Chapter-6- SUMMARY AND CONCLUSION

- Summary of present work
- Conclusion
- Scope for the future work
- Errata
Summary of Present Work, Conclusion: and Scope for the Future Work.

6.1. Summary of the present work:-

In the present thesis applications of new computational tools for drug discovery are explored and the details of methodology, results, discussion and conclusions derived are presented.

Initially a brief overview of drug design methods, cyclooxygenase enzyme isoforms, various pharmacophore reported for selective cox-2 inhibition and introduction to various methods, approaches of CADD for drug discovery are highlighted.

Followed by the detailed discussion of methodology, results and discussion of applications of 3D QSAR studies using CoMFA and kNN MFA methods to 1,5 diaryl pyrazole and 4,5 diaryl imidazole series of compounds.

The overall information generated out of these studies helped us to design better, nontoxic analgesic and anti-inflammatory agents.

The NCEs were designed using information generated by QSAR studies using CombiLib tool of V-Life MDS software.

The synthetic route for the designed NCEs belonging to both, 1,5-diarylpyrazole and 4,5-diaryl imidazole pharmacophore were designed in order to synthesize the designed NCEs.

The details of synthetic methods, Optimization strategies, results of various physicochemical studies performed to establish synthetic methods, quality, quantity and structures anticipated are presented in this thesis.

The synthesized compounds were subjected to pharmacological screening for anti-inflammatory, analgesic activities and evaluation of ulcerogenicity potential of the most potent derivatives. The details of methodology, results and discussion are presented in this thesis. The most of the designed, synthesized NCEs were found to be significantly potent and non ulcerogenic.
Chapter-6 Summary of Present Work, Conclusion and Scope

All the designed NCEs were subjected to molecular docking studies using cyclooxygenase-2 isomorf, along with some standard cox-2 inhibitors like Celecoxib, SC-558, Etoricoxib, Valdecoxib, Rofecoxib etc. The results of these studies were validated by including non selective cox-2 inhibitor pharmacophore containing compounds.

The details of methodology, results and discussion important binding site/residues etc. are discussed here in the chapter molecular Docking Studies.

The overall outcome of the project i.e. conclusion and summary of all chapters is highlighted here in this section at the end of dissertation.

The list of research publications presented in various conferences/seminars/workshops and/or published in various periodicals/journals is included in the statements in the annexure.

The publications/presentations at various conferences have already endorsed the importance of findings of these studies indicating importance and the quality of the research work required in this field of NSAIDs to aid human beings to get adequately optimized medicine and to increase level of patient compliance measures.

The scope for future research work is also discussed in brief.

6.2. Conclusion:-

One of the major objectives set was to cross verify the results obtained by 2D and 3D QSAR studies (Dry Lab Work) and results obtained by synthetic and pharmacological work (Wet Lab Work). Looking at the results of both dry lab work and wet lab work it can be concluded that the the molecular modeling studies resulted in significantly correlating results.

ii). Second objective was to endorse the correctness of the rationale based on which the NCEs were designed. It proved to be a very correct.
iii). Third objective was to find out the probable binding sites and binding modes of the designed COX-2 inhibitors with COX-2 enzyme. Based on results of molecular Docking studies, we could conclude possible modes and locus of interactions of the synthesized NCEs.

iv). Last objective was to conclude whether the results of the research attempted were really sufficient to optimize the selected pharmacophores for selective COX-2 inhibitor by comparing the binding interactions of designed COX-2 inhibitors with those of Celecoxib, Rofecoxib and SC-558. As optimization of the pharmacophore required for selective COX-2 inhibition is the central objective of the present dissertation work. Yes the present research work was sufficient to optimize the pharmacophore requirement for a potent anti-inflammatory activity of the compounds under investigation.

6.3 Scope for the future work:

1). The synthesized compounds were found to have promising analgesic, anti-inflammatory activities compared to the standard drug Celecoxib. The compounds were designed using results of QSAR studies and only required functional groups were substituted so as to develop selective, nontoxic compounds.

All the synthesized compounds were found to be either nontoxic or very less toxic ulcerogenic compared to nonselective COX-2 inhibitor, Diclofenac acid. This observation endorsed our belief that these compounds might act as selective inhibitor of cyclooxygenase-2 (COX-2) owing to their structural similarity. Therefore these compounds were subjected to assay of their ability to selectively inhibit COX-2 enzyme to obtain further insights of selective binding to COX-2 enzyme.

