Biochemical, Toxicological and Anti-inflammatory Studies of Some Newly Synthesized Compounds and Herbal Drugs

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6. SUMMARY AND CONCLUSION

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain and inflammation, particularly for different types of arthritis. They have a long and fascinating history, with the use of aspirin derived from willow bark stretching back into the Assyrian culture. In twenty-first century we are faced with both the challenge of balancing the benefits and side effects of these drugs and the exciting prospect of new, safer agents with comparable efficacy. The history of nonsteroidal anti-inflammatory drugs (NSAIDs) stretches back to the use of extracts of willow bark in prehistory and extends forward to an exciting frontier of technological advances and new indications for the use of aspirin and the nonaspirin NSAIDs.

NO-NSAIDs, are a new class of non-steroidal anti-inflammatory drug (NSAID) developed with the intention of providing greater safety than existing NSAIDs. These compounds were first described by John Wallace and colleagues[36]. NO-NSAID are hybrid compounds generated by the fusion of an existing NSAID with a nitric oxide (NO)-donating moiety by chemical means, usually by ester linkage. NO-NSAIDs retain the anti-inflammatory efficacy of NSAIDs *via* inhibition of cyclooxygenase (COX) while arguably improving upon gastric and vascular safety, most likely *via* vasorelaxation, inhibition of leukocyte adhesion and reduction of apoptosis, all known effects of NO.

The natural antiinflammatory agents omega-3 EFA, white willow bark, turmeric (curcumin), green tea, and *Boswellia* have all been shown to have various abilities to block certain parts of arachidonic pathway. Pycnogenol, cat's claw, and capsaicin also have anti-inflammatory effects associated with their action on the NF-κB pathway. PGD-2 = prostaglandin D2; PGG = prostaglandin G; PGH = prostaglandin H.

This present study covers anti-inflammatory pharmacological profile of 93 newly synthesis compounds, semi synthetic drug BCOV and 9 herbal drugs extract compared with the standard drugs valdecoxib and Indomethacin, using *in vitro*, *ex vivo* and *in vivo* experimental models.

Chapter 1 gives an overview about how complex can be the Inflammation process is, right from acute inflammation to chronic inflammation e.g. Rheumatism, it also contains different class of anti-inflammatory drugs and their side effects e.g. gastrointestinal side effects and CVS complications.

In chapter 2, the introductory chapter, includes scope and the objective of this thesis, it also uncovers the complications and limitations of the present NSAIDs. Synthetic approaches based upon chemical modification of oxadiazoles have been

taken with the aim of improving safety profile and in turn therapeutic window of this oxadiazoles. Several studies have described the derivatization of the carboxyl ate function of representative oxadiazoles with less acidic azoles, viz. 1,2,5- oxadiazole, Triazole, etc. which resulted in an increased anti-inflammatory activity with reduced ulcerogenic. Furthermore, it has been reported in the literature that certain compounds bearing oxadiazole nucleus possess significant anti-inflammatory activity. In our attempt to discover new, safer and potent agents for treatment of inflammatory diseases, we have replaced the carboxylic acid group of less acidic heterocycle, 1,2,5- oxadiazole, in order to accentuate potency and reduce GI toxicities associated with the parent ring due to its free –COOH group. The compounds designed so were found to possess much significant analgesic-anti-inflammatory profile with significant reduction in potential for ulcerogenic toxicities.

Synthesis and study of metal complex(BCOV) with active drugs as ligand such as Curcumin is a research area of increasing interest for inorganic, pharmaceutical and medicinal chemistry and has concentrated much attention as an approach to new drug development. The goal is to prepare new compounds with better or different pharmacological profile than that of the free ligand. Herbal drugs may answer for chronic inflammatory condition like arthritis and inflammatory bowel disease because they are safe and effectively cure the disease from root level on long term treatment

Chapter three covers all the materials and methodology used in the present studies it includes *in vitro* cyclooxygenase inhibition, acute and chronic *in vivo* studies, safety pharmacological studies including acute and sub acute toxicity testing.

The series of 1,2,5- oxadiazole compounds described in present study showed preferential COX-2 inhibition over COX-1 inhibition. The enzymatic and *in vivo* data on isomeric 3-[4-Methanesulphonylphenyl]-4-phenyl analogue MCR-363 indicates more potent than any other substitutes in the series of compounds. MCR 363 also shows excellent anti-inflammatory and analgesic properties in every efficacy model. The studies aimed at modulating the extended safety and efficacy in the parent 1,2,5-oxadiazole-N-oxide ring resulted in Nitric oxide donor nature and absence of –COOH group in any structure provides double sward safety. Amongst new series compounds specially methoxy(-OMe) substituted compounds MCR-207, MCR-292, MCR-320, MCR-322, MCR-363 demonstrate an excellent overall profile in all *in vitro* and *in vivo* models. Selected methoxy substituted compounds (e.g.MCR-363) were superior to the

