, J=15), 7.63 (d, 2H, Ar-H, J= 8.4), 8.01 (s, 1H, triazole ring proton). ^{13}C-NMR (120MHz, $CDCl_3$): 20.38 (OCH_2CH_3), 57.90 (OCH_2), 64.12, (OCH_2CH_3), 101.90 (CH_2 piperine) 119.13-150.24 (Ar-C), 166.18 (C=O). ESI-MS m/z: 442 $[M+Na]^+$. Anal. Calcd. for $C_{23}H_{21}N_3O_5$: C, 65.86; H, 5.05; N, 10.02; found C,65.10; H, 5.26; N, 10.35.

5.4.15. 5-Benzo[1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(2,4-di-chloro-phenyl)-1H-[1,2,3]trizol-4-ylmethyl ester ***(3o)*****:** White powder; yield: 67%; M.p: 168-169^{o}C; R_f : 0.50; FT-IR (cm^{-1} KBr); 1677, 1570, 1505, 1412. ^{1}H-NMR (300 MHz, $CDCl_3$, δ_{ppm}): 5.34 (s, 2H, CH_2), 5.87 (s, 2H, CH_2), 5.91 (s, 1H, CH=CH-CH=CH), 6.58-6.92 (m, 5H, Ar-H), 7.35-7.53 (m, 4H, Ar-H), 8.01 (s, 1H, triazole ring proton). ^{13}C-NMR (120MHz, $CDCl_3$): 57.90 (OCH_2), 102.10 (CH_2 piperine) 120.43-151.24 (Ar-C), 166.98 (C=O). ESI-MS m/z: 466 $[M+Na]^+$. Anal. for $C_{21}H_{15}Cl_2N_3O_4$: C, 56.77; H, 3.40; N, 9.46; found C, 56.89; H, 3.77; N, 9.98.

5.4.16. 5-Benzo[1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(3,4-di-chloro-phenyl)-1H-[1,2,3]trizol-4-ylmethyl ester ***(3p)*****:** White powder; yield: 66%; M.p: 171-172^{o}C; R_f : 0.50; FT-IR (cm^{-1} KBr); 1671, 1577, 1525, 1418. ^{1}H-NMR (300MHz, $CDCl_3$, δ_{ppm}): 5.41 (s, 2H, CH_2), 5.95 (s, 2H, CH_2), 6.00 (s, 1H, CH=CH-CH=CH), 6.72-7.01(m, 5H, Ar-H), 7.63 (s, 2H, Ar-H), 7.47 (dd, 1H, Ar-H, J=10.8 J=15), 7.92 (s 1H, Ar-H), 8.09 (s,1H, triazole ring proton). ^{13}C-NMR (120MHz, $CDCl_3$): 57.80 (OCH_2 piperine), 101.18 (CH_2 piperine) 121.33-151.84 (Ar-C), 166.16 (C=O). ESI-MS m/z: 466 $[M+Na]^+$. Anal. Calcd. for $C_{21}H_{15}Cl_2N_3O_4$: C, 56.77; H, 3.40; N, 9.46; found C,56.98; H, 3.56; N, 10.01.

5.4.17. 5-Benzo[1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(3,4-di-methyl-phenyl)-1H-[1,2,3]trizol-4-ylmethyl ester ***(3q)*****:** White powder; yield: 62%; M.p: 164-166^{o}C; R_f : 0.52; FT-IR (cm^{-1} KBr); 1679, 1575, 1535, 1419. ^{1}H-NMR (300MHz, $CDCl_3$, δ_{ppm}): 2.38 (s, 6H, CH_3), 5.48 (s, 2H, CH_2), 5.97 (s, 2H, CH_2), 6.04 (s, 1H, CH=CH-CH=CH), 6.60-7.15(m, 7H, Ar-H), 7.31(m, 1H, Ar-H), 7.59 (dd, 1H, Ar-H, J=10.8, J=15.3), 8.15 (s, 1H, triazole ring proton). ^{13}C-NMR (120MHz, $CDCl_3$): 22.19 (CH_3), 58.45 (OCH_2), 101.40 (CH_2 piperine) 120.56-148.23 (Ar-C), 166.68 (C=O). ESI-MS m/z: 404 $[M+H]^+$. Anal. Calcd. for $C_{23}H_{21}N_3O_4$: C, 68.47; H, 5.25; N, 10.42; found C,68.89; H, 5.39; N, 10.47

5.4.18. 5-Benzo[1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(4-nitro-phenyl)-1H-[1,2,3]trizol-4-ylmethyl ester ***(3r)*****:** White powder; yield: 56%; M.p: 180-181^{o}C; R_f : 0.50; FT-IR (cm^{-1} KBr); 1887, 1587, 1515, 1423. ^{1}H-NMR (300MHz, $CDCl_3$, δ_{ppm}): 5.43 (s, 2H, CH_2), 5.95 (s, 2H, CH_2), 6.01 (s, 1H, CH=CH-CH=CH), 6.72-7.01(m, 7H, Ar-H), 7.55 (dd, 1H, Ar-H, J=10.8 J=15), 8.01(d 1H, Ar-H, J=9.0), 8.22 (s, 1H, triazole ring proton), 8.44 (d, 1H, Ar-H, J=9). ^{13}C-NMR (120MHz, $CDCl_3$): 56.65 (OCH_2), 101.20 (CH_2 piperine) 118.97-148.28 (Ar-C), 165.67 (C=O). ESI-MS m/z: 421 $[M+H]^+$. Anal. Calcd. for $C_{21}H_{16}Cl_2N_4O_6$: C, 60.00; H, 3.84; N, 13.33; found C,60.27; H, 3.56; N, 13.78.

5.4.19. 5-Benzo[1,3]dioxol-5-yl-penta-2,4-dienoic acid 1-(3-nitro-phenyl)-1H-[1,2,3]trizol-4-ylmethyl ester ***(3s)*****:** White powder; yield: 52%; M.p: 185-186^{o}C; R_f : 0.50; FT-IR (cm^{-1} KBr); 1687, 1589, 1514, 1419. ^{1}H-NMR (300 MHz, $CDCl_3$, δ_{ppm}): 5.35 (s, 2H, CH_2), 5.87 (s, 2H, CH_2), 5.91 (s, 1H, CH=CH-CH=CH), 6.63-6.92 (m, 5H, Ar-H), 7.40 (dd, 1H, Ar-H, J=10.8 J= 15), 8.11-8.26 (m, 4H, ArH), 8.54 (s, 1H, triazole ring proton). ^{13}C-NMR (120 MHz, $CDCl_3$): 56.35 (OCH_2), 102.70 (CH_2 piperine), 119.87-149.78 (Ar-C), 165.34 (C=O). ESI-MS m/z: 421 $[M+H]^+$. Anal. Calcd. for $C_{21}H_{16}N_4O_6$: C, 60.00; H, 3.84; N, 13.33; found C,60.34; H, 3.95; N, 13.39.

6. Pharmacology

6.1. Anti-inflammatory activity

The anti-inflammatory activity was carried out by the reported method [26]. Animals were divided into twenty one groups of five animals each. One group kept as a positive control group was orally administered with standard indomethacin (20mg/kg) and other group kept as a negative control group and administered with 0.5% carboxymethyl cellulose solution. The

remaining groups were test groups and administered orally with synthesized compounds at dose of 20mg/kg b.w. A freshly prepared solution of carrageenan (1.0% in sterile 0.9% NaCl solution) in a volume of 0.1 ml was injected subcutaneously into the subplantar region of the right hind paw after 1 h of administration of the test sample. Right hind paw volume was measured at 3 h and 5 h after carrageenan injection with the help of digital plethysmometer. The percent anti-inflammatory activity was calculated according to the formula given below.

$$\% \text{ Anti-inflammatory Activity} = [V_C - V_t / V_C] \times 100$$

Where, V_t represents the mean increase in paw volume in rats treated with test samples and V_C represents the mean increase in paw volume of rats in control group.

6.2. Molecular Docking studies on TNF-α

Crystallized structure of 2AZ5 was chosen from Protein Data Bank and used as target for molecular docking studies. 2AZ5 structure was imported in Schrodinger using Protein Preparation Wizard. Missing hydrogen and were added using prime interface. Undesired water molecules were removed. The protein was then optimized and minimized to give low energy and structurally correct target protein. As the target protein had already the site for reference ligand, the grid was generated by selecting the ligand as the reference ligand. Finally the grid was validated and was used for further docking with new unknown ligands to predict their docking score. Chemical structures were drawn in maestro and geometrically refined by LigPrep module. In this module 2-D structures were converted into 3-D structures, which were further subjected to OPLS-2005 force field to generate single low energy 3-D structure for each input structure. During this step chiralities were maintained. Docking was carried using Schrodinger Glide software with Extra precision and XP descriptor information. This generates favourable ligand poses which are further screened through filters to examine spatial fit of the ligand in the active.