2). The most potent compounds can be further subjected to acute and chronic toxicity studies.

3). If found promising in above mentioned screening methods then further trials can be initiated.
Design of New Chemical Entities

As Potential

Anti-inflammatory Agents Using QSAR Approach.

A

Synopsis submitted to the University of Pune of the dissertation for the degree of

DOCTOR OF PHILOSOPHY

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In

Pharmaceutical Chemistry

University of Pune

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Title: Design of New Chemical Entities as Potential Anti-Inflammatory Agents Using QSAR Approach

Introduction: - Inflammatory musculoskeletal diseases have existed long before the recognition of rheumatology as a distinct medical discipline. Descriptions of gout were made by both Hippocrates and the ancient Babylonians over 2000 years ago. Ankylosing spondylitis and osteoarthritis (OA) are known to have occurred for over a 1000 years. Gastric intolerance and toxicity of these remedies have also been recognized from an early stage, necessitating the search for less toxic anti-inflammatory agents. Thus, a variety of nonsteroidal anti-inflammatory drugs (NSAID) were developed, starting in 1949 with phenylbutazone. Unfortunately, gastrointestinal (GI) toxicity did not diminish with the introduction of these newer agents.¹

There is consensus for use of NSAIDS in inflammatory arthropathies such as rheumatoid arthritis (RA), seronegative spondyloarthropathies, and crystalline arthropathies. Prostaglandins (PGs) elicit a variety of important beneficial and untoward biological responses. Among the undesirable properties of prostaglandins are their ability to induce pain, fever and symptoms associated with the inflammatory response. Nonsteroidal anti-inflammatory drugs (NSAIDs) block the formation of prostaglandins by inhibiting cyclo-oxygenase and have analgesic, antipyretic, and anti-inflammatory activity. However treatment with NSAIDs particularly chronic, often leads to disruption of beneficial prostaglandin-regulated processes, most importantly gastro protective prostaglandin synthesis. The annual risk for developing a serious GI complication while on NSAID therapy is estimated to be 1.3% for RA patients and 0.73% for OA patients². The mortality rate attributed to NSAID related gastro duodenal toxicity is 0.22% per year.

288
Although the individual risk for GI complications is relatively low, the high prevalence of NSAID use translates into substantial population morbidity and mortality.

In the past it was thought that cyclooxygenase (COX, Prostaglandin H Synthase) is a bifunctional enzyme catalyzing the first two steps in biosynthesis of prostaglandins (PGs) from the substrate, arachidonic acid. This led to the widely held notion that inhibition of cyclooxygenase would unavoidably lead to both beneficial and detrimental effects. However recently it was observed that cyclooxygenase activity dramatically increases in inflammatory states and that cellular COX activity can be induced by inflammatory cytokines and endotoxins.

This suggested that a second form of COX exists as an inducible form (COX-2) that is expressed during inflammatory conditions, along with a constitutive form (COX-1) that produces physiologically important PGs and is present in tissues such as the gastrointestinal tract and and kidney and is responsible for maintaining normal physiological functions and the PGs produced by this enzyme play a protective role. The expression of COX-2 is affected by various stimuli such as mitogens, oncogenes, tumor promoters, and growth factors.

Recently a number of selective inhibitors of COX-2 were shown to possess anti-inflammatory activity with little or no gastric side effects. To date, two distinct structural classes of molecules have been reported as selective inhibitors of COX-2. First, Members of the methane sulfonamide class of COX-2 inhibitors viz Nimesulide, Diflumidone, Flosulide and second, Coxibs, the tricyclic COX-2 inhibitors like Celecoxib, Rofecoxib, Valdecoxib, Etoricoxib, Lumaricoxib etc are a few of the many worth mentioning.
Development of selective anti-inflammatory agents:
Currently used NSAIDS like Diclofenac, Indomethacin, Naproxen, Ketoprofen and Ketorolac have major drawback of GI irritation and ulceration which is attributed at least in part to COX-1 inhibition occurring with long exposure or at higher doses.

Traditional NSAIDs

Diclofenac Acid

Indomethacin

Ketorolac

Ibuprofen

Coxib class of COX-2 Inhibitors.

