The Thai Journal of Pharmaceutical Sciences

Volume 39
Issue 2 *2015*

Article 1

1-1-2015

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Sukumar Bepary

Ge Hyeong Lee

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Original Article

TJPS
The Thai Journal of Pharmaceutical Sciences
39 (2), April-June 2015: 35-63

In silico docking and pharmacophoric analysis of 3-indolyl pyridine derivatives toward cyclooxygenase-2

Sukumar Bepary[1, 2*], Ge Hyeong Lee[2], Hee-Jong Lim[2] and In Kwon Youn[3]

[1]*Department of Pharmacy, Jagannath University, Dhaka-1100, Bangladesh*
[2]*Medicinal Chemistry Research Division, Korea Research Institute of Chemical Technology, Yuseong-gu, Jhang-dong, Daejeon, South Korea*
[3]*Department of Chemistry, Pai Chai University, Seo-gu, Doma-dong, Daejeon, South Korea*

Abstract

A series of 3-indolyl pyridine derivatives having anti-inflammatory activities were docked into the indomethacin binding pocket of cyclooxygenase-2 enzyme. In this *in silico* study, the possible orientations of the molecules in the binding site of the receptor as well as the binding patterns of the molecules were identified. The observed orientations were compared with indomethacin along with the probable interactions points. Finally, based on these observations, some structural changes have been proposed to increase the binding interactions with the receptor for achieving more potent anti-inflammatory compounds, which will further be applied in the future research activities.

Keywords: Docking, Binding mode, Activity, Receptor, Ligand, Indolyl pyridine, Anti-inflammatory, Cyclooxygenase

Introduction

Drug discovery is a complex and costly endeavor and thus worldwide the pharmaceutical industry is under growing pressure from the gradually increasing research and development (R&D) costs for a number of years. Based on the data obtained from Eli Lilly [1], any single new drug molecular entity will incur an average cost of $280 million, which is as high as 33% of the total average cost per successfully discovering and launching of a new drug product. At the same time, to complete this preclinical study the cycle time is around 4.5 years. Whatever it is, there are reports mentioning that the total budget for drug design and development related activities has been increased 30-fold since 1970s. Many Pharmaceuticals devote more than $5 billion per year to R&D, with over $30 billion per year of cumulative spending, greater than the total NIH budget of $28 billion [2]. Thus, there appears some sort of inefficiencies in the R&D activities. According to the published reports [2, 3], approximately 80 – 90 % of drugs that reach the clinical testing phase fail to make it to market. Thus, the drug discovery and development process should be more efficient, especially in terms of total time and cost.

Correspondence to: Sukumar Bepary, Department of Pharmacy, Jagannath University, Dhaka-1100, Bangladesh
Tel: +88 02 9515035; FAX: +88 02 7113752
Email: sukumarsb@yahoo.com

Received: 13 March 2015
Revised: 15 June 2015
Accepted:24 June 2015

Academic Editor: Supakarn Chamni

Identifying a novel "druggable" protein target is a critical first step for a successful and efficient drug discovery effort. Knowing the three dimensional structure of a protein often provide insights into the molecular basis of the protein's biological function, its relationship to a particular disease, and also the detailed information on the sequence and structural characteristics that govern the ligand binding interactions. Building a drug discovery effort based on these structural information promises to help in the identification of novel therapeutic targets, in the discovery of new lead compounds, and in the optimization of drug-like properties to improve the efficacy and safety.

In addition, since any high-throughput screening produces a significant amount of negative data (most ligands do not bind or inhibit a protein), the *in silico* screening method can be exploited initially to reduce these negative data thereby increasing the number of potential binders in any compound library. Molecular docking is a computational tool that predicts the binding site location and conformation of a compound when bound to a protein [4-6]. During the prediction of protein–ligand co-structures, molecular docking programs calculate a binding score that allows the selection of the best ligand poses. The binding score is typically based on a combination of geometric and energetic functions such as, bond lengths, dihedral angles, van der Waals forces, Lennard-Jones and electrostatic interactions, in conjunction with empirical functions unique to each specific docking program [7-10]. Ligands of any compound library are also frequently ranked in order of their ligand-protein interactions based on the binding energies predicted after docking the compounds into the target protein. The virtual or *in silico* screening of a library composed of the thousands of theoretical compounds can be accomplished in a day with minimal cost [11, 12], thereby significantly accelerating the hit identification and optimization process.

