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Optimization of Naltrexone Diclofenac Codrugs for Sustained Drug Delivery Across Microneedle-Treated Skin

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Abstract

Purpose—The purpose of this work was to optimize the structure of codrugs for extended delivery across microneedle treated skin. Naltrexone, the model compound was linked with diclofenac, a nonspecific cyclooxygenase inhibitor to enhance the pore lifetime following microneedle treatment and develop a 7 day transdermal system for naltrexone.

Methods—Four different codrugs of naltrexone and diclofenac were compared in terms of stability and solubility. Transdermal flux, permeability and skin concentration of both parent drugs and codrugs were quantified to form a structure permeability relationship.

Results—The results indicated that all codrugs bioconverted in the skin. The degree of conversion was dependent on the structure, phenol linked codrugs were less stable compared to the secondary alcohol linked structures. The flux of naltrexone across microneedle treated skin and the skin concentration of diclofenac were higher for the phenol linked codrugs. The polyethylene glycol link enhanced solubility of the codrugs, which translated into flux enhancement.

Conclusion—The current studies indicated that formulation stability of codrugs and the flux of naltrexone can be enhanced *via* structure design optimization. The polyethylene glycol linked

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DISCLOSURES

Audra L. Stinchcomb is a significant shareholder in and Chief Scientific Officer of AllTranz Inc., a specialty pharmaceutical company involved in the development of transdermal formulations for MN delivery.

naltrexone diclofenac codrug is better suited for a 7 day drug delivery system both in terms of stability and drug delivery.

Keywords

codrugs; drug delivery; microneedle; stability; transdermal

INTRODUCTION

Codrugs or mutual prodrugs are two active pharmaceutical ingredients (API) linked *via* a covalent linkage to form a single chemical moiety (1). They are primarily synthesized to solve drug delivery issues like stability and solubility of the parent compounds or to enhance permeation of one or both active moieties across biological barriers by modification of the chemical structure (2). Codrugs and prodrugs are pharmacologically inactive in their modified form; however they are bioconverted back to parent drugs either by chemical or enzymatic hydrolysis in formulation, when crossing biological barriers, or in the plasma (1,2).

The overall goal of the current project was to optimize codrug structures in order to develop a sustained release transdermal drug delivery system for alcohol addiction. Naltrexone (NTX), the model compound (MW-341.4, log P-2.0) is a μ-opioid receptor antagonist approved for alcohol and opioid addiction treatment (3). 6β-Naltrexol (NTXol) (MW-343.4, log P-0.68), the active metabolite of NTX, has also been shown to have therapeutic efficacy for alcohol addiction (4,5). The current available dosage forms for NTX are oral and an extended release intramuscular injection. Both forms have their own drawbacks. The oral dosage form has compliance issues in an addict population due to daily dosing and gastrointestinal side effects (5,6). The extended release intramuscular dosage form has been associated with serious injection site reactions and difficulty with removal in case of emergency opiate requirements (7,8).

NTX can potentially benefit from an alternative delivery route like transdermal drug delivery. This noninvasive technique allows delivery of drugs directly into the systemic circulation and bypasses gastrointestinal side effects. The transdermal route is also known to be patient-friendly and comparatively more effective in an addict population with compliance issues (9). However, NTX doesn't cross the stratum corneum (SC) barrier at a therapeutically relevant rate by passive diffusion alone (10). The SC, the topmost layer of the skin is formed of dead keratinized epidermal cells and a lipid matrix, and forms the most important barrier to transport of xenobiotics across the skin (11).

Previous work, in both animal models and humans, has shown that by using small micron-scale needles or microneedles (MN), it is possible to deliver NTX at a rate that provides plasma concentrations in the lower end of the therapeutic range (10). MNs are a physical enhancement technique used to permeablize the skin by creating micropores. The effectiveness of this technique for enhancement of transdermal drug delivery has been established in the literature over the past decade and a half (12,13). Using the 'poke (press) and patch' approach for MN treatment drug can be delivered across treated skin for 48–72 h under occlusion. The drug delivery window can be further enhanced by local application of a non-steroidal anti-inflammatory drug, diclofenac sodium (DIC) (MW-296.1, log P-4.5) (3,14,15). Due to physicochemical incompatibility of the two molecules in formulation, daily or alternate day application of DIC was required to keep micropores open for a 7 day period (16,14). The incompatibility in formulation also leads to a significant decrease in flux of NTX across micropores in the presence of DIC, as compared to NTX alone, due to precipitation issues upon co-administration (16).

The codrug approach was thereafter used to combine NTX and DIC into a single chemical moiety with the goal of solving the formulation issue. The bioconversion of the properly designed new chemical entities in the skin or plasma should not be a problem since it has been previously shown that prodrugs/codrugs are bioconverted in the skin, both during passive delivery and MN enhanced delivery (17-20). The hypothesis was that following bioconversion in the skin, DIC would act locally and keep the pores open facilitating systemic delivery of NTX. A proof of concept study was conducted using a NTX-DIC 3-O-ester linked codrug to look at stability, solubility, transdermal flux and local skin concentration of the codrugs and the parent drugs (21). Although pharmacokinetic profiles were not obtained from the study, pore visualization techniques were used to verify the presence of micropores in the skin at the end of 7 days, using the codrug formulation compared to absence of micropores for the NTX control. These studies confirmed that the codrug approach was useful for development of a 7 day system, however further optimization was required. The codrug also had a formulation half-life of around 4–5 days, and the solubility and transdermal flux were an order of magnitude lower compared to the most optimized formulation for NTX delivery across MN treated skin (22,23).

In the current study, four codrugs of NTX/NTXol and DIC have been compared to look at structure *vs*. stability, solubility, transdermal flux and bioconversion in the skin. The codrugs were synthesized either using the 3-OH (phenol position) on NTX or the 6-OH (secondary alcohol) position on NTXol, and the −COOH group on diclofenac. Ester, carbamate or amide linkers were used to join the two molecules either directly, or with the help of a polyethylene glycol (PEG) linker. Furthermore, hydrochloride salts of promising codrugs were synthesized and tested to develop a formulation for *in vivo* pharmacokinetic studies.

