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Preparation and Characterization of Mutual Prodrugs of Ibuprofen

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Ibuprofen was esterified with paracetamol and salicylamide to give two mutual prodrugs. The purity of the compounds was confirmed by TLC. Prodrugs were characterized on the basis of elemental analysis, IR spectroscopy, [^{1}H] NMR spectroscopy and mass spectroscopy. The physicochemical properties such as solubility in water and organic solvents like methanol, ethanol, acetone, chloroform and ether, and partition coefficient in octanol-water, octanol-hydrochloric acid buffer (pH 1.2), and in octanol-phosphate buffer (pH 7.4) were determined. The results indicate that the prodrugs are more lipophilic than parent drug i.e. ibuprofen and their dissolution rate was pH-dependent.

Gastrointestinal side effects constitute the most frequent of all the adverse reactions of NSAIDs. The reactions 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. The development of GIT ulceration and haemorrhage induced by NSAIDs is due to the inhibition of prostaglandin synthesis. Endogenous prostaglandins are known to have a cytoprotective action on gastric mucosa. Prostaglandins help to regulate acid secretion and maintain mucosal integrity against stress, variety of chemicals and thermal injury[1,2].

NSAIDs are used for long term therapy of arthritis and other rheumatic diseases. There is always a need for safer NSAIDs and 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[3,4].

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The gastric side effects of ibuprofen is attributed to the presence of free –COOH group and inhibition of endogenous prostaglandins. Therefore, blocking this group by synthesizing functional derivatives of carboxylic acid may reduce these side effects[5]. Earlier reports revealed that the most prevalent approach for preparing a prodrug of NSAIDs is to alter the –COOH group common to most of them[6-11].

The present work is aimed at the concept of drug design 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. Therefore, in addition to characterization of the proposed structures, the physicochemical parameters like solubility, partition coefficients and dissolution rates of the two prodrugs were studied.

MATERIALS AND METHODS

Melting points were determined in a Toshniwal electrically heated apparatus and are 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 (λ_{max}) were determined on GBC Cintra-10 UV/Vis double beam spectrophotometer using 10 mm matched quartz cells. The IR spectra were recorded on a Jasco V-530 FTIR Spectrophotometer, ^{1}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 ibuprofen with paracetamol (IBU-PA):

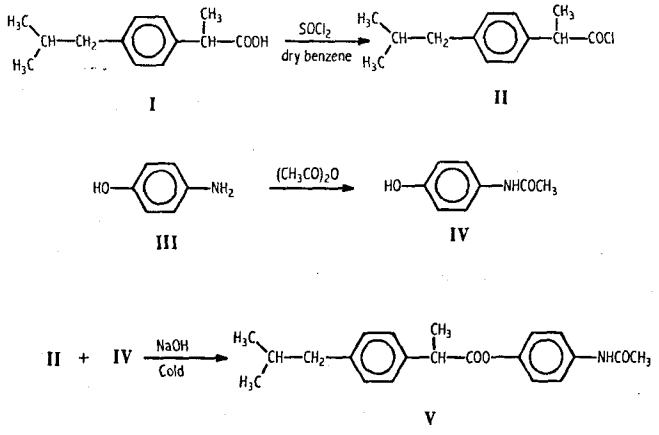
The synthesis involved the conversion of ibuprofen into its acid chloride (II). The acid chloride of ibuprofen was reacted with paracetamol (IV) in alkaline medium according to Scheme 1.

Preparation of acid chlorides of ibuprofen (II):

Ibuprofen (I) (10.3 g, 0.05 mol) and thionyl chloride (4.5 ml, 0.06 mol) in benzene (30 ml) were refluxed for 2 h, until the evolution of hydrogen chloride and sulphur dioxide ceased. The excess of thionyl chloride and benzene were distilled off, to give white crystalline product (II), m.p. 60-62°, yield 84%.

