Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3439–3442

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Stability Study of a Modified Phenylpropionic Acid Linker-Based Esterase-Sensitive Prodrug

Xiaoping Song and Teruna J. Siahaan*

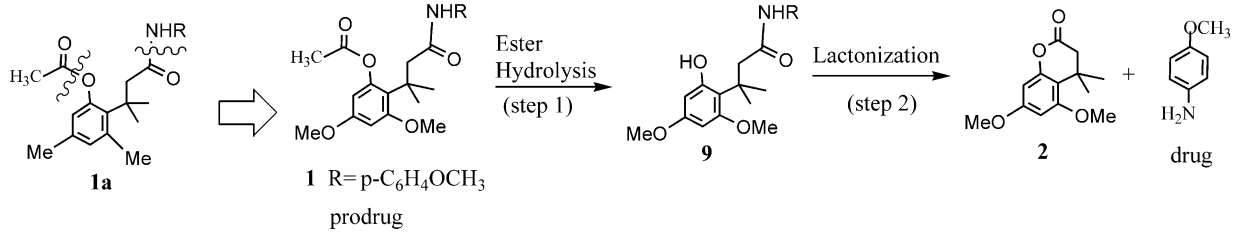
Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66047, USA

Received 3 July 2002; accepted 15 August 2002

Abstract—An esterase-sensitive amide prodrug **1** with a modified phenylpropionic acid linker was synthesized. The prodrug can be converted to the drug using isolated porcine esterase and human plasma. Paraoxon, an esterase inhibitor, can inhibit prodrug-to-drug conversion. The conversion of prodrug **1** was via phenol intermediate **9** followed by a lactonization reaction to give lactone **2** and the drug.

Prodrug strategies have been developed and utilized to transiently alter the physicochemical properties of drugs. Previously, we have used cyclic prodrugs to enhance cell membrane permeation and metabolic stability of peptides and peptidomimetics.[1–7] These cyclic prodrugs were formed by linking the C- and N-termini of the peptide using three different linkers, such as acyloxy(alkoxy),[1–3] coumarinic acid[4–5] and phenylpropionic acid[4–7] linkers. Conversion of these cyclic prodrugs to the parent drugs was initiated by esterase hydrolysis of the ester bond between the peptide and the linker followed by a fast chemical reaction (Scheme 1). Unfortunately, the coumarinic acid- and phenylpropionic acid-based cyclic prodrugs have low water solubility, largely due to their hydrophobicity. Therefore, there is a need to modify these linkers to balance the solubility and membrane permeation of the cyclic prodrugs.

In this work, the phenylpropionic acid (**1a**) linker was modified to increase its hydrophilicity by substituting the dimethyl groups with dimethoxy groups at positions-3 and -5 of the aromatic ring to give a linker used in prodrug **1** (Scheme 1). In the future, the methoxy groups at position-3 or -5 can be used as a handle for other functional groups to balance the solubility and membrane permeation of the prodrugs. However, this modification may change prodrug-to-drug conversion properties of this linker. Thus, we synthesized a model amide prodrug **1** using the modified linker (Scheme 1).



Scheme 1.

*Corresponding author. Tel.: +1-785-864-7327; fax: +1-785-864-5736; e-mail: siahaan@ku.edu

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00750-3

Finally, the rate of prodrug-to-drug conversion was evaluated in isolated esterase solution and human plasma in the absence and presence of paraoxon, an esterase inhibitor. The chemical stability study of the cyclic prodrug **1** was conducted in phosphate buffer, pH 7.4.

The synthesis of prodrug **1** was initiated by alkylation of the 3,5-dimethoxy phenol at the 2-position with 3,3-dimethyl acrylate in CH_3SO_3H at 70 °C to produce lactone **2** in 40% yield (Scheme 2). The use of CH_3SO_3H gave a better yield of lactone **2** than did H_2SO_4, which was used previously.[8] Reduction of lactone **2** with $LiAlH_4$ produced diol **3** in 70% yield. The primary alcohol in **3** was selectively protected with *t*-butyl dimethylsilyl group (TBDMS) to give compound **4** in 78% yield. The phenolic group in compound **4** was acetylated using acetic anhydride to yield 81% of compound **5**. Treatment of compound **5** with a mixture of acetic acid, water and THF removed the TBDMS-protecting group, resulting in compound **6** in 63% yield. The primary alcohol in compound **6** was oxidized to aldehyde **7** with PCC in CH_2Cl_2; further oxidation of the aldehyde with $KMnO_4$ gave an acid **8** in 65% yield. Finally, a coupling reaction between carboxylic acid **8** and 4-methoxy aniline using DCC produced the desired prodrug **1** in 87% yield.[9]

