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The effect of substitution patterns on the release rates of opioid peptides DADLE and [Leu^5]-enkephalin from coumarin prodrug moieties

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Abstract

A coumarin-based prodrug system has been developed in our laboratory for the preparation of esterase-sensitive prodrugs of amines, peptides, and peptidomimetics. The drug release rates from this prodrug system were found to be dependent on the structural features of the drug moiety. The effect of the phenyl ring substitutions on the release kinetics of such prodrugs of model amines was examined recently and it was found that appropriately positioned alkyl substituents on the phenyl ring could help to facilitate the release. Aimed at further understanding the structure–release rate relationship of the coumarin-based cyclic prodrugs, we synthesized and examined a series of substituted coumarinic acid derivatives of opioid peptides, DADLE, and [Leu^5]-enkephalin.

Keywords: Coumarin; Cyclic prodrug; Opioid peptide; Esterase; Structural effect

1. Introduction

Many biologically active compounds do not make for good pharmaceutical candidates due to their lack of permeability across biological barriers such as the blood

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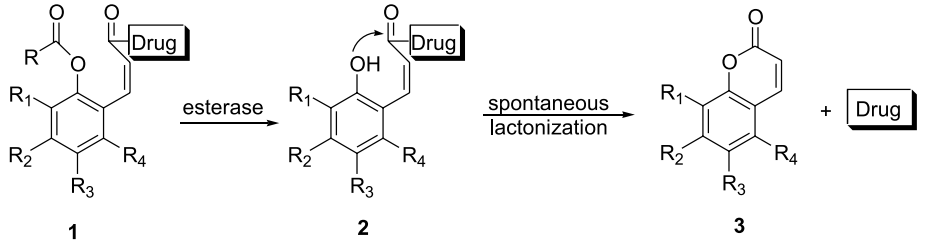
brain barrier or the intestinal mucosa [1,2]. The lack of permeability of these compounds can be caused by such undesirable physicochemical properties as charge, hydrogen bonding potential, and hydrophilicity. Temporarily masking these undesirable traits is one approach to facilitate drug transport across biological barriers. Prodrugs [3] are utilized for the purpose of aiding the facilitation of drug permeation across biological barriers by optimizing the parent drug's physicochemical properties. Modification of these adverse properties, either by enhancing the chemical stability, altering the aqueous solubility, or improving upon the bioavailability, all while maintaining the inherent pharmacological properties of the parent drug, allows for increased membrane permeability across biological barriers.

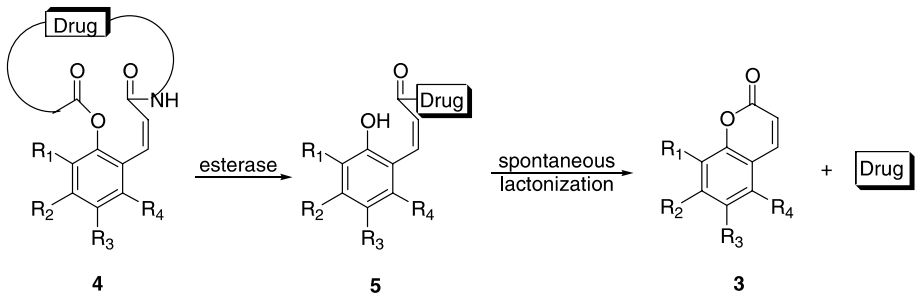
An innovative coumarin-based prodrug system has been developed in our laboratory for the preparation of esterase-sensitive prodrugs of amines [4–8], peptides [9–11], and peptidomimetics [12–14]. This design takes advantage of the facile lactonization of coumarinic acid and its derivatives (Scheme 1). In such a strategy, a latent nucleophile can be unmasked using an esterase triggering mechanism that initiates a lactonization reaction to release the parent drug. Using this prodrug strategy, we have prepared esterase-sensitive prodrugs of model amines [4–8], cyclic prodrugs (Scheme 2) of opioid peptides [9–11], and peptidomimetic glycoprotein IIb/IIIa antagonists [12–14] with greatly improved membrane permeability.

With the demonstration of the feasibility of this prodrug system, it became important for us to address one practical issue, i.e., the ability to fine-tune the release rates so that the release time profile can be modulated to suit the need of individual drugs. In our earlier studies, we have found that the release rates of the coumarin-based prodrugs are largely dictated by the rate of lactonization, which in turn depends on the structural features of the drug moieties [4,6]. The release rates are affected by the pK_a and the steric features of the amines to be released. Ideally, one would like to be able to modulate the release rates independent of the structural properties of the drug moiety. To address this problem, we have examined the effect of the phenyl ring substituents on the release rates of esterase-sensitive prodrugs of model amines and found that introduction of methyl substitutions *ortho* to the alkyl side chain and the phenol hydroxyl group results in significant increases in release rates [7,8].

Opioid peptides are potential candidates for further development as therapeutic agents [15,16]. However, in many cases the clinical development of these opioid peptides has been hindered due to their metabolic lability and poor cell mem-



Scheme 1. A coumarin-based esterase-sensitive prodrug system.



Scheme 2. A coumarin-based cyclic prodrug system.

brane permeation characteristics [17]. Potentially the unfavorable biopharmaceutical properties of opioid peptides could be transiently modified using prodrug strategies [3]. Recently, our laboratories have prepared cyclic prodrugs of peptides [9–11] as a way to modify the physicochemical properties of the molecules sufficiently to overcome these biopharmaceutical barriers. Aimed at further understanding the structure–release rate relationship of the coumarin-based cyclic prodrugs, we synthesized a series of substituted coumarinic acid derivatives and the release rates of these prodrugs were examined to investigate the substitution effect.

2. Experimental

2.1. General methods

All ^{1}H and ^{13}C NMR spectra were recorded at 300 or 400 MHz with TMS as the internal standard. Column chromatography was performed using silica gel (200–400 mesh) from Aldrich. Commercially available starting materials and reagents were purchased from Aldrich. Amino acids were purchased from Novabiochem. Tetrahydrofuran (THF) was distilled from sodium and benzophenone; dichloromethane (DCM) was distilled from CaH_2. A Shimadzu 1601 UV–Vis spectrophotometer was used for the esterase kinetic studies. Atlantic Microlab, Norcross, Georgia performed elemental analyses. The Mass Spectroscopy Laboratories at both University of Kansas and North Carolina State University carried out mass spectral analyses. Compounds **3a** [18], **7a–b** [8], and **8a–b** [8] were prepared as reported in the literature. The tetrapeptides **17** [11] and **18** [11] were synthesized following the standard peptide synthesis procedure as reported previously.

2.2. Purified esterase kinetic studies

The esterase kinetic studies were carried out following procedures reported earlier using PLE [4,6,9].