Rofecoxib

Celecoxib
Sulphonamaide Class of COX-2 Inhibitors

Further the differential tissue distribution of cyclo-oxygenase-1 (COX-1) and cyclo-oxygenase-2 (COX-2) provides a rationale for the development of selective COX-2 inhibitors as anti-inflammatory agents that lack the GI side effects exhibited by traditional nonsteroidal anti-inflammatory agents (NSAIDs). The various methods used for the development of new chemical entity are

1. Exploration of Natural Products.
2. Quantitative Structure Activity Relationship (QSAR)
4. High throughput screening.
5. Structure Based Drug Design.
6. Analog Based Approach.

In the present work we have explored QSAR and structure based drug design in order to design new chemical entities as potential anti-inflammatory agents.

1. **AIMS AND OBJECTIVES:-**
   
The process of design of new and potent chemical analogues is attempted by well established methods called Structure Based Drug Design (SBDD) and Analogue Based Drug Design. In the SBDD method, docking of small molecules is made with crystallographically determined structure of target protein or enzyme. The latter method involves generation of physicochemical
descriptors of molecules represent as contours in CoMFA and 3D grid plots in case of k Nearest Neighbour Molecular Field Analysis (kNN MFA) are popular approaches which have been exclusively applied for discovery of new chemical entities as potential drugs.

Present research work has following aims and objectives in order to develop

1) 2D and 3-D QSAR studies:

A. 1, 5 Diaryl pyrazole series:
   a) 2D and 3-D QSAR studies of selected 1, 5 Diaryl pyrazole series of compound by (CoMFA).
   b) 2D and 3-D QSAR studies of selected 1, 5 Diaryl pyrazole series of compound by k Nearest Neighbor (kNN) method by Simulated Annealing kNN Molecular Field Analysis (SA-kNN MFA) and Stepwise variable selection kNN MFA (SW-kNN MFA).

B. 1, 5 Diaryl imidazole series:
   a) 2D and 3-D QSAR studies of selected imidazole series using SA kNN MFA.
   b) 2D and 3-D QSAR studies of selected imidazole series using SW kNN MFA.

2. Molecular Docking studies of designed compounds in to cyclooxygenase

3. Design of New Chemical Entities (NCEs) based on QSAR results containing 1, 5- diaryl pyrazole and 1, 5 diaryl imidazole pharmacophore.

4. Synthesis of designed New Chemical Entities (NCEs).

5. Pharmacological studies of synthesized compounds
   b) Evaluation of analgesic activity.
Assay.

1.3-2D QSAR studies:
In principle, a 3D QSAR study should have two functions. The derivation of a statistically significant and highly predictive QSAR model that allows to estimate and rank new compounds to be synthesized and for the design process even more important, the provision of an easily interpretable graphical tool that denotes those areas of known inhibitors that require a particular physicochemical property to increase affinity and selectivity.

Computational methods play a crucial role in the process of drug design. The potential of computer-aided drug design has been greatly enhanced by the development and application of three-dimensional quantitative structure-activity relationships. Such 3D QSAR methods help to derive a correlation between 3D molecular structures and biological activity and to propose some potent compounds with the least efforts and maximum information.

In the present study, a pharmacophore based drug design; 3D QSAR method by molecular field analysis has been applied to a series of 1, 5-diaryl-pyrazoles and 1, 5-diaryl imidazole derivatives exhibiting selective COX-2 inhibitory activity.

Molecular property fields are evaluated between a probe atom and each molecule of a data set at the intersections of a regularly spaced grid. For this, a widely used CoMFA method has been applied to calculate steric and electrostatic properties according to Lennard – Jones and Coulomb potentials using SYBYL software.

This series of compounds has also been studied by Simulated Annealing; StepWise variable selection methods based on kNearest Neighbour (kNN) molecular field analysis methods.
All inhibitors were modeled with SYBYL. Initial geometric optimizations were carried out using the standard Tripos force field, with a 0.001 Kcal/mol energy gradient convergence criterion and a distance-dependent dielectric constant employing Gasteiger charges\textsuperscript{12}. Further geometric optimizations were performed using MOPAC with the AM-1 Hamiltonian and derived MOPAC charges were used for the subsequent analysis\textsuperscript{13}. The final geometry of the molecular skeleton is very similar to that of Celecoxib\textsuperscript{14}.

Fragment 1 (For Series-I) and Fragment 2 (For Series-II) are common templates to all the molecules that were considered in this study and the molecules were aligned with respect to these fragment, using the simple alignment method in SYBYL.