Ligand poses which pass through initial screening are subjected to evaluation and minimization of grid approximation. Scoring is then carried on energy minimized poses to generate Glide score.

6.3. TNF-α Assay

RAW 264.7 cells were cultured in RPMI-1640 medium supplemented with 10% FBS, penicillin (100 units/mL), and streptomycin sulfate (100m g/mL) in a humidified atmosphere of 5% CO_2. The cells were harvested with trypsin-EDTA and diluted to a suspension in fresh medium. The cells were then seeded in 96-well plates with 2.1×10^5 cells/well, and allowed to adhere for 1 h. Then the medium was induced with 100 μg/ml LPS (lipopolysaccharide), test samples (20μM), and incubated for 24 h. The supernatant (50 μL) was then transferred into a 96-well ELISA plate and TNF-α level were quantified by ELISA kits according to the manufacturer's instructions.

6.4. MTT assay for Cell Viability

RAW 264.7 cells (2×10^5) were cultured in 96-well plate containing DMEM supplemented with 10% FBS. The cells were stimulated with 20μM test compounds in the presence of 100 μg/ml LPS for 24 h. Then, the cells were washed twice with DPBS and incubated for 2h at 37^{o}C with 100 μl of 0.5 mg/ml MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide). The medium was discarded and 100 μl dimethyl sulfoxide (DESI-MSO) was added to it. The absorbance was recorded at 570nm using a microplate reader after 30 min incubation.

6.5. Antinociceptive activity

The analgesic activity was carried out by writhing test method using the previously reported method [27]. Swiss albino mice (35–40 g) of either sex were divided into seven groups with each group containing five animals. Group I was taken as control and received CMC suspension only, group II received standard drug indomethacin and rest of the groups were orally administered with synthesized compounds at a dose of 20mg/kg. After 30 min of test samples administration, 0.1% acetic acid solution was given to mice intraperitoneally. The number of muscular contractions was counted over a period of 10 min after acetic acid injection. The data represents the total number of writhes observed during 10 min and is expressed as writhing numbers.

6.6. *Ulcerogenic activity*

The ulcerogenic study was carried out by earlier reported method [28]. Those compounds which showed significant anti-inflammatory activity were further evaluated for their ulcerogenic effects. Control group rats were orally given a suspension of 1% carboxymethylcellulose only. The standard group and test samples were orally administered at a dose of 60mg respectively which is three times of the dose used for anti-inflammatory activity. Animals were sacrificed after 5 h of dosing of standard group and test samples.

6.7. Lipid peroxidation assay

Lipid peroxidation assay was carried out by reported method [29]. The gastric mucosa was scraped with two glass slides and weighed (100 mg) and homogenized in 1.8 mL of 1.15% ice cold KCl solution. One milliliter of suspension medium was taken from the supernatant, 0.5 mL of 30% trichloroacetic acid followed by 0.5 mL of 0.8% thiobarbituric acid reagent were added to it. The tubes were covered with aluminum foil and kept in a shaking water bath for 30 min at 80°C. After 30 min, tubes were taken out and kept in ice cold water for 10 min. These

were then centrifuged at 3000 rpm for 15 min. The absorbance of supernatant was read at 540 nm at room temperature.

6.8. Immunohistochemistry

The paw tissues were fixed in formalin and embedded in paraffin. Sections of 5 µm thickness were cut onto poly-lysine coated glass slides. Sections were deparafinized three times (5 min) in xylene followed by dehydration in graded ethanol and finally rehydrated in running tap water. For antigen retrieval, sections were boiled in 10mM citrate buffer (pH 6.0) for 5-7 min. Sections were incubated with hydrogen peroxide for 15 min to minimize non-specific staining and then rinsed three times (5 min each) with 1X PBST (0.05% Tween-20). Blocking solution was applied for 10 min and then sections were incubated with diluted (1:100) primary anti-bodies, purified rabbit polyclonal anti-NF-κB antibody (BioLegend), rabbit polyclonal anti-COX-2 antibody (Bio Vision) and polyclonal anti-TNF-α antibody (Bio Vision) overnight at 4^{o}C in humid chamber. Further processing was done according to the instructions of Ultra Vision plus Detection System Anti-Polyvalent, HRP/DAB (Ready-To-Use) staining kit (Thermo scientific system). The peroxidase complex was visualized with 3,3-diaminobenzidine (DAB). Lastly the slides were counterstained with haematoxylin, cleaned in xylene, dehydrated with ethanol and after DPX mounting microscopic (BX 51 Olympus) analysis was done at 40X magnification.[30]

Supplementary data

The supplementary data has been provided with this article

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References

1. P. K. Warrier, Indian Spices 18 (1981) 32.
2. V.S. Parmar, S.C. Jain, K.S. Bisht, R. Jain, P. Taneja, Phytochemistry. 46 (1997) 597-673
3. N. Pathak, S. Khandlewal, Eur. J. Pharmacol 160 (2006) 576-582.
4. E.S. Sunila, G. Kuttan, . J. Ethnopharmacol. 90 (2004) 339-346.
5. A. Balkrishna, Ayurved Jadi Buti Rahasya. 5th ed.Divya Prakashan Patanjali Yogpeeth, Maharishi Dayanand Gram, Haridwar, India. (2008) 362-400.
6. M. Ibrahim, K.M. Nane, A. Anjum, World J. Gastroenterology. 16 (2008) 2566-2571
7. S. Panda, A. Kar, Horm.Metab.Res. 35 (2003) 523.
8. Li.S. Wang, Li.W. Koike, K. Nikaido, T. Wang, J. Asian Nat. Prod. Res. 9 (2007) 421-430.
9. O. Ibrahim, K.A. Mahrous, E.H.E. Fars, M. A. Kamal, Abdulmalik, H.N. Steven, Int. J. Med. Sci. 7 (2010) 232-239.
10. G.R. Jorge, T.L. David, Geriatr. Nephrol. Uro. 7 (1997) 51-57.
11. H.H. Tan, W.M.C. Ong, S.H. Lai, W.C. Chow, Sing. Med. J. 48 (2007) 582-585.
12. N.K.V.M.R. Kumara, In WHO Symposium. University of Ruhuna, Sri Lanka, (2001) 12-14.
13. K. M. Banu, A. Dinakar, C. Ananthanarayanan, Indian J. Pharm. Sci, 4 (1999) 202.
14. S. Haider, A. Sarwar, H. Hamid, S. Shafi, A. Nargotra, P. Mahajan, S. Nazreen, M. Kalle, C. Kharbanda, Y. Ali, A. Alam, A. Panda, Eur. J. Med.Chem. 70 (2013) 579-588

