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SUBSTITUTED COUMARINS AS ESTERASE-SENSITIVE PRODRUG MOIETIES WITH IMPROVED RELEASE RATES

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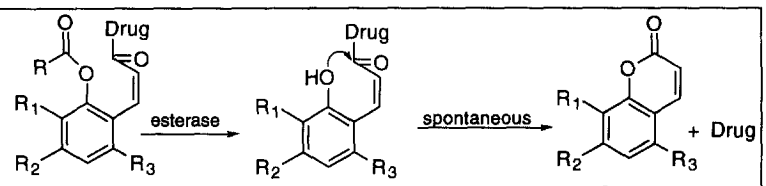
Abstract: Our laboratory has recently reported a coumarin-based prodrug system for the preparation of esterase-sensitive prodrugs of amines, peptides, and peptidomimetics. However, the release from this prodrug system was undesirably slow for some drug moieties. In this report, we describe the synthesis and evaluation of several substituted coumarin-based prodrugs of model amines with significantly increased release rates.

Introduction

Recently, our laboratory has developed a novel coumarin-based prodrug system for the preparation of esterase-sensitive prodrugs of amines,[1,2] peptides,[3–7] and peptidomimetics,[8–10] which are otherwise difficult to make.[11–16] The design takes advantage of the facile lactonization of *cis*-coumarinic acid and its derivatives **2** (Scheme 1).[17,18] In such a strategy, a latent nucleophile can be unmasked using an esterase triggering mechanism that, in turn, initiates a lactonization reaction to release the parent drug (Scheme 1). Using this prodrug strategy, we have prepared esterase-sensitive prodrugs of model amines (Scheme 1),[1,2] esterase-sensitive cyclic prodrugs of opioid peptides[3–6] and peptidomimetic glycoprotein IIb/IIIa antagonists[8,9] with greatly improved membrane permeability. We have also prepared an esterase-sensitive cyclic prodrug of tirofiban,[19–22] an FDA approved antithrombotic drug that can only be administered through the iv route. The coumarin-based prodrug of tirofiban, however, showed greatly improved oral bioactivities in preliminary studies in dogs, further demonstrating the clinical potential of this coumarin-based prodrug approach.[23]



1 2 3

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

During the course of our studies of the bioreversibility of the coumarin-based prodrug system using purified porcine liver esterase (PLE) and pig plasma, we noticed that all the coumarin-based prodrugs of either model amines or peptides did not release the "drug" moiety at the same rate.[1–3,24] The slow lactonization (Scheme 1) was found to be a major reason contributing to the slow release of some compounds. To further understand the effects of different structural features on the release kinetics, we undertook the effort to understand the effect of the amine drug moiety on the release kinetics.[2] It was found that the release rates depend on the pKa and the steric features of the amines to be released.[2] Amines with lower pKa's tend to be

