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Dual Inhibition of COX-2 and 5-LOX by Novel 4-Aminoacetanilide Derivatives: Insights from Synthesis, Bioassays, and Molecular Docking

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INTRODUCTION & AIM

The treatment of inflammatory conditions is still a desperate medical need and the treatment is mostly based on conventional drugs i.e. NSAIDs, steroids, etc. which possess inadequate safety profile.

NSAIDs are involved in the non-selective inhibition of COX-1 pathway and, are therefore responsible for side effects like gastrointestinal ulcer and renal toxicity.

- COX-2 inhibition is associated with cardiovascular side effects; the blockade of COX-2 redirects the inflammatory pathway towards LOX pathway which enhances the production of leukotrienes and lipid mediators and cause more side effects as these mediators are involved in inflammation (by leukocyte activation and adhesion to vascular endothelium) and in the pathogenesis of bronchial asthma and edema formation.
- Hence, targeting multiple Arachidonic acid pathways whilst maintaining normal physiological function, is being suggested as an effective treatment strategy.
- The simultaneous inhibition of COX-2 and 5-LOX is an emerging research trend in the field of medicine.
- The acetamide derivatives are being reported in numerous research studies to exhibit diverse medicinal effects i.e. analgesia, anti-inflammatory activity, anti-pyretic activity, anti-hyperglycemic activity, anti-oxidant activity, anti-cancer activity, anti-convulsant activity, and anti-microbial activity.

The present study is focused on the following objectives:

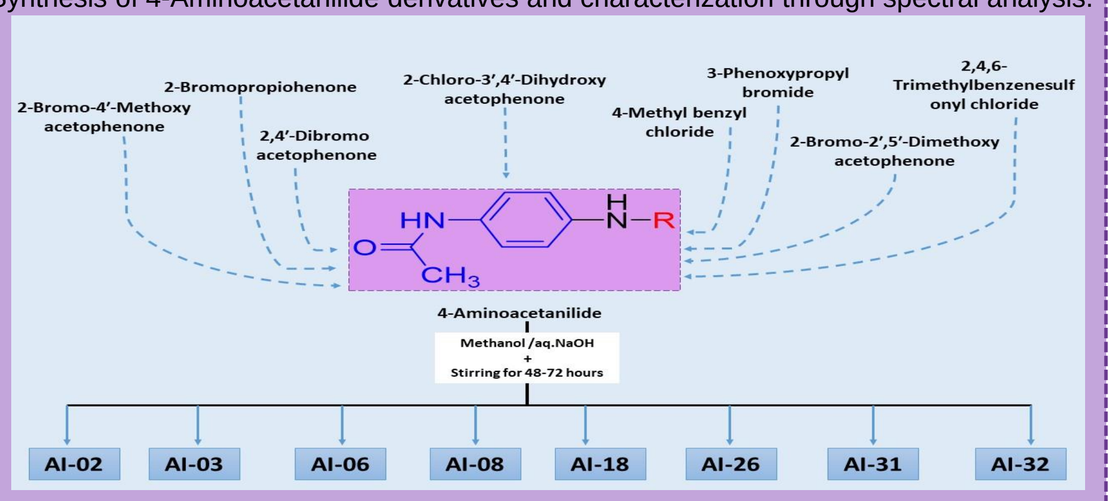
- **Design and synthesis of novel 4-Aminoacetanilide derivatives**
- **Investigation of the potential of proposed ligands against COX and LOX activity**
- **SAR studies of the synthesized derivatives based on their in-silico and in-vitro analysis**