MATERIALS AND METHODS

Chemicals

NTX HCl and NTX base were purchased from Mallinckrodt (St. Louis, MO), DIC acid from AK Scientific, Inc. (Mountain view, CA) and DIC sodium salt from TCI America (Portland, OR). Water was purified using a NANOpure Diamond™, Barnstead water filtration system. Propylene glycol was purchased from Sigma (St. Louis, MO). Sodium acetate and acetic acid were obtained from Fisher Scientific (Fairlawn, NJ). 1-Octanesulfonate, sodium salt was obtained from Regis Technologies, Inc (Morton Grove, IL). Trifluroacetic acid (TFA), triethylamine (TEA) and acetonitrile (ACN) were obtained from EMD chemicals (Gibbstown, NJ). Unless otherwise noted, all other reagents for codrug synthesis were obtained from Sigma Aldrich (St. Louis, MO).

Synthesis of the Codrugs

Codrug I and hydrochloride salt of codrug I were synthesized following previously reported procedures (21).

Codrug II—Benzyl bromide (0.522 mL, 4.375 mmol) was added to a suspension of 6β-NTXol (500 mg, 1.458 mmol) and potassium carbonate (1 g, 7.3 mmol) in acetone (10 mL). The reaction mixture was then refluxed for 2 h before it was concentrated under reduced pressure. The resulting crude product was further purified by column chromatography on silica gel using dichloromethane-methanol mixture to obtain compound **1** as a white solid (500 mg, 79%). A mixture of compound **1** (500 mg, 1.153 mmol), DIC acid (685 mg, 2.306 mmol), and DMAP (7 mg, 0.06 mmol) were dissolved in dichloromethane (50 mL) and cooled to −78°C in an acetone/dry ice bath under N_2. To the reaction mixture, *N*,*N*'-dicyclohexylcarbodiimide (685 mg, 2.306 mmol) in dichloromethane (20 mL) was then added with vigorous stirring. After 1 h, the reaction mixture was allowed to warm up to

room temperature and concentrated under reduced pressure. The resulting crude product was purified by column chromatography on silica gel using hexane-ethyl acetate mixture to yield compound 2 as a white solid (260 mg, 32%). Catalytic hydrogenation of compound **2** (100 mg, 0.140 mmol) over a Pd/C catalyst provided Codrug II as a solid (80 mg, 92%).

Codrug III—Triethylamine (0.123 mL, 0.879 mmol) was added to a suspension of NTX·HCl (200 mg, 0.586 mmol) and 4-Nitrophenyl chloroformate (130 mg, 0.644 mmol) in dichloromethane (5 mL). The resulting solution was stirred for 4 h at room temperature, and then concentrated under reduced pressure to yield an oilic crude product. The crude product was purified by column chromatography on silica gel using dichloromethane-methanol mixture to provide compound **3** as a light yellow solid (292 mg, 66%). To a dichloromethane solution containing compound **3** (61 mg, 0.143 mmol) and DIC-EBE (73 mg, 0.143 mmol) was added DMAP (35 mg, 0.286 mmol). The reaction mixture was then stirred for 1 h at room temperature and then concentrated under reduced pressure. The resulting crude product was purified by column chromatography on silica gel using dichloromethane/methanol solvent systems to obtain codrug III as a light yellow solid (78 mg, 69%).

Codrug III hydrochloride salt—To a dichloromethane solution of codrug III (100 mg, 0.126 mmol), 1 M HCl was slowly added and continuously stirred for 10 min. The resulting codrug III salt was filtered and dried to yield codrug III·HCl as a white solid (95 mg, 91%).

Codrug IV—To a suspension of 6β-NTXol (300 mg, 0.875-mmol) and di-tert-butyl dicarbonate (210 g, 0.962 mmol) in acetone (20 mL) was added potassium carbonate (604 mg, 4.373 mmol) with vigorous stirring. The reaction mixture was then refluxed for 1 h and concentrated under reduced pressure. The resulting crude product was further purified by column chromatography on silica gel using dichloromethane-methanol solvent systems to provide compound **4** as a white solid (340 mg, 88%). To a suspension of compound **4** (340 mg, 0.766 mmol) and 4-Nitrophenyl chloroformate (232 mg, 1.149-mmol) in dichloromethane (10 mL) was added DMAP (280 mg, 2.298 mmol). The resulting mixture was stirred for 1 h at room temperature and subsequently concentrated under reduced pressure to yield the crude product, which was further purified by column chromatography on silica gel using dichloromethane/methanol solvent systems to obtain compound **5** as a light yellow solid (445 mg, 95%). Next, *N*, *N*-Diisopropylethylamine (0.623 mL, 3.58 mmol) was added to a suspension of compound **5** (436 mg, 0.716 mmol) and DIC-EBE (458 mg, 1.074 mmol) in dichloromethane (10 mL) and stirred for 1 h at room temperature. After the reaction mixture was concentrated under reduced pressure, the resulting crude product was further purified by column chromatography on silica gel using dichloromethane/ methanol solvent systems to obtain compound **6** as a white solid (455 mg, 71%). Trifluoroacetic acid (1 mL) was added to a solution of compound **6** (300 mg, 0.334 mmol) in dichloromethane (10 mL). The resulting solution was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane-methanol mixture to afford Codrug IV as a semi solid (120 mg, 45%).

HPLC Assay

All codrugs and parent drugs were quantified using HPLC. The HPLC instrument used consisted of a Waters™ e2695 separation module, a 2489 UV/Visible detector and Empower™3 software. A Perkin Elmer Spheri5 VL C18 column (5 μ, 220×4.6 mm) and a C18 guard column (15×3.2 mm) were used with the UV detector set at a wavelength of 280 nm/215 nm. The mobile phase consisted of 70:30(v/v) ACN: (0.1% TFA with 0.065% 1-octane sulfonic acid sodium salt, adjusted to pH 3.0 with TEA aqueous phase). Samples

were run at a flow rate of 1.5 ml/min. All codrugs and parent drugs were quantified using standard curves in the range of 100–10,000 ng/ml.

Stability Studies

The stabilities of all codrugs were determined in 0.3 M acetate buffer pH 5.0 at 32°C. For stability studies drug was presolubilized in ACN. The solution was then used to spike prewarmed buffer at 32°C (100 μl in 10 ml of buffer, <1% total ACN concentration). One hundred μl samples were obtained at regular time intervals and the reaction was terminated using 900 μl of ACN. All samples were stored at −80°C until analysis using HPLC. Samples were stable in −80°C verified using quality control standards (data not shown). Data for all stability studies were analyzed using pseudo first-order kinetics. All studies were run in triplicate.

