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Synthesis and hydrolytic behavior of two novel tripartate codrugs of naltrexone and 6β-naltrexol with hydroxybupropion as potential alcohol abuse and smoking cessation agents

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Abstract—A codrug approach for simultaneous treatment of alcohol abuse and tobacco dependence is considered as very desirable because of substantial evidence that smoking is increased significantly during drinking, and that smoking is regarded as a behavioral 'cue' for the urge to consume alcohol. The purpose of this study was to design and synthesize codrugs for simultaneous treatment of alcohol abuse and tobacco dependence. Two novel tripartate codrugs of naltrexone (NTX) and naltrexol (NTXOL) covalently linked to hydroxybupropion (BUPOH) were synthesized (**25** and **26**, respectively), and their hydrolytic cleavage to the parent drugs was determined. These codrugs were generally less crystalline when compared to NTX, or NTXOL, as indicated by their lower melting points, and were expected to be more lipid-soluble. Also, the calculated *c*log*P* values were found to be higher for the codrugs compared to those for NTX and NTXOL. The studies on the hydrolysis of the codrugs provided good evidence that they could be efficiently converted to the parent drugs in buffer at physiological pH. Thus, these codrugs are likely to be cleaved enzymatically in vivo to generate the parent drugs, and are considered to be potential candidates for simultaneous treatment of alcohol abuse and tobacco dependence.

1. Introduction

The prodrug approach is commonly used to improve physicochemical, biopharmaceutical, and drug delivery properties of therapeutic agents. Ideally, an inactive pro-moiety is covalently attached to the parent molecule, and the resulting prodrug is converted to the parent drug in the body before it exhibits its pharmacological effect.[1–3] A codrug, or mutual prodrug, consists of two different synergistic drugs within a single chemical entity. The two drugs may be connected either directly or by means of a cleavable covalent linker. Many diseases are treated by a combination of therapeutic agents that are co-administered in separate dosage forms.[4] However, there are potential advantages in delivering the co-administered agents as a single chemical entity. For example, improved delivery and pharmacokinetic properties compared to a physical mixture of the two drugs, and improved targeting of the drugs to specific sites of action.[5] Numerous codrugs have been described in the literature,[6–8] and the antibiotic drug sultamicillin (Unasyn Oral) is marketed as a synergistic codrug combination of ampicillin and the β-lactamase inhibitor, penicillanic acid sulfone.[9,10] We have recently reported on bipartate codrugs of ethacrynic acid with atenolol and timolol as novel antiglaucoma drugs,[11] and on antiangiogenic tripartate codrugs of 5-fluorouracil and trihydroxy steroid (THS).[12]

Simultaneous treatment of alcohol abuse and tobacco dependence is considered very desirable because of substantial evidence that smoking is increased significantly during drinking.[13] It also appears that drinking is increased in smokers and that a cigarette smoke environment is a behavioral 'cue' for the urge to consume alcohol;[14–21] thus, each addiction exacerbates the other.[14–18] The biochemical basis for this common co-morbidity may be related to the mediation of the dopamine-activating and reinforcing properties of alcohol via central nicotinic acetylcholine receptors.[19] As a consequence of this, alcoholics may also be at a much higher risk of developing tobacco-related diseases.[20] In this respect, opiate addicts also exhibit a high prevalence

Keywords: Alcohol Abuse; Hydroxybupropion; Naltrexone; β-Naltrexol; Codrugs; Transdermal delivery.
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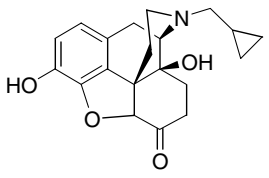
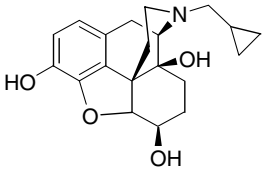
of tobacco dependency and associated co-abuse problems, and would also benefit from a co-therapy approach.[22]

Naltrexone (NTX, **1**) (Fig. 1) is an opioid antagonist used in the treatment of opiate addiction and alcohol dependence.[23] However, NTX is a hepatotoxin that has been demonstrated to have low oral bioavailability, and adverse side effects, such as abdominal pain, constipation, nausea, and vomiting. β-Naltrexol (NTXOL, **2**) (Fig. 1) is the active metabolite of NTX.[24,25] Bupropion (BUP, **3**) (Fig. 1) is an antidepressant medication and a therapeutic agent currently used in the treatment of nicotine dependence as a smoking cessation agent. Hydroxybupropion (BUPOH, **4**) (Fig. 1) is the major active metabolite of BUP, and is believed to contribute significantly to its antidepressant activity, as well as the smoking-cessation properties of BUP.[26–29] It is likely that BUP may be acting as a nicotinic receptor antagonist, and that this property is responsible for the smoking cessation properties of the drug.[27] BUP and BUPOH are drug molecules that are considered ideally suitable, from both a chemical and pharmacological perspective, for covalent linkage to NTX and NTXOL to afford clinically useful codrug entities for the treatment of both alcohol abuse and tobacco dependence.

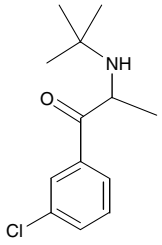
The purpose of this present study was to design and synthesize a series of novel NTX and NTXOL tripartate codrugs covalently linked to either BUP or BUPOH via an enzymatically cleavable linker moiety, and to determine if these codrugs are efficiently hydrolyzed to the parent drug molecules at physiological pH. This will provide initial mechanistic rationale for carrying out a more extensive and in-depth pharmacokinetic study in animals, including the potential for drug–drug interactions. The codrug molecules were specifically designed to determine if such codrugs could improve pharmacotherapy both for alcohol abuse and tobacco dependency, by providing a single, clinically effective, codrug dosage form to treat both co-dependent conditions.



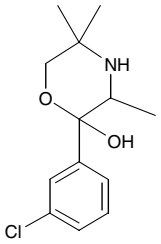
1, Naltrexone (NTX)



2, 6-β-Naltrexol (NTX)



3, Bupropion (BUP)



4, Hydroxybupropion (BUPOH)

Figure 1. Chemical structures of naltrexone (NTX, **1**), 6-β-naltrexol (NTXOL, **2**), bupropion (BUP, **3**), and hydroxybupropion (BUPOH, **4**).

Thus, the ultimate therapeutic goal of this approach is to identify a codrug that can be efficiently hydrolyzed to afford therapeutically effective levels of the two parent drugs in the body. The conversion of the highly crystalline and high melting NTX to a covalently linked codrug with BUP or BUPOH should afford a lower melting and more lipid soluble molecule.