Preparation of prodrug (IBU-PA) (V):

Paracetamol was prepared by reported method from p-aminophenol (III)[12]. Paracetamol (IV) (7.53 g, 0.05 mol) was dissolved in cold sodium hydroxide (40 ml 5% w/v) in a conical flask equipped with magnetic stirrer. Acid chloride of ibuprofen (II) (11.34 g, 0.05 mol) was made into slurry in



I II III IV V

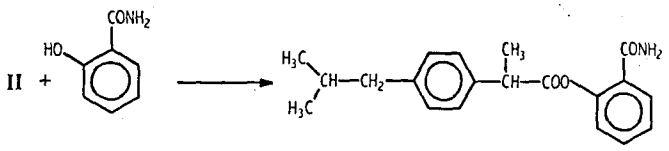
Scheme 1: Preparation of IBU-PA.

I is ibuprofen, II is acid chloride of ibuprofen, III is p-aminophenol, IV is paracetamol and V is IBU-PA, conjugate of ibuprofen with paracetamol.

acetone (approximately 20 ml), and was taken in a separating funnel. The slurry was then added slowly dropwise 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 (IBU-PA; V) was purified by recrystallization from ethanol:water (3:1), (yield, 80.72%; m.p. 86-88°); λ_{max}: methanol-243 nm, hydrochloric acid buffer (pH 1.2)- 224 nm, phosphate buffer (pH 7.4)[13]- 224 nm, R_f- 0.74, benzene:methanol (4:1), elemental analysis for $C_{21}H_{25}NO_3$: C %, (calcd. 74.27) found 74.44; H %, (7.36) 7.42 , N %, (4.12) 4.10; IR (KBr): 3390 cm^{-1} (-NH str. amide), 2960 cm^{-1} (-CH str. alkane), 1720 cm^{-1} (C=O str. ester), 1690 cm^{-1} (C=O, str. amide I), 1540 cm^{-1} (C-N str, N-H band amide II), 1360-1380 cm^{-1} (isopropyl split doublet), 1240 cm^{-1} (C-O str. ester). ^{1}H NMR: δ 0.89 (6H, d, J=5Hz, -CH(C<u>H</u>$_3$)$_2$), δ 0.92 (3H, d, J=5Hz, (-CH(C<u>H</u>$_3$)-), δ 1.55 (2H, d, J=7Hz, -C<u>H</u>$_2$-), δ 1.88 (1H, m,-C<u>H</u>(CH$_3$)$_2$-), δ 2.07 (3H, s, COC<u>H</u>$_3$), δ 3.90 (1H, q, J=5Hz, (-C<u>H</u>(CH$_3$)-), δ 7.15-7.07 (4H, Ar<u>H</u>, ibuprofen), δ 7.29-7.23 (4H, Ar<u>H</u>, paracetamol), δ 7.73 (1H, S, -N<u>H</u>COCH3), Mass: m/z- 339 ($C_{21}H_{25}NO_3$, M$^+$).

Preparation of prodrug of ibuprofen with salicylamide (IBU-SAL):

This prodrug was synthesized according to Scheme 2. Salicylamide (VI) (5.25 g, 0.05 mol) was dissolved in hot water. To this solution, the slurry of acid chloride of ibuprofen (II) (11.34 g, 0.05 mol) in acetone as prepared in case of IBU-PA was added in dropwise fashion. The product which got separated was recrystallized from ethanol to give (IBU-SAL) (VII), (yield, 81.30%; m.p. 108-110°); λ_{max}: methanol – 304 nm, hydrochloric acid buffer (pH 1.2)- 300 nm, phosphate buffer (pH 7.4)- 305 nm, R_f- 0.70, benzene:methanol (4:1). Elemental analysis for $C_{20}H_{23}NO_3$: C%,(calcd 73.75) Found 74.04; H%, (7.06) 7.05; N%, (4.30) 3.89. IR (KBr): 3398 cm^{-1} (-NH str. amide), 2953 cm^{-1} (-CH str. alkane), 1730 cm^{-1} (C=O str. ester), 1676 cm^{-1} (C=O str. amide I), 1630 cm-1 (C-N str+N-H bend amide II), 1360-



VI VII

Scheme 2: Preparation of IBU-SAL.

II is acid chloride of ibuprofen, VI is salicylamide and VII is IBU-SAL, conjugate of ibuprofen with salicylamide.