2.3. 1-N-Boc-D-leucyl-2-[(Z)-3-(1-tert-butyl-1,1-dimethylsilyloxy)-1-propenyl]-3,6-dimethylphenol (**9a**)

To a solution of Boc-D-Leu-OH hydrate (1.37 g, 5.50 mmol) in dry dichloromethane (50 mL) was added *N*,*N*′-dicyclohexylcarbodiimide (1.16 g, 5.62 mmol) at room temperature under N_2 atmosphere. The reaction mixture was then put into an ice bath (0 °C). To this cold solution was added **8a** (1.34 g, 4.58 mmol) in 25 mL of dry dichloromethane and DMAP (0.29 g, 2.37 mmol). After stirring for 1 h, the reaction was taken out of the ice bath and stirred overnight at room temperature. Dichloromethane (100 mL) was added to the residue, which was washed with 1 N HCl (2 × 40 mL), 5% $NaHCO_3$ (35 mL), and water (35 mL). The organic layer was then dried over $MgSO_4$, filtered, and evaporated to afford the crude product. The residue was purified by column chromatography (2:1 hexane/dichloromethane) to give **9a** as a white solid (1.96 g, 85%). ^{1}H NMR ($CDCl_3$) δ 0.03 (6H, s), 0.86 (9H, s), 0.99 (6H, d, $J = 6.1$ Hz), 1.26 (1H, m), 1.46 (9H, s), 1.75 (2H, m), 2.13 (3H, s), 2.19 (3H, s), 4.00 (2H, d, $J = 4.9$ Hz), 4.48 (1H, m), 5.11 (1H, brs), 5.86 (1H, m), 6.11 (1H, d, $J = 11.3$ Hz), 6.98 (1H, d, $J = 7.8$ Hz), 7.04 (1H, d, $J = 7.8$ Hz). MS (FAB) m/z 506.3 ($M^+ + H$). *Anal*. Calcd for $C_{28}H_{47}NO_5Si$: C, 65.95; H, 9.22; N, 2.85. Found: C, 65.82; H, 9.26; N, 2.79.

2.4. 1-N-Boc-L-leucyl-2-[(Z)-3-(1-tert-butyl-1,1-dimethylsilyloxy)-1-propenyl]-3,6-dimethylphenol (**10a**)

In a manner similar to the preparation of **9a**, Boc-L-Leu-OH hydrate (0.88 g, 3.52 mmol, in 35 mL dry DCM), DCC (1.11 g, 5.38 mmol), **8a** (0.93 g, 3.18 mmol, in 20 mL dry DCM), and DMAP (0.20 g, 1.62 mmol) were treated to afford **10a** as a clear oil (1.52 g, 95%). ^{1}H NMR ($CDCl_3$) δ 0.00 (6H, s), 0.86 (9H, s), 0.99 (6H, d, $J = 6.3$ Hz), 1.46 (9H, s), 1.64 (1H, m), 1.77 (2H, m), 2.13 (3H, s), 2.19 (3H, s), 4.00 (2H, d, $J = 5.1$ Hz), 4.45–4.53 (1H, m), 5.12 (1H, brs), 5.82–5.90 (1H, m), 6.12 (1H, d, $J = 11.1$ Hz), 7.01 (2H, dd, $J_1 = 7.8$ Hz, $J_2 = 18.9$ Hz).

2.5. 1-N-Boc-D-leucyl-2-[(Z)-3-hydroxy-1-propenyl]-3,6-dimethylphenol (**11a**)

To a solution of **9a** (1.94 g, 3.84 mmol) in THF (30 mL) was added water (30 mL), and then followed by addition of acetic acid (90 mL) dropwise. The mixture was stirred at room temperature for 3 h and then evaporated to remove THF, water, and acetic acid. Ethyl acetate (100 mL) was added to the residue, which was washed with 5% $NaHCO_3$ (3 × 40 mL), and water (2 × 40 mL). The ethyl acetate layer was dried over $MgSO_4$, concentrated, and purified by column chromatography (6:1 hexane/ethyl acetate) to afford **11a** as light yellow oil (1.38 g, 91%). ^{1}H NMR ($CDCl_3$) δ 1.00 (6H, d, $J = 3.9$ Hz), 1.45 (9H, s), 1.60 (1H, m), 1.81 (2H, m), 2.04 (3H, s), 2.13 (3H, s), 3.93 (2H, d, $J = 5.9$ Hz), 4.49 (1H, m), 4.95 (1H, d, $J = 8.3$ Hz), 5.96 (1H, m), 6.22 (1H, d, $J = 11.2$ Hz), 6.98 (1H, d, $J = 7.7$ Hz), 7.05 (1H, d, $J = 7.2$ Hz). MS (FAB) m/z 392.2 ($M^+ + H$). *Anal*. Calcd for $C_{22}H_{33}NO_5$: C, 67.49; H, 8.50; N, 3.58. Found: C, 67.54; H, 8.61; N, 3.52.

2.6. 1-N-Boc-L-leucyl-2-[(Z)-3-hydroxy-1-propenyl]-3,6-dimethylphenol (**12a**)

In a manner similar to the preparation of **11a**, **10a** (1.52 g, 3.01 mmol, in 30 mL THF), water (30 mL), and acetic acid (90 mL) were reacted to give **12a** as a light yellow oil (1.12 g, 95%). ^{1}H NMR ($CDCl_3$) δ 1.00 (6H, d, $J = 5.6$ Hz), 1.45 (9H, s), 1.63 (1H, m), 1.79 (2H, m), 2.14 (6H, d, $J = 5.6$ Hz), 3.93 (2H, d), 4.49 (1H, m), 4.96 (1H, m), 5.97 (1H, m), 6.23 (1H, d, $J = 11.6$ Hz), 7.02 (2H, dd, $J_1 = 7.6$ Hz, $J_2 = 23$ Hz).

2.7. 1-N-Boc-D-leucyl-3,6-dimethyl-2-[(Z)-3-oxo-1-propenyl]phenol (**13a**)

To a solution of **11a** (1.36 g, 3.34 mmol) in dry dichloromethane (50 mL) was added in one portion 85% activated manganese (IV) oxide (1.16 g, 11.6 mmol), which was dried with a drying tube containing anhydrous $CaSO_4$. Then every 1 h manganese (IV) oxide (1.16 g, 11.6 mmol) was added in 1 portion for a period of 5 h. The reaction mixture was filtered through a Celite plug, washed with dichloromethane, and evaporated to afford **13a** as yellow oil (1.20 g, 89%). ^{1}H NMR ($CDCl_3$) δ 0.97 (6H, d, $J = 8.3$ Hz), 1.44 (9H, s), 1.52–1.64 (1H, m), 1.71–1.79 (2H, m), 2.16 (3H, s), 2.24 (3H, s), 4.45 (1H, m), 4.78 (1H, d, $J = 8.6$ Hz), 6.15–6.22 (1H, m), 7.05 (1H, d, $J = 7.8$ Hz), 7.15 (1H, d, $J = 7.8$ Hz), 7.34 (1H, d, $J = 11.3$ Hz), 9.49 (1H, d, $J = 8.3$ Hz). MS (FAB) m/z 390.3 ($M^+ + H$). *Anal.* Calcd for $C_{22}H_{31}NO_5$: C, 67.84; H, 8.02; N, 3.60. Found: C, 67.91; H, 8.08; N, 3.65.