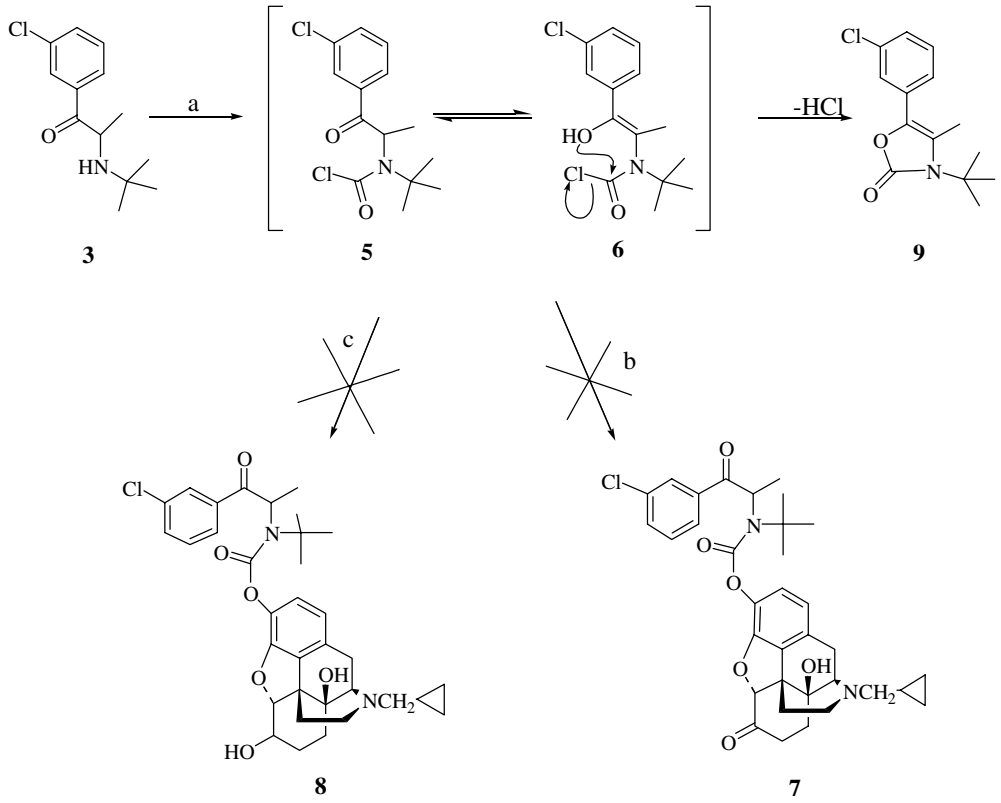
2. Results and discussion

2.1. Chemistry

A codrug is formed by chemical conjugation of two or more drugs via a suitably designed labile linker unit. The drugs are usually linked via linker moieties, such as ester, carbonate, amide, carbamate, etc., which are then cleaved enzymatically to regenerate the active drug molecules at a required site in the body. One advantage is that often, when the two drugs are chemically linked together in the codrug structure, the resulting physicochemical properties of the codrug are superior to those of the individual parent drugs. Thus, careful design of the codrug entity can afford a unique product that may have superior physicochemical properties for drug delivery, compared to those of the individual drug entities themselves, leading to improved pharmaceutical properties. In addition, there are also other factors, such as the ability to control drug delivery by appropriate design of the hydrolyzable linker(s) connecting the two drug entities, and the obvious effect that simultaneous delivery of the two drugs, as one chemical entity, will have on the pharmacokinetics of each respective drug.

In efforts to synthesize the carbonate codrugs **7** and **8** (Scheme 1), which are codrugs of BUP covalently linked to either NTX or NTXOL, initial coupling of the two molecules utilizing phosgene as the linker precursor was attempted. First, N-acylation reaction conditions (Scheme 1) utilizing bupropion and phosgene under a variety of conditions and solvents in the presence of triethylamine, followed by addition of NTX, afforded the stable cyclic product **9** and unreacted NTX. When BUP was reacted with phosgene in the absence of NTX, **9** was formed in good yield, rather than the expected chlorocarbonyl intermediate **5** (Scheme 1). This result indicates that the chlorocarbonyl intermediate **7** is not stable enough to be isolated, and its half-life appears to be too short for the reaction with NTX tooccur. The formation of **9** can be explained by **5** existing in an equilibrium mixture with its enol tautomer **6**, which then undergoes rapid intramolecular O-acylation to form **9**.

Hussain[30] and Nelson[31] have reported the synthesis of a number of 3-*O*- and 6-*O*-ester analogs of NTX and NTXOL. These methodologies do not require protection of the C-14 hydroxyl group, due to its unreactivity, which results from significant steric hindrance around this functional group. Under the reaction conditions

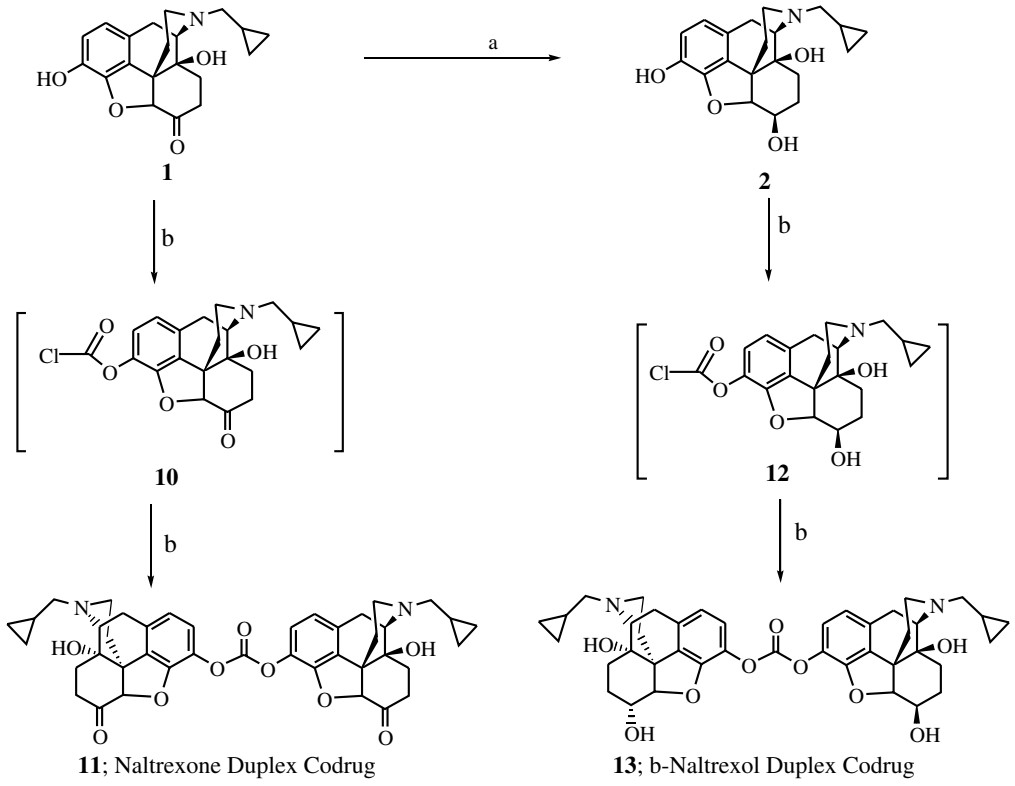


Scheme 1. Reagents and conditions: (a) CH_2Cl_2 or THF, base = TEA or pyridine, 0 °C, argon; (b) CH_2Cl_2, TEA, 0 °C, argon, naltrexone; (c) CH_2Cl_2, TEA, 0 °C, argon, β-naltrexol.

described by Hussain et al.,[30] O-acylation of NTX in the presence of base is regiospecific for the more reactive C-3 phenolic group (as the phenolate anion). Similarly, O-acylation of NTXOL in the presence of base is regiospecific for the 3-OH group.[32] Thus, no prior protection of either the 14- or 6-β-OH group in either NTX or NTXOL is required for regiospecific 3-O-acetylation of these molecules. With this in mind, in an alternative strategy, the synthesis of codrugs **7** and **8** via intermediate **10** (Scheme 2), which results from the reaction of NTX and phosgene in the presence of triethylamine, was attempted. Under these conditions, the reaction of NTX with phosgene afforded exclusively the dimeric 3-*O*, 3′-*O* carbonate ester of NTX (**11**, Scheme 2) rather than the expected NTX-3-*O*-chlorocarbonyl intermediate **10**.[33] Also, when this reaction was carried out in the presence of BUP, both **11** and **9** were formed, and no codrug could be detected in the reaction mixture. The use of a variety of different reaction conditions in these reactions failed to yield the desired intermediate **10**, affording only **11** in good yield. Similarly, when NTXOL was utilized in place of NTX, the reaction with phosgene resulted in the formation of the corresponding dimeric 3-*O*, 3′-*O* carbonate ester of NTXOL, **13** (Scheme 2).

Efforts to synthesize codrugs **14**–**17** (Fig. 2) of BUPOH with NTX or NTXOL were also explored. The hemiketal form of BUPOH, **4**, exists in equilibrium with its ring-opened form, **18** (Scheme 3). Thus, if the hemiketal form of BUPOH, **4**, is reacted with phosgene, then either the chlorocarbamate intermediate **19** (Scheme 3), the chlorocarbonate intermediate **20** (Scheme 3), or both, could be formed, either of which if coupled with NTX or NTXOL would afford codrugs **14**–**17** (Fig. 2). Also, there is a possibility that the formation of **19** might be followed by ring opening, leading to the formation of **21** (Scheme 3). On the other hand, if the ring-opened form of BUPOH, **18**, reacts with phosgene, the chlorocarbamate intermediate, **21**, would be expected to be formed. If **21** is stable enough to couple to NTX or NTXOL, it may undergo subsequent cyclization to the hemiketal form, resulting in the formation of codrugs **14**–**17**. Also, BUPOH may be initially *N*-chlorocarbonylated to afford **21**, which, like the corresponding BUP analog, **6**, can exist in equilibrium with its enol tautomer, **22** (Scheme 3), followed by rapid intramolecular O-acylation to **23** (Scheme 3). When equimolar amounts of BUPOH and phosgene were reacted under basic conditions, the phosgene–BUPOH intermediate **23** (Scheme 3) was formed as the major product in the reaction. Compound **23** could be isolated, purified, and fully characterized.