Donor Solution Preparation and Solubility Determination

Donor solution was prepared for all codrugs in 0.3 M acetate buffer pH 5.0 containing 10% propylene glycol (PG). Excess drug was added to the above mixture, vortexed, sonicated for 10 min and left overnight at 32°C (The only exception, codrug I was incubated in donor solution for an hour before dosing due to stability issues). The choice of the donor was based on previous studies using codrugs and flux of model compounds across MN treated skin (20,21). For solubility determination, codrug solution was removed from the donor, filtered using a 0.2 μm, 500 μl centrifugal filter and injected onto the HPLC after suitable dilution with ACN. Saturation solubility was determined for all codrugs except hydrochloride salt of codrug III due to limited availability of codrug. All solubility determinations were carried out in triplicate.

Melting Point Determination of Codrugs

Differential scanning calorimetry (DSC) was used to estimate melting point of parent drugs and codrugs in the range of 50–300°C. The apparatus used was a DSC 2920 with a DSC refrigerated cooling system from Texas Instruments (TA Instruments, New Castle, DE). Two to five mg of drug was weighed into hermetic aluminum pans and sealed with a lid. Drug was heated @10°C/min from 50 to 300°C. Data was collected using Thermal Advantage and analyzed using Universal Analysis 2000 softwares from TA Instruments. Melting point was determined from the inflection point of the melting curve.

Diffusion Studies

All diffusion studies were carried out using full thickness Yucatan miniature pig skin. Studies were approved by the IACUC protocols at University of Kentucky and University of Maryland, Baltimore. The skin was obtained from euthanized animals, dermatomed to a thickness of 1.4–1.8 mm and stored at −20°C until the day of the experiment. MN's for this study were obtained from Dr. Mark Prausnitz's laboratory at the Georgia Institute of Technology. A 5 MN in plane array was used for *in vitro* studies (Fig. 1). Each MN was 750 μm long, 200 μm wide and 75 μm thick and the interneedle spacing was 1.35 μm. For all experiments skin was cut into small rectangular pieces. They were then placed on a wafer of Sylguard® 184 silicone elastomer to mimic the underlying tissue and treated with a 5 MN array, 20 times to generate a total of 100 MN pores per diffusion cell (within the 0.95 cm^2 active area). The permeation experiments were carried out using a PermeGear® flow through system (In-line, Riegelsville, PA). The receiver solution used was water alkalified to pH 7.4 containing 20% ethanol at 37°C. The flow rate was set at 1.5 ml/h and the temperature of the diffusion cells was maintained at 32°C to mimic physiological skin surface conditions. Each cell was charged with 250 μl of the relevant codrug or parent drug for control cells and receiver solution was collected in 6 h increments for a total of 48 h. At

the end of the study the cells were removed and drug was quantified both in the receiver solution and skin samples using HPLC. For the receiver solution, steady state flux values were determined for NTX/NTXol from the codrugs using Fick's first law of diffusion. The cumulative amount permeated was plotted as a function of time and the slope of the steady state portion of the curve was used to determine flux. Permeation profiles did not reach steady state in the 48 h timeframe for DIC and codrug, so cumulative amount of drug permeated was quantified, where applicable. Receiver samples were concentrated, if required by evaporating 1 ml of the receiver solution under nitrogen at 37°C followed by reconstitution in 100 μl of ACN. For quantification of the drug concentration in the skin samples, the active permeation area was dissected out and cut into small pieces. The concentration of parent drugs and codrug in the skin was determined by incubating the skin in 10 ml of ACN overnight at 32°C and analyzing the solution by HPLC after appropriate dilutions. All permeation studies were carried out in triplicate, at a minimum.

Statistical Analysis

Data for all experiments were reported as mean ± standard deviation. Statistical analysis of data was carried out with Students' *t*-test and one way ANOVA with post hoc Tukey's pairwise tests, if required, using GraphPad Prism® software, version 5.04 software. $P<0.05$ was considered to be statistically significant.

RESULTS

Synthesis of Codrugs

Physical properties of parent drugs and codrugs are reported in Table I. Structures of all codrugs and intermediates are provided in Figs. 2 and 3. The synthesis of codrug I, base and salt form has been previously reported (21). Codrugs II, III and IV and hydrochloride salt form of codrug III were synthesized successfully. The structures were verified using ^{1}HNMR and HRMS.

Codrug II—^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41–7.24 (m, 10 H),7.09 (m, 1H), 6.96–6.92 (m, 3H), 6.72 (m, 1H), 6.57–6.54 (m, 2H), 5.31 (2H, s), 5.12–5.07 (m, 3H), 4.79–4.71 (m, 2H), 4.04 (m, 2H), 3.86 (s, 2H), 3.49 (m, 2H), 3.08–2.99 (m, 2H), 2.64–2.56 (m, 2H), 2.39–2.18 (m, 4H), 2.09–1.95 (m, 7H), 1.81 (m, 1H), 1.71–1.60 (m, 12H), 1.49–1.43 (m, 2H), 1.37–1.27 (m, 6H), 1.17–1.11 (m, 7H), 0.84 (m, 1H), 0.52 (m, 2H), 0.12 (m, 2H)

Codrug III—^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.78 (s, 1H), 7.32 (m, 2H), 7.12–7.07 (m, 2H), 6.97–6.93 (m, 2H), 6.86 (t, 1H, *J*=8.5 Hz), 6.71–6.66 (m, 3H), 6.60 (m, 1H), 6.49 (d, 1H, *J*=8.5 Hz), 5.98 (m, 1H), 4.69 (m, 2H), 3.64–3.44 (m, 13H), 3.19 (s, 2H), 3.10–3.00 (m, 4H), 2.72–2.56 (m, 4H), 2.44–2.31 (m, 8H), 2.17–2.12 (m, 3H), 1.90–1.26 (m, 8H), 0.86 (m, 2H), 0.56 (m, 4H), 0.15 (m, 4H)