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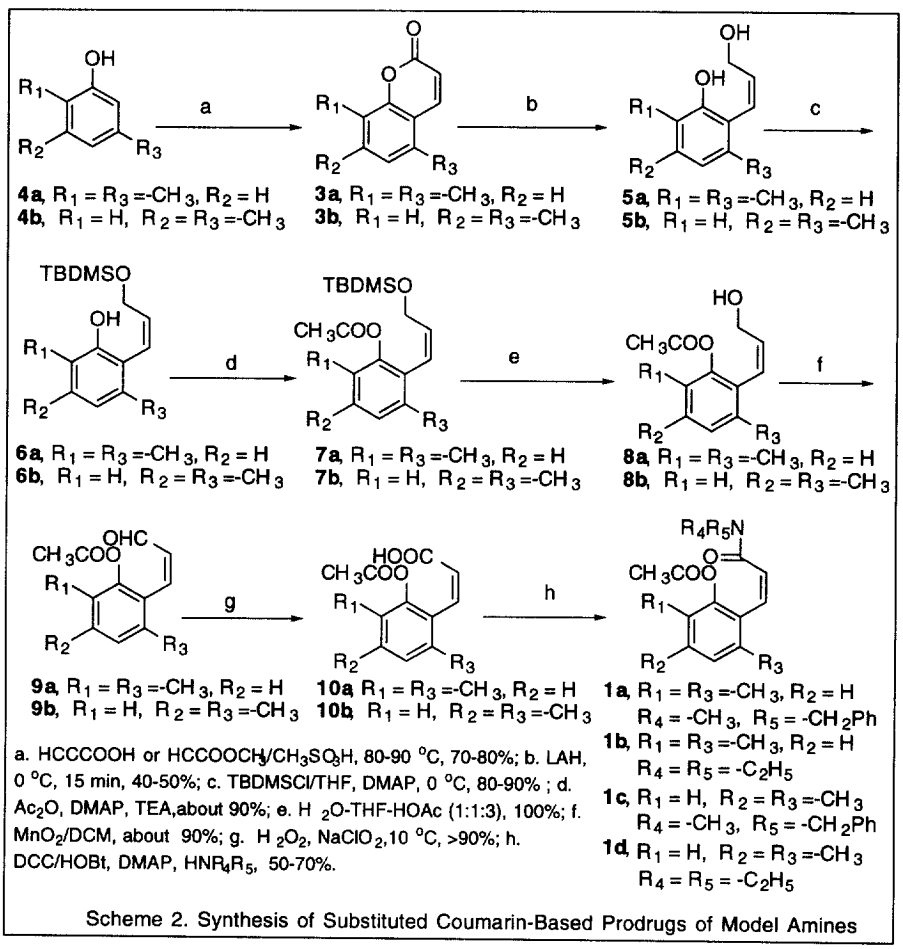
released at faster rates. Steric hindrance on the amine part tends to slow down the release. Consequently, the release of all primary amine studied was fast with half-lives of less than 50 min. However, the release was much slower with secondary amines. For example, while the half-life for the release of benzylamine from the coumarin-based prodrug was about 16 min, the half-life for the release of diethylamine was about 190 min.[2] The slow release of secondary amines will limit the application of this prodrug system to making prodrugs of only primary amines. This slow release of secondary amines is because secondary amines generally have higher pKa's than primary amines due to the electron-donating effect of the second alkyl group and are generally more sterically hindered.[2] Aimed at finding ways to fine-tune this coumarin-based prodrug system to allow for the manipulation of the release rates for amines and peptides, we examined the effect of substituents on the phenyl ring on the release kinetics. Four compounds (Scheme 2, **1a–d**) with methyl substitutions at different positions on the phenyl ring were synthesized. The release rates of these prodrugs were studied using PLE in a phosphate buffer solution, pH 7.4. It was found that introduction of methyl substitutions ortho to the alkyl side chain and the phenol hydroxyl group results in significant increases in release rates.



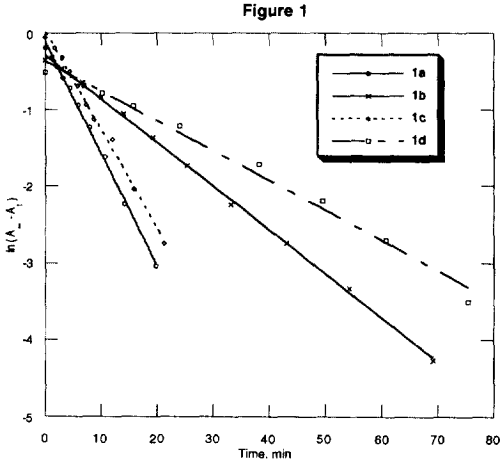
Scheme 2. Synthesis of Substituted Coumarin-Based Prodrugs of Model Amines

Results and Discussion

The objectives of the study are (1) to see whether the release rates can be modified through the introduction of substituents on the phenyl ring of the prodrug moiety and (2) to find ways to increase the release rates for secondary amines from the prodrug system. Conceivably, the release rates can be modified by changing the electronic and/or steric features of the system. Our design takes advantage of the known steric effect of alkyl substitutions ortho to the pendent side chain and the phenol hydroxyl groups, which facilitate the lactonization reactions.[17,18,25–32] Therefore, four compounds (**1a–d**, Scheme 2) were designed with methyl substitutions at the 3,5- or 3,6-positions of the phenyl ring. *N*-methylbenzylamine and diethylamine were used as the model amines for the study.

