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RESEARCH ARTICLE

A promising codrug of nicotinic acid and ibuprofen for managing dyslipidemia. I: Synthesis and *in vitro* evaluation

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Abstract

Nicotinic acid is therapeutically the optimum antihyperlipidemic agent, yet its intolerable cutaneous flushing hinders its wide clinical implication. The codrug of nicotinic acid and ibuprofen (IBP) was synthesized in the aim of overcoming the troublesome side effect of nicotinic acid by blockade of prostaglandin synthesis through released IBP, thus enhance patient's compliance. The physico-chemical properties of codrug namely solubility, partition coefficient, and pKa were determined. Its solubility in aqueous and organic solvents was highest in 0.1 M HCl and isopropanol, respectively. The kinetics of hydrolysis of the codrug and IBP 2-hydroxyethyl ester was studied in aqueous phosphate buffer solution in pH 1.2, 6.8, and 7.4 at 70°C, 80°C, and 90°C. The hydrolysis was found to be pH dependent and followed Arrhenius equation. The half-life of codrug and IBP 2-hydroxyethyl ester at 25°C in pH 7.4 was 218 days and 3 years, respectively. *In vitro* enzymatic hydrolysis of codrug and IBP 2-hydroxyethyl ester was studied in human plasma and rat liver homogenate. Codrug and IBP 2-hydroxyethyl ester exhibited faster *in vitro* enzymatic hydrolysis than *in vitro* chemical hydrolysis. The pseudo-first-order rate constants were 0.0113, 0.177 min^{-1} for codrug and 0.0006, 0.0569 min^{-1} for IBP 2-hydroxyethyl ester in human plasma and rat liver homogenate, respectively. Thus, nicotinic acid will be rapidly released from codrug to manage dyslipidemia, followed by the later release of IBP from IBP 2-hydroxyethyl ester to alleviate nicotinic acid cutaneous flushing.

Keywords: Dyslipidemia, codrug, nicotinic acid, ibuprofen, chemical hydrolysis, enzymatic hydrolysis

Introduction

Dyslipidemia is a vastly growing disease having serious implications; the most serious is being a predisposing factor for chronic heart disease[1]. Many drugs have been developed for management of dyslipidemia; however, one of these drugs currently in use is nicotinic acid, which is also known as niacin. It is the most effective and least expensive but the least tolerable[1,2].

Nicotinic acid, chemically known as 3-pyridine carboxylic acid[3], is a member of water-soluble vitamins (vitamin B_3) that was introduced into clinical practice as the first lipid-modifying drug 50 years ago[4,5]. Nicotinic acid is a potent lipid-modifying drug and is known as broad-spectrum antihyperlipidemic drug[6]. It is capable of lowering elevated plasma concentrations of triglyceride-rich very-low-density lipoprotein (VLDL) and cholesterol-rich low-density lipoprotein (LDL) and their subclasses including intermediate-density lipoprotein (IDL) and small dense LDL[6,7]. Moreover, it is an antihyperlipidemic that is capable of lowering lipoprotein (Lp (a))[8], and is comparable with fibrates in achieving significant reductions in triglyceride levels[9]. The usage of nicotinic acid has re-emerged due to its superiority over other antihyperlipidemic drugs. Its uniqueness resides in its ability to significantly increase the concentration of the cardio-protective HDL, particularly the subfraction HDL_2[10], and reduction of chylomicrons elevated levels[11].

The most prominent intolerable and dose-dependent side effect of nicotinic acid that hinders its clinical usage is cutaneous flushing[4,11]. Because nicotinic acid-related flushing appears to be a result of prostaglandin activity, usage of nonsteroidal anti-inflammatory drugs

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(NSAIDs) in controlling the troublesome nicotinic acid-related flushing, such as acetyl salicylic acid and ibuprofen (IBP) that are highly effective inhibitors of prostaglandin synthesis, has been well supported by previously conducted studies[12,13]. It was previously reported by Obertwittler et al.[14] that acetyl salicylic acid, or IBP taken before a single 500-mg dose of immediate-release nicotinic acid on an empty stomach significantly reduced flushing[14]. Furthermore, Cefali et al.[15] proved that administration of acetyl salicylic acid, either before or concomitantly with extended-release nicotinic acid, significantly reduced the incidence, intensity, and duration of flushing associated with reformulated extended-release nicotinic acid (2 g)[15].

IBP, chemically known as (±)-(*R*, *S*)-2-(4-isobutylphenyl)-propionic acid[16], is widely accepted as the best tolerated NSAID[17], exhibiting good analgesic, anti-inflammatory, and antipyretic activity with the safest gastrointestinal profile[18–20]. A recent study by Kourounakis et al. (2002) showed that IBP possesses dose-dependent antihyperlipidemic properties[21,22]. The major mechanism by which IBP exhibits its pharmacological actions is the non-selective competitive inhibition of cyclo-oxygenase enzymes[23,24].