Codrug III HCl Salt—^{1}H NMR (DMSO–d_6, 500 MHz) δ 8.66–8.58 (m, 2H), 8.08 (s, 1H), 7.96 (s, 1H), 7.47 (s, 1H), 7.08 (m, 2H), 6.77–6.74 (m, 2H), 6.61 (m, 1H), 6.63 (d, 1H, *J*=7.0 Hz), 6.41–6.36 (m, 2H), 6.28–6.22 (m, 2H), 5.85 (m, 1H), 4.70 (s, 1H), 4.58 (s, 1H), 3.61–3.56 (m, 2H), 2.82–2.79 (m, 6H), 2.71–2.50 (m, 11H), 2.30–2.23 (m, 3H), 2.09 (m, 12H), 1.74–1.59 (m, 4H), 1.09–1.07 (m, 4H), 0.67–0.64 (m, 6H), 0.27 (m, 2H), 0.20 (m, 2H), 0.10 (m, 2H), 0.00 (m, 2H)

Codrug IV—^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.62 (s, 1H), 7.33–7.31 (m, 2H), 7.17–7.15 (m, 1H), 7.09–7.08 (m, 1H), 6.96 (t, 1H, *J*=8.0 Hz), 6.88–6.86 (m, 1H), 6.76–6.69 (m, 2H), 6.65 (d, 1H, *J*=8.0 Hz), 6.49 (d, 1H, *J*=8.0 Hz), 5.28 (m, 1H), 4.51 (m, 2H), 3.76–3.47 (m, 18H),

3.08–2.97 (m, 3H), 2.59–2.57 (m, 2H), 2.35 (m, 2H), 2.18–1.88 (m, 8H), 1.60–1.58 (m, 1H), 1.46–1.25 (m, 9H), 0.82 (m, 1H), 0.51 (m, 2H), 0.11 (m, 2H)

HPLC Assay Development

The first step towards analysis of codrugs and parent drugs was development of a HPLC method. The same column, mobile phase composition and wavelength (280 nm) were used for all compounds. All codrugs and parent drugs had baseline resolution on the assay. The retention times (±0.1 min) for all compounds from the HPLC assay are reported in Table II. Standard curves for both codrugs and parent drugs were prepared in the range of 100–10,000 ng/ml for ACN samples and 100–5,000 ng/ml for extracted receiver samples. All standard curves were linear (r^2>0.999).

Stability and Solubility Studies

Formulation stability was carried out for all codrugs to determine the relative rate of conversion due to chemical hydrolysis. The results indicated that stability was significantly enhanced by using the 6-O-secondary alcohol linkage over the 3-O-phenol linkage for both codrugs I and II, and codrugs III and IV. Stability was also significantly enhanced by using the PEG covalent link and carbamate/amide linkage compared to the ester linkage, both for codrug I and III, and codrug II and IV (Fig. 4).

The solubility studies were carried out to quantify saturation solubility of codrugs in donor formulation and evaluate its effect on flux. The studies indicated that solubility of the NTX-DIC codrugs were significantly higher compared to NTXol-DIC codrugs with similar linkage. The PEG linkage enhanced solubility for codrug III and IV, as compared to codrug I and II. Solubilities of codrugs were also enhanced by using a hydrochloride salt form of the drug compared to a free base form.

Diffusion Studies

The diffusion studies were carried out to quantify the permeation of NTX/NTXol across the skin and concentration of codrugs and parent drugs in the skin. The diffusion studies indicated that flux of NTX from codrugs I salt and III were significantly higher compared to flux of NTXol from codrugs II and IV (p<0.05). The flux of NTX from codrug I salt and codrug III, and NTXol from codrug II and codrug IV were not significantly different from each other (p>0.05) (Fig. 5). Permeation of DIC and intact codrug was also observed for codrug III from the 10% PG containing formulation. Cumulative amount of DIC and codrug present in the receiver solution were 1.09±0.36 nmol and 2.89±1.0-nmol, respectively, at the end of the 48 h experiment. NTX flux was further enhanced by 2.5 fold on using the salt form of codrug III, over free base form (Fig. 6). Significantly higher permeation of DIC and intact codrug were also observed using the salt form of codrug III, they were 3.1± 0.86 nmol and 24.3±8.8 nmol, respectively.

Skin concentration of codrugs and parent drugs were quantified at the end of the experiment to evaluate the effect of codrug structure on bioconversion in the skin. The skin concentration data indicated that bioconversion was more complete for NTX-DIC codrugs compared to the NTXol-DIC codrugs. Intact codrug was present in the skin for all codrugs, whereas NTX and DIC were only quantifiable for codrugs I and III. Skin concentrations of codrugs were not significantly different among the 4 codrugs. Skin concentration of NTX was not significantly different between codrugs I and III (p>0.05). Skin concentration of DIC was significantly different between codrugs I and III (p<0.05). Skin concentration was also determined for codrug III salt to evaluate the effect of salt form on skin concentration. No significant differences were observed for NTX or DIC concentration using codrug III base or salt form (p>0.05).

Flux data from codrug I has been previously reported (21). The salt form of codrug I has been used for all comparisons for diffusion experiments in this study since the limited solubility of the free base form in 10% PG containing formulation prevented direct comparison of results.

DISCUSSION

Prodrugs and codrugs have been explored for drug delivery *via* different routes over the last few decades. The approach is mostly used to solve a drug delivery issue and the major advantage lies in the fact that chemical modification renders the new chemical entity pharmacologically inactive thus decreasing potential side effects and the need for additional pharmacological evaluation. Compared to all other routes of drug delivery, the topical/transdermal field has seen slower progress in terms of the number of new API's approved and on the market over the years (24). The most important reason being the extreme barrier property of the skin/SC. Chemical modification of the API itself to form codrugs/prodrugs provides a new approach for flux enhancement, decreasing some of the need for excess permeation enhancer supplementation. Additionally, two API can be delivered at the same time in a codrug to have a synergistic effect. A few examples of topical/transdermal codrugs that have been evaluated include NTX and bupropion for smoking cessation and alcohol addiction, retinyl ascorbate and ascorbic acid for oxidative stress, and 5 fluorouracil with triamcinolone acetonide for actinic keratosis (25,26).

The current study evaluated codrugs for pore lifetime enhancement following MN treatment. Unlike previous codrug examples in the literature where both drugs were targeted either for systemic delivery or for topical application, the NTX/NTXol codrugs are designed for systemic delivery of NTX/NTXol while DIC acts locally in the SC/epidermal layer of the skin to enhance micropore lifetime. The physicochemical properties of NTX allow systemic permeation, and a higher log P and negative charge facilitates retention of DIC in the skin (21).