All conventional nonsteroidal anti-inflammatory drugs (NSAIDs) nonspecifically inhibit both COX-1 and COX-2 at the standard anti-inflammatory doses. The beneficial anti-inflammatory and analgesic effects occur through the inhibition of COX-2. However, the gastrointestinal toxicities and the mild bleeding diathesis are caused by the concurrent inhibition of COX-1. Biologically active compounds that enable to inhibit COX-2, while sparing COX-1, are attractive and potentially represent a major advance in the treatment of rheumatoid arthritis and osteoarthritis. A number of laboratories have shown that COX-2 is induced during various inflammatorily experiments [13] and in human rheumatoid synovial tissues [14]. In addition, prostaglandins that are produced by COX-2 are indeed responsible for inflammatory indications [15]. The selective inhibition of inducible COX-2 enzyme was found to be anti-inflammatory but non-ulcerogenic in both the carrageenin-induced inflammation [16] and the adjuvant arthritis [17] in rats. The anti-inflammatory efficacy and the relatively low toxicity of selective COX-2 inhibition have been confirmed *in vitro* with human tissue assays [18, 19]. Several COX-2 inhibitors, such as, Celecoxib, Parecoxib, Valdecoxib, Etoricoxib and Lumiracoxib, have been marketed worldwide. Although gastrointestinal events are reduced, other side effects are remained as the problems of these commercial drugs. Thus we are interested to conduct further researches to discover more safe and effective anti-inflammatory agents.

According to our research works for developing the novel anti-inflammatory agents, we have planned to

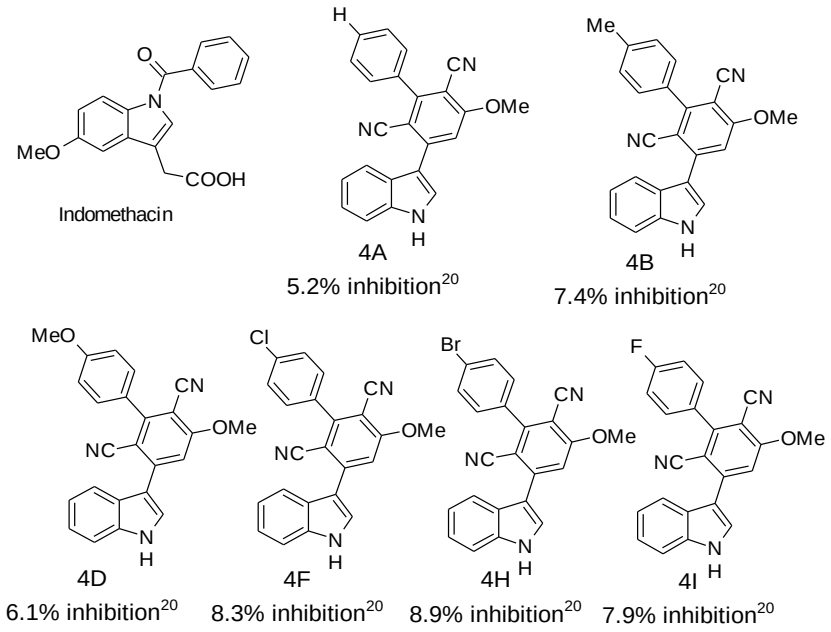

Figure 1 Indomethacin and reported 3-indolyl pyridine derivatives

in silico study of some ligands having new scaffolds thereby focusing on the structure-activity-relationships along with the possible binding modes and thus finally making a proposal for structural modification to increase the binding potential at the binding site of the cyclooxygenase-2 enzyme. Accordingly, the laboratory observations of the anti-inflammatory activity [20] regarding to 3-indolyl pyridine derivatives (Figure 1), which compared to indomethacin, a well-known anti-inflammatory agent for pain management, have been considered for this initial *in silico* study. Our results were reported herein.