METHOD

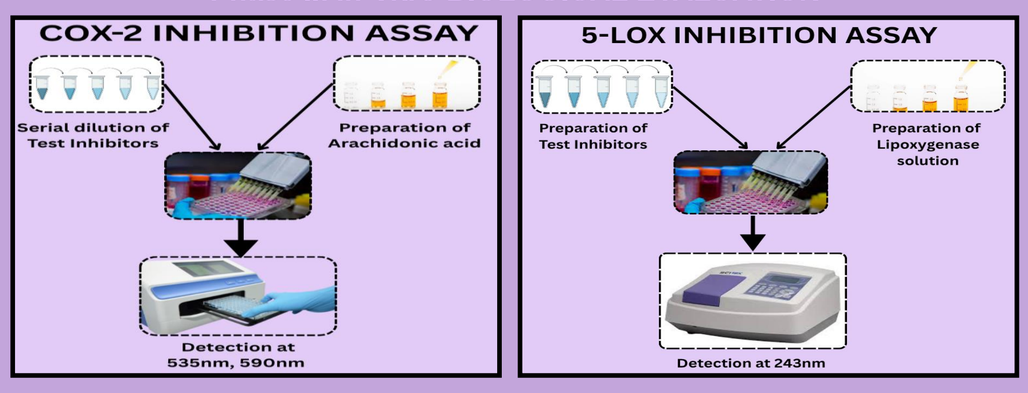
The Study is based on 3 phases:

Phase I: SYNTHESIS

Synthesis of 4-Aminoacetanilide derivatives and characterization through spectral analysis.

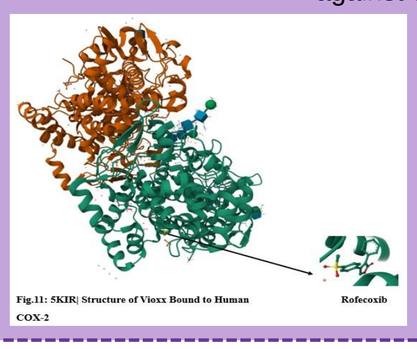


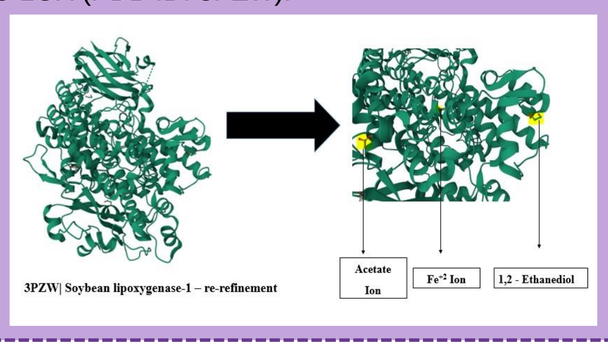
Phase II: *In-Vitro* BIOLOGICAL EVALUATION



Phase II: MOLECULAR MODELLING

MOE (Molecular Operating Environment) 2014 was used to evaluate ligand-protein interaction of AI-06 against COX-2(PDB ID: 5KIR); and interactions of AI-03, AI-06, AI-08 against 5-LOX (PDB ID: 3PZW).