Purity and structure of all codrugs were confirmed using NMR and HRMS. Quantitative analysis is an important step in the development of any drug delivery system. HPLC was used for quantification of codrug and parent drugs in stability, solubility and permeation studies. Since all compounds were evaluated using reverse phase chromatography and the same assay parameters, retention time on the HPLC can be used as a comparative estimate of hydrophobicity or log P of the molecules (27). NTX and NTXol were comparable in hydrophobicity with NTXol being slightly more hydrophilic due to the presence of an additional hydroxyl group at the 6 position compared to the ketone of NTX. DIC was the most hydrophobic of the parent molecules, which is expected from the reported log P values from Scifinder®. Comparing codrugs I and II, codrug I with a retention time of 4.9 min was more hydrophobic compared to codrug II at 4.4 min. The difference in hydrophobicity can be attributed to more significant hydrophilic interactions of a phenolic hydroxyl as compared to a ketone functional group substitution. Comparison of codrug III and IV followed the same pattern, where the retention time shifted from 3.8 min for codrug III to 3.7 min for codrug IV. Although in both cases the codrugs with the free phenolic hydroxyl had less retention on the column, the shift was much less significant for the PEG linked codrugs. This can be attributed to significant hydrophilic interactions of the PEG chain itself. Effect of PEG chain on enhancement of hydrophillicity is also confirmed by the fact that both codrug III and IV are eluted significantly earlier than codrug I and II. Since hydrophobicity/hydrophilicity play an important role in permeation of molecules across intact and MN treated skin, the comparative analysis provides an estimate of solubility and transport. For MN treated skin, it has been shown that lower viscosity aqueous formulations lead to higher flux. Higher hydrophillicity would translate into enhanced solubility in an

aqueous medium; a molecule with lower log P will be beneficial for the current delivery system in terms of higher solubility.

Codrugs/prodrugs are primarily designed to solve a drug delivery problem, but since these molecules are pharmacologically inactive chemical/enzymatic hydrolysis is imperative for bioconversion and function of these molecules. In the current project, codrugs were designed to formulate NTX and DIC in a single formulation so that precipitation of one drug on application of the other didn't lead to reduced NTX flux across the micropores. Thus, stability in formulation is a very important criterion for these codrugs. The results from the stability studies indicated that codrugs I and III (phenol linked codrugs) were less stable in formulation compared to codrugs II and IV (secondary alcohol linked codrugs). This was expected since the resonance stabilization of a phenol makes it a better leaving group compared to a secondary alcohol. Codrug III was more stable compared to codrug I, and codrug IV was more stable compared to codrug II. This can be attributed to the carbamate/amide linkage in place of the ester linkage. It has been shown in the literature that ester linked prodrugs are more susceptible to chemical hydrolysis as compared to carbamate prodrugs at a formulation pH of 5.0 (20). On the other hand, carbamate linked prodrugs are less stable at physiological pH 7.4-at similar buffer concentrations (20). The change in the rate of chemical degradation within the pH range indicates that the primary mechanism for hydrolysis of a monosubstituted carbamate in an aqueous formulation is probably *via* proton elimination and not nucleophile attack of hydroxyl groups (28). Thus employing carbamate codrugs enhances formulation stability and could be beneficial because they are known to degrade faster under physiological conditions. Since NTX/NTXol and DIC codrugs could not be linked directly with a carbamate linkage, a PEG link was used to serve the dual role of linking the molecules and enhancing solubility. PEG is one of the most commonly used topical/transdermal excipients, thus irritation, toxicity and side effects related to incorporation of PEG in the formulation should be limited or non-existent. Once inside the body, PEG is metabolized into a series of phase I and II metabolites, with no toxicological concern. Molecular weight of the PEG subunit does play a role in its metabolism, with PEG's >5000 showing little or no metabolism (29). Thus from the stability studies it can be concluded that codrugs II, III and IV with more than 85% intact codrug in formulation at 7 days could be used for the development of a drug delivery system. Since the stability studies were conducted in aqueous buffers, the codrugs will be expected to be more stable in a 10% PG containing formulation since hydrolysis is the primary mechanism for degradation. Once inside the body, the codrugs will be degraded by enzymatic hydrolysis in the skin, in addition to chemical hydrolysis for much faster regeneration of parent drugs. Presence of esterase, amidase, and protease, in addition to the normal phase I and II enzymes are well documented in the skin (30,31).

Solubility of drug in the donor is important for transdermal drug delivery. The drug concentration in formulation is the main driving force for transport across the skin. As stated before, MN-enhanced drug delivery with lower viscosity aqueous formulations has demonstrated significantly higher flux compared to viscous PG-rich formulations. Ten percent PG containing 0.3 M acetate buffer at pH 5.0 was chosen as the formulation based on previous studies (21,24). The solubility data indicated that the PEG link significantly enhanced solubility for both codrug III and IV, compared to codrug I and II. Solubility of codrug I and III was higher compared to II and IV, indicating that phenol linked codrugs are more soluble compared to secondary alcohol linked codrugs. This can be further explained in terms of the melting point (MP) estimates where codrug I has a lower melting point compared to codrug II. Lower MP translates into higher aqueous solubility for the unionized form of the drug (32).

Stability and solubility studies provide important information about how well drug transport might proceed, however skin permeation studies are required for estimation of flux and bioconversion in the skin. Diffusion studies were carried out using dermatomed Yucatan miniature pig skin. Yucatan miniature pig was chosen as the appropriate model based on previously conducted *in vitro in vivo* correlation (IVIVC) studies for MN enhanced transdermal delivery. The studies compared *in vitro* diffusion data to *in vivo* pharmacokinetic studies in order to evaluate the importance of Yucatan pig skin for MN studies. Modeling of the transdermal pharmacokinetic data using *in vitro* flux as an input parameter and IV bolus data as the elimination parameter helped in estimation of the optimum skin thickness required for IVIVC for the MN geometry used in this study. For passive transdermal studies, skin thickness of 200–300 μm is considered optimum, however the MN are 750 μm long, very thin skin leads to a release rate study across the skin as opposed to permeation across a dermal barrier. A skin thickness of 1.4–1.8 mm was found to be optimum for MN enhanced delivery and used for the current study (33,34). The diffusion studies indicated that the flux of NTX from codrug I and III was significantly higher compared to NTXol flux from codrug II and IV. This can be attributed to the higher formulation stability of the NTXol codrugs; significant amounts of intact codrug were found in the skin, and no parent drugs were quantified at the end of the study suggesting that NTXol was not released efficiently from the codrug to generate transdermal flux. The difference in physicochemical properties of NTX and NTXol could also be responsible for reduced flux of NTXol. Since NTXol has an additional hydroxyl group following conversion, H-bonding potential of NTXol is higher. It has been shown in the literature that the higher the number of hydrogen bonds, the lower the transdermal flux across intact skin (35). The skin concentration study also showed that DIC was only present in the skin for the NTX codrugs. Since the presence of DIC in the skin is imperative for its local effect on pore lifetime enhancement, the NTXol codrugs would not be able to keep micropores open beyond 48–72 h in the absence of DIC. Also, significantly lower flux from these codrugs as compared to the NTX codrugs is a disadvantage. The skin concentration of DIC is significantly different between codrug I and III. This can be explained with the help of mass spectrometry data (not shown) which indicates that DIC is not completely bioconverted in the skin from codrug III. A mixture of DIC (MW: 296) and DIC + PEG linker conjugate (MW: 452) was found in the receiver and skin samples by mass scan and ESI-/ESI + ionization. Since an accurate estimate of local skin concentration of DIC required for pore lifetime enhancement is not available, it would be beneficial to test codrug III *in vivo* to see if it is efficacious. If not, the linker at the DIC end of the molecule will have to be optimized further for faster release of DIC.