1380 cm^{-1} (isopropyl split doublet), 1250 cm^{-1} (C-O str. ester). ^1H NMR: δ 0.89 (6H, d, J=5Hz -CH($C\underline{H}_3$)$_2$), δ 0.92 (3H,d,J=5Hz –CH($C\underline{H}_3$)-), δ 1.55(2H,d,J=7Hz –$C\underline{H}_2$-), δ 1.88 (1H, m-, C<u>H</u>-(CH_3)$_2$), δ 3.88 (1H, q, J=5Hz –C<u>H</u>(CH_3)-), δ 6.47 (2H, br, -CO<u>N</u>H$_2$); δ 7.12 (4H, Ar<u>H</u> ibuprofen), δ 7.22-7.45 (4H, Ar<u>H</u>, salicylamide) Mass: m/z- 324.53 ($C_{20}H_{23}NO_3$ M^+).

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±1° for overnight[14,15]. The solvent was filtered through Whatman filter paper and extracted three times with 5 ml of organic solvents (chloroform for IBU-PA and ether for IBU-SAL). 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 dissolved 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 μl), UV/Vis photodiode array detector (model SDP–MIOA vp) and Stainless Steel column (250 x 6.4 mm, 5 μ, Phenomenex, Inc. USA) packed with C_{18} Hypersil. Pure acetonitrile of HPLC grade (M/s Ranbaxy) was used as solvent. The flow rate was maintained at 1 ml/min. The detection was performed at 240 nm. The retention time of ibuprofen, IBU–PA and IBU–SAL was 3.19 min, 3.85 min and 3.84 min, respectively.

Solubility in organic solvents:

Solubilities of the compounds were found out 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°) 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 the weight of the residue was determined. The solubility in respective solvent was calculated as mg/ml[14,15]. 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° 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°. 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 above after suitably taking into organic phase. The partition co-efficient was calculated as, partition coefficient= concentration of drug in organic phase/that in aqueous

TABLE 1: SOLUBILITY AND PARTITION- COEFFICIENT DATA OF THE SYNTHESIZED MUTUAL PRODRUGS OF IBUPROFEN

Solvent	Solubility (mg/ml)		Systems	Partition – coefficient	
	IBU-PA	IBU-SAL		IBU-PA	IBU-SAL
Water	0.81	0.89	Octanol-Water	8.35	4.70
Methanol	145	130			
Ethanol	190	178	Octanol-HCl buffer (pH 1.2)	116.85	34.95
Acetone	605	470			
Chloroform	580	97	Octanol-phosphate buffer (pH 7.4)	3.12	1.43
Ether	630	482			

Partition–coefficient of ibuprofen in octanol/water is 3.50, IBU–PA is the conjugate of ibuprofen with paracetamol and IBU–SAL is the conjugate of ibuprofen with salicylamide.

phase. Results are shown in Table 1.

DISSOLUTION RATE STUDIES

In vitro dissolution studies of IBU–PA and IBU–SAL were carried out in VDA–6 Veego dissolution rate apparatus (six station). Drug pellets (100 mg; 12 mm) were prepared using hydraulic pressure (Shimadzu, Japan) by compressing at 8 ton/in^2 pressure for two minutes. The pellets were placed in the wire basket and suspended in the vessel containing 1000 ml. of dissolution medium at 37±1°.

The dissolution media were, hydrochloric acid buffer (pH 1.2) and phosphate buffer (pH 7.4). The hydrochloric acid buffer (pH 1.2) and phosphate buffer (pH 7.4) were prepared as per I.P.[13] The baskets were rotated at 100 rpm. Five millilitres of the sample was withdrawn at each time interval and replaced with equal volume of fresh dissolution medium. Prodrug concentration in the sample was determined by HPLC method as detailed above after suitably taking into organic phase. The release profiles were plotted as percentage drug dissolved Vs time and depicted in fig. 1–3. The values of correlation coefficient (r), dissolution rate (k) and the time to dissolve 50% drug ($t_{50\%}$) are given in Table 2.