15. Allais, J. Meier, Ger. Offen. 181 (1969) 546.

16. M.D. Chen, S.J. Lu, G.P. Yuag, S.Y. Yang, X.L. Du, Heter. Comm. 6 (2000) 421-427.

17. E.A. Sherement, R.I. Tomanov, E.V. Trukhin, V.M. Berestovitskaya, Russ. J. Org. Chem. 40 (2004) 594

18. R. Meier, E.P. Eidem, US. 4789680 (1986).

19. A. Passannanti, P. Diana, P. Barraja, F. Mingoia, A. Lauria, G. Cirrincione, Heterocycles. 48 (1988) 1229.

20. M. Jilino, F.G. Stevens, J. Chem. Soc, (1998) 1677.

21. C.R. Pradeep, G. Kuttan, Immnupharm. Immnotoxic. 25 (2003) 337-346.

22. B. L. Slomiany, J. Piotrowski, A. Slomiany, Scan. J. Gastero. 32 (1997) 638-642.

23. G.D. Diana, J.J. Nitz, E.P. Eidem, 566199 (1993).

24. Y.J. Surh, K.S. Chun, H.H. Cha, S.S. Han, Y.S. Keum, K.K. Park, S.S. Lee, Mutat. Res. (2001) 243–268.

25. T.W. Chung, S.K. Moon, Y.C. Chang, K. J.H. Lee, Y.C. Cho, G. Kim, S.H. Kim, G.J. Kim, FASEB. J. 14 (2004) 1670–1681.

26. C.A. Winter, E.A. Risley, G.W. Nuss, Proceed. Soc. Exp. Biol. Med. 111(1962) 544-546.

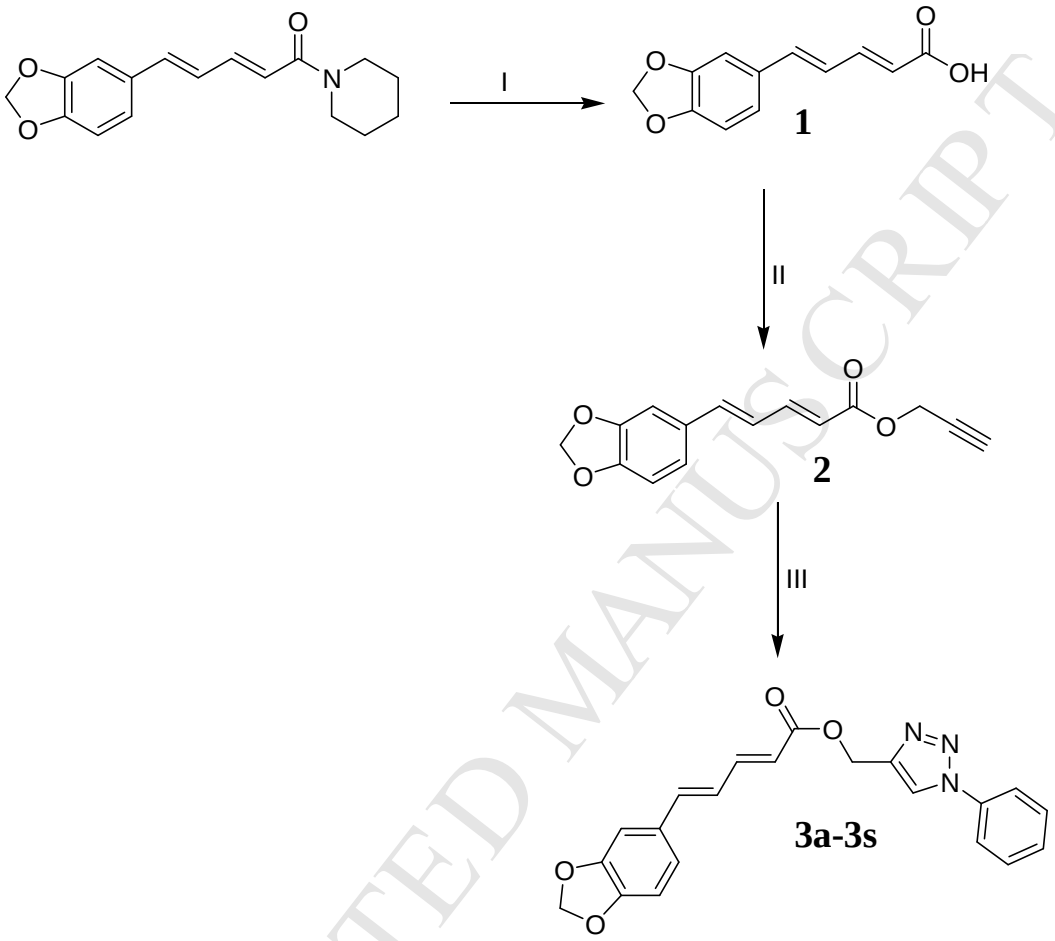
27. R. Koster, M. Anderson, E.J. Beer, Fed. Proceed. 18 (1959) 412–416.

28. H.G. Vogel, H.W. Vogel, Springer-Verlag: Berlin Heidelberg, New York, (1997) p. 1231

29. H. Ohkawa, N. Ohishi, K. Yagi, Anal. Biochem. 95 (1979) 351–358.

30. H. Saba Zuhair, Y. Kamaruddin Mohd, M. Suzana, Y. Yasmin Anum Mohd, 8 (2013) 1-8

Scheme I:



Reagents and Conditions: (I) Alcohol, KOH, reflux; (II) $CeCO_3$, dry THF, reflux; (III) CH_3COONa, NaN_3, Ar-N_3.

Table1: Anti-inflammatory activity of synthesized compounds.

Compounds	**R**	**Change in paw volume(ml) mean±(SEM)**		**% inhibition**	
		3h	5h	3h	5h
Control	-	1.68±0.029	1.70±0.020	-	-
(Indomethacin)	-	0.608±0.032**	0.54±0.023**	72.05	77.02
Piperine	-	0.676±0.022*	0.670±0.013*	53.69	54.72
3a	H	1.12±0.028*	1.11±0.014**	36.98	38.51
3b	4-F	0.87±0.017*	0.85±0.025*	55.47	57.02
3c	4-Cl	0.83±0.017*	0.81±0.022*	57.53	59.72
3d	4-Br	0.89±0.023*	0.87±0.017*	53.56	55.53
3e	2-F	0.68±0.014**	0.65±0.023**	68.19	70.81
3f	2-Cl	0.58±0.017**	0.56±0.022**	74.52	76.71
3g	2-Br	0.56±0.020**	0.51±0.025**	76.12	80.40
3h	3-Cl	0.71±0.016**	0.69±0.015**	64.72	67.81
3i	4-CH_3	1.01±0.025*	0.98±0.029*	46.22	49.09
3j	O-CH_3	0.91±0.017*	0.88±0.029*	51.50	54.59
3k	4-C_2H_5	0.83±0.010*	0.80±0.011*	57.53	60.00
3l	4-OCH_3	0.88±0.016*	0.86±0.020*	53.97	55.94
3m	2-OCH_3	0.76±0.009**	0.72±0.022**	61.64	65.00
3n	4-OC_2H_5	1.10±0.023*	1.14±0.034	32.87	36.48
3o	2,4-di-Cl	0.64±0.023**	0.622±0.04**	69.31	71.21
3p	3,4-di-Cl	0.632±0.023*	0.588±0.08**	69.58	72.97
3q	3,4-di-CH_3	0.68±0.020**	0.66±0.016**	67.94	70.27
3r	4-NO_2	1.39±0.020*	1.37±0.013	19.58	22.07
3s	3-NO_2	1.44±0.021*	1.46±0.011*	15.61	15.40

Data is analyzed by one way ANOVA followed by Dunnett's 't' test and expressed as mean ± SEM from five observations where * $p < 0.05$, ** $p < 0.01$

Table2: Docking results of synthesized compounds.

Ligands	Glide score	Glide energy	M.W	QPlog Po/w	QPlogS	PSA
3a	-5.80	-40.12	375.38	4.17	-5.31	87.20
3b	-3.82	-38.22	393.37	4.44	-5.68	86.31
3c	-4.73	-37.15	409.82	4.71	-6.07	86.31
3d	-3.58	-40.32	454.27	4.78	-6.19	86.31
3e	-5.59	-42.36	393.37	4.43	-5.57	85.81
3f	-5.78	-42.15	409.82	4.70	-5.93	78.51
3g	-5.81	-41.80	454.27	4.78	-6.04	85.34
3h	-5.61	-43.98	409.82	4.70	-6.06	86.31
3i	-4.28	-34.69	389.41	4.52	-5.91	86.31
3j	-4.64	-38.93	389.41	4.49	-5.92	87.19
3k	-5.09	-37.30	403.43	5.14	-6.71	86.32
3l	-3.18	-33.97	405.40	4.23	-5.46	95.49
3m	-5.15	-36.96	405.40	4.27	-5.46	92.33
3n	-3.30	-39.91	419.43	4.27	-5.28	94.06
3o	-5.67	-43.63	444.27	3.44	-5.33	85.39
3p	-5.32	-41.25	444.27	3.11	-4.35	80.32
3q	-5.13	-39.76	403.43	4.78	-6.37	87.19
3r	-4.09	-37.59	420.38	3.41	-5.35	132.11
3s	-2.81	-38.75	420.38	3.44	-5.33	131.19
Indomethacin	-5.02	-33.09	357.00	4.28	-5.31	84.32
Piperine	-4.42	-24.68	285.00	3.25	-3.44	47.98

Table 3: Analgesic activity of active compounds

Group	Number of writhes in 10 min	% Protection
Control	92.8 ± 1.49	-
Indomethacin	39.4 ± 1.16**	57.43
3e	45.8± 1.85*	48.70
3f	45.8± 1.85**	50.64
3g	42.6 ± 1.63**	54.09
3o	51.0 ± 1.87*	45.04
3p	46.4±1.69**	50.00
3q	51.2±1.28*	44.93

Data is analyzed by one way ANOVA followed by Dunnett's 't' test and expressed as mean ± SEM from five observations where * $p < 0.05$, ** $p < 0.01$

Fig captions

Fig1: Docking images of active compounds (**3g, 3f**), standard drug indomethacin and piperine respectively.

Fig 2: Effect of synthesized compounds on LPS induced TNF-α cytokine level in the RAW 264.7 cell line.

Fig 3: Cytotoxicity assay of active compounds.

Fig4: Histopathology of rat stomach in Albino Wistar rats, photomicrographs (**20X**) of stomach wall of the animal groups administered with compound **3g, 3f, 3p, 3o** standard drug indomethacin and control group respectively.

Fig5: Lipid peroxidation assay of active compounds.