These results clearly indicate that the ring-opened and cyclized (hemiketal) forms of BUPOH are in dynamic equilibrium under the conditions of the initial acylation reaction with phosgene (Scheme 3), although the possibility that **22** might be generated by ring opening of **19**, if it is formed, cannot be excluded. Unlike the corresponding BUP analog, **9**, intermediate **23** has a terminal



Scheme 2. Reagents and conditions: (a) NaOH, formamidinesulfinic acid, 80–85 °C, 1.5 h; (b) $COCl_2$, CH_2Cl_2, TEA, 0 °C, argon.

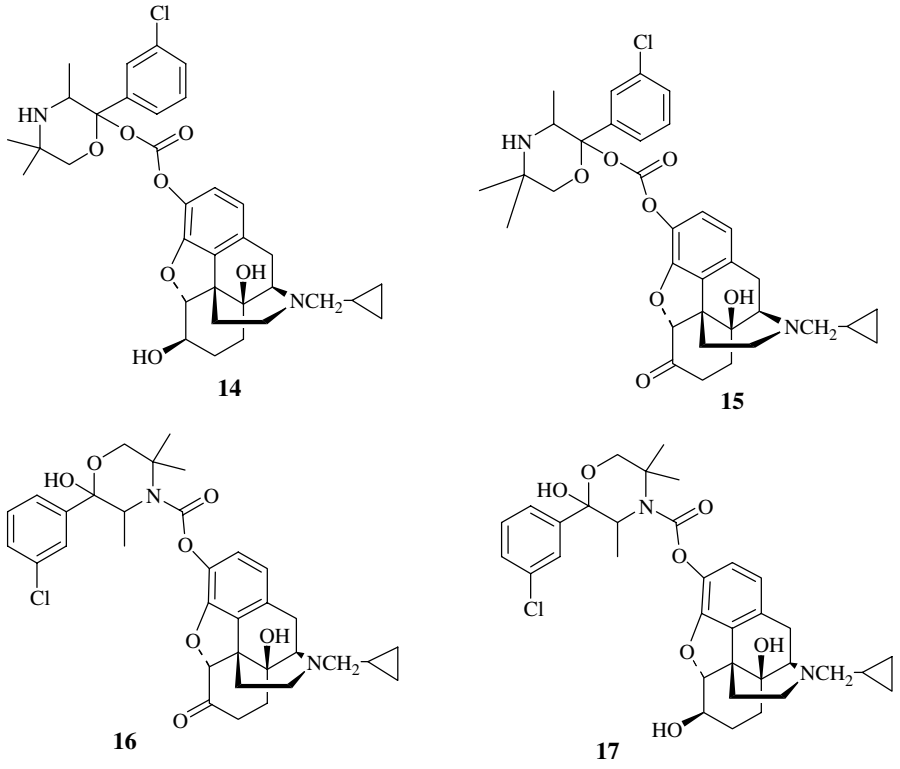
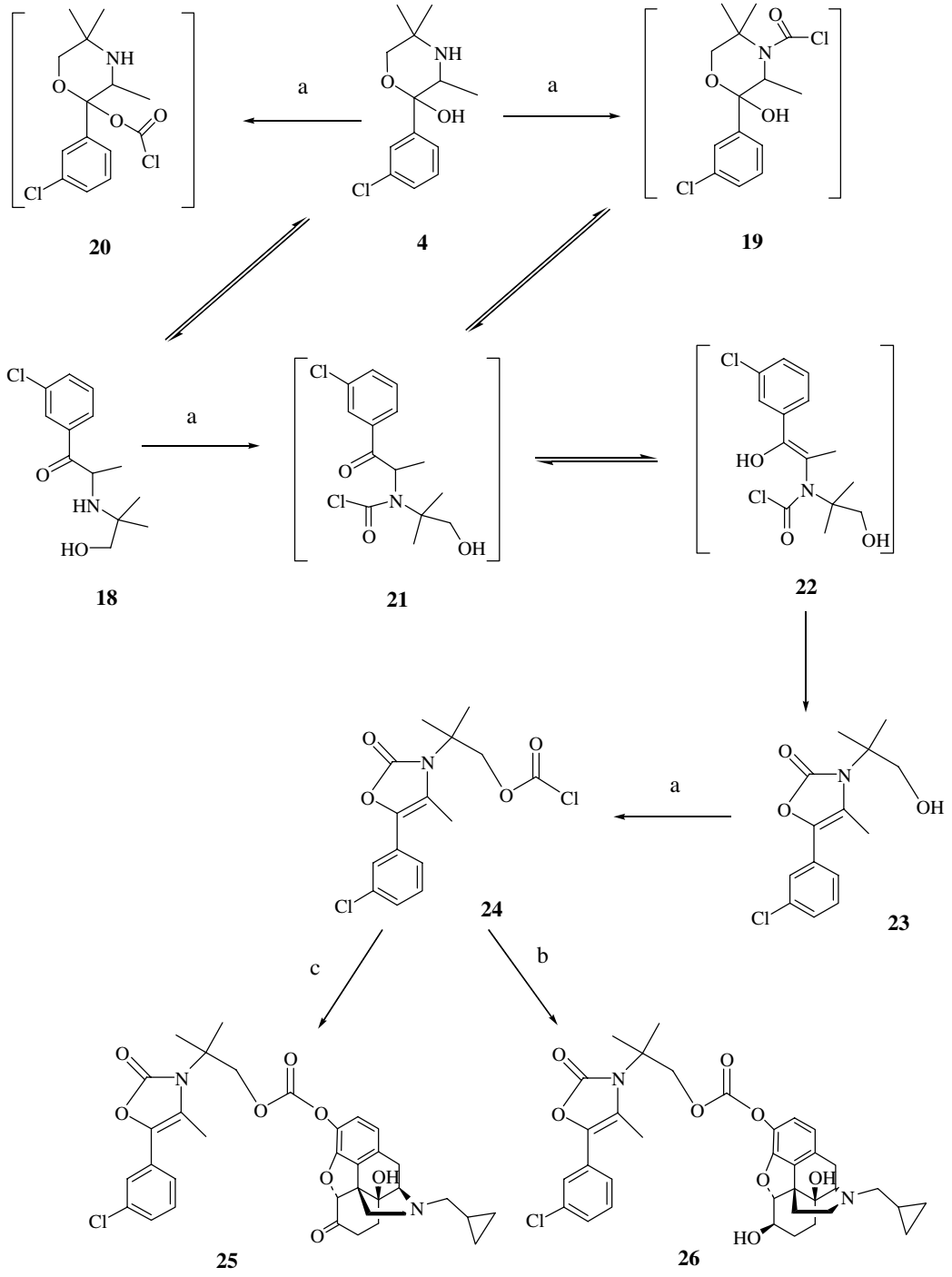


Figure 2. Proposed codrugs of hydroxybupropion with naltrexone and β-naltrexol.

hydroxyl group that can be utilized for linking with NTX or NTXOL via a carbonate moiety. Furthermore, the cyclic carbonate analog of BUPOH, **23**, is expected to be enzymatically cleaved to BUPOH in vivo. Thus, this modified BUPOH entity was considered to be a valid substitute for BUPOH in BUPOH–NTX or



Scheme 3. Reagents and conditions: (a) $COCl_2$, CH_2Cl_2, TEA, 0 °C, argon; (b) NTXOL, CH_2Cl_2, TEA, 0 °C, argon; (c) NTX, CH_2Cl_2, TEA, 0 °C, argon.

BUPOH–NTXOL codrug molecules. Also, when BUPOH was reacted with excess phosgene under basic conditions, the intermediate **24** (Scheme 3) was the major product in the reaction. Intermediate **24** was isolated and fully characterized, and afforded the modified codrug **25** (Scheme 3) on treatment with NTX in dichloromethane.

In a similar manner, the desired NTXOL–BUPOH codrug **26** (Scheme 3) was obtained when compound **24** was reacted with NTXOL in dichloromethane in the presence of triethylamine. These results again indicate that the ring-opened and cyclized (hemiketal) forms of BUPOH are in dynamic equilibrium under the conditions of the initial N-chlorocarbonylation reaction with phosgene (Scheme 3).