RESULTS AND DISCUSSION

Mutual prodrugs in the form of esters of ibuprofen with paracetamol and salicylamide were synthesized. Thin layer chromatography was performed on pre-coated silica gel G glass plates using benzene: methanol (4:1) solvent system to ascertain the purity of these compounds. The compounds gave single spots. The structures of the synthesized compounds were confirmed by elemental analysis, infra-red spectroscopy, ^{1}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 ^{1}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 solubilities of compounds were determined in water, methanol, ethanol, acetone, chloroform and ether. A perusal of the Table 1 showed the solubilities of the prodrugs to be more in organic phases as compared to aqueous phase. The highest solublities of both, i.e. IBU–PA and IBU–SAL in ether suggest the lipoidal nature of them. The solubility of

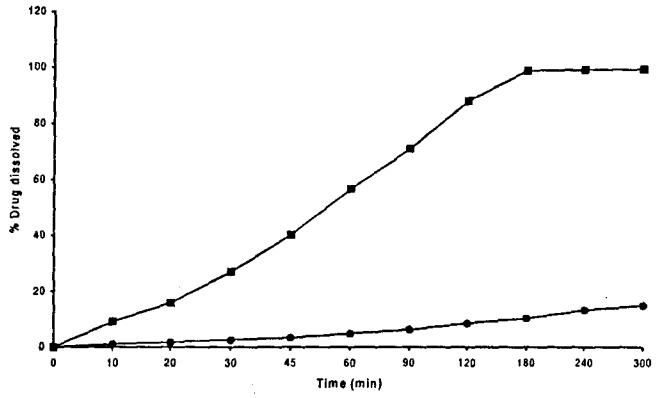


Fig. 1: Dissolution rate profile of ibuprofen.

Dissolution rate studies of ibuprofen were performed at 37° at pH 1.2 (-●-) and pH 7.4 (-♦-).

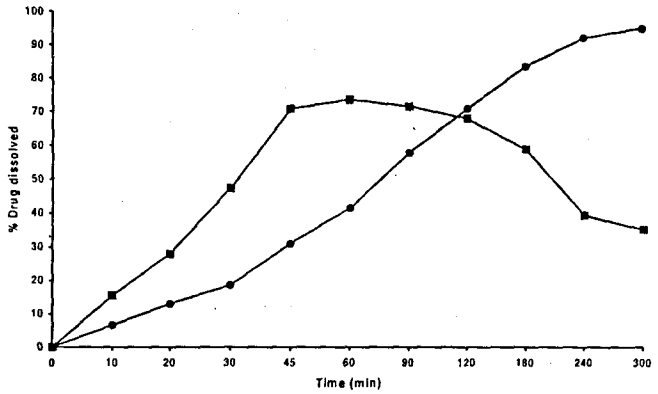


Fig. 2: Dissolution rate profile of IBU-PA.

Dissolution rate studies of IBU-PA were performed at 37° at pH 1.2 (-●-) and pH 7.4 (-♦-).

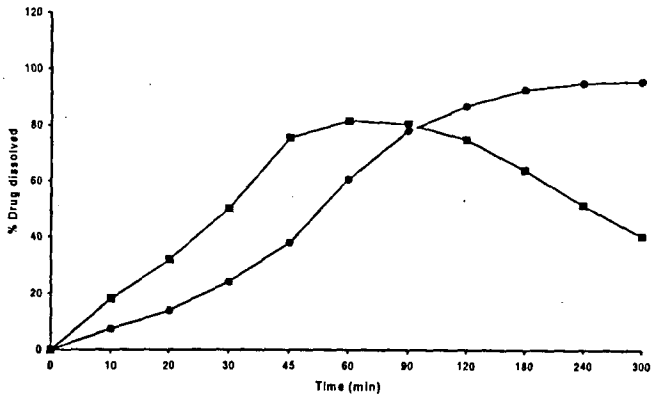


Fig. 3: Dissolution rate profile of IBU-SAL.

Dissolution rate studies of IBU-SAL were performed at 37°, at pH 1.2 (-●-) and pH 7.4 (-♦-).