2.8. 1-N-Boc-L-leucyl-3,6-dimethyl-2-[(Z)-3-oxo-1-propenyl]phenol (**14a**)

In a manner similar to the preparation of **13a**, **12a** (1.12 g, 2.86 mmol, in 45 mL dry DCM), and 85% activated manganese (IV) oxide (1.00 g × 6, 9.8 mmol × 6) were reacted to afford **14a** as a yellow crystal (0.90 g, 81%). ^{1}H NMR ($CDCl_3$) δ 0.97 (6H, dd, $J_1 = 2.4$ Hz, $J_2 = 6.4$ Hz), 1.44 (9H, s), 1.61 (1H, s), 1.77 (2H, m), 2.16 (3H, s), 2.23 (3H, s), 4.43 (1H, m), 4.78 (1H, d, $J = 8.4$ Hz), 6.18 (1H, m), 7.05 (1H, d, $J = 8.0$ Hz), 7.15 (1H, d, $J = 7.6$ Hz), 7.34 (1H, d, $J = 10.8$ Hz), 9.48 (1H, d, $J = 8.0$ Hz).

2.9. (Z)-3-[2-(1-N-Boc-D-leucyl)-3,6-dimethylphenyl]-2-propenonic acid (**15a**)

A solution of 80% sodium chlorite (0.27 g, 3.0 mmol) in 3.9 mL water was added dropwise over 2 h to a stirred solution of **13a** (0.45 g, 1.17 mmol) in 1.8 mL acetonitrile, sodium phosphate (54 mg) in 1.8 mL water, and 30% hydrogen peroxide (0.18 mL), while its temperature was kept at 10 °C with an ice-water bath. The reaction mixture was then stirred at room temperature (~2 h) until there was no more oxygen evolution seen (monitored with a bubbler). A small amount of sodium thiosulfite was added to destroy the unreacted HOCl and H_2O_2. The solution was acidified with 1 N HCl to pH 1–2. The mixture was extracted with ethyl acetate (60 mL). The ethyl acetate layer was then washed with saturated NaCl (2 × 60 mL) and dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography (20:1 DCM/MeOH) to afford **15a** as a white solid (0.46 g, 97%). ^{1}H NMR ($CDCl_3$) δ 0.99 (6H, d, $J = 5.9$ Hz), 1.45 (9H, s), 1.59 (1H, m),

1.71–1.82 (2H, m), 2.12 (3H, s), 2.22 (3H, s), 4.50 (1H, m), 4.93 (1H, d, $J = 8.9$ Hz), 6.13 (1H, d, $J = 12.2$ Hz), 6.82 (1H, d, $J = 12.0$ Hz), 7.01 (1H, d, $J = 7.7$ Hz), and 7.08 (1H, d, $J = 7.8$ Hz). MS (FAB) m/z 406.3 ($M^+ + H$). *Anal.* Calcd for $C_{22}H_{31}NO_6$: C, 65.17; H, 7.71; N, 3.45. Found: C, 65.21; H, 7.67; N, 3.40.

2.10. (Z)-3-[2-(1-N-Boc-L-leucyl)-3,6-dimethylphenyl]-2-propenonic acid (**16a**)

In a manner similar to the preparation of **15a**, 80% sodium chlorite (0.46 g, 5.11 mmol) in 6.8 mL water, **14a** (0.90 g, 2.31 mmol, in 3.2 mL acetonitrile), sodium phosphate (94 mg) in 3.2 mL water, and 30% hydrogen peroxide (0.35 mL) were reacted to give **16a** as light yellow solid (0.78 g, 83%). ^{1}H NMR ($CDCl_3$) δ 0.99 (6H, dd, $J_1 = 1.5$ Hz, $J_2 = 6.3$ Hz), 1.46 (9H, s), 1.60 (1H, m), 1.77 (2H, m), 2.17 (3H, s), 2.23 (3H, s), 4.51 (1H, m), 4.92 (1H, d, $J = 9.3$ Hz), 6.13 (1H, d, $J = 12.3$ Hz), 6.81 (1H, d, $J = 12.0$ Hz), 7.01 (1H, d, $J = 7.8$ Hz), 7.09 (1H, d, $J = 7.5$ Hz).

2.11. Linear precursor (**19a**)

Under N_2 atmosphere, an ice-cold (0 °C) solution of compound **15a** (0.59 g, 1.43 mmol) in 12 mL of dry THF was reacted with DCC (0.85 g, 4.12 mmol). After 5 min, HOBt (0.37 g, 2.74 mmol) was added to the reaction mixture. After 20 min, a solution of H_2N-Tyr-D-Ala-Gly-Phe-OtBu **17** (0.81 g, 1.57 mmol) in 20 mL dry THF was added, followed by the addition of DMAP (0.06 g, 0.49 mmol). After the solution stirred at 0 °C for 1 h, the mixture was taken out of the ice bath and allowed to stir at room temperature for 3 h. The resulting mixture was dissolved in 100 mL ethyl acetate. The ethyl acetate solution was extracted with 10% citric acid (2×30 mL), 5% $NaHCO_3$ (2×30 mL), and saturated NaCl (2×30 mL). The ethyl acetate layer was dried and concentrated and the residue was purified by gradient column chromatography (60:1 DCM/MeOH to 20:1 DCM/MeOH) to afford **19a** as a white solid (0.73 g, 57%). ^{1}H NMR (CD_3OD) δ 0.99 (6H, dd, $J_1 = 6.3$ Hz, $J_2 = 8.1$ Hz), 1.10 (3H, d, $J = 7.2$ Hz), 1.36 (9H, s), 1.46 (9H, s), 1.71 (1H, m), 1.80 (2H, m), 2.08 (3H, s), 2.14 (3H, s), 2.81 (2H, m), 3.02 (2H, d, $J = 6.9$ Hz), 3.64 (1H, m), 3.82 (2H, m), 4.17 (2H, m), 4.37 (1H, m), 4.51 (1H, m), 6.20 (1H, d, $J = 11.7$ Hz), 6.62–6.69 (3H, m), 6.92–6.98 (3H, m), 7.06 (1H, d, $J = 8.1$ Hz), 7.21–7.27 (6H, m). MS (FAB) m/z 900.5 ($M^+ + H$). *Anal.* Calcd for $C_{49}H_{65}N_5O_{11}$: C, 65.39; H, 7.28; N, 7.78. Found: C, 65.12; H, 7.36; N, 7.72.