Materials and methods

Protein preparation: The compounds subjected in these studies have been reported to show the anti-inflammatory activity considering indomethacin as the positive control. The published PDB entry, 4COX, is the X-ray crystallographic data of cyclooxygenase-2 enzyme complexed with indomethacin as the ligand and thus was chosen here as the receptor protein for this study. The selected PDB had a resolution of 2.90Å. Water molecules, ligands and other hetero atoms were removed from the protein molecule. The chain A was taken for docking of the ligands. The missing residues were also added by using the Swiss PDB Viewer (SPDBV, version - 4.10). Energy minimization was performed by using the same SPDBV software. The energy-minimized protein was then converted to the PDBQT file by using the Autodock Tools (version - 1.5.6). These PDBQT files were taken for the docking [21] by Autodock Vina (version - 1.1.2).

Ligand preparation: The reported novel 3-indolyl pyridine derivatives [20] with anti-inflammatory potency have been considered for the initial *in silico* study. Additionally, for making logical observations on the structure-activity-relationship, some compounds have been selected based on the substitution patterns on the 4-phenyl ring (Figure 1). The ligand structures of the molecules were drawn in ChemSketch (Version - 12) where the outputs were saved as the mol2 files. Those output mol2 files were then converted to the PDB files by using the Accelrys Discovery Studio 4.0 Client and SPDBV (version - 4.10). In the final stage, the PDB structures were further converted to the PDBQT file for docking with the Autodock Vina (version - 1.1.2).

Docking using Autodock Vina: Docking simulations were performed here for predicting the protein–ligand interactions by using the Autodock Vina (version - 1.1.2). Some of the selected compounds were subjected to repeat docking simulation for ensuring the reproducibility and consistency of our approach. The docking process involved a conformational search for compound which complements a target binding site, with the aim of identifying the best matching pose along with the active site to perform docking. Note that the stability of docked ligand-protein complex was due to hydrogen bonding and non-polar interactions like, van der Waals forces.

The 4COX PDB entry is the X-ray crystallographic structure of cyclooxygenase-2 (prostaglandin synthase-2) complexed with a non-selective inhibitor, indomethacin. Thus, the pocket for indomethacin binding was taken as the target ligand binding site. The default parameters of the automatic settings were used to set the generic algorithm parameters. The docked conformations with the lowest energy (highest affinities) were selected to analyze the mode of binding.

Results

For searching the binding modes, the targeted compounds were docked individually and the highest affinity binding modes were superimposed to compare with indomethacin (Figures 2). In most of the cases (discussed in the following section), the overall alignments were comparable with that of indomethacin (element color). The compounds 4A (red), 4B (magenta), 4D (yellow), 4F (hot-pink), 4H (raspberry) and 4I (pink) were found to have consistent SAR profile, where they had the indole moieties, pyridine rings and 4-phenyl groups oriented in a similar position as that of the 4-chlorophenyl ring, indole moiety and the acetic acid function of indomethacin, respectively.

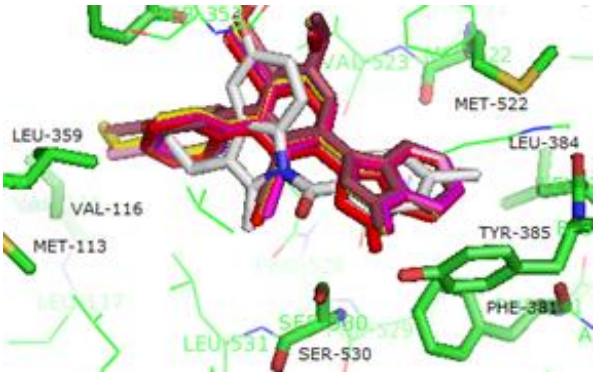

Figure 2 Orientation and possible interactions of 3-indolyl pyridine derivatives while comparing with Indomethacin (Element color, stick model) as observed after docking in the receptor site of COX-2 enzyme by applying the Autodock Vina software (4A-red, 4B-magenta, 4D-yellow, 4F-hot-pink, 4H-raspberry and 4I-pink).

As observed from the figure 2, all of these compounds were achieving the orientations where the different moieties were aligned in a similar fashion as those of indomethacin. Thus, this scaffold appeared to be interesting to perform in depth *in silico* study for exploring additional ligand-protein interaction possibilities.