Prodrug is a pharmacologically inactive derivative of a parent drug molecule that requires spontaneous, chemical, or enzymatic transformation *in vivo* to release the active drug[25]. Mutual prodrug is a type of carrier-linked prodrug, where the carrier used is another biologically active drug instead of some inert molecule. Mutual prodrug approach offers a very fruitful area of research and an efficient tool for improving the clinical and therapeutic effectiveness of a drug that is suffering from some undesirable properties hindering its clinical usefulness otherwise[26]. For instance, sultamicillin, a double ester of sulbactam plus ampicillin, has been synthesized to extend the antibacterial activity of the latter to include some beta-lactamase-producing strains of bacteria that would otherwise be resistant[27,28]. Also, prodrug of atorvastatin salt with ranitidine[29], prodrug of atorvastatin with amlodipine[30], and diverse nicotinic acid derivatives[31] were made for the purpose of achieving two therapeutic responses by one dosage form.

Alkyl nicotinates have been of great interest due to hydrolytic conversion to nicotinic acid in the body; therefore, they are classified biologically as active pyridine derivatives[32]. They are mainly intended for topical and transdermal delivery[33]. For example, tetradecyl nicotinate delivers nicotinic acid at a sustained rate at the site of delivery, stratum corneum, where it is readily converted to nicotinic acid and the free alcohol[34,35]. Aminocarbonyloxymethyl esters of nicotinic acid are considered superior over amidomethyl esters, which are only slowly hydrolyzed in human plasma, due to almost instantaneous liberation of nicotinic acid. Currently, there is evaluation of similar derivatives containing an ionizable functionality to increase aqueous solubility[36]. Fluorinated esters of nicotinic acid have also been synthesized to enhance the solubility of nicotinic acid for pulmonary administration[37].

The majority of prodrugs made on carboxylic acids such as IBP are of the ester type. They are aimed to temporarily mask the carboxylic acid group of NSAIDs, thus reduction or suppression of gastrointestinal toxicity[38,39]. The attractiveness of using esters comes from the ready availability of a diverse set of alcohols and phenols and the ubiquitous distribution of esterases and peptidases to liberate the active principle in the *in vivo* setting[40]. Glyceride prodrugs[39], alkyl glucopyranoside[38] and guaiacol esters[40], and amide conjugates with ethylenediamine and benzathine of IBP[41] were all found to resist acid-catalyzed hydrolysis in stomach but liberate IBP freely in intestine. Thus, IBP gastric ulceration is suppressed without adversely affecting its anti-inflammatory and analgesic activity.

The main objective of this work is to synthesize a codrug or a mutual prodrug of nicotinic acid and IBP that retains its pharmacological potency but masks the cutaneous flushing, which is a major side effect of nicotinic acid therapy precipitating patient incompliance. The rationales for the selection of this codrug in particular is dependent on the concurrent clinical usage of nicotinic acid along with IBP to alleviate nicotinic acid cutaneous flush[13,42,43]. Also the rationale for the use of ethylene glycol as a safe spacer was based on a marketed etofibrate which is a codrug of nicotinic acid and etofibric acid with ethylene glycol spacer[44].

Methods

Materials

Reagents used such as the bromoethanol, nicotinyl chloride hydrochloride, and all chemicals used in the preparation of the buffer were all of analytical or reagent grade and the solvents were of high-performance liquid chromatography (HPLC)-grade obtained from Aldrich Chemical Company (USA), ACROS Chemicals (Belgium), and Scharlau Chemical (Spain). IBP was kindly provided by the Jordanian Pharmaceutical Manufacturing Company (Jordan). Water used in the HPLC procedure was deionized. Melting points were determined using Stuart Scientific-melting point apparatus (UK). ^{1}H and ^{13}C-NMR spectra were obtained using a 400 MHz Bruker Avance Ultrashield Spectrometer (Switzerland). NMR data are reported in ppm using automatic calibration to the residual proton peak of the solvent, $CDCl_3$ or MeOH. The splitting pattern abbreviations are as follows: *s*, singlet; *d*, doublet; *t*, triplet; *m*, multiplet; and *dd*, doublet of doublet. Electron spray ionization (ESI) mass spectra of synthesized compounds were obtained using Applied Biosystems-MDS SCIEX API 3200 LC/MS/MS system (USA). IR spectra were recorded on Shimadzu IR Affinity-1 FT-IR (Japan) using KBr disks. Elemental analysis was obtained using Euro EA3000 Elemental Analyzer (Italy). TLC analysis was performed on Albet aluminum thin layer chromatography (TLC) plate, Aluminum, Silica 60, and UV254 (Spain).

Synthesis

2-hydroxyethyl 2-(4-isobutylphenyl) propanoate (1)

