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## Synthesis and Physicochemical Characterization of Mutual Prodrug of Indomethacin

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**Abstract:** Indomethacin, which causes gastrointestinal side effect and that, may be minimized by design and development of mutual prodrugs. It involves combining two different pharmacophores with similar pharmacological activities to give synergistic action. The drug (indomethacin) was esterified with paracetamol to prepare mutual prodrug. The structure of the prodrug was confirmed on the basis of IR,  $^1\text{H}$  NMR, mass spectroscopy and elemental analysis. Various physicochemical properties such as solubility in water and organic solvents like chloroform, ether, acetone, methanol and ethanol and partition coefficient in octanol-water, octanol-hydrochloric acid buffer (pH 1.2) and in octanol phosphate buffer (pH 7.4) were determined. In acid pH, the mutual prodrug cannot undergo dissociation and only can undergo in intestinal pH. The mutual prodrug may have devoid of gastric irritation in GIT due to its unionized form in stomach and ionized form in intestine and may have synergic action due to two drugs are combined together. The results indicate that the prodrug dissociate in the intestinal pH because of less partition coefficient in octanol phosphate buffer.

**Key words:** Mutual prodrug, indomethacin, NSAIDS, paracetamol, gastrointestinal toxicity

### INTRODUCTION

The therapeutic efficacy of currently available non-steroidal anti-inflammatory drugs is significantly limited by associated gastrointestinal toxicity, which causes a higher incidence of morbidity in long term NSAID users (Gabriel *et al.*, 1991). The tendency of many acidic drugs to accumulate in the stomach walls soon after oral absorption has been considered as a contributing factor to G.I. irritation (Vane and Botting, 1987). In addition, cyclooxygenase (COX) inhibition, which is the principal mechanism of anti-inflammatory properties of NSAIDs has also been associated with nephrotoxicity and side effects (Warner *et al.*, 1999; Kincaid-Smith, 1986). The reaction range in both severity and frequency from relatively mild to the more serious and in some cases may develop life-threatening states, which lead to GIT ulceration and haemorrhage (Shrivastava *et al.*, 2003a; Jackson and Jason, 2001). In the case of non-steroidal anti-inflammatory drugs containing the carboxylic group (COOH) such significantly ionized at physiological pH of stomach. The result is that such NSAIDs are poorly absorbed through lipid-water membrane barriers and are irritating. There is always a need for safer NSAIDs and the research efforts are going on for developing safer NSAIDs. The prodrug approach is one of the most promising ones among these. In recent years, there has been an increasing interest in the design and development of mutual prodrugs, which involves combining of two different pharmacophores with similar pharmacological activities to give synergistic action (Fukuhara *et al.*, 1996; Sheha *et al.*, 2002). Earlier reports showed that the most prevalent approach for preparing a prodrug of NSAIDs is to alter the -COOH group common to most of them (Brian *et al.*, 1998; Vijay Kumar and Gilbert, 1996).

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The gastric side effects of indomethacin are attributed to the presence of free -COOH group and inhibition of endogenous prostaglandins (Shrivastava *et al.*, 2003b). Many indomethacin prodrug i.e farnesil ester (Mishima *et al.*, 1990), octyl ester (Ogiso *et al.*, 1994), etc and some mutual prodrug such as flurbiprofen-H<sub>2</sub> antagonist (Fukuhara *et al.*, 1996), naproxen-propyphenanone (Sheha *et al.*, 2002), etc were reported in literature.

The current work is targeted at the concept of designing drug through conjunction of two different pharmacophores having similar pharmacological activities. The physicochemical properties of a drug play a major role in the design, development of formulations and bioavailability. Thus in addition to characterization of the proposed structure, the physicochemical parameters like partition coefficient, solubility of the prodrug were also studied.

## MATERIALS AND METHODS

Melting points were determined in Jindal electrically heated melting point apparatus and were uncorrected. The TLC of the compounds was performed on silica gel G coated glass plates with benzene: methanol (4:1) as solvent. Iodine vapors and UV lights were used as detecting agent. The absorbance maxima ( $\lambda_{\text{max}}$ ) were determined on Shimadzu Pharmaspec UV-1700UV/Vis double beam spectrophotometer using 10 mm matched quartz cells. The IR spectra were recorded on a Jasco FTIR-470 plus spectrophotometer, <sup>1</sup>H NMR spectra were recorded on a Varian VRx-300 (300 MHz) instrument using TMS as an internal standard and mass spectra were recorded on API/Qstar Pulsar (Perkin Elmer/SIEX, USA) by electron bombardment technique and electrospray ionization technique.

