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Molecular docking, synthesis and biological screening of mefenamic acid derivatives as anti-inflammatory agents

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ABSTRACT

Drug induced gastrointestinal ulceration, renal side effects and hepatotoxicity are the main causes of numerous Non-Steroidal Anti-inflammatory Drugs (NSAIDs). Cyclooxygenase-2 (COX-2) inhibitors discovered to decrease the gastrointestinal issues, but unfortunately, most of them are associated with major cardiovascular adverse effects. Along these lines, various new strategies and frameworks were developed wherein basic alterations of the present medications were accounted for. The aim of the study was to prepare derivatives of mefenamic acid to evaluate anti-inflammatory activity with fewer adverse reactions. In this study, molecular docking investigations of outlined derivatives were done utilizing Protein Data Bank (PDB ID-4PH9). Synthesis of heterocyclic compounds was carried out utilizing Dicyclohexylcarbodiimide/4-Dimethylaminopyridine (DCC/DMAP) coupling. Acute toxicity prediction was performed using free online GUSAR (General Unrestricted Structure-Activity Relationships) software. The study indicated most of the compounds under safe category. *In-vitro* pharmacological assessment of heterocyclic compounds was done for COX-1 and COX-2 enzymes for the determination of selectivity. *In vivo* pharmacological screening for anti-inflammatory activity and ED_{50} value were determined utilizing carrageenan induced rat paw edema. Gastro intestinal safety study was carried out on selected compounds and found to be devoid of any gastric ulcer toxicity. Most of the compounds indicated high scores as compared to standard during molecular modelling, analysis and displayed interactions with active amino acids of a COX-2 enzyme. The pharmacological screening uncovered that compound substituted with p-bromophenyl indicated maximum potency.

1. Introduction

Anti-inflammatory drugs are used to treat pain and inflammation associated with musculoskeletal muscle and joints. Inflammation is a result of an increase in the level of prostanoids which are also responsible for the protection of the gastric mucosal membrane (Kalgutkar et al., 2002). Cyclooxygenase (COX) enzyme is involved in the rate-limiting step for the synthesis of different prostaglandins and thromboxanes from arachidonic acid (Marnett et al., 1999). The COX-1 enzyme is responsible for the cytoprotection and the COX-2 enzyme is inducible and responsible for the biosynthesis of Prostaglandins in inflammatory tissues (Dogné et al., 2006; Kalgutkar et al., 2002; Marnett et al., 1999). Unfortunately, anti-inflammatory agents discovered till now suffer from major side-effects. Classical NSAIDs are associated with adverse effects like gastric ulceration, hepatotoxicity, anemia etc. In order to overcome these side effects various selective COX-2 inhibitors were discovered. As a result, celecoxib (Celebrex®) (Penning et al., 1997), rofecoxib (Vioxx®), (Prasit et al., 1999) valdecoxib (Kalgutkar et al., 2000) and etoricoxib (Riendeau et al., 2001) were marketed as selective COX-2 inhibitors for the treatment of inflammation (Bansal et al., 2014). However, in the year 2004, Merck & Co., voluntarily withdrawn rofecoxib from the market due to severe cardiovascular adverse events. The adverse cardiovascular toxicity related to selective COX-2 inhibitors is due to the increased level of TXA_2 in platelet (Patrono and Baigent, 2014). Kalgutkar et al. synthesized various derivatives of classical NSAIDs (Aljadhey et al., 2010). They have synthesized various amide and ester derivatives of meclofenamic acid and indomethacin, which showed selectivity for the COX-2 enzyme. There are several reports which revealed that derivative preparation of well-known NSAIDs showed better results (Kalgutkar et al., 2000; Talley et al., 2000; Woods et al., 2001). Moreover, the gastric irritation associated with NSAIDs and COX-2 inhibitors is free of the route of the administration totally, mitigating any rationalization that removing the carboxylic acid moiety would bring about less gastro-

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intestinal irritation due to some sort of local effect. The points of interest offered by the utilization of the particular COX-2 inhibitors urged to evaluate the pharmacological movement of novel heterocyclic compounds that could be potential anti-inflammatory agents. Hence, preparation of amide derivatives of NSAIDs can be utilized as the classical approach for the development of more efficacious anti-inflammatory agents, which is our real objective.

2. Materials and methods

2.1. Molecular docking studies

Docking was used to predict both ligand orientation and binding affinity with the COX-2 enzyme. The response of designed molecules at the specific active site of the crystallographic structure of the protein was determined. SYBYL-X 1.2 software was used to build all the compounds and energy minimization was carried out using a conjugate gradient algorithm with a gradient convergence value of 0.01 kcal/Mol Å. Partial atomic charges were calculated using the Gasteiger Huckel method. To examine the binding affinities of the designed compounds with the COX-2 enzyme, docking of these compounds using GOLD 5.2 was performed. PDB ID- 4PH9 with a resolution of the 1.8 Å cocrystal structure was downloaded from RCSB (Research Collaboratory for Structural Bioinformatics) protein data bank for docking study. The result of docking analysis was obtained in terms of GOLD score and was compared with the standard drug (Ganga Reddy et al., 2016).