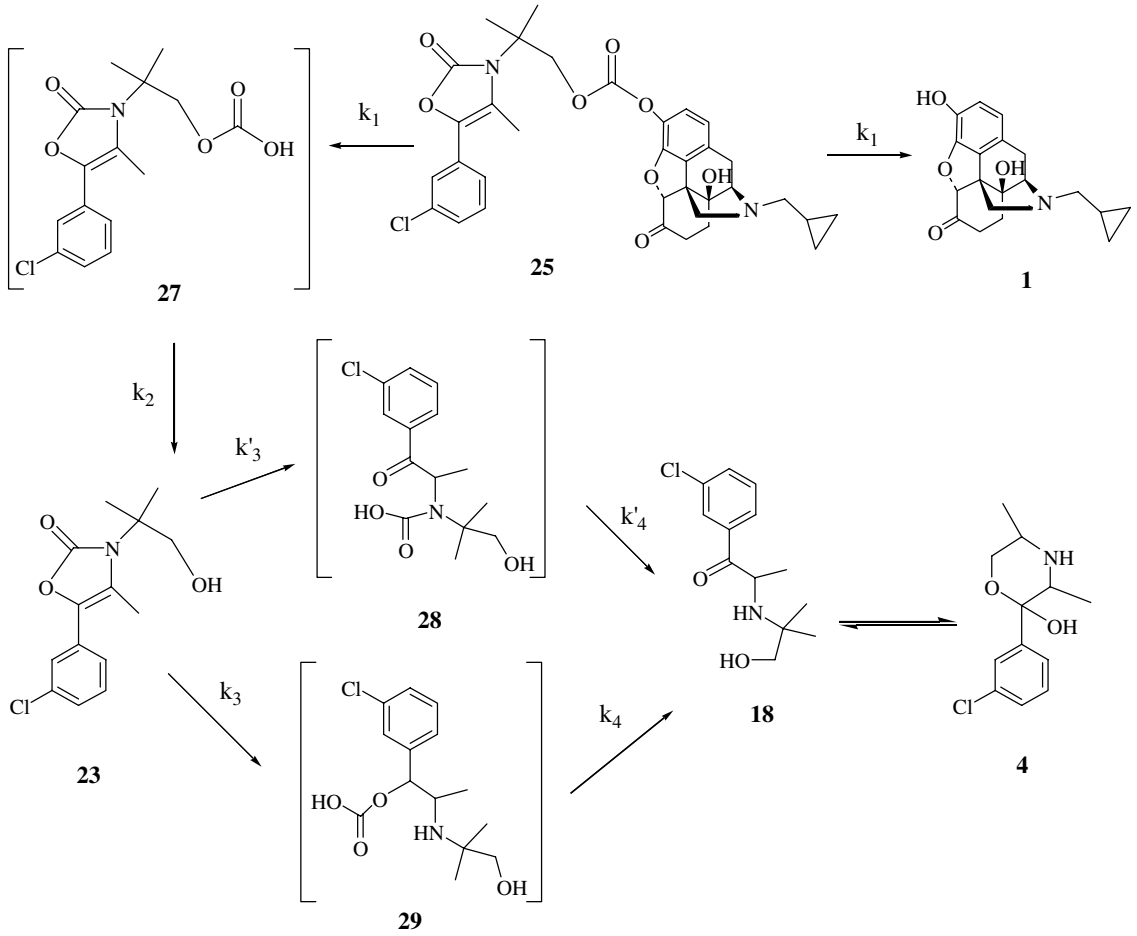
2.2. Hydrolysis studies on codrugs 25 and 26

In order to determine if codrugs **25** and **26** have the potential to be enzymatically cleaved in vivo to generate BUPOH and NTX, and BUPOH and NTXOL, respec-

tively, initial studies on the hydrolysis of these codrugs in buffer at physiological pH were carried out to provide evidence that the parent drugs are capable of being generated by hydrolytic cleavage of the codrug. The detailed in vivo evaluation and pharmacokinetics of the codrugs described herein are currently under investigation, and are outside the scope of the current study; the results from these studies will be reported in a further communication.[34]

To determine whether codrugs **25** and **26** could be hydrolyzed to the parent drugs, hydrolysis studies were carried out at physiological pH (pH 7.4) using isotonic phosphate buffer. These carbonate codrug molecules should be susceptible to hydrolytic cleavage, and we anticipated the hydrolytic conversion to proceed as illustrated in Scheme 4. From previously reported studies,[35,36] the codrug **25** is expected to hydrolyze to NTX and intermediate **27**, which will then hydrolyze rapidly to give **23**. Thus, the intermediate **27** will not accumulate, and the rate of appearance of **23** should show pseudo-first-order kinetics. Also, from similar kinetic studies that have been reported on structurally related oxazolidino analogs,[37] the intermediate **23** is expected to hydrolyze to BUPOH through pseudo-first-order kinetics by hydrolytic cleavage of the 2,4-oxazolidine ring in **23**. Furthermore, the intermediate **23** is expected to hydrolyze through initial cleavage of the CO–O moiety to give the transient carbamic acid intermediate, **28**; this unstable intermediate will not accumulate, and will be rapidly N-decarboxylated to BUPOH, as literature reports indicate.[38–40] However, hydrolysis of **23** to **29** followed by O-decarboxylation to give BUPOH cannot be ruled out as an alternative cleavage mechanism, as has been suggested by others.[38–45]

The chemical stability of the codrug **25** in isotonic phosphate buffer at pH 7.4 was studied over 4 days. Concentration versus time curves for the appearance of the parent drugs NTX and BUPOH, and the disappearance of the carbonate codrug **25** are shown in Figure 3. The pseudo-first-order rate constants k_1 and k_3 (k'_3) were calculated to be 0.020 and 0.015 h^{-1}, respectively, for the generation of NTX and BUPOH. The rate of appearance of NTX was the same as the rate of disappearance of the codrug, indicating that there is no detectable intermediate involved in the direct formation of NTX from codrug **25**. Whereas, the rate of formation of BUPOH is slower than the disappearance of the codrug, and follows a sigmoidal time curve, suggesting the presence of a relatively stable intermediate, which was subsequently identified as intermediate **23**. However, a summation of the molar equivalents of BUPOH and intermediate **23** formed from the hydrolysis of



Scheme 4. A schematic diagram showing stepwise hydrolytic cleavage of the carbonate codrug **25** into NTX and BUPOH in isotonic phosphate buffer, pH 7.4.

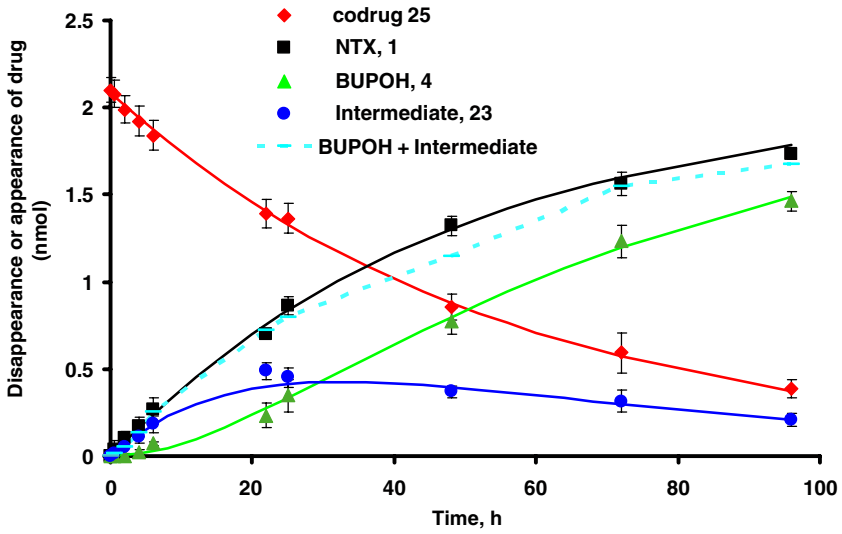


Figure 3. A hydrolytic profile of the carbonate codrug **25** showing the hydrolysis into NTX and BUPOH in isotonic phosphate buffer, pH 7.4, at 32 °C.

codrug **25** indicated stoichiometry with the formation of NTX, as represented by the dashed line in Figure 3. This indicates that, apart from **23**, there is no other stable intermediate involved in the formation of NTX and BUPOH from codrug **25**.

Thus, codrug **25** is hydrolyzed in isotonic phosphate buffer (pH 7.4, 32 °C) and efficiently releases the parent drugs, NTX and BUPOH. However, the rate of formation of BUPOH is somewhat slower than that of NTX, due to the relatively slower rate of hydrolysis of intermediate **23**. Nevertheless, we believe that codrug **25** will be susceptible to enzymatic cleavage in vivo to generate BUPOH and NTX, and the rate of this conversion should be even more efficient than that seen in buffer. Thus, codrug **25** is a potential candidate for the treatment of both alcohol abuse and tobacco dependency.

