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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 5 h 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%**).

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 hepato-toxicity [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.

Abbreviations: TNF-α, tumor necrosis factor; COX, cyclooxygenase; LPO, lipid peroxidation; NASIDs, non steroidal anti-inflammatory drug.

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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 7.99–8.54 ppm along with methylene protons appearing at δ 5.35–5.87 ppm. The double doublet of H-3 appeared in the range of δ 7.47–7.49 ppm and the singlet of H-4 appeared in the range of δ 5.91–6.05 ppm (Fig. 1). 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 3 h and **80.40%** inhibition at 5 h) as compared to the standard drug indomethacin (**72.05%** at 3 h and **77.02** at 5 h).

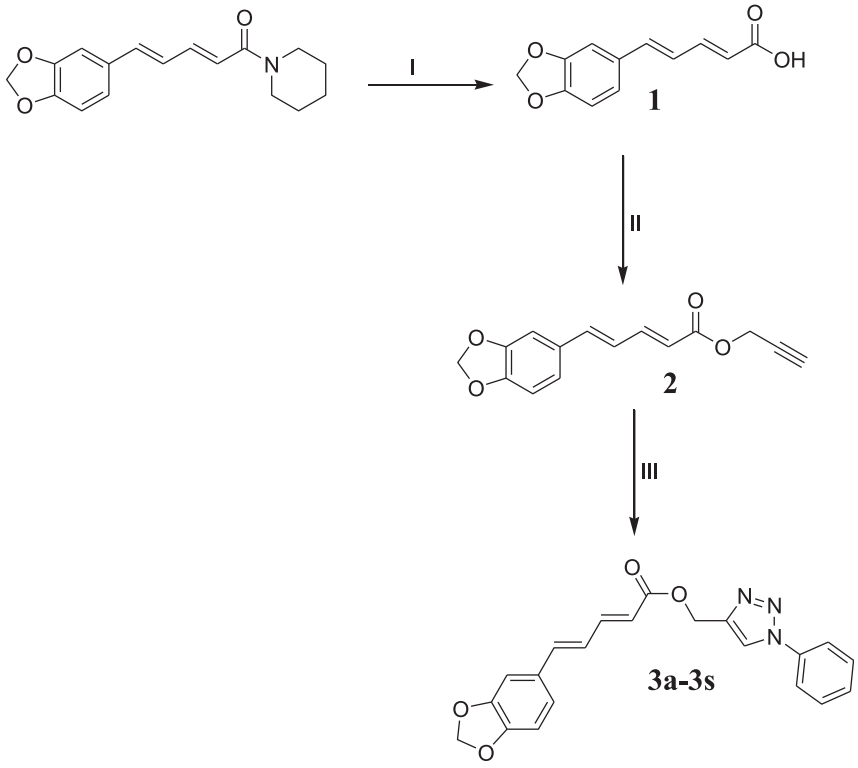
The compounds **3e**, **3f**, **3o**, **3p**, and **3q** also showed significant anti-inflammatory activity (**70.81%**, **76.71%**, **71.21%**, **72.97%**, and **70.27%**) respectively which is comparable to standard drug indomethacin at 5 h of inflammation (**77.02%**) but higher than that of piperine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



Scheme 1. Reagents and Conditions: (I) Alcohol, KOH, reflux; (II) $CeCO_3$, dry THF, reflux; (III) CH_3COONa, NaN_3, Ar−N_3.