Alignment was made by different methods such as field fit, RMS fit and multfit methods.

A) **Comparative Molecular Field Analysis Studies (CoMFA):**

CoMFA fields were generated using the standard Tripos field and 3D-QSAR analysis was performed by the PLS method\textsuperscript{15}. For each cross-validated CoMFA analysis, the minimum \( \sigma \) value was set to 2 to expedite calculations. For the non cross-validated CoMFA analyses, the minimum \( \sigma \) value was set to 0. The steric and electrostatic field energies were calculated using a sp\textsuperscript{3} carbon probe atom with a +1 charge. CoMFA models were generated from training set data and these models were evaluated on the basis of cross validated \( r^2 \) conventional \( r^2 \) F-value
and predictive $r^2$ values. The initial analysis of 19 molecules resulted in models with low predictive and correlative properties. The original data set was modified by inclusion of 3 more compounds and this data set was reanalyzed. This analysis resulted in CoMFA models with better predictive and correlative properties. The information generated in this 3D-QSAR study was then utilized for the design of more potent analogues.

B) 3D QSAR studies with Simulated Annealing k-Nearest Neighbor Molecular Field Analysis (kNN MFA) Method

Both the series were subjected to 3D QSAR using the principle of kNN method. In the present studies Simulated Annealing was employed to develop the field points. All the 22 molecules were aligned using same fragment as used for CoMFA studies of 1, 5-diphenyl template as shown in Fragment 1.

The number of molecules in training and test set were kept same as described in CoMFA methods. The statistical results were improved by using stepwise variable selection method.

C) 3D QSAR studies with k-Nearest Neighbor Molecular Field Analysis (kNN MFA) with Stepwise (SW) variable selection Method

Both the series were subjected to 3D QSAR using the principle of kNN method. In the present studies Stepwise descriptor selection method was employed to develop the field points. The results obtained, that is predictability of stepwise variable selection method are better than those obtained by CoMFA and Simulated Annealing models.

Results and Discussion: The relative contributions of steric and electrostatic parameters are 0.375 and 0.625 in both of the KNN methods whereas it was 0.37 and 0.63 in COMFA method indicating that the results of all these methods are complementary to each other and the contribution of electronic parameters is
significantly related to COX-2 inhibitory activity of the selected series of compounds.

2. Molecular Docking Studies using COX-2 Enzyme\(^\text{17}\).

Computer-assisted drug design (CADD) approach has contributed to the successful discovery of several novel enzyme inhibitors, including inhibitors of thymidylate synthase, HIV-1 Protease, purine nucleoside phosphorylase inhibitors\(^\text{18}\), COX-2\(^\text{19}\), and fructose 1, 6-bisphosphatase. In each case, CADD has been used to predict the binding affinity of an inhibitor designed from a lead compound.

New Chemical Entities were designed (series-I to SV-8) based on results of QSAR studies were docked into the COX-2 active binding site using GLIDE\(^\text{20}\). Similarly NCEs comprising imi-1 to imi-8) in Series-II were docked into COX-2 enzyme in order to ensure accuracy of results of 3D QSAR and wet lab studies (synthetic and pharmacological studies). The docking scores were compared with results of QSAR and wet lab work and a significant correlation was found in all these studies, indicating correctness of our rationale for design of NCEs. The results of 3D QSAR Studies and the results of wet lab work were found to correlate with the results of docking studies.

3. Design of New Chemical Entities:

Based on the findings of QSAR studies some potent New Chemical Entities (NCEs) Were designed from 1,5 diaryl pyrazole and 1,5 diaryl imidazoles pharmacophores. These designed NCEs were subjected to docking studies in COX-2 active binding site.