Similarly, hydrolytic studies with codrug **26** were also carried out, and the corresponding rate constants k_1 and k_3 were calculated to be 0.024 and 0.014 h^{-1}, respectively. Thus, both codrugs **25** and **26** appear to hydrolyze rapidly at the carbonate linker moiety to afford NTX or NTXOL followed by hydrolytic cleavage of the resulting common cyclic BUPOH intermediate **23**, to generate BUPOH. This comparable hydrolytic behavior of codrugs **25** and **26** is expected, since the only difference between codrugs **25** and **26** is at the C-6 position, which is a carbonyl moiety in NTX, and a β-hydroxy group in NTXOL. This structural difference is not expected to affect the rate of cleavage of the carbonate linker to afford NTX/NTXOL.

2.3. Physicochemical properties of the codrugs

A more qualitative basis for the design of codrugs to enhance delivery of NTX or NTXOL is to improve the physicochemical properties essential for increased permeation through biological membranes. Some of the physicochemical properties of codrugs **25** and **26** were measured and compared to those for NTX or NTXOL, and these are shown in Table 1.

Table 1. Physicochemical properties of NTX, NTXOL, and the carbonate codrugs **25** and **26**

Compound	MW	Mp (°C)	*c*log *P*[a]	Half-life, $t_{1/2}$ (h)[b]
1	341.40	175.7 ± 1.20	0.36	Stable
2	343.42	187.76 ± 2.62	0.83	Stable
4	255.74	124.40 ± 1.60	2.87	Stable
25	649.13	137.00 ± 1.41	3.23	36.68 ± 2.88
26	651.15	159.50 ± 2.12	3.71	28.88 ± 2.82

[a] Derived from Daylight® Software.
[b] Studied in isotonic phosphate buffer, pH 7.4, at 32 °C.

The melting points of the codrugs were measured because this physical property can be easily related to drug solubility properties.[24,41] As shown in Table 1, the calculated *c*log *P* values were found to be higher for the codrugs compared to those for the corresponding parent drugs. The *c*log *P* value provides a way of estimating the lipophilicity of a drug, and the higher the *c*log *P* value the more lipophilic the drug. Another important physicochemical property necessary for enhancement of bioavailability is rapid bioconversion of the codrug to the corresponding parent drugs. Rapid bioconversion rates of codrugs are represented by short half-lives in isotonic phosphate buffer (pH 7.4/32 °C), and these values can be significantly increased in vivo, because in addition to hydrolysis, the codrugs are also susceptible to enzymatic action by esterases. In addition to regeneration of the active parent drugs, rapid bioconversion of the codrugs in plasma causes an enhancement of the concentration gradient across biological membranes, and consequently leads to an increased potential of the drug to cross the biological barrier.

3. Conclusions

Two novel codrugs of NTX and NTXOL with BUPOH (**25** and **26**) have been synthesized, and the kinetics of

their hydrolysis has also been studied. Both codrugs were hydrolyzed in isotonic phosphate buffer (pH 7.4/32 °C) and efficiently released the parent drugs. Thus, the two codrugs **25** and **26** are predicted to be cleaved enzymatically in vivo to generate BUPOH and NTX, and BUPOH and NTXOL, respectively, representing potential candidate drugs for the treatment of both alcohol abuse and tobacco dependency.

4. Experimental section

4.1. General methods

All purchased solvents and reagents were used without further purification. Phosgene was purchased from Fluka Chemie AS. (CAUTION!!! Care must be exercised in the handling of phosgene). Flash column chromatography was carried out using ICN SilicTech 32–63, 60 silica gel. TLC analyses were carried out on EMD Chemicals Inc. glass plates precoated with 250 μm silica gel 60 F_{254}. Melting points were determined on a Fisher Scientific melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectrometric analyses were recorded on a Varian spectrometer, operating at 400.1 and 299.9 MHz, respectively. Chemical shifts are reported in parts per million (δ) using TMS as the internal standard. The splitting pattern abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, and br = broad. Mass spectra were recorded on a JEOL JMS-700T MStation or on a Bruker Autoflex MALDI-TOF MS. GC-mass spectra were recorded on an Agilent 6890 GC incorporating an Agilent 7683 autosampler and an Agilent 5973 MSD.

4.2. Liquid chromatography–mass spectrometry identification of synthetic products

Different drugs were identified in different experiment under varying mobile phase compositions. Chromatography was performed on a Waters Symmetry® C18 (2.1 mm × 150 mm, 5 μm) column at 35 °C using a mobile phase consisting of acetonitrile: 2 mM ammonium acetate (32:68 v/v for NTX, NTXOL, BUP, and BUPOH; 80:20 v/v for **25** and **26**; or 60:40 v/v for **23**) at a flow rate of 0.25 mL/min. A Waters Symmetry® C18 (2.1 mm × 10 mm, 3.5 μm) guard column was also used. The LC–MS system consisted of a Waters Alliance 2695 HPLC pump (Waters, Milford, MA, USA), a Waters Alliance 2690 autosampler, and a Micromass ZQ detector (Waters, Milford, MA, USA) that utilized electrospray ionization (ESI) for ion production. Selected ion monitoring (SIM) was performed in the positive mode, with dwell time set at 0.30 s. Capillary voltage was 4.5 kV and cone voltage was 30 V. The source block and desolvation temperatures were 120 and 250 °C, respectively. Nitrogen was used as a nebulization and drying gas at flow rates of 50 and 450 L/h, respectively. LC–MS data are as follows, for NTX *m/z* 342 (M^+, retention time: 4.80 min), NTXOL *m/z* 344 (M^+, retention time: 3.22 min), **25** *m/z* 650 (M^+, retention time: 3.51 min), **26** *m/z* 282 (M^+, retention time: 3.13 min), and **23** *m/z* 652 (M^+, retention time: 4.21 min).

4.3. Synthesis of 6-β-naltrexol (2)

NTXOL base was synthesized by a modification of a previously published method.[42] To a suspension of NTX free base (10.22 g, 30.0 mmol) under argon was added 100 mL (enough to afford complete dissolution) of 7.22 M aqueous NaOH. This alkaline solution of NTX was treated drop-wise at ambient temperature over 20 min with 13.0 g (120 mmol) of formamidinesulfinic acid dissolved in 200 mL of 7.22 M aqueous NaOH. After the addition was complete, the solution was heated and stirred at 80–85 °C for 1.5 h when silica gel TLC analysis indicated that the reaction was complete. The reaction mixture was cooled (ice bath) and then treated drop-wise under argon with a solution of ammonium chloride (15.40 g, 288 mmol) in distilled water (100 mL). The aqueous mixture was extracted with 5×100 mL of $CHCl_3$, the combined organic extracts were filtered through a pad of Na_2SO_4 and evaporated in vacuum to afford the crude product (free base) as a foam, which was dissolved in 20 mL of warm (50 °C) ethyl acetate and diluted to 60 mL with warm (40 °C) *n*-hexane. Crystallization occurred spontaneously on cooling. The crystals were collected by filtration, washed with 2 × 10 mL of cold ethyl acetate/*n*-hexane (1:3 volume/volume), and oven-dried in vacuo at 60 °C to give 9.12 g (89% yield) of NTXOL as a white solid, mp 175–177 °C (lit. [42] mp 188–190 °C). The NMR spectral data were consistent with the previously published data.[30] ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.71 (d, J = 8.1 Hz, 1 H), 6.56 (d, J = 8.1 Hz, 1 H), 4.55 (d, J = 6.1 Hz, 1H), 3.57 (m, 1H), 3.16–3.02 (m, 2H), 2.70–2.58 (m, 2H), 2.37 (d, J = 6.4 Hz, 2H), 2.33–2.22 (m, 1H), 2.16–2.06 (m, 1H), 2.02–1.88 (m, 1H), 1.72–1.55 (m, 2H), 1.54–1.44 (m, 1H), 1.38–1.24 (m, 1H), 0.82 (m, 1H), 0.60–0.50 (m, 2H), 0.20–0.10 (m, 2H) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz): δ 142.5, 140.5, 132.0, 123.1, 118.1, 117.0, 95.4, 71.7, 69.7, 61.8, 58.5, 46.9, 43.6, 30.4, 29.7, 27.3, 22.2, 9.3, 3.8, 3.6 ppm. LC–MS: *m/z* 344 (M^+) single peak at t_R = 3.22 min.