2.12. Linear precursor (**20a**)

In a manner similar to the preparation of **19a**, **16a** (0.77 g, 1.92 mmol, in 16 mL dry THF), DCC (1.13 g, 5.47 mmol), HOBt (0.48 g, 3.55 mmol), H_2N-Tyr-Gly-Gly-Phe-OtBu (**18**, 1.05 g, 2.12 mmol, in 30 mL dry THF), and DMAP (0.08 g, 0.66 mmol) were reacted to afford **20a** as a yellow oil (0.98 g, 58%). ^{1}H NMR (CD_3OD) δ 0.99 (6H, dd, $J_1 = 6.6$ Hz, $J_2 = 10.8$ Hz), 1.37 (9H, s), 1.45 (9H, s), 1.70 (1H, m), 1.81 (2H, m), 2.08 (3H, s), 2.13 (3H, s), 2.85 (2H, m), 3.03 (2H, m), 3.59 (1H, m), 3.75–3.93 (2H, m), 4.29 (1H, m), 4.38 (1H, m), 4.53 (2H, m), 6.15

(1H, d, $J = 12.0$ Hz), 6.65 (2H, d, $J = 8.1$ Hz), 6.71 (1H, d, $J = 8.4$ Hz), 6.90–6.99 (3H, m), 7.08 (2H, t, $J = 5.1$ Hz), 7.21–7.27 (5H, m).

2.13. Cyclic prodrug of DADLE (**21a**)

Compound **19a** (0.27 g, 0.30 mmol) was treated with a solution of 1:1 TFA:DCM (45 mL) under N_2 atmosphere for 5 h at room temperature. The mixture was concentrated and dried under vacuum pump for 1 h. The residue was then dissolved in 200 mL dry DCM and 4.2 mL DMF. Bop-Cl (0.72 g, 2.83 mmol) and 0.52 mL TEA were added into the solution and the reaction was stirred for 18.5 h at room temperature. The reaction mixture was concentrated, dissolved in ethyl acetate (100 mL), and extracted with water (30 mL), 10% citric acid (30 mL), 5% $NaHCO_3$ (30 mL), and water (3×30 mL). The ethyl acetate layer was then dried and concentrated to afford crude product. Purification by column chromatography (20:1 DCM/MeOH) yielded final product **21a** as a white solid (55 mg, 25%). ^{1}H NMR (CD_3OD) δ 1.02 (6H, dd, $J_1 = 6.8$ Hz, $J_2 = 18.0$ Hz), 1.12 (3H, d, $J = 7.2$ Hz), 1.72 (2H, m), 1.86 (1H, m), 2.19 (3H, s), 2.22 (3H, s), 3.21 (2H, m), 3.34 (1H, d, $J = 2.0$ Hz), 3.39 (1H, s), 3.49 (1H, m), 3.84 (1H, d, $J = 15.6$ Hz), 4.09 (1H, m), 4.19 (1H, d, $J = 6.8$ Hz), 4.39 (1H, m), 4.49 (1H, m), 6.19 (1H, m), 6.67 (1H, m), 6.79 (2H, t, $J = 12.4$ Hz), 6.89 (1H, d, $J = 8.4$ Hz), 7.04 (1H, brs), 7.14–7.32 (7H, m). MS (FAB) m/z 726.3 ($M^+ + H$). *Anal.* Calcd for $C_{40}H_{47}N_5O_8$: C, 66.19; H, 6.53; N, 9.65. Found: C, 66.40; H, 6.52; N, 9.57.

2.14. Cyclic prodrug of Leu-enkephalin (**22a**)

In a manner similar to the preparation of **21a**, **20a** (0.26 g, 0.29 mmol) was treated with a solution of 1:1 TFA:DCM (45 mL) under N_2 atmosphere for 3.5 h at room temperature. Upon concentration, the residue (in 200 mL dry DCM and 4.2 mL DMF), Bop-Cl (0.69 g, 2.69 mmol), and 0.5 mL TEA were reacted to afford **22a** as a white solid after column chromatography followed by recrystallization (0.028 g, 14%). ^{1}H NMR (CD_3OD) δ 1.01 (6H, dd, $J_1 = 6.0$ Hz, $J_2 = 18.4$ Hz), 1.77 (2H, m), 1.90 (1H, m), 2.12 (3H, s), 2.22 (3H, s), 2.52 (1H, m), 2.99 (1H, m), 3.13 (1H, m), 3.25 (1H, m), 3.41 (1H, d, $J = 3.2$ Hz), 3.83 (2H, m), 4.32 (2H, m), 4.48 (1H, q, $J_1 = 6.4$ Hz, $J_2 = 9.2$ Hz), 4.78 (1H, m), 6.21 (1H, d, $J = 12.0$ Hz), 6.68 (1H, m), 6.72 (1H, d, $J = 12.0$ Hz), 6.93–7.03 (3H, m), 7.06 (1H, d, $J = 8.0$ Hz), 7.13 (1H, d, $J = 8.0$ Hz), 7.21–7.30 (6H, m).

2.15. 1-N-Boc-D-leucyl-2-[(Z)-3-(1-tert-butyl-1,1-dimethylsilyloxy)-1-propenyl]-4-methylphenol (**9b**)

In a manner similar to the preparation of **9a**, Boc-D-Leu-OH hydrate (0.55 g, 2.2 mmol, in 30 mL dry DCM), DCC (0.90 g, 4.4 mmol), **8b** (0.50 g, 1.8 mmol, in 10 mL dry DCM), and DMAP (0.11 g, 0.90 mmol) were treated to afford **9b** as a clear oil (0.69 g, 79%). ^{1}H NMR ($CDCl_3$) δ 0.04 (6H, s), 0.89 (9H, s), 1.00 (6H, d, $J = 6.4$ Hz), 1.46 (9H, s), 1.62 (1H, m), 1.78 (2H, m), 2.34 (3H, s), 4.27 (2H, dd, $J_1 = 1.6$ Hz, $J_2 = 6.4$ Hz), 4.51 (1H, m), 4.96 (1H, d, $J = 8.8$ Hz), 5.86 (1H, m), 6.34 (1H, d, $J = 12.0$ Hz), 6.94 (1H, d, $J = 8.0$ Hz), 7.03 (1H, s), 7.08 (1H, d, $J = 8.4$ Hz).