Synthesis. The preparation of the original coumarin-based prodrugs started with the reductive opening of the lactone ring of coumarin.[1,2] The same method was used for the synthesis of substituted coumarin-based prodrugs used in this study (Scheme 2). In this approach, substituted coumarins **3a,b** need to be synthesized. There are several procedures reported for the preparation of substituted coumarins using methods ranging from palladium-catalyzed addition[33] to Wittig reactions[34–37] and other reactions.[38–40] We took a Friedel–Crafts type of reaction approach for the synthesis of substituted coumarins **3a,b** by heating the corresponding substituted phenol **4** with propiolic acid or its ester in methanesulfonic acid to give the desired substituted coumarins **3** in 70–80% yields (Scheme 2). We studied this reaction using both propiolic acid and its methyl ester and found that both gave similar yields. Then the substituted coumarins were reduced using lithium aluminum hydride (LAH) at 0 °C to give the ring-opened diols **5** in about 40–50% yields. The primary hydroxyl groups of diols **5** were selectively protected as silyl ethers by reacting with *tert*-butyldimethyl silyl (TBDMS) chloride to give **6** in 80–90% yields. The acetylation of the free phenol hydroxyl group of **6** was accomplished by reaction with acetic anhydride in the presence of triethyl amine (TEA) and 4-dimethylaminopyridine (DMAP) in about 90% yields. The free primary hydroxyl group of **8** after deprotection of the silyl group using acetic acid was converted to the carboxyl group in two steps. The oxidation to the aldehydes **9** was accomplished using manganese dioxide in dichloromethane (DCM) in about 90% yield. Conversion of the aldehydes **9** to the carboxylic acids **10** was accomplished in over 90% yield by oxidation with hydrogen peroxide in the presence of sodium chlorite under acidic conditions.[41] The free acids **10** were then coupled with *N*-methylbenzylamine and diethylamine using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) as the activating reagent in the presence of hydroxybenzotriazole (HOBt) and DMAP to give the model prodrugs **1a–d**.[42]

Figure 1



Esterase kinetics. The prodrugs **1a–e** were designed to release the model amine drugs after esterase-catalyzed hydrolysis of the phenol ester bond (Scheme 1). PLE was used as the enzyme trigger as described in previous studies.[1–3] Briefly the reaction was followed with UV.[2] Upon incubation with PLE in phosphate buffer (0.05 M, pH 7.4, 37 ± 0.5 °C) at an enzyme

concentration of about 1 unit/mL, all model prodrugs **1a–d** quickly released the amine moiety as designed (Table 1). The pseudo-first-order rate constants were determined from linear curve-fittings (Figure 1). Data from the first 4–5 half-lives were used.

The objectives of this study were to see whether the release rate could be modified through the introduction of substituents and whether the methyl substitutions could help to enhance the release rates as designed. As shown in Table 1, with the dimethyl substituted model prodrug systems **1a–d**, significant release rate enhancements were observed compared with the un-substituted model prodrugs **1e–f**.[2] For example, the release rates for *N*-methylbenzylamine in the coumarin-based model prodrug system **1e** ($R_1 = R_2 = R_3 = H$) had a half-life of about 32 min (Table 1). With the dimethyl-substituted model prodrug systems **1a** and **1c**, the release was about fivefold faster with half-lives of 4.8 and 6.3 min, respectively. With the model prodrug of diethylamine, the rate enhancements were even more significant. The release half-life of the coumarin-based prodrug of diethylamine **1f** ($R_1 = R_2 = R_3 = H$) was about 188 min (Table 1). Whereas, with the dimethyl-substituted model prodrug systems **1b** and **1d** the release was about 10- to 16-fold faster with half-lives of 11.4 and 18.8 min, respectively. The different release rates for *N*-methylbenzylamine and diethylamine are mostly due to their different electronic features as discussed previously.[2] The data presented in Table 1 indicates that methyl substitutions on the phenyl ring were able to increase the release rates as designed. Therefore, we have developed substituted coumarin-based prodrugs which allow for the modification of the release rates through the introduction of different substituents on the phenyl ring of the prodrug system. Such systems should be useful for the preparation of prodrugs of amines, peptides and peptidomimetics with different release profiles.