4.4. Synthesis of bupropion hydrochloride

Bupropion was synthesized by a modification of a previously published method.[43] 12.24 g (72.6 mmol) of *m*-chloropropiophenone was dissolved in 25.0 ml of methylene chloride in a 50-ml round-bottomed flask. A few drops of 1.0 M solution of Br_2 in methylene chloride were added with stirring and the reaction mixture was briefly warmed to initiate the reaction (as judged by the disappearance of the color of the bromine). Then, the flask was placed in an ice bath and 11.6 g (72.5 mmol) of bromine in methylene chloride solution was added drop-wise with stirring. The methylene chloride was removed by distillation. Thirty-five milliliters of *tert*-butylamine and 25 ml NMP were added, and the flask was heated in a 50–60 °C water bath with stirring for 10 min. The contents of the flask were transferred to a separatory funnel, 100 ml of 10% w/v aqueous sodium carbonate was added, and the mixture was extracted with diethyl ether (3× 50 ml). The combined ether extracts were washed with water (3× 50 ml), then brine solution (50 ml), dried over anhydrous K_2CO_3,

and transferred to a beaker chilled in an ice bath. A 20:100 v/v mixture of concentrated HCl and isopropyl alcohol was added drop-wise with stirring until the contents were acidic. The desired product was filtered at the pump, washed with diethyl ether, and dried to afford BUP–HCl as a white solid (20.1 g, 95% yield), mp 236–238 °C (lit. [44] mp 233–234 °C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.75 (d, J = 12.3 Hz, 1H), 8.63 (br, 1H), 8.27 (s, 1H), 8.17 (d, J = 7.8 Hz, 1H), 7.85 (d, J = 7.8 Hz, 1H), 7.69 (d, J_1 = 7.8 Hz, J_2 = 7.8 Hz, 1H), 5.32 (qt, J = 7.2 Hz, 1H), 1.53 (m, J = 7.2 Hz, 3H), 1.32 (s, 9H) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz): δ 203.60, 136.56, 133.83, 132.94, 130.70, 127.87, 126.98, 52.10, 50.31, 29.39, 22.18 ppm.

4.5. Synthesis of bupropion free base (2)

1.0 g of BUP–HCl was dissolved in a minimum amount of water in a 250 ml flask. The contents of the flask were transferred to a separatory funnel, to which 20 ml of 10% w/v aqueous sodium carbonate was added, and the mixture was extracted with methylene chloride (3× 50 ml). The combined methylene chloride extracts were washed with water (3× 50 ml), then brine solution (50 ml), dried over anhydrous K_2CO_3, filtered and the filtrate stripped down under reduced pressure on a rotary evaporator to give the desired product as a yellow oil (7.9 g, 90% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.90 (s, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.48 (d, J = 7.8 Hz, 1H), 7.37 (t, J = 7.8 Hz, 1H), 4.24 (qt, J = 7.2 Hz, 1H), 1.19 (d, J = 7.2 Hz, 3H), 0.97 (s, 9H) ppm; MS: m/z 240 (M^+). LC–MS: m/z 240 (M^+) single peak at t_R = 8.40 min.

4.6. Synthesis of hydroxybupropion (4)

The racemate[45] of BUPOH [(+/−)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol] was synthesized by the following procedure. 10.2 g (60.5 mmol) of *m*-chloropropiophenone was dissolved in 25.0 ml of methylene chloride in a 50-ml round-bottomed flask. A few drops of a 1.0 M solution of Br_2 in methylene chloride were added with stirring and the reaction mixture was briefly warmed to initiate the reaction (as judged by the disappearance of the color of the bromine). Then, the flask was placed in an ice bath, and 11.6 g (72.5 mmol) of bromine in methylene chloride was added drop-wise with stirring. The methylene chloride was removed by distillation. Forty milliliters of 2-amino-2-methyl-1-propanol and 25 ml of NMP were added, and the flask was heated in a 50–60 °C water bath with stirring for 50 min. Then, the contents of the flask were transferred to a separatory funnel, 100 ml of 10% w/v aqueous sodium carbonate solution was added, and the mixture was extracted with methylene chloride (3× 50 ml). The combined methylene chloride extracts were washed with water (3× 50 ml), then brine solution (50 ml), dried over anhydrous K_2CO_3, and reduced to a small volume under reduced pressure on a rotary evaporator. The desired product was precipitated by trituration with excess *n*-pentane. The resulting solid was filtered at the pump, and further purified by recrystallization from a methylene chloride: *n*-pentane mixture to afford BUPOH as a white solid (17.3 g, 92% yield), mp 123–126 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.58 (s, 1H), 7.44 (m, 1H), 7.27–7.25 (m, 2H), 3.76 (d, J = 11.1 Hz, 1H), 3.36 (d, J = 11.1 Hz, 1H), 3.12 (q, J = 6.6 Hz, 1H), 1.34 (s, 3H), 1.01 (s, 9H), 0.77 (d, J = 6.6 Hz, 3H) ppm; MS: m/z 256 (M^+). LC–MS: m/z 256 (M^+), single peak at t_R = 3.96 min.

4.7. Synthesis of 3-*tert*-butyl-5-(3-chloro-phenyl)-4-methyl-oxazolidin-2-one (9)

A solution of phosgene (20% w/w in toluene, 10.0 ml, 20.0 mmol) was cooled to 0 °C in an ice-bath under argon. To this stirred solution was added a mixture of BUP (1.0 g, 4.2 mmol) and triethylamine (1.7 ml, 12.5 mmol) in 20.0 ml of methylene chloride. After stirring for 48 h, the excess phosgene and solvents were removed in a stream of argon. The resulting residue was dissolved in methylene chloride and washed with 5% hydrochloric acid ((2× 60 ml), water (50 ml), and then brine solution (20 ml). The organic phase was then separated and dried over anhydrous sodium sulfate, filtered, and the solvent stripped off under reduced pressure and dried to afford **9** as a brown oil (1.1 g, 90% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.46–7.28 (m, 4H), 2.39 (s, 3H), 1.68 (s, 9H) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz) δ: 154.3, 134.7, 133.9, 130.2, 130.0, 127.9, 126.6, 124.7, 121.1, 58.7, 29.8, 14.0 ppm. LC–MS: m/z 265 (M), single peak at t_R = 3.12 min.

4.8. Synthesis of naltrexol duplex codrug (13)

A solution of 1.6 ml phosgene (3.0 mmol, 20% w/w in toluene) was cooled to 0 °C in an ice-bath under argon. To this stirred solution was added a mixture of NTXOL (120 mg, 0.35 mmol) and triethylamine (50 μl, 0.36 mmol) in 20.0 ml of methylene chloride. After stirring for 20 h, the excess phosgene and solvents were removed in a stream of argon. The resulting residue was dissolved in methylene chloride and washed with water (2× 50 ml), 10% w/v aqueous sodium carbonate solution (2× 40 ml), then brine solution (50 ml). The organic phase was then separated and dried over anhydrous sodium sulfate, filtered, and the filtrate reduced to a small volume under reduced pressure on a rotary evaporator. The desired product was precipitated by trituration with excess *n*-pentane. The resulting solid was filtered at the pump, and further purified by recrystallization from a mixture of methylene chloride and *n*-pentane to afford **13** as a white solid (102 mg, 82%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.97 (d, J = 8.0 Hz, 1H), 6.68 (d, J = 8.0 Hz, 1H), 4.58 (d, J = 6.0 Hz, 1H), 3.62–3.50 (m, 1H), 3.16–3.02 (m, 2H), 2.70–2.58 (m, 2H), 2.37 (d, J = 6.4 Hz, 2H), 2.33–2.22 (m, 1H), 2.16–2.06 (m, 1H), 2.02–1.88 (m, 1H), 1.72–1.55 (m, 2H), 1.54–1.44 (m, 1H), 1.38–1.24 (m, 1H), 0.82 (m, 1H), 0.60–0.50 (m, 2H), 0.20–0.10 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz): δ 150.79, 146.87, 134.18, 133.61, 131.51, 121.68, 119.12, 97.56, 72.42, 70.27, 62.08, 59.39, 47.40, 43.75, 30.82, 29.70, 25.38, 23.16, 9.55, 4.14, 3.98 ppm. LC–MS: m/z 713 (M^+), single peak at t_R = 18.15 min.