2.16. 1-N-Boc-L-leucyl-2-[(Z)-3-(1-tert-butyl-1,1-dimethylsilyloxy)-1-propenyl]-4-methylphenol (**10b**)

In a manner similar to the preparation of **9a**, Boc-L-Leu-OH hydrate (0.55 g, 2.2 mmol, in 30 mL dry DCM), DCC (0.90 g, 4.4 mmol), **8b** (0.44 g, 1.6 mmol, in 10 mL dry DCM), and DMAP (0.11 g, 0.90 mmol) were treated to afford **10b** as a clear oil (0.64 g, 83%). ^{1}H NMR ($CDCl_3$) δ 0.04 (6H, s), 0.89 (9H, s), 1.00 (6H, d, $J = 6.8$ Hz), 1.46 (9H, s), 1.62 (1H, m), 1.78 (2H, m), 2.36 (3H, s), 4.27 (2H, dd, $J_1 = 1.6$ Hz, $J_2 = 6.4$ Hz), 4.51 (1H, m), 4.96 (1H, d, $J = 8.4$ Hz), 5.86 (1H, m), 6.35 (1H, d, $J = 11.6$ Hz), 6.94 (1H, d, $J = 8.0$ Hz), 7.03 (1H, s), 7.08 (1H, d, $J = 8.0$ Hz). ^{13}C NMR ($CDCl_3$) δ 18.50, 21.15, 22.03, 23.12, 25.08, 26.12, 28.54, 41.83, 52.49, 60.40, 80.15, 121.92, 124.48, 129.28, 130.98, 133.97, 135.67, 146.11, 155.67, 172.06. MS (FAB) m/z 492.3 ($M^+ + H$). *Anal.* Calcd for $C_{27}H_{45}NO_5Si$: C, 65.95; H, 9.22; N, 2.85. Found: C, 65.95; H, 9.32; N, 2.96.

2.17. 1-N-Boc-D-leucyl-2-[(Z)-3-hydroxy-1-propenyl]-4-methylphenol (**11b**)

In a manner similar to the preparation of **11a**, **9b** (0.62 g, 1.26 mmol, in 15 mL THF), water (15 mL), and acetic acid (45 mL) were reacted to give **11b** as yellow oil (0.47 g, 99%). ^{1}H NMR ($CDCl_3$) δ 1.00 (6H, d, $J = 5.7$ Hz), 1.46 (9H, s), 1.61 (1H, m), 1.75 (2H, m), 2.34 (3H, s), 4.20 (2H, d, $J = 6.4$ Hz) 4.48 (1H, m), 4.94 (1H, d, $J = 8.1$ Hz), 5.94 (1H, m), 6.40 (1H, d, $J = 11.3$ Hz), 6.92 (1H, s), 6.95 (1H, d, $J = 5.4$ Hz), 7.10 (1H, d, $J = 8.3$ Hz). ^{13}C NMR ($CDCl_3$) δ 21.09, 21.96, 23.14, 25.08, 28.50, 41.48, 52.46, 59.67, 80.36, 121.78, 125.67, 128.73, 129.39, 131.11, 133.57, 135.90, 145.93, 155.78, 172.46. MS (FAB) m/z 378.3 ($M^+ + H$). *Anal.* Calcd for $C_{21}H_{31}NO_5$: C, 66.82; H, 8.28; N, 3.71. Found: C, 66.44; H, 7.94; N, 3.54.

2.18. 1-N-Boc-L-leucyl-2-[(Z)-3-hydroxy-1-propenyl]-4-methylphenol (**12b**)

In a manner similar to the preparation of **11a**, **10b** (0.57 g, 1.16 mmol, in 13.5 mL THF), water (13.5 mL), and acetic acid (40 mL) were reacted to give **12b** as a light yellow oil (0.37 g, 85%). ^{1}H NMR ($CDCl_3$) δ 1.00 (6H, d, $J = 5.8$ Hz), 1.46 (9H, s), 1.61 (1H, m), 1.79 (2H, m), 2.34 (3H, s), 4.20 (2H, d, $J = 6.1$ Hz) 4.48 (1H, m), 4.93 (1H, d, $J = 8.4$ Hz), 5.96 (1H, m), 6.40 (1H, d, $J = 11.5$ Hz), 6.92 (1H, s), 6.95 (1H, d, $J = 5.4$ Hz), 7.10 (1H, d, $J = 8.1$ Hz).

2.19. 1-N-Boc-D-leucyl-4-methyl-2-[(Z)-3-oxo-1-propenyl]phenol (**13b**)

In a manner similar to the preparation of **13a**, **11b** (0.40 g, 1.06 mmol, in 15 mL dry DCM) and 85% activated manganese (IV) oxide (0.40 g × 6, 4.0 mmol × 6) were treated to afford **13b** as a light yellow oil (0.35 g, 88%). ^{1}H NMR ($CDCl_3$) δ 1.00 (6H, dd, $J_1 = 2.4$ Hz, $J_2 = 6.0$ Hz), 1.45 (9H, s), 1.61 (1H, m), 1.78 (2H, m), 2.37 (3H, s), 4.48 (1H, m), 4.86 (1H, d, $J = 8.0$ Hz), 6.19 (1H, m), 7.03 (1H, d, $J = 8.0$ Hz), 7.16 (1H, s), 7.23 (1H, m), 7.54 (1H, d, $J = 11.6$ Hz), 9.82 (1H, d, $J = 8.0$ Hz).

2.20. 1-N-Boc-L-leucyl-4-methyl-2-[(Z)-3-oxo-1-propenyl]phenol (**14b**)

In a manner similar to the preparation of **13a**, **12b** (0.31 g, 0.83 mmol, in 15 mL dry DCM) and 85% activated manganese (IV) oxide (0.30 g × 6, 3.0 mmol × 6) were reacted to afford **14b** as a light yellow oil (0.25 g, 82%). ^{1}H NMR ($CDCl_3$) δ 1.00 (6H, dd, $J_1 = 2.0$ Hz, $J_2 = 6.0$ Hz), 1.45 (9H, s), 1.61 (1H, m), 1.78 (2H, m), 2.37 (3H, s), 4.48 (1H, m), 4.86 (1H, d, $J = 7.6$ Hz), 6.18 (1H, m), 7.03 (1H, d, $J = 8.0$ Hz), 7.16 (1H, s), 7.23 (1H, m), 7.54 (1H, d, $J = 11.2$ Hz), 9.82 (1H, d, $J = 8.0$ Hz). ^{13}C NMR ($CDCl_3$) δ 20.99, 21.99, 23.09, 25.15, 28.52, 41.45, 52.67, 80.43, 116.84, 122.48, 127.24, 131.69, 131.87, 132.30, 136.22, 143.49, 146.59, 155.68, 171.96, 192.69. MS (FAB) m/z 376.2 ($M^+ + H$). *Anal.* Calcd for $C_{21}H_{29}NO_5$: C, 67.18; H, 7.79; N, 3.73. Found: C, 67.40; H, 7.76; N, 3.63.

2.21. (Z)-3-[2-(1-N-Boc-D-leucyl)-4-methylphenyl]-2-propenonic acid (**15b**)

In a manner similar to the prepatation of **15a**, 80% sodium chlorite (0.19 g, 2.10 mmol) in 2.6 mL water, **13b** (0.35 g, 0.93 mmol, in 1.2 mL acetonitrile), sodium phosphate (36 mg) in 1.2 mL water, and 30% hydrogen peroxide (0.12 mL) were reacted to give **15b** as a yellow oil (0.27 g, 75%). ^{1}H NMR ($CDCl_3$) δ 0.99 (6H, d, $J = 4.8$ Hz), 1.45 (9H, s), 1.61 (1H, m), 1.77 (2H, m), 2.32 (3H, s), 4.48 (1H, m), 4.95 (1H, d, $J = 8.8$ Hz), 6.05 (1H, d, $J = 12.4$ Hz), 6.86 (1H, d, $J = 12.8$ Hz), 6.97 (1H, d, $J = 8.4$ Hz), 7.13 (1H, d, $J = 8.8$ Hz), 7.23 (1H, s).