IBP (5.00 g, 24.24 mmol) were dissolved in acetonitrile at reflux of 80°C, and to it potassium carbonate (5.02 g, 36.40 mmol) and drop-wise 2-bromoethanol (4.54 g, 36.4 mmol) were added, and the reaction was maintained at reflux for 3 days. The reaction progress was followed up by TLC employing 50% ethyl acetate/hexane system. Upon reaction's completion, the reaction mixture was filtered and evaporated. The remaining residue after evaporation was dissolved in ethyl acetate then washed with cold 1 N sodium hydroxide solution (300 mL × 3). The organic layer was then dried over sodium sulfate (Na_2SO_4) and the solvent was evaporated to obtain a colorless oil (5.46 g, 90%) which was used for the next step without further purification. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 7.22 (2H, *d*, *J* = 8 Hz, *Ar-H*$_2$), 7.12 (2H, *d*, *J* = 8 Hz, Ar-*H*$_2$), 4.20 (2H, *m,* CO_2CH_2), 3.75 (3H, *m*, *CH*CH_3 and *CH*$_2$OH), 2.45 (2H, *d, J* = 8 Hz, *CH*$_2$), 1.75– 1.95 (1H, *m*,-*CH* $(CH_3)_2$), 1.50 (3H, *d*, *J* = 8 Hz, -CH*CH*$_3$), and 0.90 (6H, *d, J* = 8 Hz, –CH*(CH*$_3$*)*$_2$). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 177.8, 143.4, 140.3, 132.1, 129.8, 68.9, 63.9, 47.8, 47.7, 32.9, 25.1, and 21.2. IR (KBr): 3444 (OH), 2954 (–C(=O)OH), 2931, 2868, and 2848 (sp^3 C-H), 1735 (C = O), 1456 (Ar C = C), 1201 and 1166 (C-O), and 1070 (C-C(O)-C). LC-MS (ESI) m/z: MH + 1 (251.2, 100.0%), MH + 2 (252.3, 19.06%), MH + 3 (253.3, 2.59%).

2-(2-(4-isobutylphenyl) propianoyloxy) ethyl nicotinate hydrochloride salt (2)

IBP 2-hydroxyethyl ester, 1, (5.47 g, 21.85 mmol) was dissolved in dichloromethane at 25°C, and to it nicotinyl chloride hydrochloride (3.89 g, 21.85 mmol) and drop-wise triethylamine (4.42 g, 43.7 mmol) were added, and the reaction was allowed to proceed at 25°C for 5 days. The reaction progress was followed up by TLC analysis employing 25% ethyl acetate/hexane system in an ammonia atmosphere. Upon reaction's completion, the reaction's mixture was washed by cold 1 N sodium hydroxide solution (300 mL × 3). The organic layer was then dried over Na_2SO_4 and the solvent was evaporated to yield the codrug as brownish-orange oil, (6.75 g, 87%). Hydrochloride salt of the codrug was prepared by simultaneous addition of 500 mL of cold distilled water and two equivalents of cold 0.1 N methanolic HCl (31.58 g, 38 mmol). The resulting solution was treated with ethyl acetate, then filtered and the solid material was crystallized from ethyl acetate. All the experiments conducted were on the hydrochloride salt of the codrug. M.P.: 133 – 135°C. ^{1}H-NMR (MeOD, 400 MHz): δ = 9.25 (1H, *s*, *–NHAr*), 9.10 (1H, *d*, *J* = 4 Hz, *-ArH*), 8.90 (1H, *d, J* = 8 Hz, *–ArH*), 8.20 (1H, *dd*, *J* = 8 Hz, *-ArH*), 7.20 (2H, *d*, *J* = 8 Hz, *Ar-H*$_2$), 7.00 (2H, *d*, *J* = 8 Hz, Ar–*H*$_2$), 4.40 – 4.70 (4H, *m*, CO_2CH_2 and *–CH*$_2$OH), 3.75 (1H, *m*, *CH*CH_3), 2.35 (2H, *d, J* = 8 Hz, *CH*$_2$), 1.70 – 1.80 (1H, *m,* –*CH*CH_3), 1.45 (3H, *d*, *J* = 8 Hz, –CH*CH*$_3$), and 0.85 (6H, *d, J* = 4 Hz, *–CH*$(CH_3)_2$). ^{13}C-NMR (MeOD, 100 MHz): δ = 175.6, 162.3, 147.4, 145.8, 143.7, 141.3, 138.7, 130.7, 129.9, 128.6, 127.9, 65.4, 62.6, 45.7, 45.5, 31.0, 22.3, and 18.4. IR (KBr): 3394 (OH), 2985 and 2385 (-C(=O)OH), 1732 (C = O), 1460 (Ar C = C), 1377 (CH_3), 1290 (C–O), 1166 (C-C(O)-C) cm^{-1}, 744 (*o,* C-H), and 678 (*m*, C-H). LC-MS (ESI) m/z: MH + 1 (356.2, 100.0%), MH + 2 (357.2, 34.29%), and MH + 3 (358.2, 6.38%); Elemental analysis (% calculated), C: 64.36, H: 6.69 and N: 3.57; (% found), C: 65.11, H: 6.73 and N: 3.67.

HPLC analysis

The HPLC system, used for quantitation of codrug, IBP 2-hydroxyethyl ester, and IBP, consisted of a SCL-10 AVP system controller, LC-10 ADVP liquid chromatograph pump, DGV-14 AVP degasser, SPD-M 10 AVP diode array detector, SIL-10 ADVP auto injector, and CTO-10 ASVP column oven connected to a computer prepared with appropriate software (Shimadzu, USA). Chromatographic separation and quantitative analysis were performed under isocratic reversed-phase conditions using Purosphere RP-C18 column (125 × 4 mm, 5 µm) (Merck, Darmstadt, Germany). The mobile phase employed for isocratic elution was a mixture of acetonitrile and 0.02 M of phosphate buffer pH 6 (55:45 v/v) at a flow rate of 2 mL/min. The mobile phase was filtered through 0.45 µm regenerated cellulose membrane filter. The UV detection wavelength and column oven temperature was set at 226 nm and at 27°C ± 1, respectively. In the same chromatographic run, the codrug and two compounds, the parent drug IBP and its 2-hydroxyethyl ester, were detected at different retention times, namely 6.6, 2.1, and 2.8 min (±0.1), respectively. This method has been recently developed and validated for determination of the codrug and its hydrolyzed products[45].