As shown in figure 2, compounds 4A, 4B, 4D, 4F, 4H and 4I had the indole NH functions close to the -OH of TYR-385 residue of the enzyme and thus indicating a polar interaction site within the receptor. Their 4-phenyl

groups were close to the VAL-116 and LEU-359, thereby indicating possibility of additional non-polar interactions. Relatively tiny space favors the better accommodation of smaller methyl group as compared to methoxy group (4B *vs* 4D) and thus 4B (7.4 % inhibition after 1 h) was found [20] to be more effective than 4D (6.1 % inhibition after 1 h) as shown in figure 1. Polar 3–CN groups of the docked compounds (4A, 4B, 4D, 4F, 4H and 4I) were close to SER-530 thereby indicating possibility of another polar interaction site. Their indole phenyl groups were projected into the relatively non-polar space – adjacent to MET-522, PHE-381 and LEU-384. Unsubstituted phenyl ring at the 4-position of the central pyridine ring (4A) gave the compound lacking the non-polar interactions with LEU-359 and VAL-116 and probably thus resulted in less inhibitory potential (5.2 % inhibition after 1 h). On the other hand, 4F, 4H and 4I, having an electro-negative atom at the para position, possibly have a special polar interaction with the relatively remote thiomethyl group of the flexible side chain of MET-113 residue. Accordingly, relatively larger halogen atoms appeared to have more access to this interaction point. This was proved [20] by the inhibitory potential of the halogen residues (Br > Cl > F: 8.9 %, 8.3 % and 7.5 % inhibition after 1 h respectively).

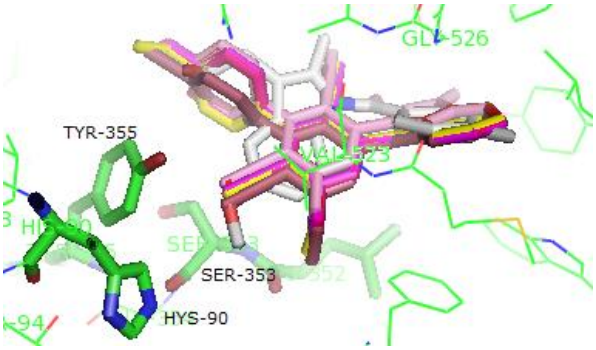

Figure 3 Interaction at the receptor site of COX-2 enzyme (Indomethacin-element color, 4A-red, 4B-magenta, 4D-yellow, 4F-hot-pink, 4H-raspberry and 4I-pink).

The docked compounds had the indole –NH functions having access to TYR-385 (Figure 2) and the 3-CN groups on the central pyridine rings having access to SER-530. There was available space in the receptor site where the 5-CN and 6-OMe groups were oriented (Figure 3). This space was targeted for exploring possibilities of additional polar interactions with residues like, TYR-355, SER-353, and HIS-90 (interaction was found in this site with some other docked molecules not mentioned here). At the same time, the para position of the 4-phenyl group also appeared as an important site for modification, where small non-branched groups may be potentially involved in further interactions with residues like, VAL-116 and LEU-359 while ensuring the polar interactions with relatively remote MET-113 residue. Thus it seems that by these simple modifications the compounds will get much higher inhibitory potential against the cyclooxygenase-2 enzyme. Obviously, there also remains encouraging spaces for further substitutions especially at the meta and para position of the 4-phenyl moiety of the central pyridine ring as well as on the terminal unsubstituted indole moiety.

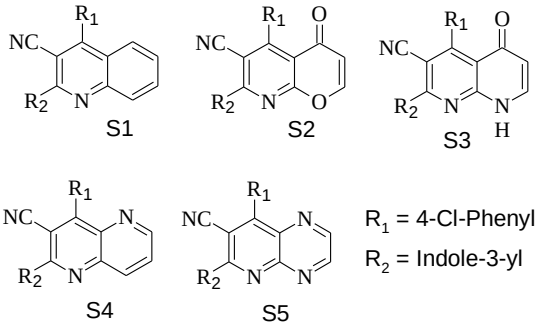

Figure 4 Modified 3-indolyl pyridine scaffolds considered in the *in silico* study

To exploit the available space adjacent to TYR-355, HIS-90 and SER-353, where the 5–CN and 6-OMe groups are extended, some fused ring systems were considered. Accordingly, some modifications were done to introduce the fused ring system in place of the single pyridine ring (Figure 4). Herein, all of the molecules were taken with the same 4-chlorophenyl substitution. As shown in figure 5, all the scaffolds were found to occupy the binding site of the enzyme with the same orientation. In addition, the orientations of these fused ring containing derivatives were similar to those of the initial 3-indolyl pyridine derivatives (4F has been shown by the element color).