### Preparation of Prodrugs of Indomethacin with Paracetamol (INDO-PA)

The synthesis involved the conversion of indomethacin (I) into its acid chloride by thionyl chloride and the acid chloride of indomethacin (II) was reacted with paracetamol (III) in alkaline medium according to Fig. 1.

### Preparation of Acid Chlorides of Indomethacin (II)

Indomethacin (I) (2.5 g, 0.006 mol) and thionyl chloride (0.6 mL, 0.0072 mol) in benzene (3.5 mL) were refluxed for 2 h, until the evolution of hydrogen chloride and sulphur oxide ceased. The excess of thionyl chloride and benzene were distilled off, to give brown crystalline product (II).

### Preparation of Prodrug (INDO-PA) (IV)

Paracetamol (III) (0.625 g, 0.006 mol) was dissolved in cold sodium hydroxide (10 mL 5% w/v) in a conical flask equipped with magnetic stirrer. Acid chloride of indomethacin (II) (11.34 g, 0.05 mol) was made slurry in acetone (approximately 20 mL) and was taken in a separating funnel. The slurry was then added slowly drop wise to a cold solution of paracetamol and stirred continuously. After complete addition, stirring was continued for a further period of 30 min. The precipitate, which got separated, was filtered off and washed several times with distilled water. The product (INDO-PA; IV) was purified by recrystallization from benzene: methanol (8:2), (Yield 82%; m.p. 145-150°C, R<sub>f</sub> 0.74, benzene: methanol (4:1); UV:  $\lambda_{\text{max}}$ : methanol: 243 nm, hydrochloric acid buffer (pH 1.2)-224 nm, phosphate buffer (pH 7.4)-224 nm, elemental analysis for: C% (calcd. 66.06%) found 66.12%; H%, (4.72%) 4.48%, N%, (5.71%) 5.69%; IR (KBr)  $\nu$  cm<sup>-1</sup>: 3400 (-NH), 2960 (aliphatic-CH), 1690 (ester C = O) 1540 (C-N), 1240 (C-O). <sup>1</sup>H NMR (TMS)  $\delta$  ppm: 2.10 (S, 3H, COCH<sub>3</sub>), 3.81 (S, 3H, (-OCH<sub>3</sub>)), 2.42 (S, 3H, CH<sub>3</sub>), 3.63 (S, 2H, -CH<sub>2</sub>C(=O)O), 7.5-7.13 (ArH, 4H, paracetamol), 7.75-7.46 (ArH, 4H, indomethacin), 8.01 (s, 1H, NHCOCH<sub>3</sub>), Mass m/z-490 (C<sub>27</sub>H<sub>23</sub>N<sub>2</sub>ClO<sub>5</sub>, M<sup>+</sup>).