Determination of physico-chemical properties of the codrug

Solubility in aqueous and organic solvents

The aqueous solubility of codrug was determined at 25°C, in aqueous 0.05 M phosphate buffer solution at pH 1.2, pH 6.8, and pH 7.4 as well as 0.1 M HCl and water, whereas for the organic solubility of codrug, it was similarly determined in methanol, isopropanol alcohol, acetonitrile, and octanol. Excess amounts of codrug were added to 1 mL of each of the previously mentioned aqueous and organic solvents. They were continuously shaken for 24 h using shaking water bath equilibrated at 25°C. The saturated solutions were filtered using millipore filters 0.45 µm and the concentration of codrug was determined by HPLC.

Apparent partition coefficient and pKa

The apparent partition coefficient expressed as log P_{app} of codrug was determined at 25°C, between n-octanol and aqueous 0.05 M phosphate buffer solution at pH 1.2, pH 6.8, and pH 7.4, as well as 0.1 M HCl. n-octanol was first saturated with each of the aqueous solutions by

vigorous stirring using magnetic stirrer for 24 h. Log P_{app} was measured by dissolving 5 mg of codrug in 2 mL of pre-saturated n-octanol and then added to a glass screw-capped test tube containing equal-volume of aqueous solution. The test tube was shaken at 25°C in water bath for 24 h at all pH values followed by centrifugation at 4000 rpm, 25°C for 5 min to ensure complete separation of the two phases. The concentration of codrug in aqueous and organic layer was analyzed by HPLC. The experiments were performed in triplicate. The partition coefficient was calculated by dividing the concentration of codrug in the n-octanol layer by its concentration in aqueous phase. pKa was theoretically calculated using ACD pKa/DB version 5.0 Software (Advanced Chemistry Development Inc., Toronto, Canada).

In vitro chemical hydrolysis of codrug in aqueous phosphate buffer

The rates of the chemical hydrolysis of the compounds were determined in aqueous phosphate buffer solution at pH 1.2, 6.8, and 7.4 (0.05 M, ionic strength was adjusted to 0.13 with NaCl) at 70, 80, and 90°C. Due to poor solubility of codrug in buffer solution, 40% isopropanol alcohol was added to the buffer solutions as a co-solvent. The reactions were initiated by preparing 100 μg/mL solutions of codrug in hydrolysis medium, which consists of pre-heated 0.05 M phosphate buffer of pH 1.2, pH 6.8, and pH 7.4 along with the co-solvent. The obtained solutions were equally divided into screw-capped test tubes followed by placement in thermostatically controlled water bath at 70, 80, and 90°C. At appropriate time intervals, samples were withdrawn, cooled with iced water, and immediately analyzed by HPLC for remaining codrug, IBP 2-hydroxyethyl ester, and formed IBP. All experiments were carried out in triplicate. The rate of hydrolysis was determined from the linear plot of ln codrug or ln IBP 2-hydroxyethyl ester concentration remaining versus time.

In vitro enzymatic hydrolysis of codrug in human plasma and rat liver homogenate

Hydrolysis of the codrug in human plasma

The rate of hydrolysis for codrug in 80% human plasma diluted with phosphate buffer saline (0.05 M, pH 7.4) was determined at 37°C. Human blood samples were obtained from healthy volunteers at Hematology Department, King Abdullah University Hospital. The blood was pooled and drawn into a test tube containing EDTA. After centrifugation at 1000 g for 10 min, the plasma was taken and used for the codrug analysis. The reactions were initiated by dissolving an appropriate amount of the codrug in phosphate buffer and adding it to human plasma, which was kept at 37°C. The solutions were kept in a water bath at 37°C, and 150 μL of human plasma/buffer mixture were withdrawn at certain time intervals and added to 450 μL absolute methanol in eppendorf tubes to quench the reaction and precipitate the proteins. Immediately after mixing and centrifugation for 5 min at 4000 rpm, the supernatant was analyzed for the remaining codrug, IBP 2-hydroxyethyl ester, and formed IBP by HPLC.

Hydrolysis of the codrug in rat liver homogenate

Preparation of rat liver homogenate

Rat liver homogenates were prepared by anesthetizing three adult male Wistar rats from Animal House at Jordan University of Science and Technology, weighing 230 ± 10 g, with ether. Then they were euthanized by cervical dislocation[46]. The livers were rapidly removed, washed with cold freshly prepared phosphate buffer saline (PBS) pH 7.4, and sliced into small pieces. Homogenate was prepared by homogenizing 2 g of liver pieces with 10 mL PBS (0.1 M, pH 7.4) for 5 min at 30,000 rpm in ice bath using a homogenizer to obtain 20% liver homogenate. The homogenate was centrifuged for 20 min at 5000 rpm and 4°C, and then the supernatant was collected and stored at –20°C until used. Animal experiments were approved by the Committee on Care and Use of Experimental Animal Resources, Faculty of Veterinary Medicine at Jordan University of Science and Technology.