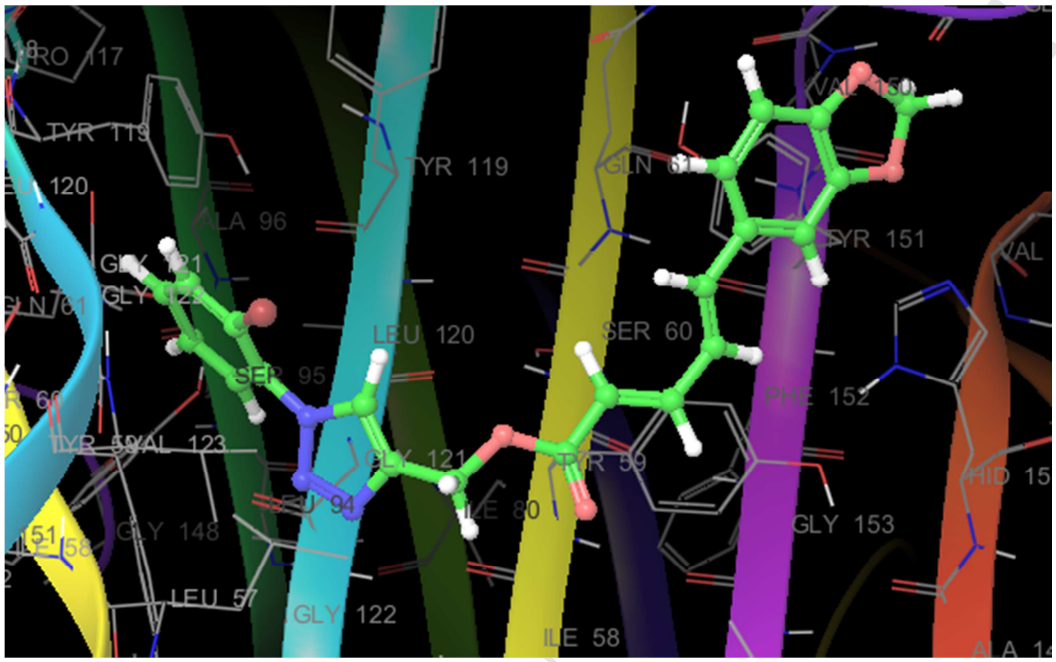
Fig6.a: Paw tissue expressions of COX-2 activation. Representative photomicrographs magnification (40X) Group I (only control), Group II (only carrageenan), Group III (carrageenan + standard drug Indomethacin), Group IV (carrageenan +**3g**) GroupV (carrageenan+ **3f).**

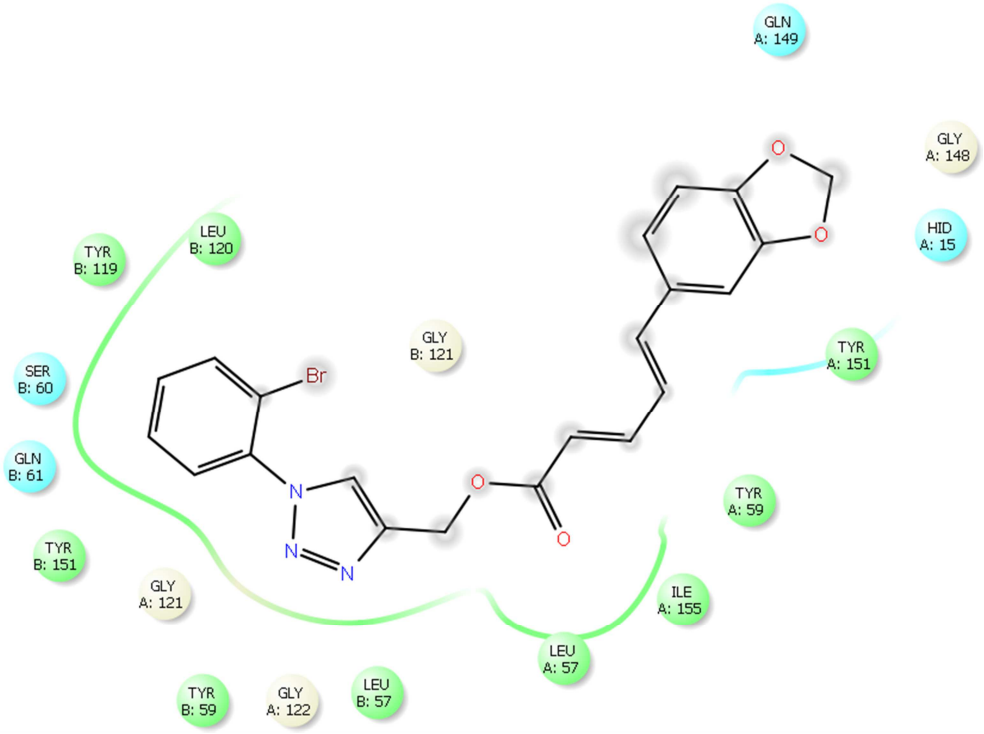
Fig6.b: Paw tissue expressions of NFkB activation. Representative photomicrographs magnification (40X) Group I (only control), Group II (only carrageenan), Group III (carrageenan + standard drug Indomethacin), Group IV (carrageenan +**3g**) Group (carrageenan+ **3f).**

Fig6.c: Paw tissue expressions of TNF-α activation. Representative photomicrographs magnification (40X) Group I (only control), Group II (only carrageenan), Group III (carrageenan + standard drug Indomethacin), Group IV (carrageenan +**3g**) Group (carrageenan+ **3f).**

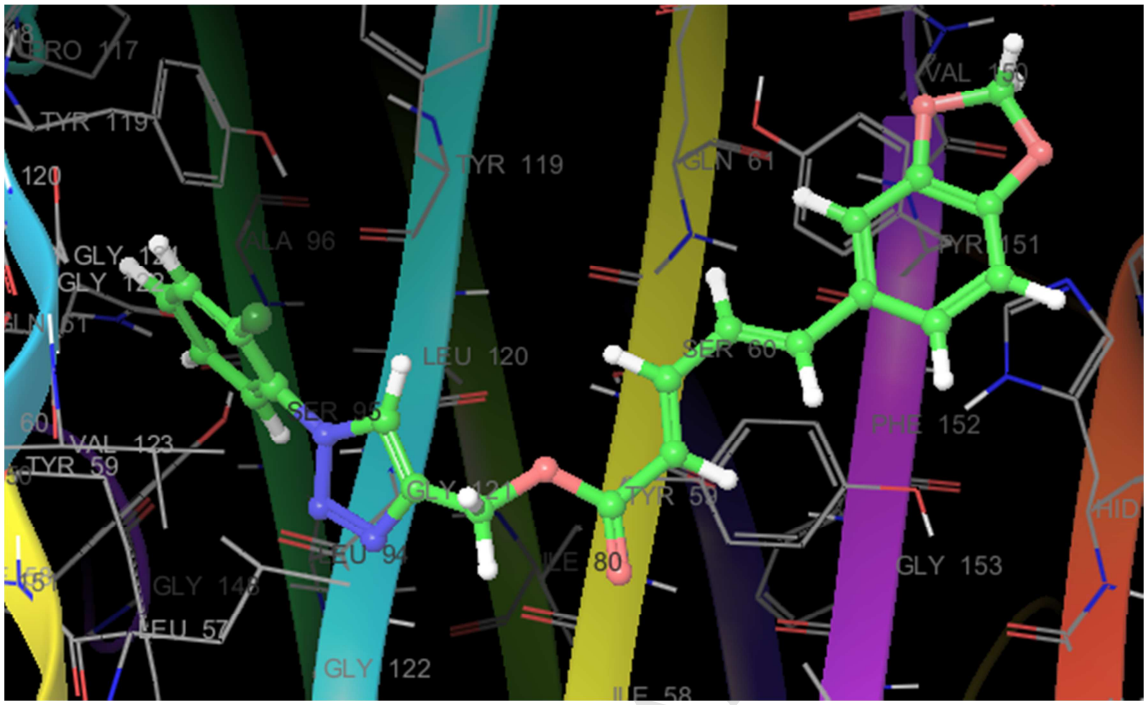
Fig1: Docking images of active compounds (**3g, 3f**), standard indomethacin and piperine respectively.