The applicability of this linker was appraised by studying the prodrug-to-drug conversion in isolated esterase or human plasma solution. For chemical stability, prodrug **1** was incubated in 50 mM phosphate buffer with ionic strength of 0.15 at pH 7.4 and 37 °C. The disappearance of prodrug **1** and the appearance of 4-methoxy aniline and lactone **2** were monitored by HPLC. In buffered solution, prodrug **1** was degraded in a pseudo-first-order fashion with stoichiometric production of lactone **2** and 4-methoxy aniline (Fig. 1). The half-life of prodrug **1** is 3529 min, which is comparable to the half-life (4039 min) of parent prodrug **1a** (Scheme 1).[8,10] In this degradation, the intermediate **9** (Scheme 1) was not observed because it was rapidly converted to lactone **2** and 4-methoxyaniline after hydrolysis of the ester. This result is consistent with the conversion of phenylpropionic acid-derived prodrugs previously reported by Borchardt et al.[8,10]

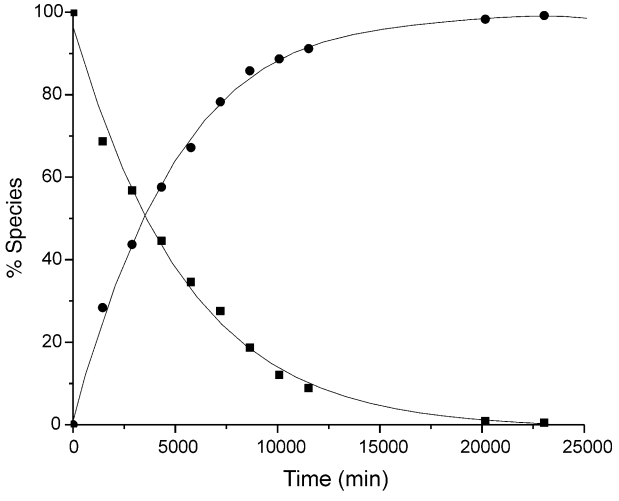


Figure 1. The stability of prodrug **1** in phosphate buffer at pH 7.4. It shows the entire time course for the disappearance of prodrug **1** (■) and the appearance of product **2** (●).

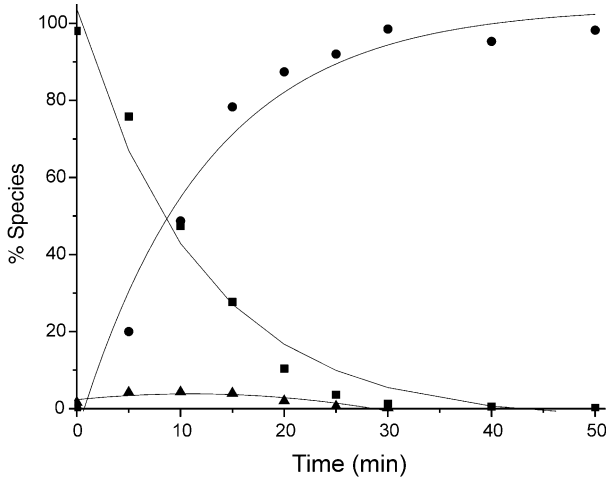
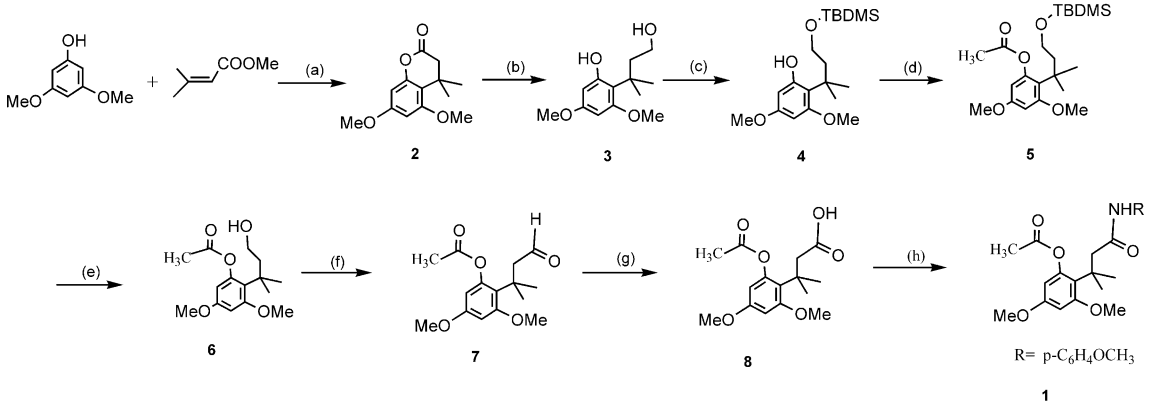