Hydrolysis of the codrug in rat liver homogenate

The same method applied in human plasma was adopted to examine the hydrolysis of codrug in rat liver homogenate. The rate constants of hydrolysis of codrug and liberated IBP 2-hydroxyethyl ester were determined from the slope of the linear plot of Log (residual) codrug and IBP 2-hydroxyethyl ester concentration versus time in the same manner employed in hydrolysis of codrug in human plasma.

Results and discussion

Chemistry

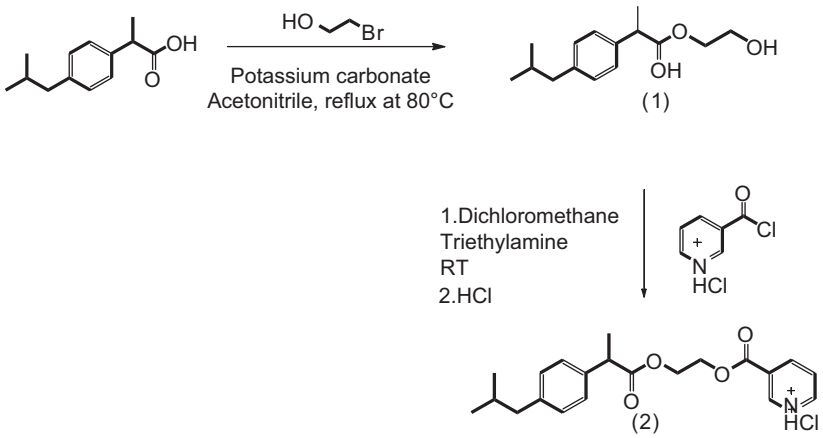
Preparation of the hydroxyalkyl ester of IBP allows for the subsequent connection of nicotinyl chloride hydrochloride to obtain the desired nicotinic acid–IBP codrug. The synthesis of IBP 2-hydroxyethyl ester is illustrated in Scheme 1. IBP and potassium carbonate were both dissolved in acetonitrile at reflux of 80°C and 2-bromoethanol was added to the stirring solution. Reaction follow-up revealed a slow but steady formation of one product that required 3 days to reach completion.

The resultant IBP 2-hydroxyethyl ester was used subsequently as a starting material in the synthesis of the 2-(2-(4-isobutylphenyl) propianoyloxy) ethyl nicotinate hydrochloride salt as shown in Scheme 1. Formation of the hydrochloride salt of nicotinic acid—IBP codrug followed by crystallization from ethyl acetate was a practical and cost effective way of purification.

Physico-chemical properties of the codrug

Solubility in aqueous and organic solvents

Solubility measurements of codrug in aqueous and organic solvents were determined after 24 h



Scheme 1. Synthesis of 2-(2-(4-isobutylphenyl) propianoyloxy) ethyl nicotinate hydrochloride codrug.

Table 1. Solubility in aqueous and organic solvents of codrug hydrochloride salt.

Solvents	Solubility (mg/mL) ± S.D.*	Solubility (mmol/mL) ± S.D.*
0.1 M HCl (pH 1)	1.234 ± 0.027	3.147 ± 0.069
0.05 M Phosphate buffer pH 1.2	0.499 ± 0.008	1.272 ± 0.019
Water (pH 4.6)	0.152 ± 0.026	0.387 ± 0.066
0.05 M Phosphate buffer pH 6.8	1.50 ± 0.10 (μg/mL)	3.827e-03 ± 0.0002
0.05 M Phosphate buffer pH 7.4	0.80 ± 0.11 (μg/mL)	2.041e-03 ± 0.0003
Methanol	1.087 ± 0.003	2.774 ± 0.007
Isopropanol alcohol	3.905 ± 0.057	9.963 ± 0.146
Acetonitrile	3.199 ± 0.008	8.165 ± 0.019
Octanol	0.402 ± 0.067	1.026 ± 0.171

*Standard deviation ($n = 3$).

equilibration in each of the selected solvents and are summarized in Table 1. It can be observed by comparing the parent compounds' solubility[47–50] to that of codrug that codrug's solubility trend resides between nicotinic acid and IBP. For instance, the aqueous solubility of codrug in water, which is 0.15 mg/mL, is less than that of nicotinic acid, 15 mg /mL[51], yet more than IBP, 1.74×10^{-4} mg/mL[48]. The solubility of codrug in organic solvents, namely isopropanol alcohol and acetonitrile, was the highest, respectively. Because the codrug is apparently poorly water soluble, the usage of the chemically inert isopropanol alcohol as a co-solvent in the preparation of standard solutions for chemical hydrolysis was necessary.

Apparent partition coefficient and pKa

The apparent partition coefficient of codrug was determined in distinct aqueous media after 24 h equilibration. The results obtained are presented in Table 2. It is clear from Table 2, that moving from pH 1.2 to 6.8 and 7.4, increases the apparent partition coefficient and the opposite is true when moving from pH 1.2 to 0.1 M HCl. This is well correlated with the solubility trend of codrug. Solubility of codrug was 5-folds more at pH 7.4 than at pH 6.8, 27-folds more at pH 1.2 than pH 7.4, and 2-folds higher at 0.1 M HCl than pH 1.2. Further, the codrug is a weak base, as indicated by its calculated pKa 3.04 ± 0.20; thereby, at 0.1 M HCl and pH 1.2, it predominates in its ionized form whereas at pH 6.8 and pH 7.4 in its unionized form. Hence, the dramatic decrease in apparent partition coefficient at 0.1 M HCl compared to pH 1.2 can be utterly contributed to the full protonation of the nitrogen atom within the pyridine ring in the codrug; leading to ultimate ionization thus greatest water solubility is achieved. It can be concluded that the codrug is markedly more hydrophobic than the parent compounds, nicotinic acid and IBP. This is supported by the comparison of apparent partition coefficient values, for example, at pH 7.4, the apparent partition coefficient values are 2.17, –2.47, and 1.07 for codrug, nicotinic acid, and IBP, respectively[37,48].