Table 1. Esterase-catalyzed Release Rates for **1a–f**

Model Prodrug	R_1	R_2	R_3	R_4	R_5	$k_{obs} \times 10^2$ (min^{-1})	$t_{1/2}$ (min)
1a	$-CH_3$	-H	$-CH_3$	$-CH_3$	$-CH_2Ph$	14.4 ± 0.4	4.8 ± 0.12
1b	$-CH_3$	-H	$-CH_3$	$-C_2H_5$	$-C_2H_5$	6.1 ± 0.5	11.4 ± 1.0
1c	$-CH_3$	$-CH_3$	-H	$-CH_3$	$-CH_2Ph$	11.0 ± 0.8	6.3 ± 0.46
1d	$-CH_3$	$-CH_3$	-H	$-C_2H_5$	$-C_2H_5$	3.7 ± 0.4	18.8 ± 1.9
1e[a]	-H	-H	-H	$-CH_3$	$-CH_2Ph$	2.13 ± 0.01	32.5 ± 0.18
1f[a]	-H	-H	-H	$-C_2H_5$	$-C_2H_5$	0.37 ± 0.03	188.0 ± 12.3

[a]Data were from reference 2.

Conclusions

Our laboratory has developed a coumarin-based prodrug system for the preparation of esterase-sensitive prodrugs of amines, peptides, and peptidomimetics. The application potential of the prodrug system has been demonstrated with opioid peptides[3–7] and RGD (Arg-Gly-Asp) analogs.[8–10,23] However, the coumarin-based prodrug system does not always give the desirable time profiles for the release of the parent drugs. In this study, we have demonstrated that the release rates of the coumarin-based prodrugs of amines can be modified through the introduction of substituents on the phenyl ring of the prodrug system. The availability of these modified coumarin-based prodrug systems will help to broaden the scope of application of the coumarin-based prodrug systems for drugs with different desirable release time profiles.

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42. **1a**: ^{1}H NMR ($CDCl_3$) δ 2.12 (3H, s), 2.23 (3H, s), 2.24 (3H, s), 2.67/2.80 (3H, s/s, rotameric), 4.36/4.52 (2H, s/s, rotameric), 6.30 (1H, d), 6.58 (1H, d), 6.99 (1H, d), 7.07 (3H, brs); 7.23–7.29 (3H, brs). Anal. calcd for $C_{21}H_{23}NO_3$: C, 74.75; H, 6.87; N, 4.15. Found: C, 74.89; H, 6.98; N, 4.10.

1b: ^{1}H NMR ($CDCl_3$) δ 0.96/1.00 (6H, t/t, rotameric), 2.10 (3H, s), 2.24 (3H, s), 2.28 (3H, s), 3.16/3.32 (4H, q/q, rotameric), 6.23 (1H, d), 6.54 (1H, d), 6.96 (1H, d), 7.07 (1H, d); 7.04 (1H, d). Anal. calcd for $C_{17}H_{23}NO_3$: C, 70.56; H, 8.01; N, 4.84. Found: C, 70.48; H, 8.11; N, 4.79.

1c: ^{1}H NMR ($CDCl_3$) δ 2.21(3H, s), 2.25 (3H, s), 2.30 (3H, s), 2.65/2.80 (3H, s/s, rotameric), 4.35/4.52 (2H, s/s, rotameric), 6.28 (1H, d), 6.57 (1H, d), 6.71 (1H, d), 6.87 (1H, d); 7.04 (1H, d), 7.12 (1H, d), 7.23–7.30 (3H, brs).

1d: ^{1}H NMR ($CDCl_3$) δ 0.97/1.04 (6H, t/t, rotameric), 2.24 (6H, s), 2.28 (3H, s), 3.20/3.30 (4H, q/q, rotameric), 6.22 (1H, d), 6.53 (1H, d), 6.68 (1H, s), 6.88 (1H, s). Anal. calcd for $C_{17}H_{23}NO_3$: C, 70.56; H, 8.01; N, 4.84. Found: C, 70.64; H, 8.12; N, 4.77.