Comparing the pyridine ring containing compounds, the pyridine-fused ring containing scaffolds occupied the extra available space in a better-fit approach. This was

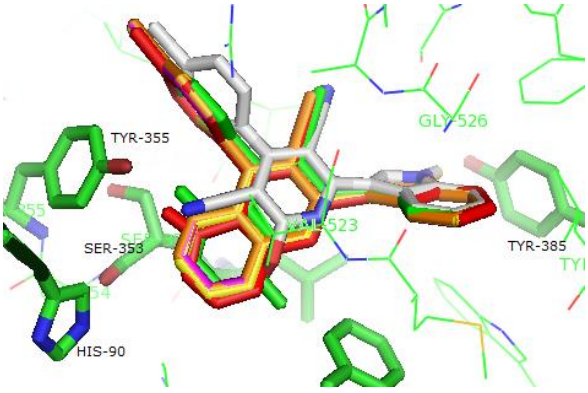

Figure 5 Orientation of modified 3-indolyl pyridine derivatives as observed from the *in silico* study (4F-element color, S1-yellow, S2-red, S3-green, S4-orange and S5-magenta)

also justified by their relative binding affinities as observed by the docking, where, the single pyridine derivatives showed the affinity values ranging from -7.6 Kcal/mol to -9.2 Kcal/mol, but the fused pyridine ring scaffolds showed these values from -8.0 Kcal/mol to -9.6 Kcal/mol.

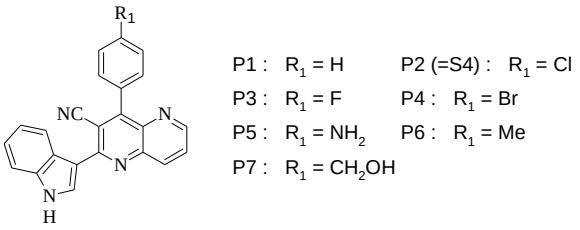

Figure 6 1,5-naphthyridine derivatives as considered in the in silico study

Among the various fused ring systems analyzed, the 1,5-naphthyridine was found to have the highest binding affinity and thus this series was taken for further analysis of the feasibility for substitution at the para position of 4-phenyl ring of the initial central pyridine moiety. Various small groups or atoms were placed accordingly (Figure 6) and the docking was done in similar ways. In this case multiple running was performed and the results were appeared to be consistent and the methods were thus found to be reproducible.

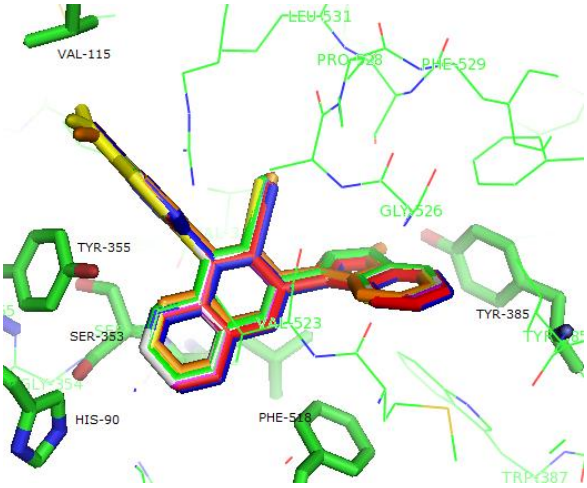

Figure 7 Orientation of 1,5-naphthyridine derivatives as observed from the *in silico* study (P1-element color, P2-red, P3-green, P4-blue, P5-yellow, P6-magenta and P7-orange)

In this final docking, the 1,5-naphthyridine derivatives were found to show the binding affinities ranging from -9.3 Kcal/mol to -9.9 Kcal/mol. The results indicated that there was a new polar interaction with TYR-355 as expected from the docking of 3-indolyl-pyridines. In addition, it was observed that all of these molecules were consistently aligned (Figure 7) in the similar orientations thereby demanding further laboratory researches.

Conclusion

In conclusion, we have identified the important binding sites through these *in silico* docking studies. At the same time, some changes have been postulated for getting compounds having better interactions with the cyclooxygenase-2 receptor. Thus, there is encouraging scopes for additional research works with the new derivatives for generating novel hit compound in this scaffold. These works are now in progress in our laboratory and will be published in time.

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