corresponding non-substituted compounds at meta or para positions in edema and particularly in the carrageenan induced paw edema model. Detailed investigation of the analogues derived from other heteroaromatic systems shows that their potency, selectivity and *in vivo* profile are greatly influenced by the substitution pattern. Diazole derived compound MCR-363 are very potent (IC_{50}) 0.47 μM and selective (IC50 COX-1/COX-2) >650) inhibitors of the human cyclooxygenase- 2 enzyme. These compounds also exhibit superb potency in the adjuvant induced arthritis model (ED50-0.6 mg/kg/p.o.) as well as in the carrageenan induced model of inflammation (ED_{50}) 13.9±2 mg/kg/p.o.). In imidazole substitutions, the –OCH3 group at C-4 position gives the optimum potency and anti-inflammatory properties. Excellent *in vivo* properties exhibited by MCR-363 and the absence of GI toxicity in the selected compounds up to 100 mg/kg/p.o. in rat demonstrates that heteroaryl modified 1,2-oxadiazole represent a series of potent anti-inflammatory agents with an improved side effect profile.

The organometallic complex Bis(Curcumino) oxovanadium - BCOV, we have chelated the vanadium centre with Curcumin. We also reported its anti dieabetic activity[154]and patented (Indian patent no. : 918 / MUM / 2001).To investigate anti-inflammatory effect of BCOV *in vitro* and *in vivo*, we evaluated the inhibitory effects of BCOV on rat carrageenan-induced paw edema and rat adjuvant-induced arthritis, respectively. BCOV shown good *in vitro* profile and effectively inhibit carrageenan-induced paw edema and adjuvant induced secondary paw swelling. In ulcerogenic effect model in rat BCOV did not showed GI toxicity even at very high dose. The results suggest that BCOV may effectively relieve acute and chronic inflammation in treatment of inflammatory disease. In conclusion, the present study clearly demonstrated that BCOV is a novel and moderately selective COX-2 inhibitor that possessed anti-inflammatory effect. It has potential therapeutic role for acute and chronic inflammatory disease.

In this study, nine herbal drugs extracts screened for its anti-inflammatory activity *in-vitro* and *in-vivo*. Some of them e.g. Banaba extract (BNB) and Pomegranate extract (PME) have shown good enzymatic COX-2 inhibition. Most potent Cox-2 inhibitory BNB, PME also showed impressive anti-inflammatory activity against rat carrageenan paw and chronic models like adjuvant induced arthritis and TNBS induced Inflammatory Bowel disease in rat. There was no ulcerogenic toxicity found even at very high dose (1g/kg) in both the extracts.

Safety Pharmacological studies on GI, CNS, and CVS with the use of different ex *vivo* and *in vivo* models, done for the selected drug candidates MCR 363, BCOV, BNB, and PME which showed anti-inflammatory activity in previous studies. These all four drugs are found quite safe in GI tract when given systemically via oral route, no ulcerogenic effect found when tested at higher doses.

To find out effect on CVS toxicity of drugs MCR 363, BCOV, BNB, PME for their effect on blood pressure, QT, heartbeats using *in vivo* model and isolated rat aorta experiment performed. These drugs did not showed any significant side effects on any of the CVS parameter, except MCR-363 showed vasodilating effect on isolated rat aorta via NO release effect. This was predicted because the MCR-363 structure has N-oxide moiety which is NO donor, hence this compound has important therapeutic cardio protective effect via NO release.

The nitric oxide releasing property of compound MCR-363 which caused relaxation of PE induced contraction in rat aorta may be due to its chemical structure(N-oxide group). This compound due to NO release property may score over other COXIB which are reported to cause CVS effect.

Different CNS safety animal models used in order to find out any alteration of on neurological and behavioral activity e.g. locomotor activity in mice, Pentobarbital induced sleeping time in mice, Haloperidol induced catalepsy in mice, Observational assessment by Irvin's method. We found that these drugs are free from CNS side effects when given via systemic route.

Data from the acute toxicity and sub acute toxicity studies indicates[189,190] that the treatment with MCR 363, BCOV, BNB, PME at the evaluated doses are not associated with obvious toxicity in both mice and rats. The body weights, hematological parameters, gross pathology, histopathology data suggest that these drugs are less threat on systemic administration. Thus, these results showed that all four drugs did not produce either significant damage in the internal organs or any sign of acute and subacute toxicity.

In conclusion, After screening 110 different compounds it is summarized that MCR 363(3-[4-Methanesulphonylphenyl]-4-phenyl 1,2,5-oxadiazole-N-oxide), BCOV (Bis[Curcumino] oxovanadium), BNB (Banaba-Lagerstroemia speciosa L— leaf extract), PME (Pomegranate- Punica Granatum-Fruit extract) have superior enzymatic Cyclooxygenase inhibition profile, impressive anti-inflammatory activity in acute and chronic models, safe and found nontoxic on single and multiple dose treatment. However after conducting more toxicity studies compounds MCR 363, BCOV, BNB, PME may be clinically evaluated for welfare of human being.