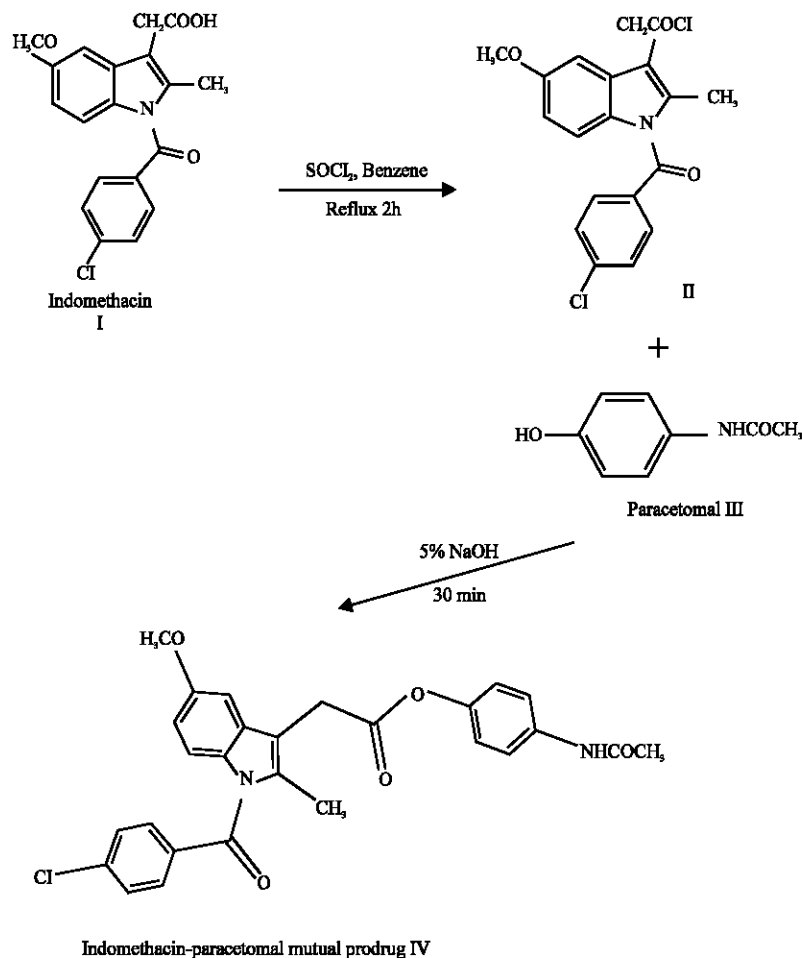


Fig. 1: Preparation of INDO-PA

#### Aqueous Solubility

The aqueous solubilities of synthesized prodrugs were determined in triplicate by taking about 200 mg accurately weighed prodrug in water (10 mL) in a vial. The vials were sealed and kept in rotatory shaker (speed 60 rpm) at  $25 \pm 1^\circ\text{C}$  for overnight. The solvent was filtered through Whatman filter paper and extracted three times with 5 mL of organic solvents (chloroform for INDO-PA). The organic phases were mixed and washed three times with distilled water (3 mL). The water extracts were discarded. Organic phase was evaporated to dryness. The residue was displayed in acetonitrile and diluted suitably to estimate the prodrug by HPLC method. The reported results given in Table 1 are average of the three readings.

HPLC was performed on instrument of M/s Shimadzu, Japan, equipped with dual piston reciprocating pump (model LC-10 AT vp), rheodyne injection system (model 7125 with loop capacity of 20  $\mu\text{L}$ ), PDA detector (model SDP-MIOA vp) and stainless steel column (250 $\times$ 6.4 mm, 5  $\mu$ , Phenomenex, Inc. USA) packed with  $\text{C}_{18}$  Hypersil. Pure acetonitrile of HPLC grade (M/s Ranbaxy) was used as solvent. The flow rate was maintained at 1  $\text{mL min}^{-1}$ . The detection was performed at 240 nm. The retention time of indomethacin, INDO-PA was 3.19 and 3.85 min, respectively.

Table 1: Solubility and partition-coefficient data of the synthesized mutual prodrug of indomethacin

Solvent	Solubility (mg mL <sup>-1</sup> )		Partition coefficient
	INDO-PA	Systems	INDO-PA
Water	0.78	Octanol-water	7.85
Methanol	156.00	Octanol-HCl buffer (pH 1.2)	114.75
Ethanol	189.00	Octanol-phosphate buffer (pH 7.4)	3.95
Acetone	590.00		
Chloroform	565.00		
Ether		645	

Partition-coefficient of indomethacin in octanol/water is 3.50, INDO-PA is the conjugate of indomethacin with paracetamol

### Solubility in Organic Solvents

Solubilities of the compounds were studied in methanol, ethanol, chloroform, acetone and ether. Compound (1 g mL) was added to solvent (5 mL) in a vial, which was tightly closed and kept in rotating shaker (speed 60 rpm) at constant temperature (25°C) for overnight. It was ensured that equilibrium was established. The solvents were filtered through Whatman filter paper and filtrate was taken in tared evaporating dish. The solvent was evaporated off and weight of the residue was determined. The solubility in respective solvent was calculated as mg mL<sup>-1</sup>. Results are shown in Table 1.

### Determination of Partition-Coefficient

The partition coefficients of synthesized prodrugs were determined in three systems i.e. octanol-water, octanol hydrochloric acid buffer (pH 1.2) and octanol-phosphate buffer (pH 7.4) at 25°C temperature. Synthesized compound (100 mg) was added to 10 mL of aqueous phase and 10 mL of organic phase was added to it. This mixture was shaken for 1 h and left for 2 h at 25°C. Layers were separated out using separating funnel. Prodrug concentration in aqueous phase, in hydrochloric acid buffer (pH 1.2) and phosphate buffer (pH 7.4) was determined by HPLC method as detailed earlier after suitably taking into organic phase. The partition co-efficient was calculated as, partition coefficient = concentration of drug in organic phase/that in aqueous phase. The obtained results are shown in Table 1.

## RESULTS AND DISCUSSION

Mutual prodrug in the form of esters of indomethacin with paracetamol was synthesized in the presence of alkaline medium. The physicochemical characteristic of the synthesized compound were done and the results showed that it is varied from the individual drugs. Thin layer chromatography was performed on pre-coated silica gel G glass plates using benzene: methanol (8:2) solvent system to ascertain the purity of these compounds. The compound gave satisfactory R<sub>f</sub> Value.