Compound 3g





Compound 3f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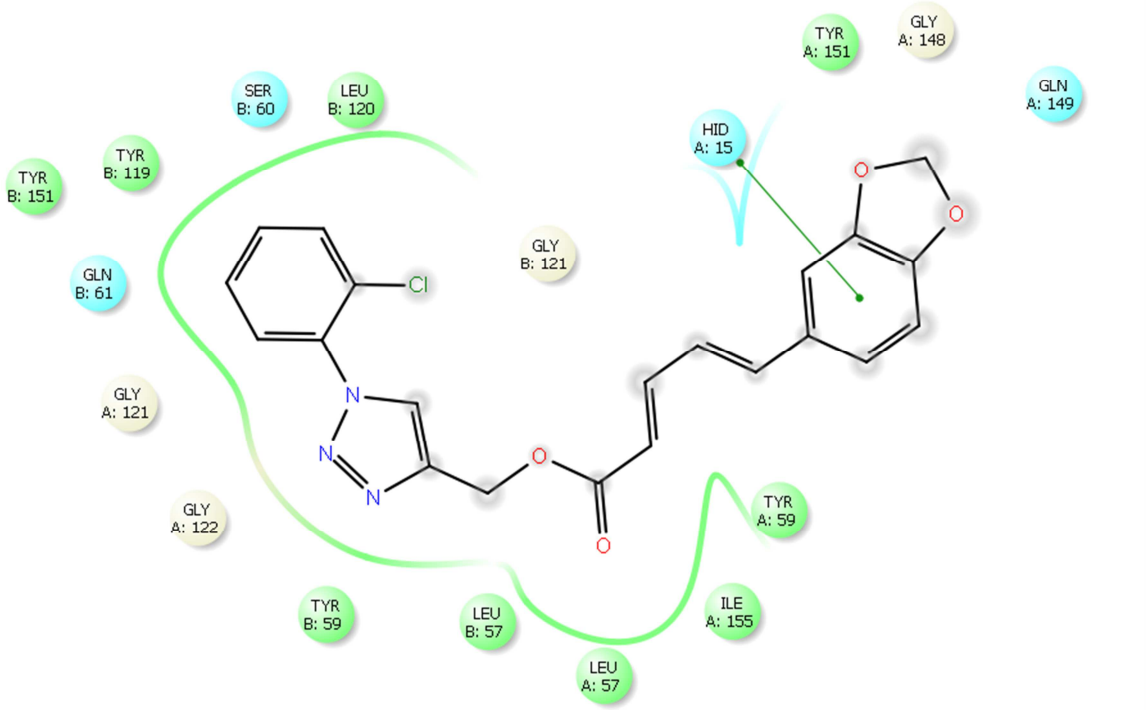
SER 60
PHE 152
GLY 153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Cl
N
N
N
O
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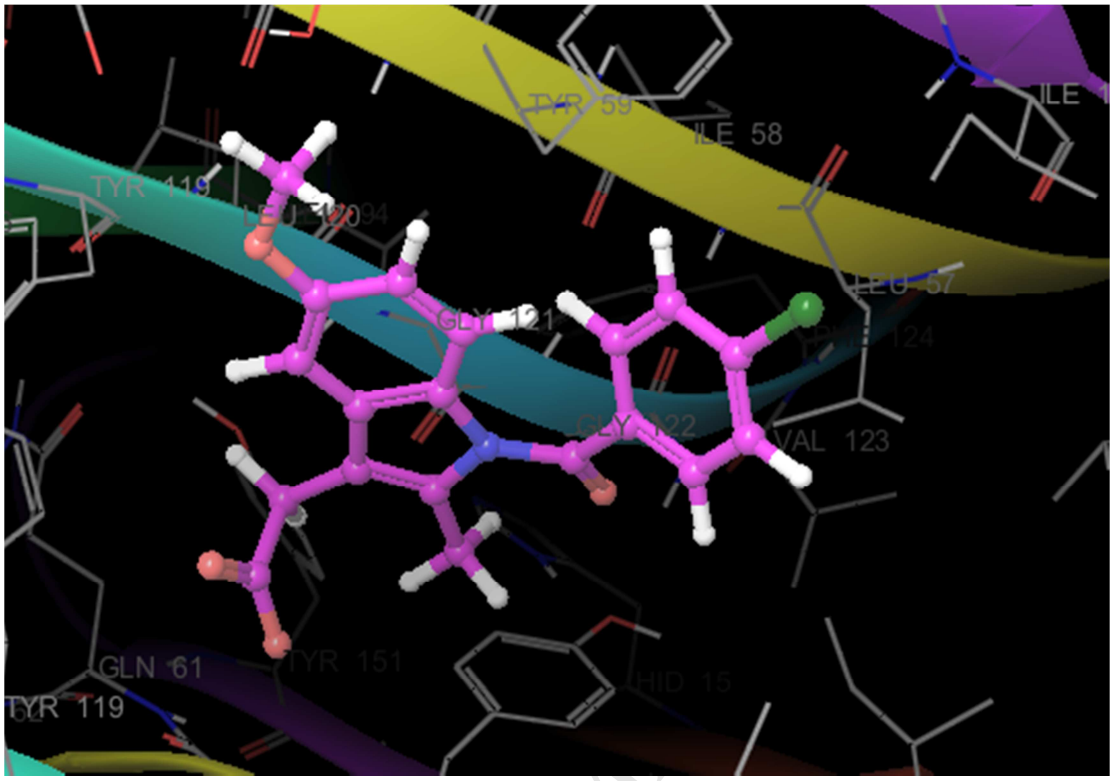
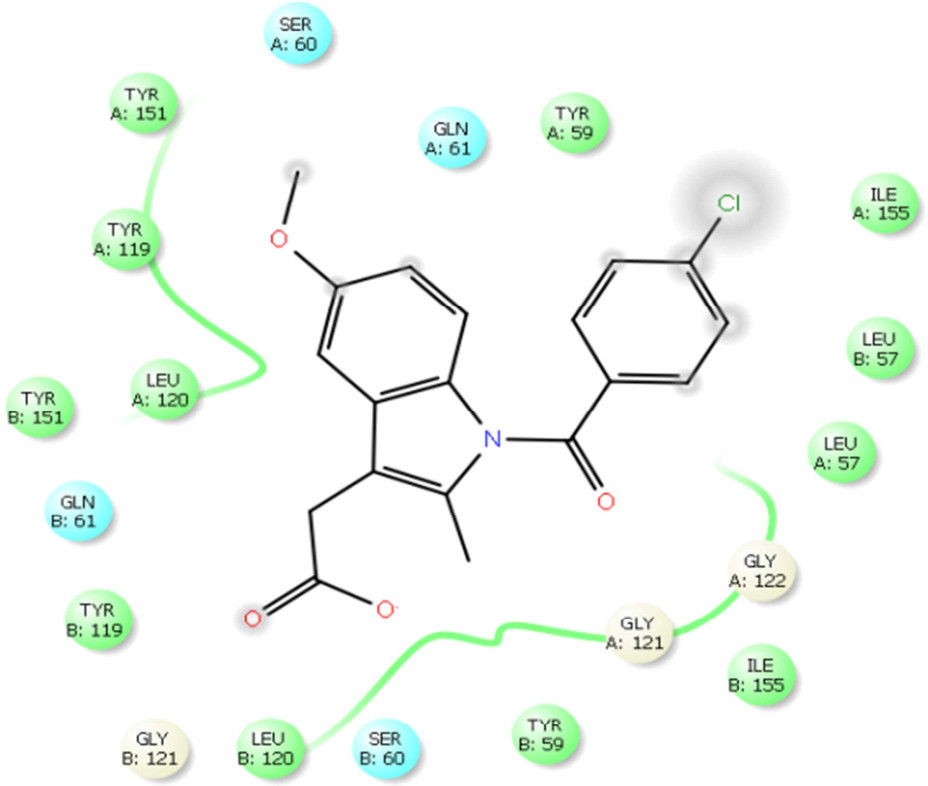
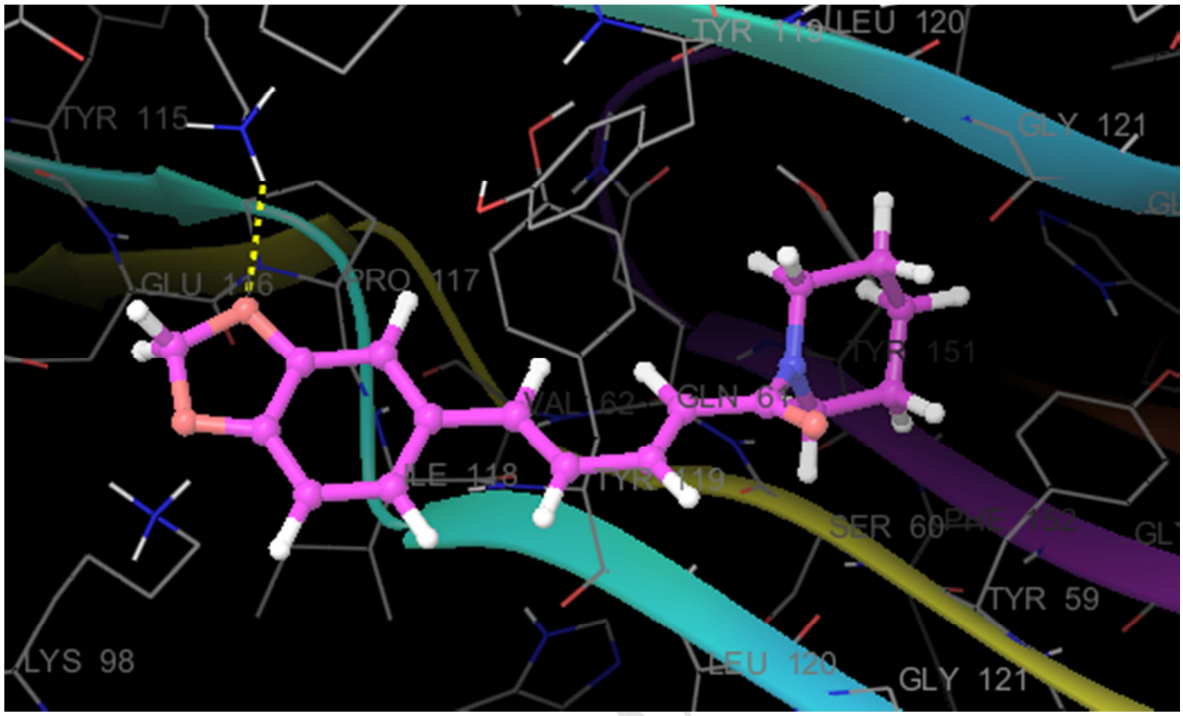
Indomethacin





