Bioorganic & Medicinal Chemistry Letters 20 (2010) 3280–3283

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel 3-*O*-pegylated carboxylate and 3-*O*-pegylated carbamate prodrugs of naltrexone for microneedle-enhanced transdermal delivery

Thirupathi Reddy Yerramreddy, Mikolaj Milewski, Narsimha Reddy Penthala, Audra L. Stinchcomb, Peter A. Crooks *

Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536, USA

ARTICLE INFO

Article history:
Received 12 March 2010
Accepted 12 April 2010
Available online 18 April 2010

Keywords:
Naltrexone prodrugs
Alcohol dependence and opioid addiction
Microneedle-enhanced transdermal delivery

ABSTRACT

A small library of novel 3-*O*-pegylated carboxylate prodrugs (**4a**–**4b**) and 3-*O*-pegylated carbamate prodrugs (**9a**–**9b**) of naltrexone were synthesized. The goal behind the design of these prodrugs was to investigate their potential for microneedle-enhanced transdermal delivery. All the synthesized 3-*O*-pegylated carboxylate prodrugs (**4a**–**4b**) and 3-*O*-pegylated carbamate prodrugs (**9a**–**9b**) of naltrexone were found to have adequate stability in a transdermal formulation and improved apparent solubility compared to naltrexone. Viscosity effects were postulated to be responsible for the observed non-linearity in the flux-concentration profile of these prodrugs.

The opioid antagonist, naltrexone (Fig. 1, **I**, NTX) is used in the treatment of opioid addiction and alcohol dependence.[1,2] Naltrexone hydrochloride is available as a 50 mg oral tablet under the trade name ReVia. Oral naltrexone therapy is associated with numerous adverse gastrointestinal effects such as abdominal pain, nausea and vomiting, and naltrexone undergoes extensive first-pass metabolism leading to poor bioavailability (5–40%), thus limiting its clinical utility.[3,4] In addition, the major challenge in naltrexone maintenance therapy has been the poor long-term patient compliance with therapy. Thus, naltrexone is the drug of choice for only very highly motivated patients.[1,5,6] To overcome this problem, several depot injections of naltrexone have been clinically investigated.[5,7] However, this relatively invasive dosage form requires the assistance of a health care professional. Hence, there is a need to develop an alternative, efficient and less invasive naltrexone delivery system to overcome these disadvantages in naltrexone therapy. Recently, transdermal drug delivery has been gaining greater popularity, and scientists have delivered numerous lipophilic drug molecules successfully via passive patches utilizing this route.[8,9] Previous work from our laboratory has focused on 3-*O*-alkyl carboxylate (Fig. 1, **II**) and 3-*O*-alkyl carbonate (Fig. 1, **III**) prodrugs of naltrexone which were investigated as lipophilic transdermal agents with improved physiochemical properties and increased skin permeation profiles, with the goal of delivering a therapeutic dose of naltrexone across human skin via a classical transdermal patch.[10–13]

Very recently, microneedle-enhanced transdermal delivery of drug molecules has been reported as a suitable drug delivery system for highly water-soluble, hydrophilic molecules.[14–17] Micrometer-size needles were shown to be long enough to penetrate the skin, effectively breaching the stratum corneum barrier, but at the same time being short enough to minimize pain sensation. In general, this process results in up to several orders-of-magnitude enhancement of transdermal drug transport.[18,19]

Previously, we have shown that although the creation of micro channels in the skin after microneedle (MN) treatment did not have a substantial effect on the delivery rates of the neutral (free-base) form of naltrexone or 6-ß-naltrexol, an improvement in the delivery of the salt form of these drugs was possible.[20] In vitro and in vivo animal studies proved the utility of the MN method of enhancement showing approximately an order-of-magnitude increase in the transdermal flux of highly water-soluble species via MN delivery over that through classical transdermal skin patch delivery.[20,21] Subsequently, a first-in-human MN study demonstrated that the combination of MN skin pretreatment and applica-

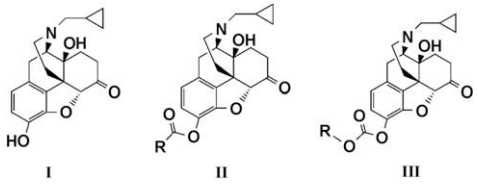


Figure 1. Structures of naltrexone (**I**), 3-*O*-alkyl carboxylate prodrugs of naltrexone (**II**), and 3-*O*-carbonate prodrugs of naltrexone (**III**).