4.9. Synthesis of hydroxybupropion phosgene intermediate 24 [5-(3-chlorophenyl)-3-(2-chlorocarbonoic-1,1-dimethylethyl)-4-methyl-oxazolidin-2-one]

A solution of phosgene (20% w/w in toluene, 15 ml) was cooled to 0 °C in an ice-bath under argon. To this stirred solution was added a mixture of BUPOH (2.00 g, 7.84 mmol) and triethylamine (3.3 ml, 24 mmol) in 20.0 ml of methylene chloride. After stirring for 48 h, the excess phosgene and solvents were removed in a stream of argon. The resulting residue was dissolved in methylene chloride and washed with water (2× 50 ml), and then brine solution (20 ml). The organic phase was separated and dried over anhydrous sodium sulfate, filtered, and the filtrate reduced to a small volume and dried under reduced pressure to afford **24** as a yellow oil (2.4 g, 90% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.38–7.28 (m, 4H), 4.65 (s, 2H), 2.34 (s, 3H), 2.24 (m, 1H), 1.75 (s, 6H) ppm.

4.10. Synthesis of hydroxybupropion: naltrexone codrug (25)

A solution of **24** (1.02 g, 2.97 mmol) in methylene chloride (20 ml) was cooled to 0 °C in an ice-bath under argon. To this stirred solution was added a mixture of NTX (1.0 g, 2.93 mmol) and triethylamine (0.45 ml, 3.23 mmol) in 20.0 ml of methylene chloride. After stirring for 48 h, the reaction mixture was washed with water (2× 50 ml), then brine solution (20 ml). The organic phase was separated and dried over anhydrous sodium sulfate and reduced to a small volume under reduced pressure. The desired product was precipitated by adding excess *n*-pentane, filtered at the pump, and washed with cold *n*-pentane. The desired product was further purified by recrystallization from a methylene chloride:*n*-pentane mixture to afford **25** as a white solid (1.5 g, 78% yield), mp 136–138 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.38–7.28 (m, 4H), 6.87 (d, J = 8.4 Hz, 1H), 6.64 (d, J = 8.4 Hz, 1H), 4.65 (s, 2H), 4.59 (s, 1H), 3.19 (d, J = 6.0 Hz, IH), 3.13–2.92 (m, 2H), 2.68 (dd, J = 5.7, 12.3 Hz, 1H), 2.59 (dd, J = 6.0, 18.9 Hz, 1H), 2.50–2.34 (m, 6H), 2.24 (m, 1H), 2.09 (m, 1H), 1.85 (m, 1H), 1.75 (s, 6H), 1.60 (m, 1H), 1.52 (m, 1H), 0.89 (m, 1H), 0.60–0.52 (m, 2H), 0.20–0.11 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz): δ 207.24, 153.96, 152.60, 147.42, 134.55, 134.04, 133.04, 130.35, 129.92, 129.86, 127.88, 126.68, 126.66, 124.79, 122.62, 121.69, 119.54, 90.85, 72.31, 70.18, 61.96, 60.33, 59.31, 50.75, 46.06, 36.24, 31.46, 30.71, 25.48, 25.36, 22.63, 14.38, 8.97, 4.54, 4.15 ppm. LC–MS: m/z 650 (M^+), single peak at t_R = 3.51 min.

4.11. Synthesis of hydroxybupropion:naltrexol codrug (26)

A solution of **24** (1.00 g, 2.91 mmol) in methylene chloride (20 ml) was cooled to 0 °C in an ice-bath under argon. To this stirred solution was added a mixture of NTXOL (1.0 g, 2.92 mmol) and triethylamine (4.5 ml, 3.23 mmol) in 20.0 ml of methylene chloride. After stirring for 48 h, the reaction mixture was washed with water (2× 50 ml), then brine solution (20 ml). The organic phase was separated and dried over anhydrous sodium sulfate and reduced to a small volume under reduced pressure. The desired product was precipitated by adding excess *n*-pentane, filtered at the pump, and washed with cold *n*-pentane. The product was further purified by recrystallization from a methylene chloride:pentane mixture to afford **26** as a white solid (1.6 g, 85% yield), mp 157–161 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.45–7.28 (m, 4H), 6.85 (d, J = 8.2 Hz, 1H), 6.65 (d, J = 8.1 Hz, 1H), 4.83 (d, J = 11.1 Hz, 1H), 4.54 (d, J = 11.1 Hz, 1H), 4.48 (d, J = 5.1 Hz, 1H), 3.58–3.42 (m, H), 3.19 (d, J = 5.4 Hz, 1H), 3.16–2.92 (m, 2H), 2.70–2.52 (m, 2H), 2.28–2.20 (m, 2H), 2.18–1.80 (m, 2H), 1.77 (s, 3H), 1.75 (s, 3H), 1.48–1.39 (m, 2H), 1.37–1.20 (m, 2H), 0.82 (m, 1H), 0.60–0.50 (m, 2H), 0.20–0.10 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz): δ 154.15, 152.74, 147.14, 134.66, 134.32, 133.88, 133.24, 131.14, 129.98, 129.87, 128.10, 126.78, 124.91, 121.83, 121.48, 118.90, 97.15, 72.46, 72.17, 70.23, 62.24, 60.33, 59.50, 47.24, 43.95, 31.24, 29.54, 25.68, 25.36, 25.30, 23.36, 14.40, 9.57, 4.35, 4.19 ppm. LC–MS: m/z 652 (M^+), single peak at t_R = 4.21 min.

4.12. Synthesis of 5-(3-chloro-phenyl)-3-(2-hydroxy-1,1-dimethylethyl)-4-methyl-oxazolidin-2-one (23)

A solution of phosgene (20% w/w in toluene, 2.2 ml, 4.2 mmol) was cooled to 0 °C in an ice-bath under argon. To this stirred solution was added a mixture of BUPOH (1.07 g, 4.2 mmol) and triethylamine (1.2 ml, 8.6 mmol) in 20.0 ml of methylene chloride. After stirring for 18 h, the excess phosgene and the solvents were removed in a stream of argon. The resulting residue was dissolved in methylene chloride and washed with water (2× 50 ml), then brine solution (20 ml). The organic phase was separated and dried over anhydrous sodium sulfate and reduced to a small volume under reduced pressure. The desired product was precipitated by adding excess *n*-pentane, filtered at the pump, and washed with cold *n*-pentane to afford 0.92 g (86% yield) of **23**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.38–7.28 (m, 4H), 3.92 (s, 2H), 2.42 (s, 3H), 2.24 (m, 1H), 1.57 (s, 6H) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz): δ 154.48, 134.91, 134.80, 130.09, 129.61, 128.21, 126.37, 124.41, 121.28, 121.28, 70.24, 62.24, 25.22, 13.47 ppm. LC–MS: m/z 282 (M^+), single peak at t_R = 3.13 min.

4.13. Hydrolysis studies on BUPOH:NTX and BUPOH:NTXOL codrugs

Methods. Standards were prepared in the concentration range 100–1000 ng/mL in Hanks' buffer. Equal amounts of the each of codrugs were distributed into labeled test-tubes (20 nmol in each tube). Care was taken to ensure that the entire codrug was dissolved and the solution remained clear. Hydrolysis of the carbonate codrug (~20 nmol) was conducted in isotonic phosphate buffer, pH 7.4, at 32 °C in a water-bath with continuous stirring. Samples were collected at predetermined time intervals and stored in the freezer at −20 °C until analyzed. All experiments were conducted in triplicate. An aliquot part (250 μL) of the hydrolysis solutions was mixed with 750 μL of acetonitrile, vortexed for 30 s,

and analyzed by HPLC for the presence of codrug and the two corresponding parent drugs, as well as for the intermediate hydrolysis product, **23**. Standards of codrug **25**, codrug **26**, NTX, 6-β-NTXOL, intermediate **23**, and BUPOH were prepared in isotonic phosphate buffer:acetonitrile (1:3, v/v) in the concentration range 0.1–2.0 μg/mL, and these solutions were used for the generation of the respective calibration curves. Sample recoveries were >95% for all the drugs, and data were corrected for the respective extraction efficiencies. A modification of the high-pressure liquid chromatographic (HPLC) assay reported by Hussain et al.[30] was used for the analysis of hydrolysis samples. The HPLC system consisted of a Waters (Milford, MA, USA) model 717 Autosampler, two model 1525 pumps, and a model 2487 dual wavelength UV absorbance detector with Millennium Chromatography software. A Brownlee C_{18} reversed-phase Spheri-5 μm column (220 × 4.6 mm) connected to a C_{18} reversed-phase guard column (15 × 3.2 mm) was utilized and detection of solutes was carried out at 215 nm. The mobile phase consisted of a mixture of acetonitrile:0.1% trifluoroacetic acid adjusted to pH 3.0 with triethylamine (50:50, v/v). The mobile phase flow rate was 1.5 mL/min and 100 μL of hydrolysis sample was injected onto the column. The retention times for each of the analytes were 14.60, 10.50, 2.86, 2.28, 5.20, and 4.56 min for codrug **25**, codrug **26**, NTX, NTXOL, intermediate **23**, and BUPOH, respectively.