Figure 2. The stability of prodrug **1** in porcine liver esterase. It shows the entire time course for the disappearance of the prodrug **1** (■), the appearance of the intermediate **9** (▲), and the appearance of product **2** (●).



Scheme 2. (a) CH_3SO_3H, 40%; (b) $LiAlH_4$, THF, 70%; (c) TBDMS–Cl, Et_3N, CH_2Cl_2, 78%; (d) $(CH_3CO)_2O$, DMAP, CH_2Cl_2, 81%; (e) HOAc, TFA/H_2O, 63%; (f) PCC, CH_2Cl_2, 70%; (g) $KMnO_4$, acetone/H_2O, 65%; (h) H_2NR, DCC, DMAP, CH_2Cl_2, 87%.

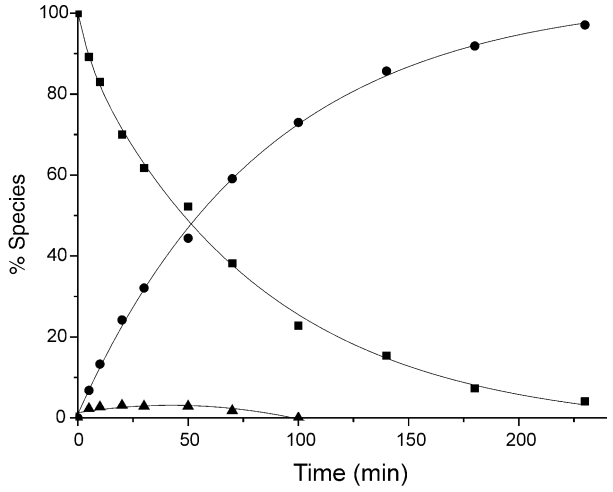


Figure 3. The stability of prodrug **1** in 90% human plasma. It shows the entire time course for the disappearance of the prodrug **1** (■), the appearance of the intermediate **9** (▲), and the appearance of product **2** (●).

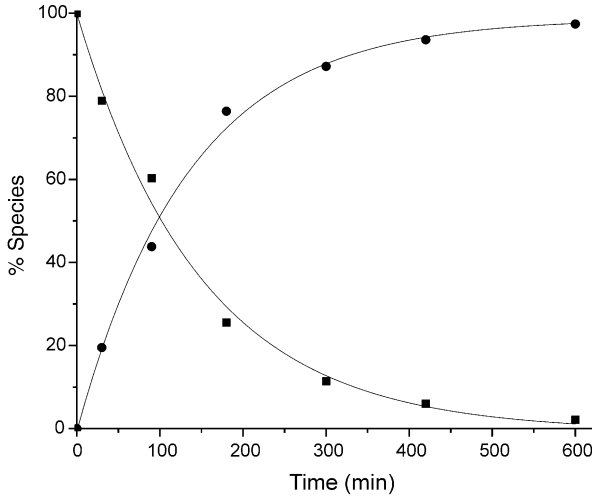


Figure 4. The stability of prodrug **1** in 90% human plasma in the presence of paraoxon. It shows the entire time course for the disappearance of the prodrug **1** (■) and the appearance of product **2** (●).

Next, the prodrug-to-drug conversion was examined using isolated porcine esterase. Incubation of prodrug **1** in porcine esterase solution converted prodrug **1** to lactone **2** and 4-methoxy aniline with a half-life of 10 min (Fig. 2). For a comparison, the half-life of the parent prodrug **1a** in porcine esterase solution was 11.9 min.[10] The enzymatic degradation of prodrug **1** exhibited pseudo-first order kinetics to completion. A small amount of intermediate **9** was observed in this enzymatic reaction, suggesting that prodrug-to-drug conversion is via intermediate **9**. Furthermore, the release of 4-methoxy aniline was due to the lactonization reaction, which gives lactone **2** as well. The presence of the intermediate **9** also suggests that the rate of ester hydrolysis is faster than that of the lactonization reaction. In addition, since no intermediate **8** (Scheme 2) was observed during the prodrug-to-drug conversion, the release of 4-methoxy aniline was not due to direct amide hydrolysis.