4. Synthesis of designed NCEs using 3-D QSAR studies and Docking studies\(^\text{21}\):-

A) The synthesis of 1, 5-diaryl pyrazoles was planned as per the route shown in scheme-1.
a. Synthesis of Intermediate-I:

\[
\begin{align*}
\text{CH}_3 & \quad \text{CO} \\
\text{R} & \quad \text{O} \\
\text{O} & \quad \text{C}_2\text{H}_5
\end{align*}
\]

\[+\]

\[
\begin{align*}
\text{OC}_2\text{H}_5 & \quad \text{CO} \\
\text{OC}_2\text{H}_5 & \quad \text{OH}
\end{align*}
\]

\[
\xrightarrow{\text{C}_5\text{H}_5\text{ONa}}
\]

\[
\begin{align*}
\text{O} & \quad \text{C}_2\text{H}_5 \\
\text{R} & \quad \text{OH}
\end{align*}
\]

b. Synthesis of Intermediate-II:

\[
\begin{align*}
\text{SO}_2\text{NH}_2 & \quad \text{SO}_2\text{NH}_2 \\
\text{NH}_2 & \quad \text{NHNH}_2
\end{align*}
\]

\[\xrightarrow{1.\text{NaNO}_2/\text{HCl}} \quad \xrightarrow{2.\text{SnCl}_2/\text{HCl}}
\]

Sulphanilamide

4-Hydrazino sulphanilamide
c. *Synthesis of Substituted 1, 5-diaryl pyrazole*:-

\[ \begin{array}{c}
\text{R} \\
\text{O} \\
\text{SO}_2\text{NH}_2
\end{array} + \begin{array}{c}
\text{NHNH}_2 \\
\text{4-Hydrazino sulfanilamide}
\end{array} \]

\[
\begin{array}{c}
\text{R} \\
\text{O} \\
\text{SO}_2\text{NH}_2 \\
\text{N-N} \\
\text{O} \\
\text{OC}_2\text{H}_5
\end{array}
\]

6 hrs Reflux, Methanol

Where
R= -H, Halogens etc

*Substituted 1, 5 diaryl pyrazole (SV-1 to SV-8)*

Scheme-1- Scheme of Synthesis of 1, 5-diaryl Pyrazoles analogues
B) The synthesis of 1, 5 diaryl imidazoles was planned as per the route shown in Scheme-2.

I) **Route-1:** a.) Synthesis of Intermediate-1

$$\text{Ar} \quad \text{CH} \quad R = \text{Ur, NO}_2 \text{ etc.}$$

Where, Ar = Phenyl, Naphyl

B) Synthesis of Intermediate 2:

$$\text{O} \quad \text{C} \quad \text{NH}_2$$

**Intermediate-1**

**Intermediate-II**
Synthesis of 1,5-disubstituted imidazoles:

\[
\text{Intermediate-1} \quad \text{Intermediate-2} \quad 4, 5\text{-Aryl Imidazole (Imi-1 to Imi-8)}
\]

Scheme-2: Synthetic route for 1, 5-disubstituted imidazoles - Route-1

II) The synthesis of 1, 5 diaryl imidazoles by Route-2

\[
\text{1, 5-disubstituted imidazoles}
\]

Scheme-2: Synthetic route for 1, 5-disubstituted imidazoles - Route-2
The reactions were monitored using TLC. The synthesized compounds were characterized using spectroscopic, chromatographic studies and elemental analyses.

5. Biological evaluation of synthesized compounds for Analgesic, Anti-inflammatory and Ulcerogenicity studies:-

The synthesized compounds were subjected to pharmacological screening for following activities.

a) Analgesic activity: Analgesic activity was evaluated using Acetic acid induced writhing model using albino mice and Diclofenac was used as standard.

b) Anti-inflammatory Activity: Anti-inflammatory Activity was evaluated using Carrageenan induced rat paw edema model using wistar albino rats; celecoxib was used as standard.

c) Ulcerogenicity Studies: The modified model of Rainsford et al was used to evaluate the ulcerogenic potential for the synthesized compounds to ensure the selectivity towards COX-2 enzyme. The stomach specimen were subjected to histopathological studies also.

d) In-Vitro Cyclo-oxygenase-2 (COX-2) selectivity assay was also performed to check selective affinity towards COX-2 enzyme.

6. Results and Discussion: The results and discussions, conclusions derived from above mentioned studies will be Presented in details in the thesis.
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Name of Student  Research Guide  Research Co-Guide

Date:  Place: Pune.
ERRATA

Page No. 39

Subheading No. 2.3 COMFA should have been written as 2.2.1

Page No. 212

PEO No. 2123 in 1st line should have been written as 83

Page No. 281

Alignment mistake in 3rd point

Page No. 281

Subtittle COX-1 should have been printed as COX-1.
Chapter-6  Summary of Present Work, Conclusion and Scope

Design of New Chemical Entities as Potential Anti-Inflammatory Agents Using QSAR Approach.