2.22. (Z)-3-[2-(1-N-Boc-L-leucyl)-4-methylphenyl]-2-propenonic acid (**16b**)

In a manner similar to the preparation of **15a**, 80% sodium chlorite (0.15 g, 1.66 mmol) in 2.2 mL water, **14b** (0.19 g, 0.51 mmol, in 1.0 mL acetonitrile), sodium phosphate (30 mg) in 1.0 mL water and 30% hydrogen peroxide (0.10 mL) were reacted to give **16b** as a yellow solid (0.16 g, 82%). ^{1}H NMR ($CDCl_3$) δ 1.00 (6H, dd, $J_1 = 2.0$ Hz, $J_2 = 6.0$ Hz), 1.46 (9H, s), 1.61 (1H, m), 1.78 (2H, m), 2.33 (3H, s), 4.49 (1H, m), 4.95 (1H, d, $J = 8.8$ Hz), 6.07 (1H, d, $J = 12.0$ Hz), 6.89 (1H, d, $J = 12.4$ Hz), 6.98 (1H, d, $J = 9.0$ Hz), 7.15 (1H, d, $J = 8.0$ Hz), 7.22 (1H, s). ^{13}C NMR ($CDCl_3$) δ 21.00, 21.96, 23.12, 25.14, 28.54, 41.46, 52.64, 80.51, 121.69, 122.34, 127.94, 130.75, 130.95, 135.68, 138.95, 145.96, 155.94, 169.38, 172.19. MS (FAB) m/z 392.2 ($M^+ + H$). *Anal.* Calcd for $C_{21}H_{29}NO_6$: C, 64.43; H, 7.47; N, 3.58. Found: C, 64.36; H, 7.37; N, 3.52.

2.23. Linear precursor (**19b**)

In a manner similar to the preparation of **19a**, **15b** (0.37 g, 0.93 mmol, in 8 mL dry THF), DCC (0.54 g, 2.6 mmol), HOBt (0.14 g, 1.03 mmol), H_2N-Tyr-D-Ala-Gly-Phe-OtBu (**17**, 0.53 g, 1.03 mmol, in 16 mL dry THF), and DMAP (0.04 g, 0.28 mmol)

were reacted to afford **19b** as a white solid (0.34 g, 41%). ^{1}H NMR (CD_3OD) δ 0.99 (6H, dd, $J_1 = 6.6$ Hz, $J_2 = 9.0$ Hz), 1.14 (3H, d, $J = 7.2$ Hz), 1.35 (9H, s), 1.46 (9H, s), 1.69 (1H, m), 1.78 (2H, m), 2.26 (3H, s), 2.81 (2H, m), 3.02 (2H, d, $J = 7.2$ Hz), 3.73 (2H, d, $J = 16.5$ Hz), 4.17–4.27 (1H, m), 4.34 (1H, m), 4.48 (1H, t, $J = 7.2$ Hz), 6.13 (1H, d, $J = 12.0$ Hz), 6.67 (2H, d, $J = 8.4$ Hz), 6.76 (1H, d, $J = 12.3$ Hz), 6.94 (3H, t, $J = 8.7$ Hz), 7.12–7.27 (7H, m). ^{13}C NMR (CD_3OD) δ 17.29, 21.05, 23.49, 26.25, 28.39, 28.94, 37.76, 38.87, 41.50, 43.54, 54.01, 56.12, 58.08, 80.97, 83.22, 116.51, 122.83, 126.84, 127.95, 128.47, 129.59, 130.65, 131.39, 131.60, 132.08, 134.14, 137.22, 138.25, 157.58, 171.46, 172.10, 172.20, 173.81, 173.88, 174.91, 175.36. MS (FAB) m/z 887.5 ($M^+ + H$).

2.24. Linear precursor (*20b*)

In a manner similar to the preparation of **19a**, **16b** (0.42 g, 1.1 mmol, in 10 mL dry THF), DCC (0.67 g, 3.2 mmol), HOBt (0.17 g, 1.3 mmol), H_2N-Tyr-Gly-Gly-Phe-OtBu (**18**, 0.59 g, 1.2 mmol, in 16 mL dry THF), and DMAP (0.04 g, 0.32 mmol) were reacted to afford **20b** as a light yellow oil (0.31 g, 33%). ^{1}H NMR (CD_3OD) δ 0.99 (6H, dd, $J_1 = 6.3$ Hz, $J_2 = 9.0$ Hz), 1.36 (9H, s), 1.46 (9H, s), 1.71 (1H, m), 1.81 (2H, m), 2.24 (3H, s), 2.77 (1H, m), 2.91 (1H, m), 3.02 (2H, dd, $J_1 = 4.8$ Hz, $J_2 = 7.8$ Hz), 3.53 (1H, d, $J = 16.8$ Hz), 3.64 (1H, d, $J = 16.8$ Hz), 3.76 (1H, d, $J = 16.4$ Hz), 3.87 (1H, m), 4.34 (2H, t, $J = 7.5$ Hz), 4.51 (1H, t, $J = 7.2$ Hz), 6.08 (1H, d, $J = 12.3$ Hz), 6.65 (2H, d, $J = 8.7$ Hz), 6.75 (1H, d, $J = 12.3$ Hz), 6.93 (3H, t, $J = 9.9$ Hz), 7.11–7.29 (7H, m). ^{13}C NMR (CD_3OD) δ 21.06, 22.00, 23.48, 26.26, 28.38, 28.95, 37.69, 38.84, 41.50, 43.35, 43.96, 54.04, 56.13, 57.72, 80.97, 83.26, 116.59, 122.83, 126.76, 128.00, 128.64, 129.61, 130.02, 130.64, 131.34, 131.62, 134.26, 137.26, 138.28, 147.63, 157.52, 158.25, 169.16, 171.33, 172.12, 172.18, 174.41. MS (FAB) m/z 873.4 ($M^+ + H$).