Finally, the conversion of prodrug **1** was evaluated in 90% human plasma for its applicability in vivo. In human plasma, the half-life of prodrug **1** is 52 min, 66 times faster than the chemical degradation (Fig. 3). This half-life was very similar to the half-life (53.7 min) of parent prodrug **1a** (Scheme 1) in plasma.[10] Similar to conversion in isolated esterase, a small amount of intermediate **9** was also observed, suggesting that ester hydrolysis is the first step of the conversion. To confirm the ester hydrolysis of prodrug **1** in human plasma, paraoxon (a potent esterase inhibitor) was used to inhibit the esterase activity in the human plasma (Fig. 4). The inclusion of paraoxon did increase the stability of prodrug **1**, and the degradation was found to be approximately 2.3 times slower. This result suggests that prodrug **1** most probably degrades in 90% human plasma by hydrolysis of the ester bond catalyzed by esterase.

In conclusion, we have synthesized prodrug **1** with a modified phenylpropionic acid promoiety. This esterase-sensitive prodrug **1** can be converted to drug by esterase hydrolysis with a similar rate to that of parent prodrug **1a**. Modification of the 3- and 5-positions of the aromatic ring in the promoiety maintained its ability to form a rapid lactonization reaction. This makes it an attractive linker for making cyclic prodrugs of peptides. In the future, the methoxy groups at the 3- and 5-positions can be utilized to balance the solubility and transmembrane permeation of the prodrug.

Acknowledgements

This work was supported by NIH grant HL-59931 and Self Faculty Scholar Award to T.J.S. from The University of Kansas. We thank Dr. Todd Williams, Mr. Robert Drake and Ms. Homigol Biesada at the Mass Spectroscopy Laboratory for their technical support.

References and Notes

1. Gangwar, S.; Pauletti, G. M.; Siahaan, T. J.; Stella, V. J.; Borchardt, R. T. *J. Org. Chem.* **1997**, *62*, 1356.
2. Bak, A.; Siahaan, T. J.; Gudmundsson, O. S.; Gangwar, S.; Friis, G. J.; Borchardt, R. T. *J. Pept. Res.* **1999**, *53*, 393.
3. Pauletti, G. M.; Gangwar, S.; Okumu, F. W.; Siahaan, T. J.; Stella, V. J.; Borchardt, R. T. *Pharm. Res.* **1996**, *13*, 1613.
4. Wang, B.; Nimkar, K.; Wang, W.; Zhang, H.; Shan, D.; Gudmundsson, O. S.; Gangwar, S.; Siahaan, T. J.; Borchardt, R. T. *J. Pept. Res.* **1999**, *53*, 370.
5. Gudmundsson, O. S.; Jois, S. D. S.; Vander Velde, D. G.; Siahaan, T. J.; Wang, B.; Borchardt, R. T. *J. Pept. Res.* **1999**, *53*, 383.
6. Wang, B.; Gangwar, S.; Pauletti, G. M.; Siahaan, T. J.; Borchardt, R. T. *J. Org. Chem.* **1997**, *62*, 1363.
7. Gudmundsson, O. S.; Nimkar, K.; Gangwar, S.; Siahaan, T. J.; Borchardt, R. T. *Pharm. Res.* **1999**, *16*, 16.
8. Amsberry, K. L.; Borchardt, R. T. *J. Org. Chem.* **1990**, *55*, 5867.
9. **1**: MS/FAB 402 $(M+H)^+$, 441 $(M+K)^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 1.58 [6H, s, 3,3-$(CH_3)_2$], δ 2.28 (3H, s, acetyl–CH_3), δ 2.87 (2H, s, 2-CH_2), δ 3.77 (3H, s, 6′-OCH_3), δ 3.79 (3H, s, 4′-OCH_3), δ 3.83 (3H, s,–OCH_3), δ 6.12 (1H, d,