Table 2. Partition coefficient of codrug hydrochloride salt in different aqueous media.

Aqueous media		Log P_{app} ± S.D.*
0.1 M HCl (pH 1)		−0.217 ± 0.057
0.05 M Phosphate buffer	pH 1.2	1.038 ± 0.025
	pH 6.8	1.559 ± 0.203
	pH 7.4	2.168 ± 0.007

*Standard deviation ($n = 3$), Log P_{app}: apparent partition coefficient.

In vitro chemical hydrolysis in aqueous phosphate buffer

The chemical hydrolysis of nicotinic acid—IBP codrug and IBP 2-hydroxyethyl ester in 40% isopropanol/aqueous buffer solutions were investigated and the results are reported in Table 3. The chemical hydrolysis of each compound followed *pseudo*-first order kinetics in which a linear relationship between time and the *ln* remaining codrug concentration was found.

Chemically, the codrug was hydrolyzed quantitatively to IBP 2-hydroxyethyl ester, which is in turn hydrolyzed to the parent compound IBP as observed in Figure 1. At constant pH, temperature, and buffer concentration, it is observed that the hydrolysis of codrug is faster than that of IBP 2-hydroxyethyl ester. This could

Table 3. Observed first-order rate constant for hydrolysis of codrug hydrochloride salt and ibuprofen 2-hydroxyethyl ester at different pH (343, 353, and 363 K).

Codrug					
pH	Temp (K)	k_{obs} (h^{-1}) ± S.D.*	R^2	$t_{0.5}$ (h)	Avg log k_{obs} (h^{-1})
1.2	343	0.010 ± 0.001	0.997	68.00	−1.996
	353	0.018 ± 0.001	0.997	39.33	−1.754
	363	0.041 ± 0.004	0.996	16.67	−1.390
6.8	343	0.013 ± 0.002	0.996	51.90	−1.873
	353	0.033 ± 0.010	0.997	21.00	−1.479
	363	0.073 ± 0.010	0.998	9.59	−1.139
7.4	343	0.034 ± 0.002	0.999	20.74	−1.475
	353	0.099 ± 0.010	0.997	6.96	−1.001
	363	0.252 ± 0.020	0.996	2.76	−0.599
Ibuprofen 2-hydroxyethyl ester					
pH	Temp (K)	k_{obs} (h^{-1}) ± S.D.*	R^2	$t_{0.5}$ (h)	Avg log k_{obs} (h^{-1})
1.2	343	0.007 ± 0.001	0.975	104.79	−2.178
	353	0.015 ± 0.004	0.986	47.50	−1.818
	363	0.020 ± 0.010	0.995	32.40	−1.649
6.8	343	0.002 ± 0.001	0.973	550.85	−2.814
	353	0.004 ± 0.001	0.982	203.75	−2.432
	363	0.024 ± 0.012	0.978	37.87	−1.622
7.4	343	0.004 ± 0.001	0.993	173.36	−2.391
	353	0.010 ± 0.001	0.994	68.90	−1.967
	363	0.025 ± 0.001	0.999	27.27	−1.595

*Standard deviation ($n = 3$).

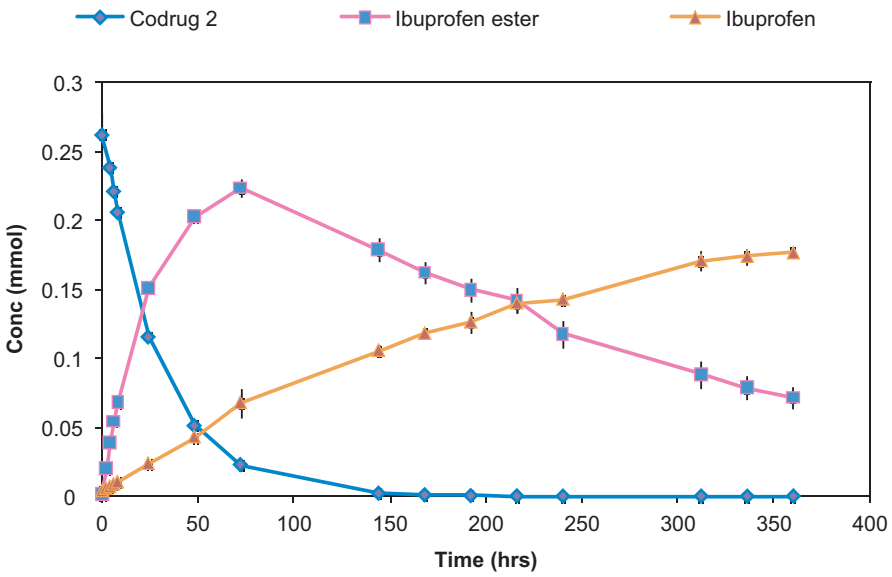


Figure 1. Explicit degradation of codrug hydrochloride salt in 0.05 M phosphate buffer pH 7.4 at 70°C.

be attributed to the presence of nicotinic acid within the codrug that is more readily labile to hydrolysis than the IBP 2-hydroxyethyl ester. The main structural feature of nicotinic acid in determining its greater susceptibility to hydrolysis is the presence of heterocyclic pyridine moiety in its molecule. The nitrogen in the pyridine moiety is nucleophilic in nature; enabling facilitated interaction with appropriate electrophiles[52]. Moreover, it had been established in literature that IBP and its esters as well as derivatives are well-known for their relatively high stability[38,53].