The hydrochloride salt form of codrug I led to a 13-fold enhancement in solubility and transdermal flux (21), but direct comparison of the effect of the salt formulation could not be evaluated due to changes in donor composition. A hydrochloride salt form of codrug III was synthesized and evaluated for further solubility and flux enhancement *in vitro*. Codrug III was chosen because formulation stability and solubility was higher as compared to codrug I. Codrugs II and IV were comparatively more stable but bioconversion, transdermal flux of NTXol, and skin concentrations of DIC were much lower. The permeation studies indicated that flux of NTX can be further enhanced using a salt form of the codrug over the free base form. A 2.5 fold enhancement in NTX flux was obtained using a sub-saturated codrug III salt as donor compared to free base. (Saturated donor could not be used due to limited availability of codrug III salt.) The permeabilities of NTX from codrug and codrug salt were not significantly different from each other, indicating that the flux enhancement was due to enhanced solubility. Skin concentrations of codrug or parent drugs were not significantly different between salt and free base. Thus salt form has no significant effect on skin concentration of the parent drugs or the codrug, when comparing data from the same donor condition, irrespective of the physicochemical properties of the molecule. This will be useful

if alternate salt forms are used to further enhance solubility (23), and additional studies for determination of local skin concentration of DIC will not be necessary. The salt form also led to higher cumulative amounts of DIC and codrug permeation compared to the base. Intact codrug diffusing through the skin will convert under physiological conditions to regenerate parent drugs, thus further contributing to plasma concentrations of NTX. The amount of DIC permeated through the skin is well below therapeutic systemic concentrations and thus should not be of any concern.

Therefore, from the structure permeability evaluation it can be concluded that NTX-DIC PEG linked codrug (codrug III) is the best choice for the current project in terms of stability, solubility, bioconversion and NTX flux. NTX requires a flux of 12–90 nmol/cm^2/h from a 25 cm^2 patch for therapeutic efficacy, based on current oral dosing and clearance. Codrug III hydrochloride salt has a flux of 10.8±2.1 nmol/cm^2/h from the currently tested sub-saturated solution, therefore further flux enhancement is expected using a saturated solution. From stability data, 85% of codrug III will be intact in formulation over a 7 day patch application period, rendering it to be a suitable molecule for *in vivo* evaluation of the codrug concept *via* pharmacokinetic studies.

The goal of the current study was to optimize the codrug structures based on flux of the parent molecules, bioconversion in the skin and determination of steady state skin concentration for codrug and parent molecules. Bioconversion of both codrugs and prodrugs in Yucatan miniature pig skin have been previously documented (20,21). However, one limitation of the study is pore closure or skin resealing, which cannot be evaluated in harvested skin. Pore closure kinetics have been evaluated *in vivo* in rats, hairless guinea pigs, Yucatan miniature pigs and humans (14-16,33,36). Rats and guinea pigs are small animal models that make pharmacokinetic evaluation and data quantification easier. Yucatan miniature pigs are more comparable to humans in terms of skin physiology, especially in dermal thickness, critical to these MN studies. Following optimization *in vitro*, the codrugs from this study will be evaluated *in vivo* either in hairless guinea pig or Yucatan miniature pig based on avaibility of codrugs and assay sensitivity for detection of permeated molecules in the plasma.

CONCLUSION

NTX/NTXol and DIC codrugs were designed and evaluated for development of a 7 day drug delivery system for alcohol addiction treatment. Stability, solubility, transdermal flux of NTX and local skin concentration of DIC were determined to develop a structure permeability relationship for the codrugs. A secondary alcohol linker enhanced stability of codrug molecules, however solubility, bioconversion, flux of NTX and local skin concentration of DIC were higher using phenol linked codrugs. Transport was further enhanced by utilizing a hydrochloride salt form of the PEG linked NTX-DIC codrug making it the most suitable candidate for further *in vivo* pharmacokinetic studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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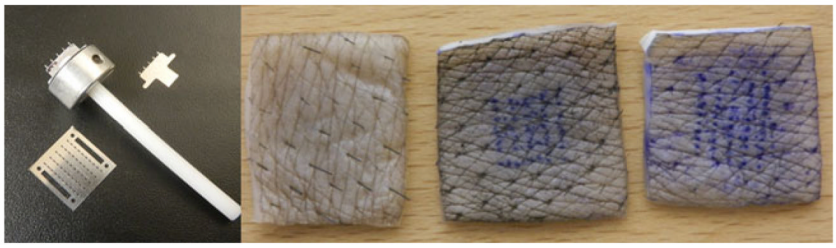

Fig. 1.

Microneedle array with 5 MN array for *in vitro* studies and 50 MN array for *in vivo* studies and applicator for 5 MN array(left panel), Yucatan miniature pig skin before treatment and following treatment with gentian violet for pore visualization (right panel).