Piperine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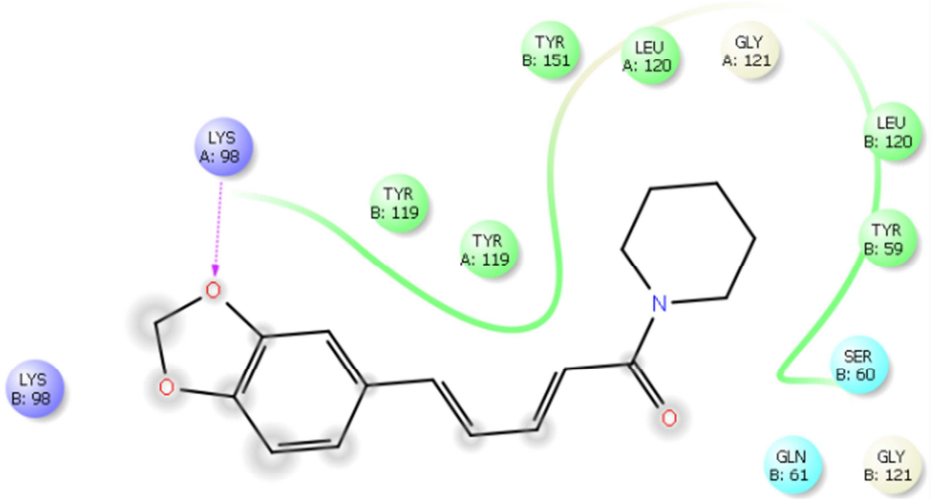
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O
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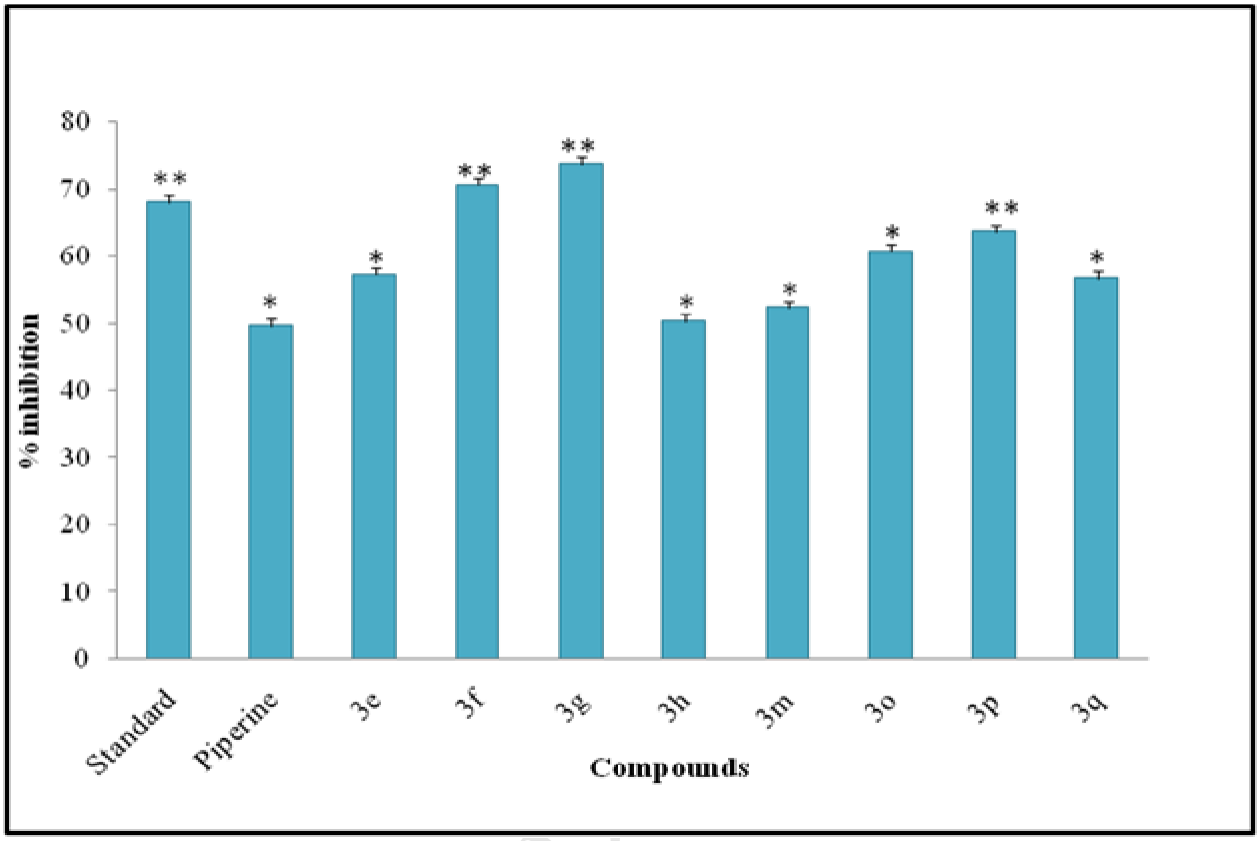
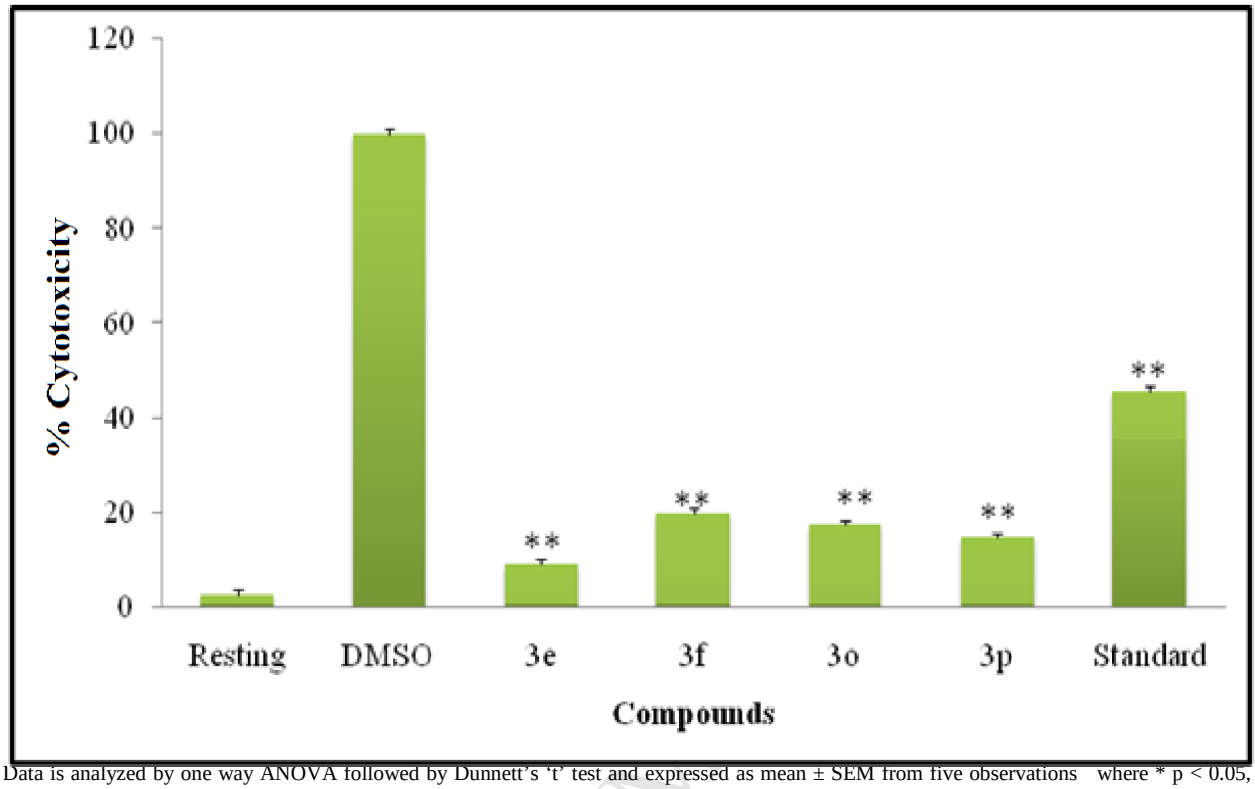
Fig 2: Effect of synthesized compounds on LPS induced TNF-α cytokine level in the RAW 264.7 cell line.