It was observed that the rate of hydrolysis of codrug and IBP 2-hydroxyethyl ester is influenced by pH. Codrug's susceptibility to hydrolysis is increased as the pH increases toward the alkaline range and vice versa is true for the hydrolysis of IBP 2-hydroxyethyl ester. Unlike IBP 2-hydroxyethyl ester, the codrug is relatively more stable at low pH values than in the alkaline pH.

The hydrolysis process was linearly affected by temperature, as the temperature increases, the hydrolysis rates increase; thus, well-fits the Arrhenius equation, as

shown in Table 3. The energy of activation and pre-exponential coefficient (A) of codrug and IBP 2-hydroxyethyl ester hydrolysis at 0.05 M phosphate buffer pH 1.2, pH 6.8, and pH 7.4 were, respectively, determined from the slope and y-intercept of the Arrhenius plot. This enabled the extrapolation of the observed first-order rate constant and half-life of codrug and IBP 2-hydroxyethyl ester at room and human body temperature, 298 K and 310 K, respectively, presented in Table 4. The half-life of codrug and IBP 2-hydroxyethyl ester remarkably decreases at a particular pH from 298 to 310 K., which is favorable. For example, at pH 7.4, the half-life of codrug is 218 and 43 days at 298 and 310 K, respectively, whereas, for IBP 2-hydroxyethyl ester, it is 3 years and 251 days, respectively.

In vitro enzymatic hydrolysis in human plasma and rat liver homogenate

The enzymatic hydrolysis of codrug and the consecutively released IBP 2-hydroxyethyl ester in human plasma and rat liver homogenate follow the same trend of the chemical hydrolysis of codrug. In other words, codrug initially rapidly degrades to release nicotinic acid and IBP 2-hydroxyethyl ester and then eventually IBP. However, the enzymatic hydrolysis of codrug and IBP 2-hydroxyethyl ester is much faster in rat liver homogenate and human plasma than the chemical hydrolysis, with fastest degradation rates in rat liver homogenate. The hydrolysis of the codrug in human plasma and rat liver homogenate was conducted to confirm that the parent drugs (nicotinic acid, IBP 2-hydroxyethyl ester, and consecutively IBP) can be effectively released from the codrug. The rates of enzymatic hydrolysis exhibited pseudo-first-order kinetics. The results are shown in Table 5.

The hydrolysis of IBP 2-hydroxyethyl ester in human plasma apparently exhibits biphasic degradation (Figure 2). This has been attributed to the different rates of hydrolysis of the two ester enantiomers (*R*- and *S*-isomers) constituting the racemic IBP 2-hydroxyethyl ester[54]. Therefore, the more rapid part of hydrolysis conveys the hydrolysis of R-enantiomeric ester, whereas the slower part represents hydrolysis of the S-enantiomeric ester. It was previously reported that plasma-catalyzed hydrolysis of various esters of IBP is attributed to plasma butyrylcholinesterase, also called pseudocholinesterase. Moreover, the esterase-like activity of human serum albumin has been shown to be enantioselective, thus this will additionally contribute to the stereoselective plasma-catalyzed hydrolysis[54]. However, the biphasic degradation of IBP 2-hydroxyethyl ester in human plasma was not observed in rat liver homogenate due to its considerably more rapid degradation in rat liver homogenate.

It can be seen that enzymatic hydrolysis of codrug and released IBP 2-hydroxyethyl ester is faster than chemical hydrolysis. For instance, Figure 3 shows that after 60 min in human plasma, 76% of codrug hydrolyzed (0.063 mmol remained from initial 0.271 mmol) yielding nicotinic acid and increasing the IBP 2-hydroxyethyl ester and IBP amounts by 14 (from 0.007 to 0.107 mmol) and 8 (from 0.01 to 0.094 mmol) times, respectively. Furthermore,

Table 5. Observed pseudo-first-order rate constant and half-life for enzymatic hydrolysis of codrug hydrochloride salt and ibuprofen 2-hydroxyethyl ester in human plasma and rat liver homogenate.

80% Human plasma		k_{obs} (min^{-1}) ± S.D.*	$t_{0.5}$ (min)
Compounds	Codrug	0.0113 ± 0.001	59.74
	Ibuprofen 2-hydroxyethyl ester	0.0006 ± 0.0001	1155.00
20% Liver homogenate		k_{obs} (min^{-1}) ± S.D.*	$t_{0.5}$ (min)
Compounds	Codrug	0.1771 ± 0.004	3.92
	Ibuprofen 2-hydroxyethyl ester	0.0569 ± 0.007	12.18

*Standard deviation ($n = 3$).