IBU-PA was found to be more (630 mg/ml) as compared to IBU-SAL (482 mg/ml) in ether. Another factor which had drawn our attention is the poor solubility of IBU-SAL in chloroform (97 mg/ml) as against IBU-PA (580 mg/ml). This may be attributed to the polarity of the amide linkage present in IBU-SAL. Polarity wise also the chloroform is the least polar out of all the organic phases tried. In all the other cases the solubility indicate the polar nature of the prodrug i.e. IBU-SAL as compared to IBU–PA.

To produce a pharmacologic response, a drug molecule has to first cross a biological membrane consisting of lipids and proteins, which acts as a barrier. The ability of a drug molecule to penetrate this barrier is based in part, on its preference for lipids (lipophilic) versus it preference for an aqueous phase (hydrophilic). A drug's partition coefficient is a measure of its distribution in a lipophilic/hydrophilic phase system, and is indicative of its ability to penetrate biological multiphase system. The partition coefficients of the compound were determined in three systems, i.e. octanol/water, octanol/hydrochloric acid buffer (pH 1.2) and octanol/Phosphate buffer (pH 7.4). The partition coefficients of compounds were found more in octanol/hydrochloric acid buffer (pH 1.2) as compared to in octanol/water and in octanol/phosphate buffer (pH 7.4). The partition coefficient of IBU-PA was found remarkably high in all system as compared to IBU-SAL. The partition coefficient of compounds was found more in octanol/water system than parent drug. This study indicates that the mutual prodrugs are more lipophilic than parent drug.

The dissolution rate is an important parameter for drug absorption. If the dissolution rate constant is greater than 1mg/min., the drug is not prone to give dissolution–rate–limited absorption problems. On the other hand, if the values are less than 0.1, such problems can definitely be anticipated, and compounds with value of dissolution rate from 0.1 to 1 mg/min. are in a gray area[16].

A perusal of the Table 2 and figs. 1-3 indicated that dissolution rate of Ibuprofen, IBU-PA, and IBU-SAL were pH-dependent. The dissolution rate of ibuprofen at pH 1.2 was very slow. In 4 h, only 14.9% drug got dissolved at pH 1.2. The dissolution rate of ibuprofen was faster at pH 7.4 whereas in 3 h, 99.4% drug got dissolved. This behavior may be attributed to ester linkage in them. The hydrolysis of an ester in acid medium is reversible whereas it is not shown in other medium.

The values of $t_{50\%}$ of IBU-PA were found to be 76.2 min at pH 1.2 and 31.7 min at pH 7.4. The dissolution followed zero-order kinetics. At pH 1.2, 94.8% drug dissolved in 300 min and at pH 7.4, 73.9% drug dissolved in 60 min. After 60 min, there was a decline in the slope of the graph, which may be attributed to the hydrolysis of prodrug. Hence later on, the dissolution rate was not found to follow zero-order kinetics. On other hand, the values of $t_{50\%}$ of IBU-SAL were found to be 53.5 min and 29.7 min at pH 1.2 and pH 7.4 respectively. The dissolution of IBU-SAL was found to follow zero- order kinetics. At pH 1.2, 95.4% drug got dissolved in 300 min. At pH 7.4, 81.3% drug got dissolved in 60 min. As in the case of IBU-PA there was a decline in the slope of the

TABLE 2: DISSOLUTION RATE STUDIES OF IBUPROFEN, IBU-PA AND IBU-SAL

S. No.	Drug	pH	r	k(%/min.)	$t_{50\%}$ (min)
1	Ibuprofen	1.2	0.9700	0.0653	-
		7.4	0.9535	0.726	53.9
2	IBU-PA	1.2	0.9829	0.573	76.2
		7.4	0.2404	1.465(up to 60 min.)	
				0.929 (up to 300 min.)	31.7
3	IBU-SAL	1.2	0.9524	0.607	53.5
		7.4	0.2994	1.621 (up to 60 min.)	
				.864 (up to 300 min.)	29.7

r is the correlation coefficient, k is the dissolution rate, $t_{50\%}$ is the time to dissolve 50% drug, IBU–PA is the conjugate of ibuprofen with paracetamol and IBU–SAL is the conjugate of ibuprofen with salicylamide.

graph at pH 7.4, which may again be due to hydrolysis of the prodrug. The results of these studies indicate that, mutual prodrugs are found to have faster dissolution rate than the parent drug.

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