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Title of Thesis: Synthesis and Characterization of Novel Organic Ligands as Therapeutic Agents

ABSTRACT

Inflammation is considered as major and natural defense mechanism of the body against any foreign antigen either external or internal. This process may vary from a localized response to a more generalized one. It is basically characterized by accumulation of leukocytes and fluids leading to swelling (edema), redness, heat and pain. During multiple inflammatory disease progression, immune cells macrophages become activated. This leads to excessive release of pro-inflammatory cytokines and mediators such as nitric oxide (NO), tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), prostaglandin E2 (PGE2) and other reactive oxygen species (ROS). Excess formation of ROS can injure a cellular arrangement, can damage nucleic acids, proteins and membrane lipids and also bases of a DNA molecule. Over decades, for the treatment of inflammatory diseases, non-steroidal anti-inflammatory drugs (NSAIDs) have been used frequently. NSAIDs inhibit prostaglandin synthesis by acting on cyclooxygenase (COX) enzyme in arachidonic acid metabolism. Long term uses of NSAIDs have been prohibited as they cause severe side effects such as gastrointestinal ulcers, haemorrahages, and nephrotoxicity etc. Research has been going, in order to discover new and safe anti-inflammatory agents. Medicinal chemists are working in the field of heterocyclic compounds as these compounds incorporate in a number of currently available NSAIDs. Heterocyclic compounds are organic compounds consisting of heteroatoms; sulphur, oxygen and nitrogen. Among them, five membered heterocyclic compounds are mostly favorable due to the wide spectrum of medicinally important activities displayed by them such as anti-inflammatory, anti-viral, anti-microbial activities and many more.

Our work deals with the synthesis and biological evaluation of two different five membered ring heterocyclic compounds as anti-oxidant and anti-inflammatory agent, in order to discover new alternatives to present treatments of inflammatory diseases. Firstly, we have synthesized 2-[5-(2-

hydrazino-2-oxo-ethyl)sulfanyl-1,2,4-triazol-1yl]acetohydrazide bearing 1, 2, 4-Triazole ring and their sixteen different derivatives comprising of different substituent's (B1-B16). Synthesized compounds undergo structural characterization using FTIR, ^{1}H NMR, ^{13}C NMR and Mass. Then, these compounds were evaluated for their anti-oxidant and anti-inflammatory potential by *in vitro*, *in vivo* and *in silico* studies. From sixteen compounds, five showed 80-90% of free radical as well as nitric oxide radical scavenging activity, comparable to standard; L-ascorbic acid. These compounds were evaluated for cytotoxicity and seems to be less toxic with an IC_{50} ≥40μM. Griess nitrite and DCFH-DA assays revealed significant inhibition of NO and ROS generation by B6 and B9 compounds. *In silico* Molecular docking and MD simulation has been performed with different interleukins (IL-1, IL-6, IL-12, TNF-α) and COX-2 enzyme that are majorly involved in the progression of various inflammatory diseases, and results illustrated that compound B6 and B9 interacts strongly with interleukins and COX-2 enzymes with very good binding affinities. Further, *in vivo* anti-inflammatory activity performed using carrageenan induced rat paw edema assay. Compound B6 showed best anti-inflammatory activity of about 64% comparable to indomethacin, standard anti-inflammatory drug. Out of 16 derivatives, B6 is proposed to be an important scaffold for synthesis of new medicinal agents in chronic inflammatory diseases.

Another five membered ring heterocyclic compounds, thiazolidinone and its thirteen derivatives were synthesized (A-M) and characterized using various spectroscopic techniques; FTIR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopy. The compounds were evaluated for their efficacy in suppressing the biomarkers of inflammation and oxidative stress *in vitro* and *in silico*. Out of thirteen derivatives, eight showed potent 75-90% free radical scavenging as well as anti-inflammatory activities, highest and comparable to standard; L-ascorbic acid. The compounds when evaluated for cytotoxicity showed an IC_{50} ≥50μM. Griess nitrite and DCFH-DA assays suggested significant inhibition of NO and ROS, particularly, by compound D and E. *In vitro* results were further confirmed through *in silico;* molecular docking and MD simulation studies. Molecular docking and MD simulation has been performed with different interleukins (IL-1, IL-6, IL-12, TNF-α) and COX-2, and results illustrated that compound E stabilizes the complexes, suggesting tight binding of the ligands and can be considered as potential drug candidate.