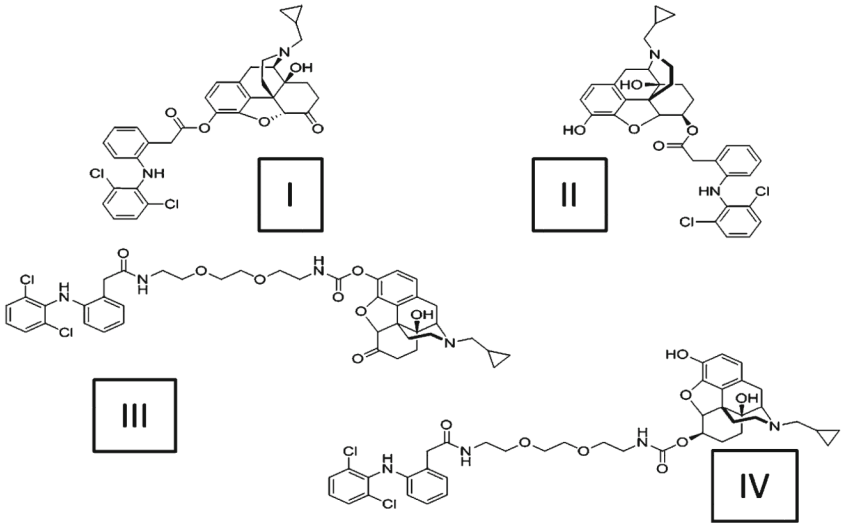

Fig. 2.
Structures of NTX/NTXol and DIC codrugs.

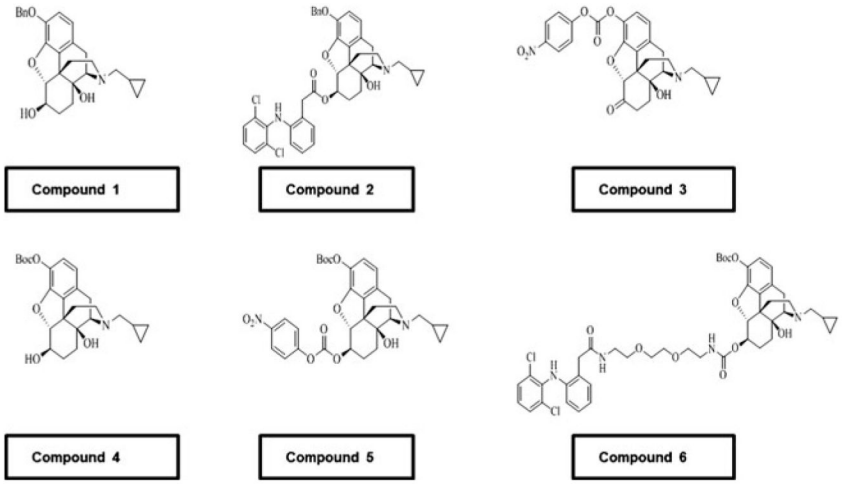

Fig. 3.
Structures of intermediates for codrug synthesis. NMR data for intermediates are provided in Supplementary Material.

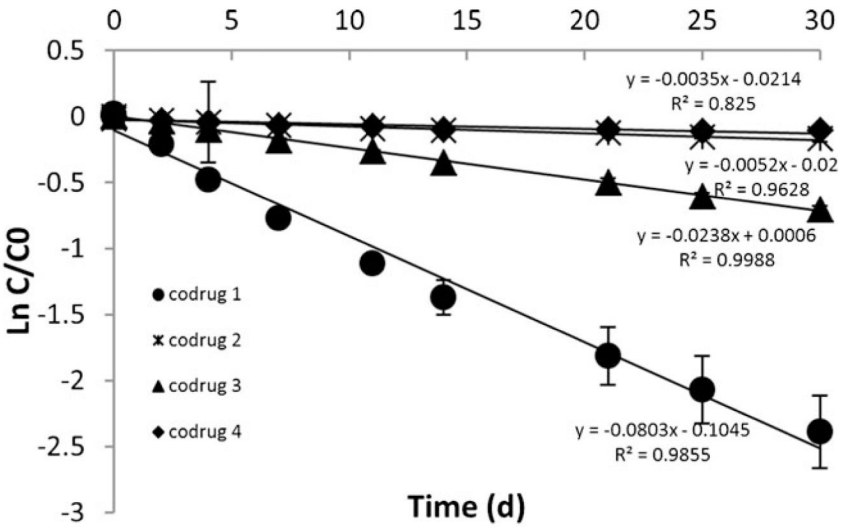

Fig. 4.

Stability of NTX/DIC codrugs in 0.3 M acetate buffer pH 5.0. Data analyzed using pseudo first-order kinetics. *n*=3 for all codrugs.