2.25. Cyclic prodrug of DADLE (*21b*)

In a similar manner to the preparation of **21a**, **19b** (0.329 g, 0.371 mmol) was treated with a solution of 1:1 TFA:DCM (45 mL) under N_2 atmosphere for 3.5 h at room temperature. Upon concentration, the residue (in 200 mL dry DCM and 4.2 mL DMF), Bop-Cl (0.81 g, 3.15 mmol), and 0.57 mL TEA were reacted to afford **21b** as a white solid (0.126 g, 48%). ^{1}H NMR (CD_3OD) δ 0.93 (6H, dd, $J_1 = 5.7$ Hz, $J_2 = 13.2$ Hz), 1.18 (3H, d, $J = 7.3$ Hz), 1.72 (3H, m), 2.28 (3H, s), 2.79 (2H, m), 3.06 (1H, m), 3.42 (1H, m), 3.70 (1H, d, $J = 17.3$ Hz), 3.96 (1H, q, $J = 7.3$ Hz), 4.36 (1H, t, $J = 7.5$ Hz), 4.60 (2H, m), 6.15 (1H, d, $J = 12.0$ Hz), 6.67 (2H, d, $J = 8.3$ Hz), 6.73 (1H, d, $J = 12.2$ Hz), 6.91 (3H, t, $J = 7.2$ Hz), 7.02 (1H, s), 7.13–7.32 (6H, m). ^{13}C NMR (CD_3OD) δ 17.05, 21.06, 21.77, 23.44, 26.02, 36.73, 37.85, 41.05, 43.96, 50.00, 51.62, 52.46, 56.29, 57.06, 116.37, 122.31, 127.58, 127.79, 128.56, 129.58, 130.36, 130.93, 131.30, 131.47, 131.57, 136.23, 137.42, 139.00, 146.68, 157.47, 167.77, 172.24, 172.64, 173.35, 174.40, 175.79. MS (FAB) m/z 712.4 ($M^+ + H$).

2.26. Cyclic prodrug of Leu-enkephalin (**22b**)

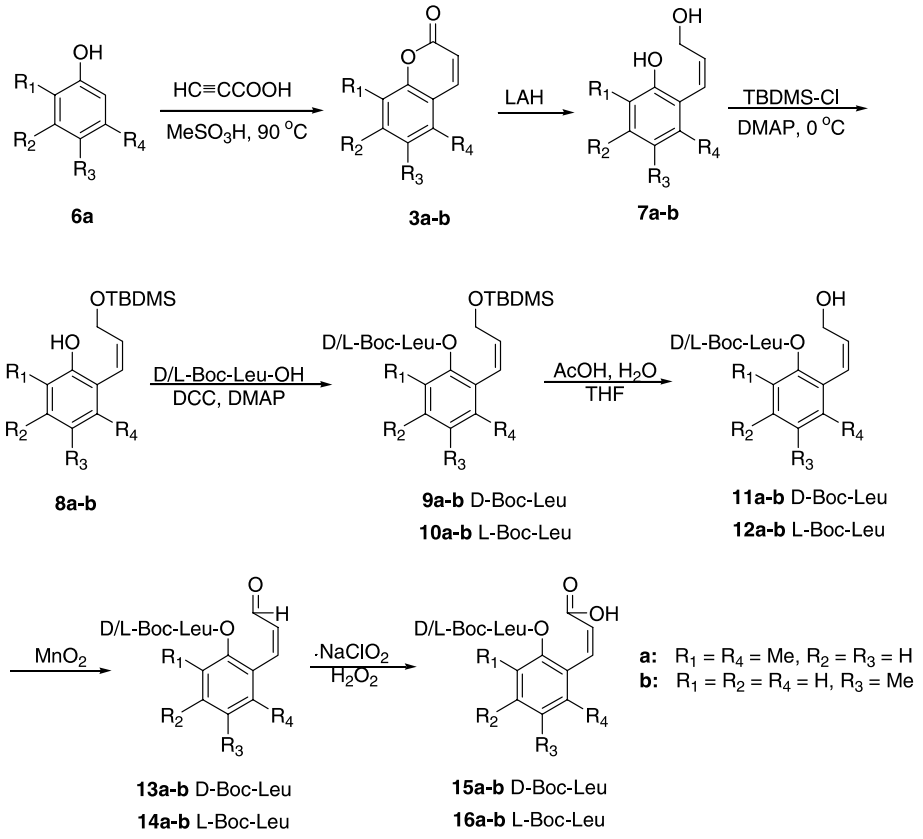
In a manner similar to the preparation of **21a**, **20b** (0.13 g, 0.15 mmol) was treated with a solution of 1:1 TFA:DCM (15 mL) under N_2 atmosphere for 3.5 h at room temperature. Upon concentration, the residue (in 144 mL dry DCM and 3 mL DMF), Bop-Cl (0.39 g, 1.53 mmol), and 0.23 mL TEA were reacted to afford **22b** as a white solid (0.026 g, 25%). ^{1}H NMR (CD_3OD) δ 1.01 (6H, dd, $J_1 = 6.0$ Hz, $J_2 = 20.0$ Hz), 1.75 (2H, m), 1.88 (1H, t, $J = 10.0$ Hz), 2.30 (3H, s), 2.63 (1H, m), 2.73 (1H, m), 3.08 (1H, m), 3.25 (1H, dd, $J_1 = 5.2$ Hz, $J_2 = 14.0$ Hz), 3.41 (1H, d, $J = 2.0$ Hz), 3.83 (2H, dd, $J_1 = 1.6$ Hz, $J_2 = 16.8$ Hz), 4.34 (1H, t, $J = 8.4$ Hz), 4.50–4.60 (3H, m), 6.19 (1H, d, $J = 12.0$ Hz), 6.67 (2H, d, $J = 8.8$ Hz), 6.77 (1H, d, $J = 12.0$ Hz), 6.93 (3H, t, $J = 8.4$ Hz), 7.03 (1H, s), 7.14 (1H, d, $J = 9.6$ Hz), 7.20–7.30 (5H, m). ^{13}C NMR (CD_3OD) δ 21.02, 21.84, 23.57, 26.17, 37.92, 38.12, 40.88, 43.85, 44.34, 53.07, 57.20, 57.29, 116.44, 122.80, 127.78, 127.98, 128.47, 129.69, 130.40, 130.78, 130.96, 131.31, 131.53, 135.45, 137.36, 138.68, 146.84, 157.57, 168.48, 171.62, 171.94, 172.53, 174.23, 174.83. MS (FAB) *m/z* 704.3 ($M^+ + Li$).

3. Results and discussion

For this study, we designed a series of compounds with methyl substitutions at different positions of the phenyl ring to achieve a thorough understanding of the effect of alkyl substitution on the release kinetics. The opioid peptides [Leu^5]-enkephalin and DADLE were used as the drug moieties for the study.