$J = 3.0$ Hz, 6′-ArH), δ 6.43 (1H, d, $J = 2.6$ Hz, 4′-ArH), δ 6.78 (2H, d, $J = 9$ Hz, Ar′H), δ 6.81 (1H, s, NH), δ 7.15 (2H, d, $J = 9.0$ Hz, Ar′H); **2**: MS/FAB 237 $(M+H)^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 [6H, s, 4, 4-$(CH_3)_2$], δ 2.59 (2H, s, CH_2), δ 3.79 (3H, s, 5-OCH_3), δ 3.82 (3H, s, 7-OCH_3), δ 6.24 (1H, d, $J = 3.0$ Hz, 6-ArH), δ 6.27 (1H, d, $J = 2.5$ Hz, 8-ArH); **3**: MS/FAB 240 M^+, 241 $(M+H)^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 [6H, s, 3, 3-$(CH_3)_2$], δ 2.13 (2H, t, $J = 7.0$ Hz, 1-CH_2), δ 3.63 (2H, t, $J = 7.0$ Hz, 2-CH_2), δ 3.75 (3H, s, 6′-OCH_3), δ 3.76 (3H, 4′-OCH_3), δ 5.93 (1H, d, $J = 3.0$ Hz, 5′-ArH), δ 6.09 (1H, d, $J = 2.5$ Hz, 7′-ArH); **4**: MS/FAB 355 $(M+H)^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.01 [6H, s, Si–$(CH_3)_2$], δ 0.86 (9H, s, *t*-butyl), δ 1.53 [6H, s, 3, 3-$(CH_3)_2$], δ 2.05 (2H, t, $J = 6.8$ Hz, 1-CH_2), δ 3.60 (2H, t, $J = 6.8$ Hz, 2-CH_2), δ 3.72 (3H, s, 6′-OCH_3), δ 3.74 (3H, 4′-OCH_3), 5.98 (1H, d, $J = 2.6$ Hz, 5′-ArH), δ 6.07 (1H, d, $J = 2.6$ Hz, 7′-ArH), δ 6.23 (1H, br, 2′-OH); **5**: MS/FAB 397 $(M+H)^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.01 [6H, s, Si-$(CH_3)_2$], δ 0.86 (9H, s, *t*-butyl), δ 1.42 [6H, s, 3, 3-$(CH_3)_2$], δ 2.08 (2H, t, $J = 6.8$ Hz, 1-CH_2), δ 2.27 (3H, s, acetyl–CH_3), δ 3.43 (2H, t, $J = 7.5$ Hz, 2-CH_2), δ 3.77 (3H, s, 6′-OCH_3), δ 3.80 (3H, 4′-OCH_3), 6.02 (1H, d, $J = 2.6$ Hz, 5′-ArH), δ 6.36 (1H, d, $J = 2.6$ Hz, 7′-ArH); **6**: MS/FAB 283 $(M+H)^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 [6H, s, 3, 3-$(CH_3)_2$], δ 2.10 (2H, t, $J = 6.9$ Hz, 1-CH_2), δ 2.29 (3H, s, acetyl–CH_3), δ 3.51 (2H, t, $J = 6.9$ Hz, 2-CH_2), δ 3.77 (3H, s, 6′-OCH_3), δ 3.82 (3H, s, 4′-OCH_3), 6.02 (1H, d, $J = 2.6$ Hz, 5′-ArH), δ 6.39 (1H, d, $J = 2.6$ Hz, 7′-ArH); **7**: MS/FAB 281 $(M+H)^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 [6H, s, 3, 3-$(CH_3)_2$], δ 2.25 (3H, s, acetyl–CH_3), δ 2.85 (2H, d, $J = 2.6$ Hz, CH_2), δ 3.78 (3H, s, 6′-OCH_3), δ 3.82 (3H, s, 4′-OCH_3), 6.08 (1H, d, $J = 2.6$ Hz, 5′-ArH), δ 6.40 (1H, d, $J = 2.6$ Hz, 7′-ArH), δ 9.54 (1H, t, $J = 2.6$ Hz, ald.); **8**: MS/FAB 296 M^+; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 [6H, s, 3, 3-$(CH_3)_2$], δ 2.29 (3H, s, acetyl-CH_3), δ 2.92 (2H, s, CH_2), δ 3.77 (3H, s, 6′-OCH_3), δ 3.81 (3H, s, 4′-OCH_3), 6.06 (1H, d, $J = 2.6$ Hz, 5′-ArH), δ 6.38 (1H, d, $J = 2.6$ Hz, 7′-ArH).

10. Amsberry, K. L.; Gerstenberger, A. E.; Borchardt, R. T. *Pharm. Res.* **1991**, *8*, 455.