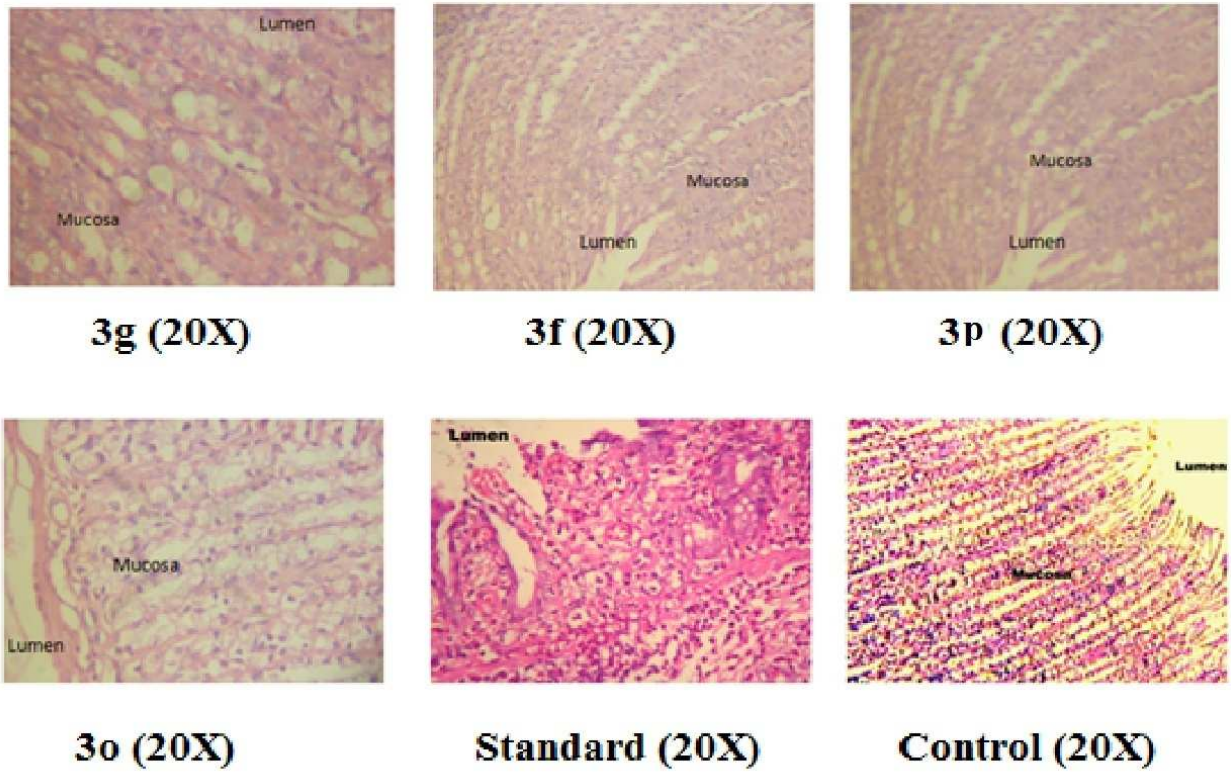
Data is analyzed by one way ANOVA followed by Dunnett's 't' test and expressed as mean ± SEM from five observations where * $p < 0.05$, **$p < 0.01$

Fig 3: Cytotoxicity assay of active compounds.



Data is analyzed by one way ANOVA followed by Dunnett's 't' test and expressed as mean ± SEM from five observations where * $p < 0.05$, ** $p < 0.01$

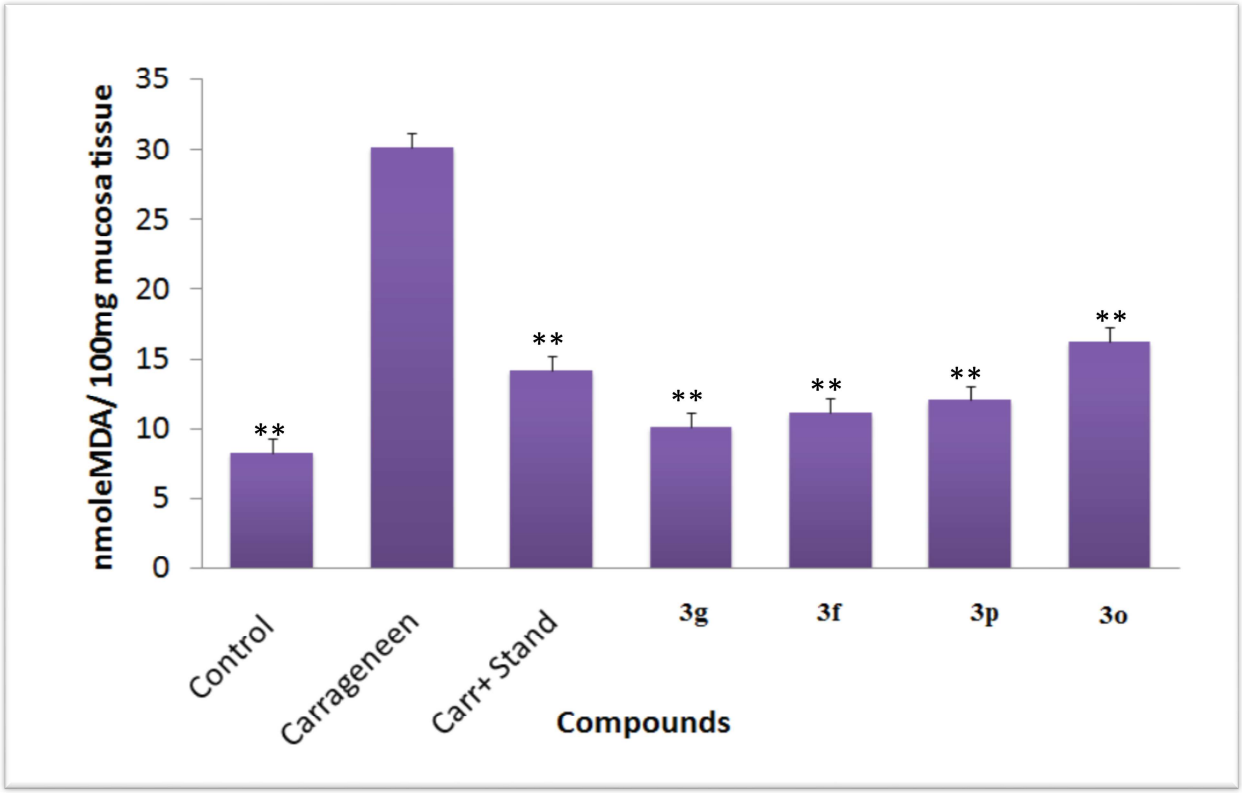
Fig4: Histopathology of rat stomach in Albino Wistar rats, photomicrographs (**20X**) of stomach wall of the animal groups administered with compound **3g, 3f, 3p, 3o,** standard drug indomethacin and control group respectively.



3g (20X) **3f (20X)** **3p (20X)**

3o (20X) **Standard (20X)** **Control (20X)**

Fig5: Lipid peroxidation assay of active compounds.