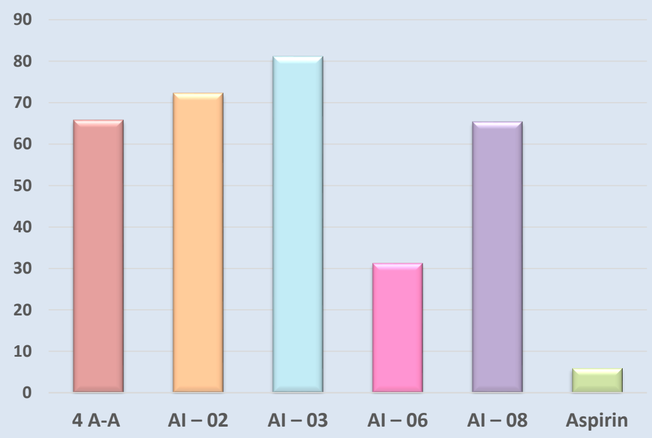




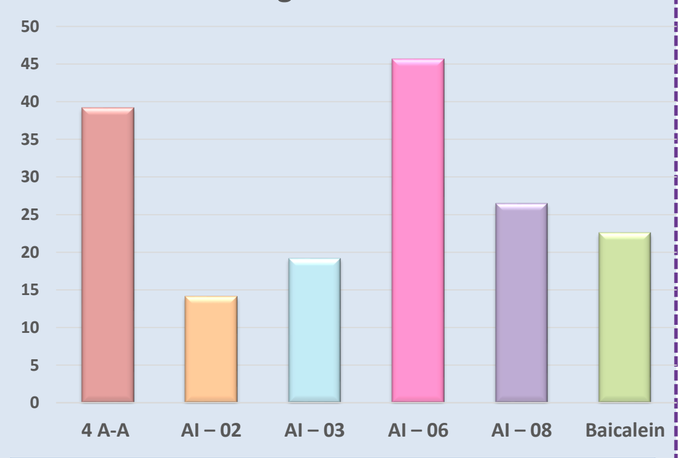
RESULTS & DISCUSSION

BIOLOGICAL EVALUATION

Graph #1: IC50 Values (μM) of Ligands & Standard against COX-2



Graph#2: IC50 Values (μM)of Ligands of against 5-LOX

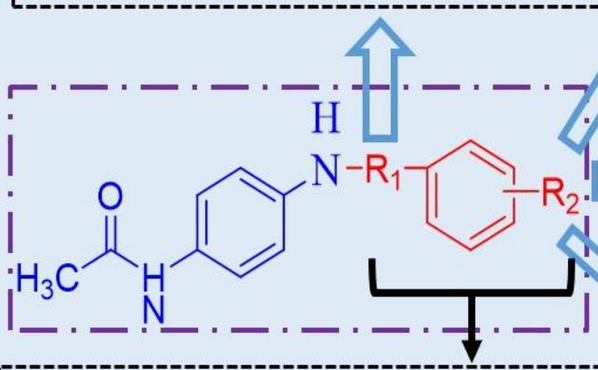


IC_{50} Values (μM) of Ligands & Standard against COX-2				
AI–02	**AI–03**	**AI–06**	**AI–08**	**Aspirin**
72.3 ± 0.15	81.1 ± 0.37	81.1 ± 0.37	65.3 ± 0.72	5.9 ± 0.17

IC_{50} Values (μM) of Ligands & Standard against LOX				
AI–02	**AI–03**	**AI–06**	**AI–08**	**Baicalein**
14.1 ± 0.32	19.2 ± 0.71	45.7 ± 0.37	26.5 ± 0.36	22.6 ± 0.11

STRUCTURE ACTIVITY RELATIONSHIP:

- Carbonyl moiety is present at R1 in AI-02, AI-06,AI-08 and is crucial for making efficient Hydrogen bond with active site residue of COX-2 (i.e. Ser530).
- Carbonyl oxygen aligns toward the Fe^{3+}–ligating region of 5-LOX but does not directly chelate; instead, it stabilizes the binding pose through hydrogen bonding and dipole interactions.



Substitution of Bromine at para position (AI-06) on the ring increases lipophilicity and enhance inhibition against COX-2 via halogen–π or halogen–carbonyl interactions.

- Methoxy group at para-position (AI-02) will enhance lipophilicity by means of favorable dipole interactions near the tunnel entrance of 5-LOX, improving binding affinity and orientation.
- It would enable hydrophobic channel occupancy and π–π stacking with aromatic residues of the LOX active site.

Substitution with Propiophenone in AI-03 here increases the chain length and add to lipophilicity. Bulky rigid lipophilic analogs can occupy the substrate channel effectively by means of van-der-Waals and π–π contacts deep in the channel as larger, more hydrophobic fused rings improve LOX binding by mimicking arachidonic acid's tail region.

Substitution with Electron-withdrawing Chelating groups: OH at both meta and para positions (AI-08) help in chelation of non-heme iron of 5-LOX.

CONCLUSION

Among the analogues, AI-06 showed highest inhibitory activity against COX-2 and showed weak inhibition of 5-LOX; with a few structural modifications, it could become a promising dual inhibitor of both COX-2 and 5-LOX. Furthermore, AI-02 demonstrated strong inhibitory activity against indicating its potential as a potent LOX inhibitor, which is further supported by molecular modelling studies. Thus, AI-02 and AI-08 may be optimized for the development of new lead molecules as dual inhibitors.

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