The structures of the synthesized compounds were confirmed by elemental analysis, infra-red spectroscopy, <sup>1</sup>H NMR spectroscopy and mass spectroscopy. Elemental analysis of the compounds was found to be within permissible limits. Infra-red spectroscopy showed the characteristic absorption bands of-NH stretching, C-O stretching and C = O vibration of these compounds. The <sup>1</sup>H NMR spectra of the synthesized compounds show chemical shifts, which are characteristic of the anticipated structures of compounds. The mass spectra of the synthesized compounds showed the parent peak confirming the molecular weight of the compounds. The mass spectra of the synthesized compound showed the parent peaks confirming the molecular weight of the compound.

The solubility of synthesized compound was determined in methanol, ethanol, acetone, water chloroform and ether. The partition coefficient of the mutual prodrug in octanol, octanol/HCl buffer (pH 1.2) and octanol/phosphate buffer (pH 7.4) reveals that partition coefficient in octanol/phosphate buffer (pH 7.4) have less (3.12) than in HCl buffer (116.85), which demonstrate that the compound

can undergo dissociation in the intestine. In acid pH, the mutual prodrug cannot undergo dissociation and only can undergo in intestinal pH. So it confirms the possibility for gastric irritation produced by indomethacin is reduced. The mutual prodrug may have devoid of gastric irritation in GIT due to unionized form in stomach and ionized form in intestine and may have synergic action due to two drugs are combined together. This preliminary work may be extended to undergo *in vitro* and *in vivo* studies to confirm the synergic action.

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#### REFERENCES

- Brian, S.F., J.H. Antony, W.G.S. Peter and R.T. Austin, 1998. Vogel's Text Book of Practical Organic Chemistry. 5th Edn., London, Addison Wesley Longman, pp: 1274-1275.
- Fukuhara, A., T. Imai and M. Otagiri, 1996. Selective disposition of flurbiprofen from a mutual prodrug with a histamine H<sub>2</sub>-antagonist to reduce gastrointestinal lesions in the rat. *Chirality*, 8: 494-502.
- Gabriel, S.E., L. Jaakkimainen and C. Bombardier, 1991. Risk for serious gastrointestinal complications related to use of non-steroidal anti-inflammatory drugs. A meta analysis. *Ann. Intl. Med.*, 115: 787-796.
- Jackson, L.R. II. and D.M. Jason, 2001. Goodman and Gilman's: The Pharmacological Basis of Therapeutics. Joel, G.H., E.L. Lee and G.G. Alfred Eds., 10th Edn., New York, McGraw Hill Medical Publishing Division, pp: 687-731.
- Kincacid-Smith, P., 1986. Effects of non-narcotic analgesic on the kidney. *Drugs*, 32: 109-128.
- Mishima, M., S. Kobayashi, S. Abe and C. Yamato, 1990. Metabolic fate of indomethacin farnesil, a prodrug of indomethacin: Characteristic biotransformation of indomethacin farnesil in rats. *Xenobiotica*, 20: 135-146.
- Ogiso, T., M. Iwaki, T. Kinoshita, T. Tanino and T. Paku, 1994. Pharmacokinetics of indomethacin octyl ester (prodrug) and indomethacin produced from prodrug. *J. Pharm. Sci.*, 83: 34-37.
- Sheha, M., A. Khedr and H. Elsherief, 2002. Biological and metabolic study of naproxen-propuphenazone mutual prodrug. *Eur. J. Pharm. Sci.*, 17: 121-130.
- Shrivastava, S.K., D.K. Jain and P. Trivedi, 2003a. Dextran-Potential drug carriers for Suprofen. *Die Pharmazie*, 58: 804-806.
- Shrivastava, S.K., D.K. Jain and P. Trivedi, 2003b. Dextran-Potential polymeric drug carriers for Flurbiprofen. *Die Pharmazie*, 58: 389-391.
- Vane, J. and R. Botting, 1987. Inflammation and the mechanism of action of anti-inflammatory drugs. *FASEB, J.*, 1: 89-96.
- Vijay Kumar and S.B. Gilbert, 1996. Modern Pharmaceutics. Banker, G.S. and C.T. Rhodes, (Eds.), 3rd Edn., New York, Marcel Dekker, Inc., pp: 611-680.
- Warner, T.D., F. Giuliano, I. Vaynovie, A. Bukasa, J.A. Mitchell and J.R. Vave, 1999. Non-steroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 era associated with human gastro-intestinal toxicity: A full *in vitro* analysis. *Proc. Natl. Acad. Sci., USA.*, 96: 7563-7568.