* Corresponding author. Tel.: +1 859 257 1718; fax: +1 859 257 7585.
E-mail address: pcrooks@email.uky.edu (P.A. Crooks).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.04.049

tion of four naltrexone hydrochloride patches afforded drug plasma levels in the lower end of the targeted therapeutic range.[22]

It is known that covalent attachment of a polyethylene glycol (PEG) unit or polymer to a drug molecule can substantially increase aqueous solubility of hydrophobic drugs and proteins.[23] In addition, PEGylation is a useful tool in the field of pharmaceutics for improving drug properties, such as enhancing stability, modifying pharmacokinetics, shielding labile molecules from proteolytic enzymes, or eliminating protein immunogenicity.[24,25] An elevated aqueous solubility was postulated to contribute to a moderate increase in flux observed for some derivatives.[26–28] Based on previous reports from our laboratory,[20] it could be anticipated that an increase in aqueous solubility achieved through PEGylation of naltrexone would favorably affect flux through MN-treated skin.

The above observations encouraged us to design and synthesize a small library of novel, highly polar, more water-soluble 3-*O*-pegylated carboxylate (Fig. 2, **4a**–**4b**) and 3-*O*-pegylated carbamate (Fig. 2, **9a**–**9b**) prodrugs of naltrexone. These analogs had terminal hydroxy, and terminal methoxy groups attached to the polyethylene glycol (PEG) moiety and were considered as hydrophilic transdermal agents with suitable physiochemical properties for delivery via MN-enhanced transdermal delivery.

The synthetic routes to the 3-*O*-pegylated carboxylate prodrugs of naltrexone, that is, 3-*O*-[3-(2-(2-methoxy-ethoxy/2-hydroxy-ethoxy)ethoxy)propanoyl]naltrexone (**4a–4b**) are illustrated in Scheme 1.

t-Butyl 3-[2-(2-methoxyethoxy/2-benzyloxyethoxy)ethoxy] propanoates (**2a**–**2b**) were prepared by Michael addition of 2-[2-(methoxy/benzyloxy)ethoxy]ethanols (**1a**–**1b**) with t-butyl acrylate in the presence of NaOH-GEB catalyst (GEB; gel-entrapped base) in *t*-butyl alcohol at 50–55 °C.[29] Deprotection of **2a**–**2b** with trifluoroacetic acid (TFA) at room temperature afforded 3-[2-(2-methoxyethoxy/2-benzyloxyethoxy)ethoxy]propanoic acids (**3a** and **3a^1**). Debenzylation of **3a^1** with Pd–C/H_2 in THF afforded 3-[2-(2-hydroxyethoxy) ethoxy]propanoic acid (**3b**). Condensation of **3a**–**3b** with naltrexone using DCC in chloroform at room temperature afforded 3-*O*-[3-(2-(2-methoxyethoxy/2-hydroxyethoxy)ethoxy)propanoyl]naltrexones (**4a**–**4b**).

N-[2-(2-(2-Hydroxyethoxy/2-methoxyethoxy)ethoxy)ethyl]phthalimides (**6a**–**6b**) were prepared by the reaction of 1-chloro-2-[2-(2-hydroxyethoxy/2-methoxyethoxy)ethoxy]ethanes (**5a**–**5b**) with potassium phthalimide in DMF under reflux conditions for 10 h. Deprotection of **6a**–**6b** with hydrazine hydrate in refluxing ethanol for 3 h afforded 2-[2-(2-hydroxy-ethoxy/2-methoxyethoxy)ethoxy]ethanamines (**7a**–**7b**). 3-*O*-(*p*-Nitrophenyloxycarbonyl)naltrexone (**8**) was prepared by the reaction of *p*-nitrophenyl chloroformate with naltrexone in the presence of triethylamine in chloroform at room temperature for 2 h. Condensation of **7a**–**7b** with 3-*O*-(*p*-nitrophenyloxycarbonyl)naltrexone (**8**) in the presence of DMAP in chloroform at room temperature for 12 h afforded 3-*O*-[2-(2-(2-hydroxyethoxy/2-methoxyethoxy)ethoxy) ethylcarbam-ic]naltrexones (**9a**–**9b**). All the synthesized compounds were characterized by ^{1}H NMR and ^{13}C NMR spectrometry and MS (ESI) analysis.[31]