3.1. Synthesis

The coumarin-based cyclic prodrugs were synthesized by the approach that uses the commercially or synthetically available coumarins with different substituents (Scheme 3). Coumarin **3b** is commercially available. However, coumarin **3a** had to be synthesized. Starting from the substituted phenol **6a**, by heating with propiolic acid in the presence of methanesulfonic acid, coumarin **3a** [18] was obtained. Then the substituted coumarins **3** were reduced to the corresponding diols **7** [8] using lithium aluminum hydride (LAH) at 0 °C. The primary hydroxyl groups of diols **7** were selectively protected using *tert*-butyldimethylsilyl (TBDMS) chloride in the presence of 4-dimethylaminopyridine (DMAP) at 0 °C to afford **8** [8]. The free hydroxyl groups of **8** were then coupled with either D-Boc-Leu-OH or L-Boc-Leu-OH using *N*,*N*′-dicyclohexylcarbodiimide (DCC) as the activating agent, in the presence of DMAP to give compounds **9** and **10**. The free allylic hydroxyl groups of **11** and **12,** after deprotection of the silyl groups using acetic acid, were converted to the carboxyl groups in two steps. The oxidation to the aldehydes **13** and **14** was accomplished using manganese (IV) oxide (MnO_2) in dichloromethane (DCM). Conversion of the aldehydes **13** and **14** to the carboxylic acids **15** and **16** was accomplished by oxidation with sodium chlorite in the presence of hydrogen peroxide under acidic conditions. The free acids were then coupled with either tetrapeptide **17**

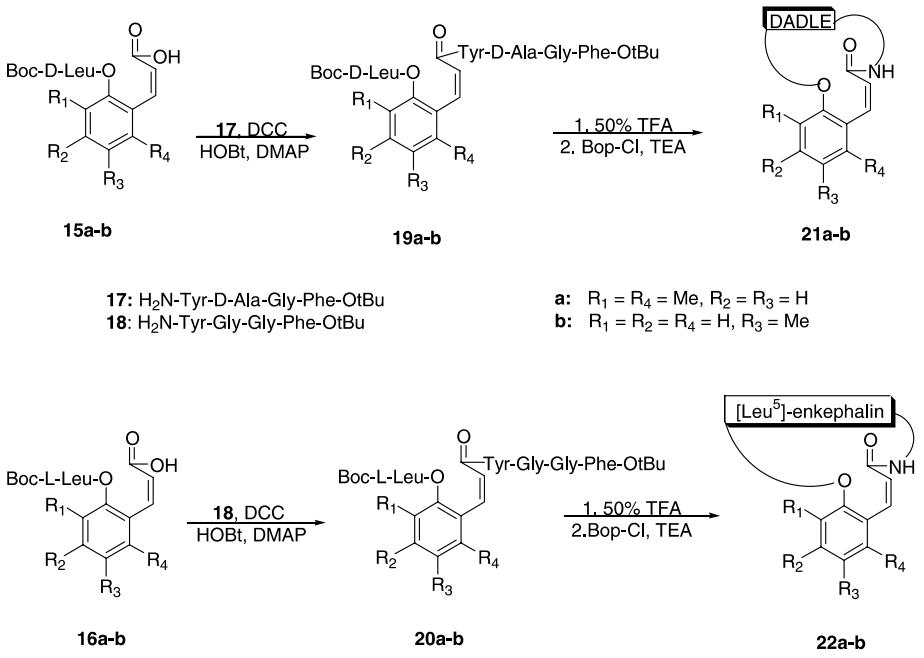


Scheme 3. Synthesis of coumarinic acid derivatives.

[11] (H_2N-Tyr-D-Ala-Gly-Phe-OtBu) or **18** [11] (H_2N-Tyr-Gly-Gly-Phe-OtBu) in the presence of DCC, 1-hydroxybenzotriazole (HOBt), and DMAP to give the linear precursor compounds **19** and **20** (Scheme 4). After deprotection of the Boc- and *t*-BuO- groups with 50% trifluoroacetic acid (TFA) in dichloromethane, the peptides were cyclized in the presence of *N*,*N*-bis[2-oxo-3-oxazolidinyl]phosphorodiamidic chloride (Bop-Cl) and triethylamine (TEA) to afford cyclic prodrugs of DADLE (**21**) and [Leu^5]-enkephalin (**22**).

3.2. Esterase kinetics

The esterase kinetic studies were carried out following procedure reported earlier using porcine liver esterases (PLE) [4,6,9]. Briefly the reaction was monitored via UV–Vis spectroscopy following the appearance of the coumarin starting materials **3**. Upon incubation with PLE in phosphate buffer (0.5 M, pH 7.4, and 37 ± 0.5 °C), all cyclic prodrugs released the opioid peptide drug as designed.



Scheme 4. Synthesis of DADLE and [Leu^5]-enkephalin cyclic prodrugs.

The objectives of this study were to see how alkyl substitutions on the phenyl ring of coumarin would affect the release rates and whether such substitutions could help to enhance the release rates as previously reported with the coumarin-based amine prodrugs. As shown in Table 1, the release rates of the phenyl substituted cyclic prodrugs were found to be slower than the non-substituted one. This is opposite to what was observed with the amine prodrugs [7,8].

It is known in our earlier studies with model amides [7,8] that methyl groups *ortho* to either the side chain alkenyl group or the phenol carboxyl group can greatly enhance the rate of the rate-limiting lactonization step through the introduction of steric congestion. However, compounds **21a** and **22a** with methyl substituents *ortho* to either the side chain alkenyl group or the phenolic carboxyl group have longer

Table 1
Esterase-catalyzed release rates of opioid peptides

Compound	R_1	R_2	R_3	R_4	$t_{1/2}$ (min)
21a	CH_3	H	H	CH_3	5636 ± 1300
21b	H	H	CH_3	H	235 ± 11
21c[a]	H	H	H	H	761 ± 69
22a	CH_3	H	H	CH_3	1950 ± 58
22b	H	H	CH_3	H	668 ± 35
22c[a]	H	H	H	H	317 ± 27

[a] Refs. [9] and [11].

half-lives compared with those of the corresponding unsubstituted ones (**21c** and **22c**) [9,11]. Most likely, the bulkiness of the peptides in cyclic prodrugs makes it more difficult for the esterase to reach the ester bond of the peptides and to catalyze the initial hydrolysis. In such an event, the rate-limiting step in cyclic prodrugs must be the esterase-catalyzed hydrolysis. Thus the steric congestion created by the substituents on the phenyl ring further hindered the access of the ester bond by porcine liver esterase. Since the steric effect imposed by the *ortho* substitution is unfavorable to the eventual release of the peptides, we synthesized the cyclic prodrugs where an electron-donating methyl group was introduced to the position *para* to the hydroxyl group (R_3). The rationale was that the increased nucleophilicity of the hydroxyl group might help to facilitate the cyclization rate. However, the anticipated results were not observed. While the release rate of DADLE prodrug **21b** increased compared to the unsubstituted one, the release of the [Leu^5]-enkephalin prodrug **22b** decreased. Overall, the release rates of the cyclic prodrugs of peptides cannot be predicted based on the substitution patterns alone. The idiosyncratic structural features of each peptide prodrug seem to play a more prominent role in determining the release kinetics in the coumarin-based prodrug system.

4. Conclusion

In order to fully understand the capabilities of the coumarin prodrug system and the substitution effect upon the release rates of opioid peptides DADLE and [Leu^5]-enkephalin, we have designed and synthesized a series of substituted coumarin derivatives to study their release kinetics. It was found that the release rates of coumarin-based cyclic prodrugs of opioid peptides are determined by the structural property of individual compounds. The substitution patterns on the phenyl ring of coumarins did not affect the release rates of opioid peptides the same way that they affected the release rate of model amines as previously reported.

Acknowledgments

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