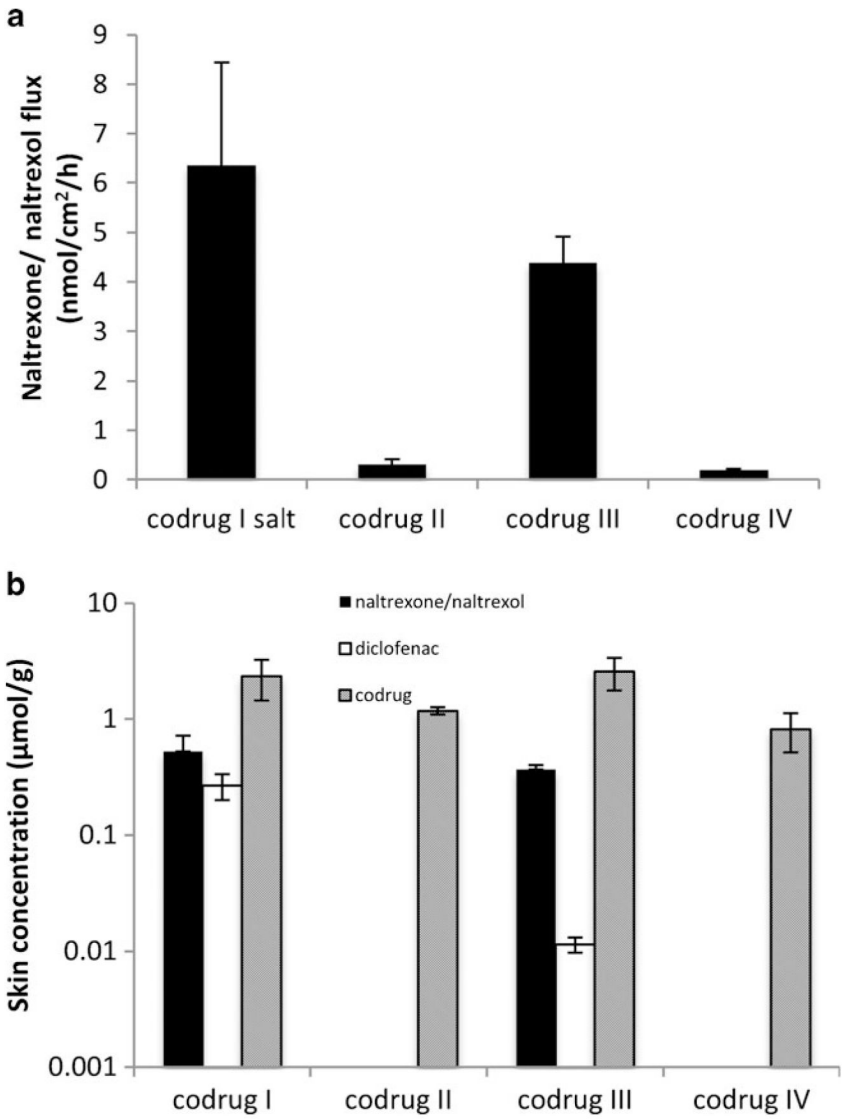

Fig. 5.

Flux of NTX/NTXol and skin concentration of parent drugs and codrugs. $n \geq 3$ for all studies. Flux of NTX from codrug I and codrug III are not significantly different ($p > 0.05$) and they are higher than NTXol flux from codrug II and codrug IV ($p < 0.05$). Skin concentration of NTX and codrug are not significantly different for I and III ($p > 0.05$). Skin concentration of DIC is significantly different ($p < 0.05$).

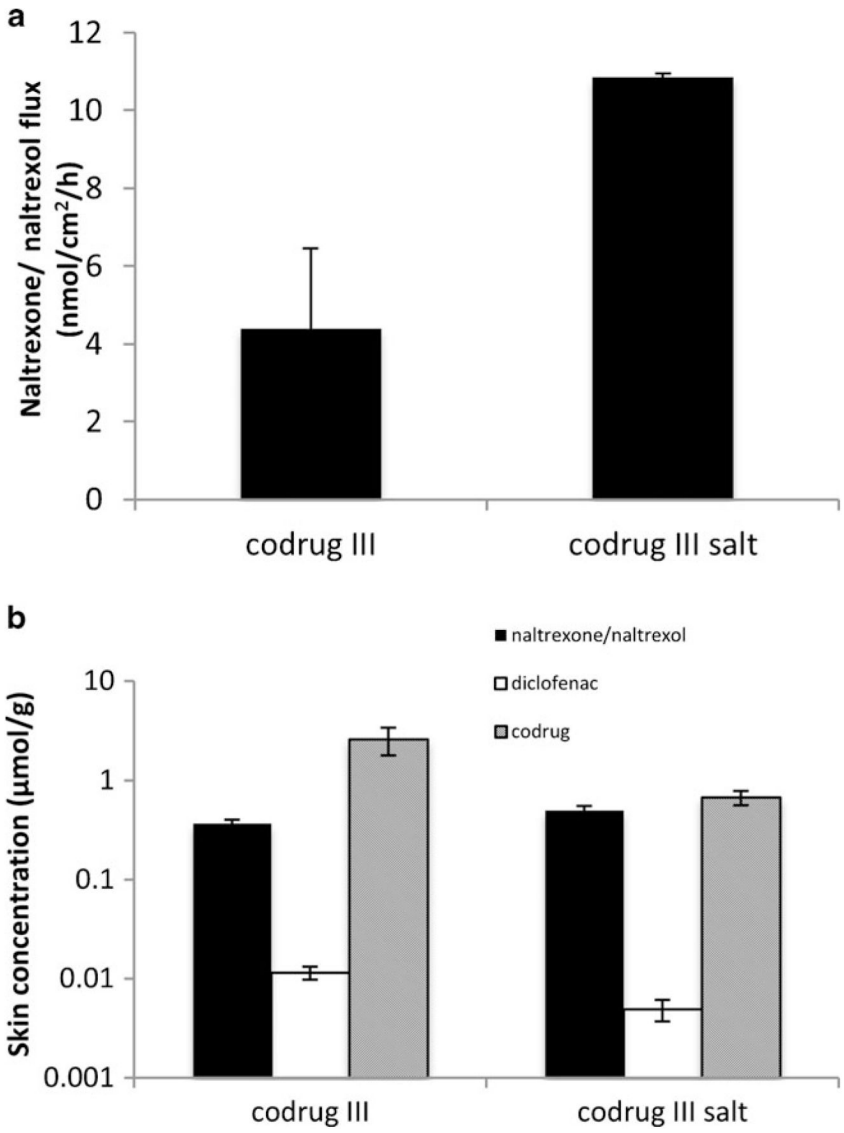

Fig. 6.

Flux and skin concentration from codrug III and codrug III salt. $n \geq 3$ for all studies. Flux of NTX was significantly higher ($p<0.05$). No significant difference in skin concentration except for codrug ($p>0.05$).

Table I

Physical Properties of Parent Drugs and Codrugs

	Molecular weight (MW)	Color/consistency	Retention time (min)	Melting point (°C)
Naltrexone	341.4	White/Solid	2.1	178
Naltrexol	343.4	Yellowish white/Solid	2.0	191.5
Diclofenac	296.15	White/Solid	2.9	184
Codrug I (hydrochloride salt)	619.53 656.0	White/Solid White/Solid	4.9	- 152.5
Codrug II	621.55	Slightly yellow/Solid	4.4	202
Codrug III (hydrochloride salt)	793.73 830.19	Yellow/Solid Yellow/Solid	3.8	-
Codrug IV	795.75	Cream/Solid	3.7	-

Table II

Physicochemical Characterization of Codrugs

	Solubility (mM)	Pseudo first order rate constant (k) (days $^{-1}$)	Stability (approx. half-life) (days)
Codrug I (hydrochloride salt)	7.23[a] 83.8±15.4	0.0803	8.72±1.05
Codrug II	-	0.0052	130.88±4.94
Codrug III (hydrochloride salt)	27.16±0.5 87.67±1.5[b]	0.0238	29.14±1.04
Codrug IV	6.8±0.2	0.0035	187.56±8.55

$n \geq 3$ for all estimates except melting point determination

[a] Codrug I solubility was determined in 43% PG, 43% ethanol containing solution (data previously reported in (21))

[b] Saturation solubility could not be reported due to limited codrug