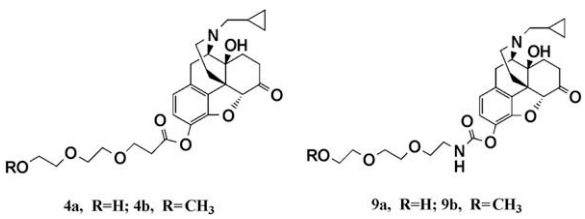
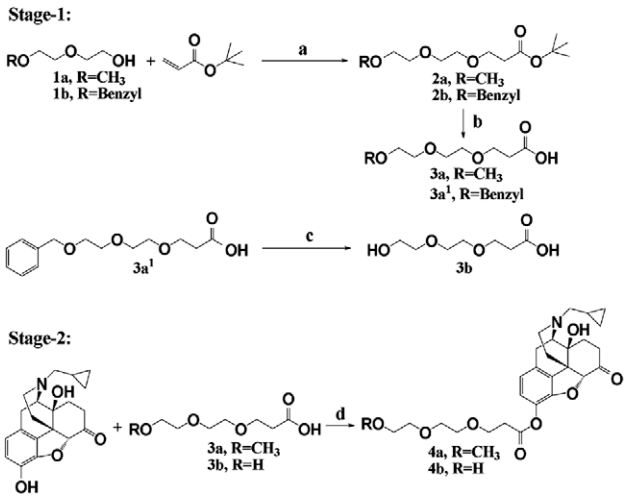
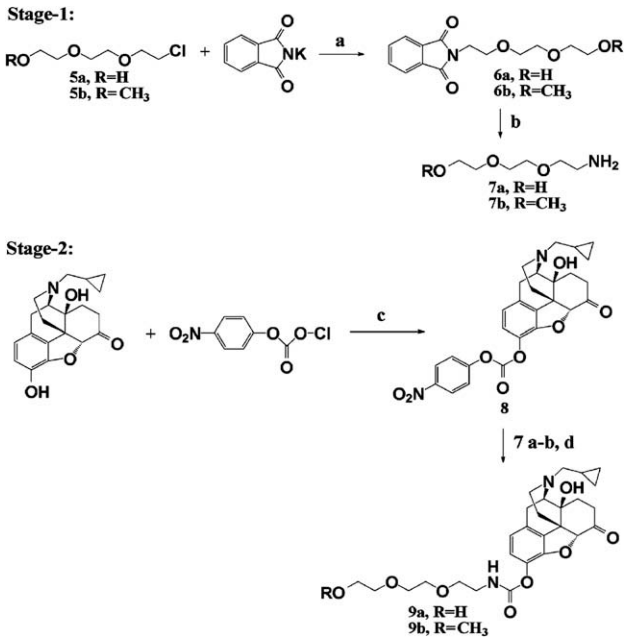


Figure 2. Structures of 3-*O*-pegylated carboxylate (**4a**–**4b**), and 3-*O*-pegylated carbamate (**9a**–**9b**) prodrugs of naltrexone.



Scheme 1. Reagents and conditions: (a) NaOH-GEB (GEB; gel-entrapped base), *t*-BuOH, 50–55 °C, 24 h; (b) TFA, rt, 12 h; (c) Pd/C, H_2, THF, 24 h; (d) DCC, DMAP, $CHCl_3$, rt, 12 h.



Scheme 2. Reagents and conditions: (a) DMF, reflux, 10 h; (b) ethanol, $N_2H_4{\cdot}H_2O$, reflux, 3 h; (c) $CHCl_3$, TEA, rt, 2 h; (d) DMAP, $CHCl_3$, rt, 12 h.

The synthetic routes to the synthesis of 3-*O*-pegylated carbamate prodrugs of naltrexone, that is, 3-*O*-[2-(2-(2-hydroxyethoxy/2-methoxyethoxy)ethoxy)ethylcarbamic]naltrexone (**9a**–**9b**), are illustrated in Scheme 2.

The apparent solubilities of naltrexone and the four naltrexone prodrugs were assessed at pH 5.0 in 0.3 M acetate buffer by equilibrium of an excess quantity of drug/prodrug in 6 mL of buffer. At pH 5.0, naltrexone and the naltrexone prodrugs are expected to be fully ionized (highly soluble) and the buffer concentration of 0.3 M allows maintenance of this pH throughout the duration of an in vitro diffusion study. The solubility data is shown in Table 1.

Adequate stability is one of the crucial characteristics of a prodrug. In the case of the microneedle-targeted route of delivery, stability in aqueous buffers at skin and physiological pHs needs to be established. The hydrolysis of both 3-*O*-pegylated carbamate prodrugs (**9a–9b**) and 3-*O*-pegylated carboxylate prodrugs (**4a–4b**)

Table 1
Solubility of naltrexone prodrugs and naltrexone at pH 5.0 in 0.3 M acetate buffer

Analog	Solubility (in mM)	Relative solubility[a]
4a	629	1.86
4b	952	2.81
9a	1035	3.06
9b	796	2.35
NTX	338	—

[a] Relative solubility represents solubility of naltrexone prodrug divided by solubility of NTX.