Data is analyzed by one way ANOVA followed by Dunnett's 't' test and expressed as mean ± SEM from five observations where * $p < 0.05$, ** $p < 0.01$

Fig6a: Paw tissue expressions of COX-2 activation. Representative photomicrographs magnification (40X) Group I (only control), Group II (only carrageenan), Group III (carrageenan + standard drug Indomethacin), Group IV (carrageenan +**3g**) GroupV (carrageenan+ **3f)**

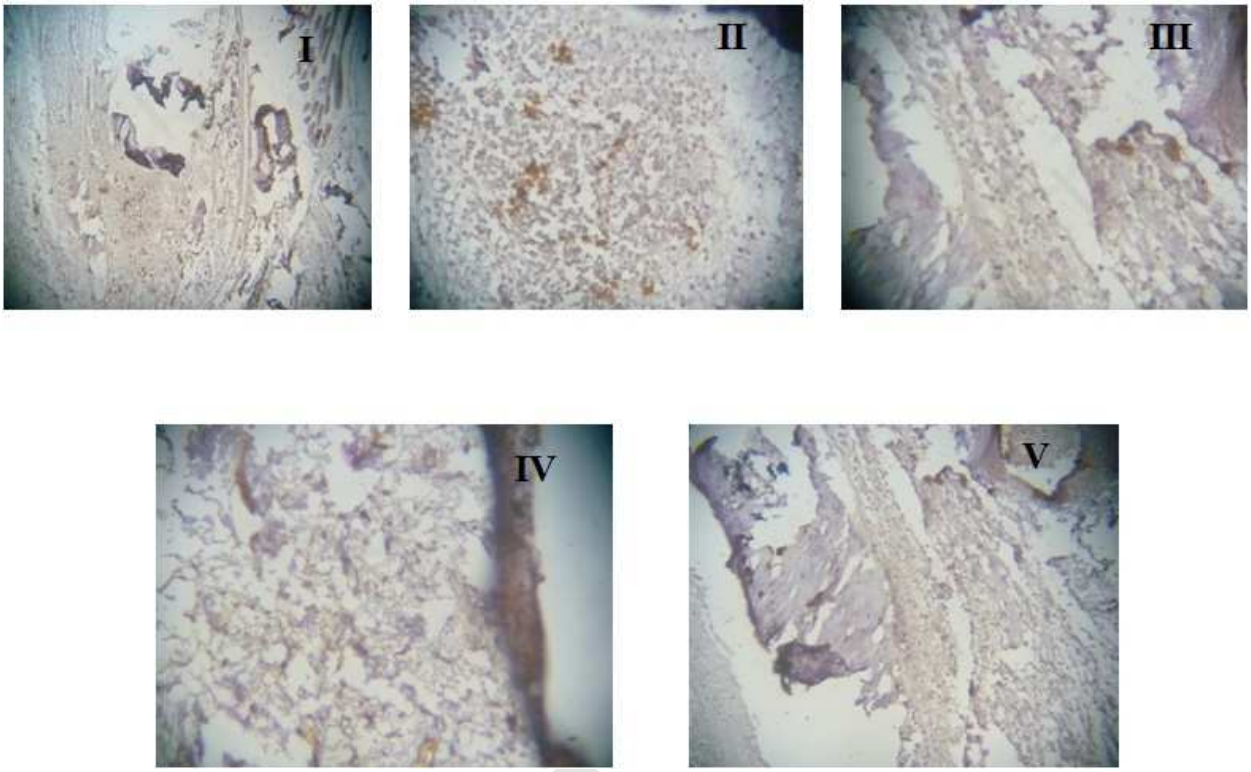


Fig6b: Paw tissue expressions of NFkB activation. Representative photomicrographs magnification (40X) Group I (only control), Group II (only carrageenan), Group III (carrageenan + standard drug Indomethacin), Group IV (carrageenan +**3g**) Group (carrageenan+ **3f)**

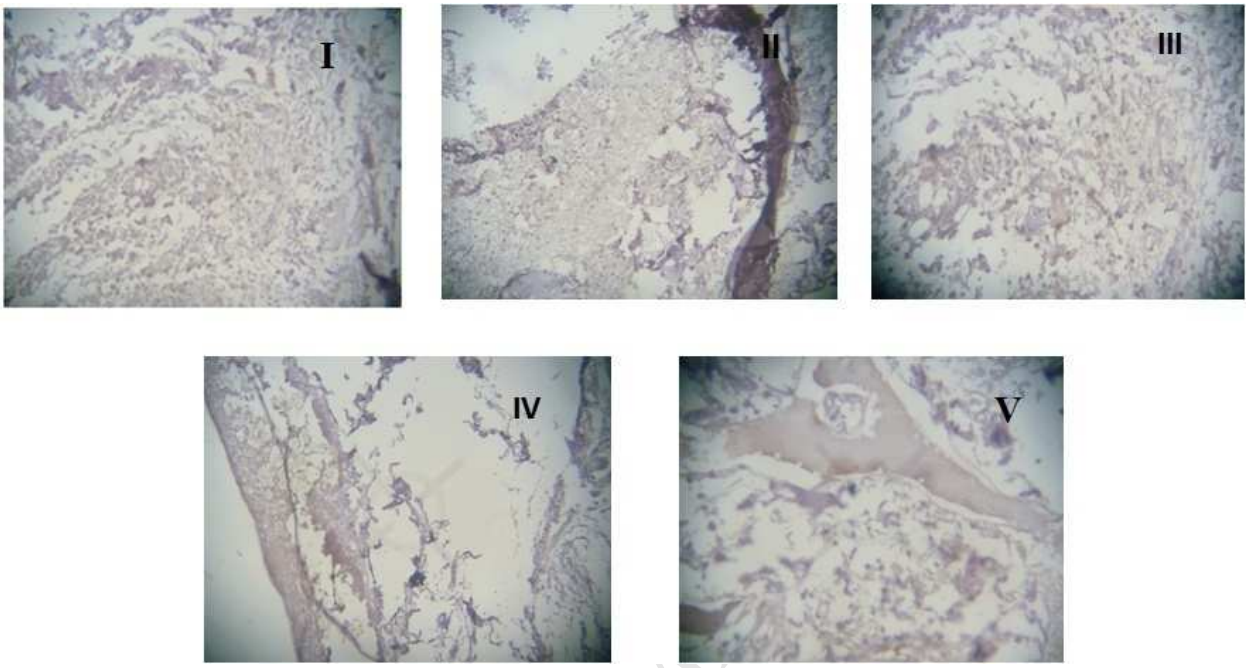
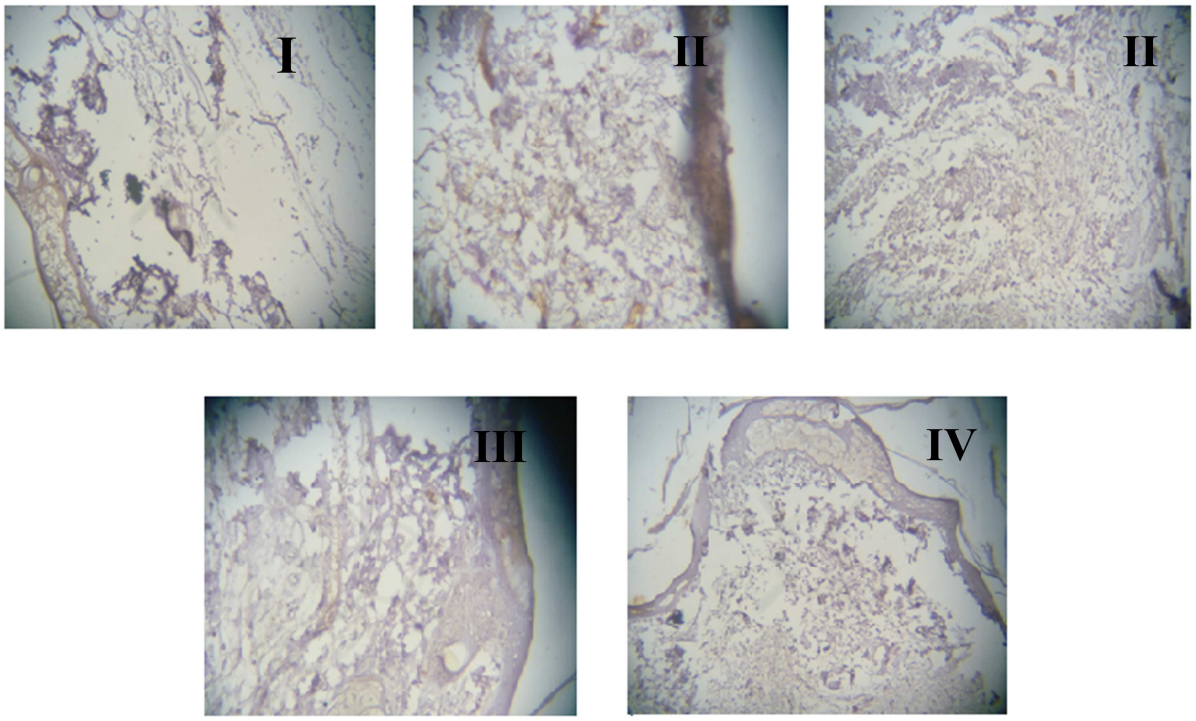


Fig6c: Paw tissue expressions of TNF-α activation. Representative photomicrographs magnification (40X) Group I (only control), Group II (only carrageenan), Group III (carrageenan + standard drug Indomethacin), Group IV (carrageenan +**3g**) Group (carrageenan+ **3f)**



Highlights

- A library of 19 novel piperine based triazoles has been synthesized.
- Compounds showed significant *in vivo* anti-inflammatory activity.
- Compounds 3g and 3f alleviated TNF-α level without showing any cytotoxicity.
- Compounds 3g and 3f significantly suppressed protein expression of COX-2 and NF-kB.
- Compounds 3g and 3f did not cause any damage to the stomach.