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Synthesis and evaluation of novel mutual prodrugs of Piroxicam

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Abstract: Therapeutic efficacy of piroxicam can be improved by retarding gastrointestinal side effects by means of temporarily modification of enolic hydroxyl group chemically. The NSAIDs such as aceclofenac, ibuprofen, mefenamic acid and naproxen were selected as promoities with the aim of getting synergistic effect through these well known pharmaco-counter parts. The targeted prodrugs are synthesized successfully and confirmed by characterization. Synthesis involved chlorination of NSAIDs and coupling of this acid chloride with piroxicam through enolic hydroxyl group to get ester derivatives. Mutual prodrugs were evaluated by hydrolysis study at different pH (acidic, neutral, alkaline) using phosphate buffer. Prodrug derivatives were found to be stable at acidic and neutral pH but prone to hydrolysis at alkaline pH. Thus the objective of the presented study was to overcome the undesirable side effects of NSAIDs. Thus, current studies confirms that the mutual prodrug approach can be applied effectively in order to achieve the purpose of raising effectiveness of piroxicam under two lines; firstly, masking of enolic hydroxyl group through acids and converting them to esters and secondly, utilizing the known NSAIDs for achieving the synergistic effect.

Keywords: Mutual prodrugs; piroxicam;NSAIDs; esters; hydrolysis.

1. Introduction

Most of the therapeutic agents in current use are associated with some unacceptable physicochemical or biopharmaceutical parameters. Non-steroidal anti-inflammatory agents (NSAIAs) have possibility of frequent adverse reactions like dyspepsia and ulceration that limits their use.[1] These NSAIDs are widely used in the treatment of pain and inflammation in many conditions, including osteoarthritis (OA) and rheumatoid arthritis (RA).[2] Succession in the side effects of gastrointestinal (GI) tract predominantly stomach ulceration, bleeding and perforation are the chief limitation in use. This is due to local action exerted by direct contact of drug with gastric mucosa.[3] The enolic hydroxyl group of piroxicam and free acidic group of aryl propionic acids plays a key role in maintaining the effectiveness and producing the gastric ulceration as well.[4] There are several approaches reported that are used to overcome the side effects of NSAIDs by chemical derivatization with retention of potency. Mutual prodrug concept involves the conjugation of two pharmacologically active compounds in which individual drug acts as promoiety. This approach has been used for minimization of GI toxicity by provisionally masking enolic group of piroxicam with acidic group of drugs. This also increase their absorption values.[5] Prodrugs of piroxicam is of profound interest for medicinal chemist as the enolic hydroxyl group can be derivatized easily and may results in diversified derivatives including ampiroxicam, one of the widely used agent.[6-8] Moreover, several prodrugs of selected NSAIDs were also reported.[9] As a part of extension of our work on mutual prodrugs,[10] and motivated by above findings, a new series of mutual prodrugs of piroxicam is designed by combining it with well known NSAIDs as aceclofenac, ibuprofen, mefenamic acid and naproxen as promoieties These therapeutic agents were selected so as to get active promoiety. Thus in the present work, the enolic hydroxyl group of piroxicam is conjugated with acidic group of propionic acid derivatives to get esters. This is for the first time mutual prodrugs of piroxicam are synthesized by taking advantage of presence of enolic hydroxyl group.

2. Result and Discussion

The present work has suggested an approach in overcoming the disagreeable properties of existing drugs by designing the mutual prodrugs. It was for the first time synthesis of mutual prodrugs of piroxicam was achieved with recognized NSAIDs. The targeted compounds PA/PI/PM and PN (Figure 1.) were synthesized (Scheme 1) and characterized successfully by converting the selected NSAIDs (aceclofenac, ibuprofen, mefenamic acid and naproxen) to their respective acid chlorides by using thionyl chloride. These NSAIDs act as masking agent for enolic hydroxyl group of piroxicam. The acid chlorides were then coupled with piroxicam through enolic hydroxyl in presence of chloroform and triethylamine. The progress