2.2. LD_{50} prediction using GUSAR software

In silico acute toxicity prediction was performed using GUSAR (General Unrestricted Structure-Activity Relationships) software. GUSAR is a free online software, which include information about the acute toxicity of around 10,000 chemical structures. The software predicts activity of compounds based on QSAR (Quantitative Structure Activity Relationship) models. The data represented as the LD_{50} values (log10 (mmol/kg) of the compounds for different routes of administration like oral, subcutaneous, intravenous and Intraperitoneal. The toxicity class of the given compound was reported, according to the OECD (Organization for Economic Co-operation and Development) classification project of chemical substance (Korobko, 2016).

2.3. Synthesis of amide derivatives of mefenamic acid ***(3a-h)***

Synthesis of amide derivatives was carried out using borosilicate glass wares. REMI rota mantle and magnetic stirrers were used for heating, refluxing as well as stirring of the reaction mixtures. Solvent recovery was done using Rotary Vacuum evaporator (Buchi type). Precoated Silica Gel TLC plates (MERCK) were used for monitoring the progress of the reaction. UV chamber was used for the determination of progress of a reaction. Melting point was measured using the paraffin bath or by melting point apparatus (VEEGO corporation). IR spectra were recorded on JASCO FTIR by KBr dispersion method. Mass Spectra were recorded on BRUKER using ESI as an ion source. ^{1}H NMR and ^{13}C NMR spectra were recorded on BRUKER 400 MHz instrument using TMS as an internal standard.

2.3.1. Synthesis of 2-(2,3-dimethylphenylamino)-N-phenylbenzamide ***3a***

To a mixture of 0.002 mol of 2-(2,3-dimethylphenylamino)benzoic acid and 0.0022 mol of aniline in DCM (dichloromethane) and DMAP (dimethyl amino pyridine) was added with continuous stirring. After 30 min of stirring at 0 °C, the cooled solution of DCC (N,N`-dicyclohexylcarbodiimide) (1.1 equiv) was added in to the above reaction mixture and allowed to stir at room temperature under the N_2 environment for 15 h. The reaction was monitored using TLC using ethyl acetate: hexane (0.5: 4.5) as the solvent system. After completion of the reaction the pale yellow product was extracted using DCM after repeated washing with brine and sodium bicarbonate.

2.3.2. Synthesis of 2-(2,3-dimethylphenylamino)-N-p-tolylbenzamide ***3b***

Same as compound **3a**; in place of aniline, p-toluidine was used (0.002 mol) and the reaction was carried out for 15 h afforded a yellow colour product of **3b**.

2.3.3. Synthesis of 2-(2,3-dimethylphenylamino)-N-(4-fluorophenyl) benzamide ***3c***

Same as compound **3a**; in place of aniline, *p*-fluoroaniline was used (0.002 mol) and the reaction was carried out for 12 h afforded a yellow colour product of **3c**.

2.3.4. Synthesis of 2-(2,3-dimethylphenylamino)-N-(2-chlorophenyl) benzamide ***3d***

Same as compound **3a**; in place of aniline, *o*-chloroaniline was used (0.002 mol) and the reaction was carried out for 15 h afforded a dark yellow colour product of **3d**.

2.3.5. Synthesis of 2-(2,3-dimethylphenylamino)-N-(4-bromophenyl) benzamide ***3e***

Same as compound **3a**; in place of aniline, *p*-bromoaniline was used (0.002 mol) and the reaction was carried out for 15 h afforded a yellow colour product of **3e**.

2.3.6. Synthesis of 2-(2,3-dimethylphenylamino)-N-(2-(piperazin-1-yl)ethyl)benzamide ***3f***

Same as compound **3a**; in place of aniline, 2-aminoethyl piperazine was used (0.002 mol) and the reaction was carried out for 15 h afforded a yellow colour product of **3f**.

2.4. Synthesis of benzimidazole and benzthiazole derivatives ***(4a-4b)***

2.4.1. Synthesis of 2-(1H-benzo[d]imidazole-2-yl)-N-(2,3-dimethylphenyl)benzenamine ***4a***

To 0.1 mol of 3g in a 100 ml round bottom flask, 0.1 mol of glacial acetic acid and Con. HCl was added. The mixture was refluxed at 80 °C for 2.5 h. After the completion of the reaction, crushed ice was added to the reaction mixture with continuous stirring. The reaction mixture was neutralized with $NaHCO_3$ until precipitates were observed. The precipitates were filtered under the vacuum and washed with cold water. The product of **4a,** was purified using column chromatography (5% ethyl acetate: hexane).

2.4.2. Synthesis of 2-(1H-benzo[d]thiazol-2-yl)-N-(2,3-dimethylphenyl)benzenamine ***4b***

Same as compound **4a**; in place of **3g**, **3h** (0.002 mol) and the reaction was carried out for 17 h afforded a pale yellow colour product of **4b**.

2.5. Pharmacological Screening

2.5.1. In vitro COX-1 and COX-2 enzymatic assay

In vitro COX-I and COX-2 inhibition assay was performed for standard and synthesized compounds. Evaluation of COX inhibitory activity was carried out using a COX colorimetric inhibitor screening assay kit (Cayman Chemicals, USA) (Jang, 1997).

The assay kit includes both ovine COX-1 and human recombinant COX-2 enzymes in order to screen isozyme-specific inhibitors. The assay measures the peroxidase activity by monitoring the appearance of oxidized TMPD (*N,N,N′,N′*-tetramethyl-*p*-phenylenediamine) at 590 nm.