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References and notes

1. Sinkula, A. A.; Yalkowsky, S. H. *J. Pharm. Sci.* **1975**, *64*, 181.
2. Stella, V. J. *Adv. Drug Delivery Rev.* **1996**, *19*, 111.
3. Fleischer, D.; Bong, D.; Stewart, B. H. *Adv. Drug Delivery Rev.* **1996**, *19*, 115.
4. Menger, F. C.; Rourk, M. J. *J. Org. Chem.* **1997**, *62*, 9083.
5. Leppänen, J.; Huuskonen, J.; Nevalainen, T.; Gynther, J.; Taipale, H.; Järvinen, T. *J. Med. Chem.* **2002**, *45*, 1379.
6. Di Stefano, A.; Sozio, P.; Cocco, A.; Iannitelli, A.; Santucci, E.; Costa, M.; Pecci, L.; Nasuti, C.; Cantalamessa, F.; Pinnen, F. *J. Med. Chem.* **2006**, *49*, 1486.
7. Jones, R. N.; Marshall, S. A.; Varnam, D. J. *Diagn. Microbiol. Infect. Dis.* **1998**, *32*, 85.
8. Bryskier, A. *Expert Opin. Invest. Drugs* **1997**, *6*, 1479.
9. Hartley, S.; Wise, R. *J. Antimicrob. Chemother.* **1982**, *10*, 49.
10. Baltzer, B.; Binderup, E.; Von Daehne, W.; Godtfredsen, W. O.; Hanson, K.; Nielson, B.; Sorensen, H.; Vangedal, S. *J. Antibiot.* **1980**, *33*, 1183.
11. Cynkowska, G.; Cynkowski, T.; Al-Ghananeem, A. M.; Guo, H.; Ashton, P.; Crooks, P. A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3524.
12. Howard-Sparks, M.; Al-Ghananeem, M. A.; Pearson, A. P.; Crooks, P. A. *J. Enz. Inhibit. Med. Chem.* **2005**, *20*, 417.
13. Mello, N. K.; Mendelson, J. H.; Palmieri, S. L. *Psychopharmacology (Berl.)* **1987**, *93*, 8.
14. Kozlowski, L. T.; Henningfield, J. E.; Keenan, R. M.; Lei, H.; Leigh, G.; Jelinek, L. C.; Pope, M. A.; Haertzen, C. A. *J. Subst. Abuse Treat.* **1993**, *10*, 171.
15. Mitchell, S. H.; Dewit, H.; Zacny, J. P. *Behav. Pharmacol.* **1995**, *6*, 359.
16. Glautier, S.; Clements, K.; White, J.; Taylor, C.; Stolerman, I. P. . *Pharmacology* **1996**, *7*, 144.
17. Smith, B. R.; Horan, J. T.; Gaskin, S.; Amit, Z. *Psychopharmacology (Berl.)* **1999**, *142*, 408.
18. Watson, W. P.; Little, H. J. *Neuropharmacology* **1999**, *38*, 587.
19. Söderpalm, B.; Ericson, M.; Olausson, P.; Blomqvist, O.; Engel, J. A. *Behav. Brain Res.* **2000**, *113*, 85.
20. Keenan, R. M.; Hatsukami, D. K.; Pickens, R. W.; Gust, S. W.; Strelow, L. J. *Psychopharmacology (Berl.)* **1990**, *100*, 77.
21. Hurt, R. D.; Offord, K. P.; Croghan, I. T.; Gomez-Dahl, L.; Kottke, T. E.; Morse, R. M.; Melton, L. J. *J. Am. Med. Assoc.* **1996**, *275*, 1097.
22. Frosch, D. L.; Shoptaw, S.; Nahom, D.; Jarvik, M. E. *Clin. Psychopharmacol. (Berl.)* **2000**, *8*, 97.
23. Volpicelli, J. R.; Alterman, A. I.; Hayashida, M. *Arch. Gen. Psychiatry* **1992**, *49*, 876.
24. Rukstalis, M. R.; Stromberg, M. F.; O'Brien, C. P.; Volpicelli, J. R. *Alcoholism: Clin. Exp. Res.* **2000**, *24*, 1593.
25. Wang, D.; Raehal, K. M.; Bilsky, E. J.; Sadee, W. *J. Neurochem.* **2001**, *77*, 1590.
26. Cooper, B. R.; Wang, C. M.; Cox, R. F.; Norton, R.; Shea, V.; Ferris, R. M. *Neuropsychopharmacology* **1994**, *11*, 133.
27. Ascher, J. A.; Cole, J. O.; Colin, J. N.; Feighner, J. P.; Ferris, R. M.; Fibiger, H. C.; Golden, R. N.; Martin, P.; Potter, W. Z.; Richelson, E. *J. Clin. Psychiatry* **1995**, *56*, 395.
28. Sanchez, C.; Hyttel, J. *Cell. Mol. Neurobiol.* **1999**, *19*, 467.
29. Slemmer, J. E.; Martin, B. R.; Damaj, M. I. *J. Pharmocol. Exp. Ther.* **2000**, *295*, 321.
30. Hussain, M. A.; Koval, C. A.; Myers, M. J.; Shami, E. G.; Shefter, E. I. *J. Pharm. Sci.* **1987**, *76*, 356.
31. Olsen, L. D.; Klein, P.; Nelson, W. L.; Yao, Y. H.; Simon, E. *J. Med. Chem.* **1990**, *33*, 737.
32. De Costa, B. R.; Ladarola, M. J.; Rothman, R. B.; Berman, K. F.; George, C.; Newman, A. H.; Mahoubi, A.; Jacobson, A. E.; Rice, K. C. *J. Med. Chem.* **1992**, *35*, 2826.
33. Hammell, D. C.; Hamad, M. O.; Vaddi, H. K.; Crooks, P. A.; Stinchcomb, A. L. *J. Controlled Release* **2004**, *97*, 283.
34. Kiptoo, P.; Hammell, D. C.; Paudel, K.; Hamad, M. O.; Crooks, P. A.; Stinchcomb, A. L. In vivo evaluation of a transdermal codrug of 6-β-naltrexol linked to hydroxybupropion in hairless guinea pigs (manuscript in preparation).
35. Cooper, G. D.; Williams, B. *J. Org. Chem.* **1962**, *27*, 3717.
36. Fife, T. H.; Hutchins, J. E. C. *J. Am. Chem. Soc.* **1981**, *103*, 4194.
37. Ellaithy, M. M.; El-Ragehy, N. A.; El-Ghobashy, M. A. *Farmaco* **2003**, *58*, 337.
38. Szeto, S. Y.; Burlinson, N. E.; Rettig, S. J.; Trotter, J. *J. Agric. Food Chem.* **1989**, *37*, 1103.
39. Szeto, S. Y.; Burlinson, N. E.; Rahe, J. E.; Oloffs, P. C. *J. Agric. Food Chem.* **1989**, *37*, 523.
40. Pothuluri, J. V.; Freeman, J. P.; Heinze, T. M.; Beger, R. D.; Cerniglia, C. E. *J. Agric. Food Chem.* **2000**, *48*, 6138.
41. Jain, N.; Yalkowsky, S. H. *J. Pharm. Sci.* **2001**, *90*, 234.
42. Chatterjie, N.; Inturrisi, C. E.; Dayton, H. B.; Blumberg, H. *J. Med. Chem.* **1975**, *18*, 490.
43. Perrine, D. M.; Ross, J. T.; Nervi, S. T.; Zimmerman, R. H. *J. Chem. Educat.* **2000**, *77*, 1479.
44. Mehta, N. B. U.S. Patent 3,819,706, 1974.
45. Morgan, P. F.; Musso, D. L.; Partridge, J. J. U.S. Patent Appl. Publ. **2003**, U.S. 20030064988 A1, April 3rd.