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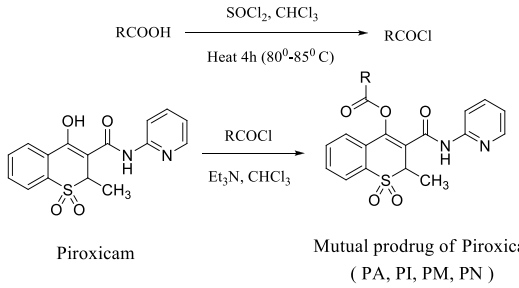

Scheme 1. RCOOH = NSAIDs (Aceclofenac/ Ibuprofen/ Mefenamic acid/ Naproxen)

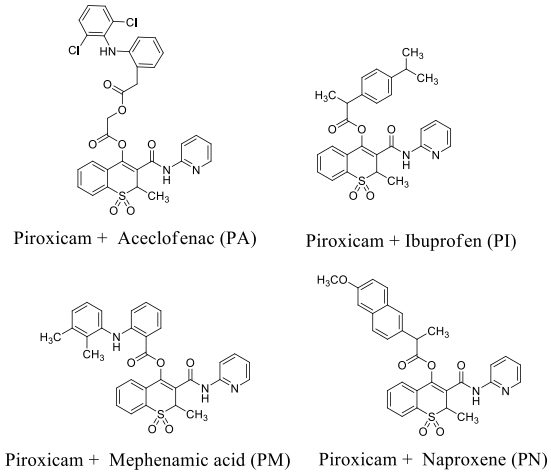

Figure 1. Structure of mutual prodrugs of Piroxicam

of reaction was monitored with the help of TLC using Petroleum ether (60^0- 80^0C) and ethyl acetate in varying proportion. Purification of synthesized compounds was done b recrystallization/column chromatography. All the spectral data is in good agreement with synthesized compounds. Infrared spectra showed the characteristics band of C=O stretching in range of 1716-1747 cm^{-1} and C-O stretching in range of 1225-1232 cm^{-1}, confirms the formation of esters. Chemical shifts in ^1H NMR spectra of synthesized compounds show absence of proton peak of carboxylic acid indicates successful masking. The mass spectra of prepared derivatives having parent peak, which is in accordance with molecular weight of said compounds.

Hydrolysis kinetics study of prodrugs was determined in acidic, neutral and alkaline pH to determine the fate of prodrugs. The available literature reveals that the essential pre-requisite for success in the use of prodrugs is that the masked compounds should be acid stable to prevent the direct contact effects with the gastric mucosa as well as the local inhibition of the prostaglandins.[11] The synthesized prodrug derivatives were estimated across the appropriate clinical range having acidic, neutral and alkaline pH values. **Figure 2** shows HPLC chromatogram of Piroxicam. The hydrolysis kinetics result by HPLC analysis showed that prodrug derivatives of piroxicam undergo chemical degradation with first order kinetics, and thus quantitatively converted to parent drug. All synthesized prodrug derivatives showed high stability in acidic condition; this indicates that the compounds passed unhydrolyzed through the stomach on oral administration.[12] On the contrary, the degradation ability of prodrugs at neutral and alkaline pH is an indication of their susceptibility for hydrolysis.

3. Conclusion

The studies showed that the mutual prodrug approach can be successfully applied in attaining the goal of increasing the therapeutic effectiveness of piroxicam under two lines; firstly, masking of enolic hydroxyl group through acids and converting them to esters and secondly, utilizing the known NSAIDs for achieving the synergistic effect. The work can be extended by studying the pharmacology for anti-inflammatory and gastroprotective effect. Thus the future prospects reside in further evaluation of activity at molecular level. Mutual prodrug approach therefore gives an opportunity to medicinal chemist for improving the clinical and therapeutic effectiveness of a drug that is suffering from unwanted properties obstructing its clinical usefulness.