Table 4. Activation energy, pre-exponential coefficient, half-lives at room temperature and body temperature of codrug hydrochlroide salt and ibuprofen 2-hydroxyethyl ester in different pHs.

Codrug						
pH	E_a[1] (kcal·mol^{-1})	A[1]	R^2	Temperature (K)	k_{obs} (h^{-1})	$t_{0.5}$ (days)
1.2	17.253	20.619	0.983	298	2.086×10^{-4}	138.45
				310	6.432×10^{-4}	44.89
6.8	20.961	26.394	0.993	298	1.295×10^{-4}	222.93
				310	5.088×10^{-4}	56.75
7.4	25.014	33.253	0.999	298	1.326×10^{-4}	217.77
				310	6.787×10^{-4}	42.55
Ibuprofen 2-hydroxyethyl ester						
pH	E_a[1](kcal·mol^{-1})	A[1]	R^2	Temperature (K)	k_{obs} (h^{-1})	$t_{0.5}$ (days)
1.2	15.118	17.189	0.965	298	2.475×10^{-4}	116.69
				310	6.639×10^{-4}	43.49
6.8	33.943	43.053	0.951	298	6.909×10^{-7}	41792.67
				310	6.334×10^{-6}	4558.74
7.4	22.724	27.765	0.999	298	2.609×10^{-5}	1106.56
				310	1.150×10^{-4}	251.06

[1]Ea: Activation energy, A: Pre-exponential coefficient.

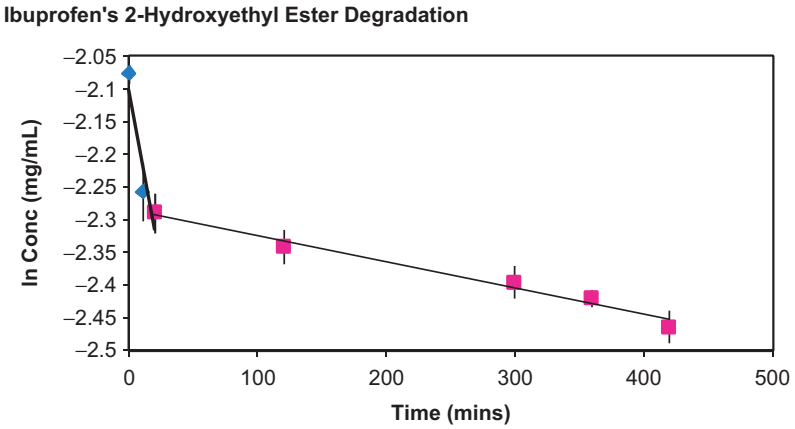


Figure 2. Enzymatic hydrolysis of ibuprofen 2-hydroxyethyl ester in human plasma.

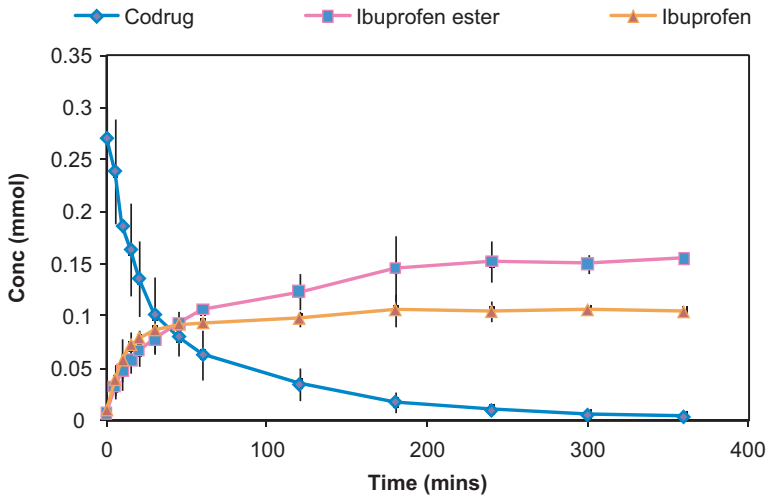


Figure 3. Explicit enzymatic hydrolysis of codrug hydrochloride salt and ibuprofen 2-hydroxyethyl ester and corresponding ibuprofen formation in human plasma.

it can be noticed that IBP 2-hydroxyethyl ester is more stable than the intact codrug.

Conclusions

The codrug of nicotinic acid and IBP was successfully synthesized and purified as the hydrochloride salt and its structure was confirmed by ^{1}H-NMR, ^{13}C-NMR, FT-IR, and Mass Spectroscopy elemental analysis. The codrug's structure was more labile at nicotinic acid linkage resulting in initial liberation of nicotinic acid followed by IBP 2-hydroxyethyl ester. The hydrolysis of the latter was found to be much slower. Hydrolysis in human plasma and rat liver homogenate showed that the codrug rapidly affords both nicotinic acid and IBP. Because the chemical hydrolysis was very slow compared to the enzymatic hydrolysis, it can be concluded that the codrug is very stable; thus, it can be formulated easily in different type of pharmaceutical formulations with reasonable and convenient shelf-lives. *In vivo* animal studies of the codrug and the physical mixture of parent drugs are being investigated to further support the achievement of nicotinic acid anti-dyslipidemic effects by its initial release and alleviation of its most troublesome side effect by the later release of IBP that will block prostaglandin synthesis that is responsible for flushing.

Declaration of interest

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