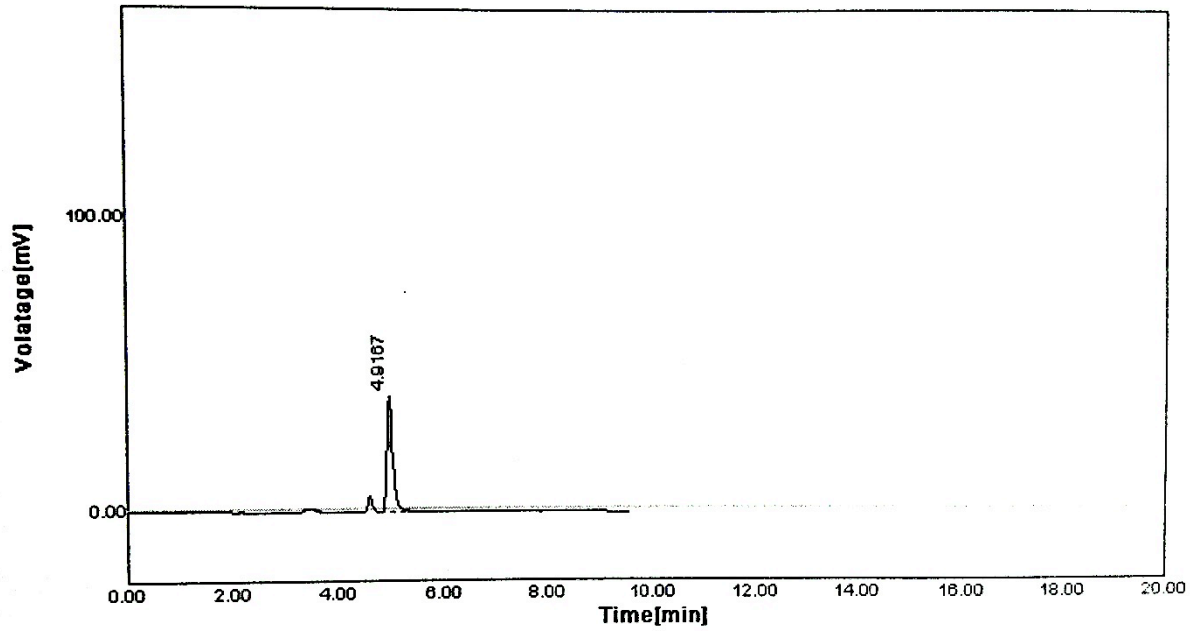

Figure 2. HPLC Chromatogram of Piroxicam showing retention time 4.91 min

4. Experimental

Materials & methods: Non-steroidal anti inflammatory agents were obtained as gift sample from Cadila Health Care Limited, Ahmadabad (India). Laboratory grade reagents are used directly. All solvents used are of analytical grade and purified as required. Open capillary method was used to determine melting point and are uncorrected. Thin layer chromatography was done on pre-coated aluminum plates using suitable mobile phase. Infra red spectra were recorded using KBr on FTIR-8400S Shimadzu. ^{1}HNMR spectra recorded on Joel-FT-NMR-300MHZ using DMSO-D_6 as solvent and TMS as internal standard. Mass spectra were recorded on Shimadzu QP1020GC-MS.

4.1. General procedure for synthesis of title compounds PA, PI, PM and PN

Mutual prodrug ester derivatives, piroxicam-aceclofenac ester (PA), piroxicam-ibuprofen ester (PI), piroxicam-mefenamic acid ester (PM) and piroxicam-naproxen ester (PN) in Figure 1 were synthesized by first converting the promoieties having acidic group into corresponding acid chlorides and followed by reaction with hydroxyl group of piroxicam as shown in Scheme 1.

Acid (5 mM) was dissolved in chloroform (6 mL) and treated with thionyl chloride (3 mL, 5 mM) and a catalytic amount of $AlCl_3$. The reaction mixture was heated for 4 h at 80-85 ^{0}C, cooled and filtered to remove unreacted acid. Solvent was evaporated. The oily product was dissolved in absolute ether, washed with water, dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated to afford acid chloride. The formation of acid chloride was confirmed by FTIR.

Acid chlorides (7 mM) in triethylamine (7 mM) was added drop wise to a solution of piroxicam (7 mM) in 42 mL chloroform at room temperature followed by stirring for 6 h. The reaction mixture was poured in to ice and extracted with chloroform (2x25 mL), the organic layer was collected, washed with 5% NaOH (20 mL) solution followed by 5% HCl (20 mL) and then using water (2x30 mL), followed by its drying over anhydrous Na_2SO_4, filtered and concentrated.[6] The residue was purified by recrystallization from Chloroform-Ethyl acetate (1:1) to afford PA, PI, PM and PN. The synthesized compounds were purified by column chromatography using silica gel G as stationary phase. The products were characterized for purity and confirmations of their structures.

PA - Yield: 73%; mp 212-214^{o}C; UV ($CHCl_3$): λ_{max} 275 nm; IR (KBr) cm^{-1}: 633 (C-Cl stretch), 2931 (C-H stretch), 1747 (C=O str. ester), 1228 (C-O str. ester); 1H NMR (300 MHz, $CDCl_3$): δ 1.48 (s, 3H, CH_3),δ 3.32 (s, 4H, -$2CH_2$),δ 7.05-7.81 (11H, aromatic); δ 8.02 (m, 1H, -NH); MS (70 eV): *m/z* 332

PI - Yield: 78%; mp 120-122^{o}C; UV ($CHCl_3$): λ_{max} 279 nm; IR (KBr) cm^{-1}: 2917 (C-H stretch), 1733 (C=O str. ester), 1225 (C-O str. ester); 1H NMR (300 MHz, $CDCl_3$): δ 1.13 (d, 12H, $4CH_3$),δ 1.94 (m, 1H, -CH),δ 3.78 (m, 1H, -CH),δ 7.09-8.36 (8H, aromatic), δ 8.53 (m, 1H, -NH); MS (70 eV): *m/z* 194

PM - Yield: 68%; mp 160-162^{o}C; UV ($CHCl_3$): λ_{max} 285, 340 nm; IR (KBr) cm^{-1}: 2926 (C-H stretch), 1716 (C=O str. ester), 1232 (C-O str. ester); 1H NMR (300 MHz, $CDCl_3$): δ 1.48-2.34 (d, 9H, $3CH_3$),δ 7.22-8.41 (11H, aromatic); δ 9.04 (m, 1H, -NH), δ 9.09 (m, 1H, -NH); MS (70 eV): *m/z* 242

PN - Yield: 75%; mp 210-212^{o}C; UV ($CHCl_3$): λ_{max} 281 nm; IR (KBr) cm^{-1}: 2924 (C-H stretch), 1738 (C=O str. ester), 1230 (C-O str. ester); 1H NMR (300 MHz, $CDCl_3$): δ 0.89 (d, 6H, $2CH_3$),δ 1.86 (m, 1H, -CH),4.02 (s, 3H, -OCH_3), δ 7.09-7.16 (10H, aromatic);δ 8.89 (m, 1H, -NH); MS (70 eV): *m/z* 332.

4.2. Evaluation of prodrugs by aqueous hydrolysis

The hydrolysis kinetics of prodrugs was studied at pH 1.2 (acidic), pH 7.4 (neutral) and pH 10.0 (alkaline) at 37^{o}C using HCl (pH 1.2), phosphate buffer (pH 7.4) and NaOH (pH 10.0). The total buffer concentration was 20 mM and constant ionic strength of 0.5M for each sample was maintained by adding KCl. Addition of samples to buffer solution initiates the hydrolysis reaction. The solutions were sealed in screw-capped glass vials and then placed into a thermostatically controlled water bath at 37 °C. At regular time intervals, the samples were withdrawn and estimated by HPLC in order to know existence of ester and hydrolyzed product as well according to method reported.[10] Pseudo first-order rate constants (K_{obs}) for the individual reactions were calculated with the help of equation, K_{obs}= *2.303/t X log (a/a-x),* Where, '*a*' is initial concentration, '*x*' is the amount of drug hydrolyzed and't' is time in minutes. The corresponding half-life ($t_{1/2}$) was then obtained from the equation: $t_{1/2} = 0.693/K_{obs}$.

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