Table 2
Hydrolysis rate constants of naltrexone prodrugs in acetate buffer at pH 5.0 and in HEPES-buffered Hanks' balanced salt solution at pH 7.4

Analog	Acetate buffer solution {k_{pH} 5.0 [h^{-1}]}	HEPES-buffered Hanks' balanced salt solution {k_{pH} 7.4 [h^{-1}]}
4a	0.00193	0.0288
4b	0.00184	0.0258
9c	0.000983	0.159
9d	0.00111	0.159

of naltrexone was investigated in acetate buffer at pH 5.0, and in HEPES-buffered Hanks' balanced salt solution at pH 7.4. The hydrolysis of the labile prodrug moieties followed pseudo first-order kinetics, and gave apparent hydrolysis rate constants. The hydrolysis rate constant values (k_{pH} 5.0 in acetate buffer; k_{pH} 7.4 in HEPES-buffered Hanks' balanced salt solution [h^{-1}]) obtained for the naltrexone prodrugs are illustrated in Table 2. The chemical hydrolysis rates of the four prodrugs were sufficiently low to produce less than 10% hydrolysis in the donor compartment throughout the 48 h duration of the diffusion study. Moreover, all four prodrugs were hydrolyzed rapidly to naltrexone at physiological pH (7.4) with the 3-*O*-carbamate naltrexone prodrug hydrolysis rates being approximately six times faster than the corresponding 3-*O*-carboxylate naltrexone prodrug hydrolysis rates.

The performance of **4a** in in vitro diffusion experiments employing a transdermal microneedle formulation has been reported elsewhere.[30] Briefly, full thickness Yucatan minipig skin (1.7 mm thickness, diffusion area 0.95 cm^2) was utilized, and pierced with solid metal, 750 mm-long microneedles before mounting the skin in a PermeGear flow-through diffusion cell system at 32 °C. Substantial non-linearity in the flux-concentration profile of **4a** was observed, ultimately resulting in decreased transport rates. It was observed that the viscosity of the donor solution of **4a** increased exponentially as a function of prodrug concentration. It has been suggested that changes in the viscosity properties of the donor solution may have a detrimental effect on delivery rates of drugs through MN-treated skin.[32] Thus, it is postulated that the non-linearity of the flux of **4a** with increasing concentration is caused by elevated viscosity of the donor solution. The other prodrugs reported herein, that is, **4b**, **9a** and **9b**, showed similar behavior to **4a** (data not published).

In conclusion, 3-*O*-pegylated carboxylate prodrugs of naltrexone (**4a–4b**) and 3-*O*-pegylated carbamate prodrugs of naltrexone (**9a–9b**) have been synthesized and fully characterized (^{1}H NMR, ^{13}C NMR, and mass spectroscopy).[31] These prodrugs had higher aqueous solubilities and showed an approximately 2–3-fold enhancement in their aqueous solubilities over naltrexone. In stability studies, these prodrugs of naltrexone were hydrolyzed rapidly at physiological pH. The 3-*O*-pegylated carbamate prodrugs had hydrolysis rates that were approximately six times faster than the hydrolysis rates of 3-*O*-pegylated carboxylate prodrugs in pH 7.4 HEPES-buffered Hanks' solution. Viscosity effects may be a confounding factor responsible for the observed non-linear relationship between transdermal flux values and prodrug concentration.[30]

Acknowledgment

This work was supported by NIH Grant R01 DA13425.

References and notes

1. Terenius, L. *Curr. Opin. Chem. Biol.* **1998**, *2*, 541.
2. Volpicelli, J. R.; Alterman, A. I.; Hayashida, M. *Arch. Gen. Psychiatry* **1992**, *49*, 876.
3. Kranzler, H. R.; Lowe, V. M.; Kirk, J. V. *Neuropyschopharmacology* **2000**, *22*, 493.
4. *PDR Generics*; 2nd Ed.; Medical Economics: Montvale, New Jersey, USA, **1996**; pp 2229–2233.
5. Rothenberg, J. L.; Sullivan, M. A.; Church, S. H.; Seracini, A.; Collins, E.; Kleber, H. D.; Nunes, E. V. *J. Subst. Abuse Treat.* **2002**, *23*, 351.
6. Volpicelli, J. R.; Rhines, K. C.; Rhines, J. S. *Arch. Gen. Psychiatry* **1997**, *54*, 737.
7. Gooberman, L. L. U. S. Patent 6,203,813, 2001.
8. Gannu, R.; Vishnu, Y. V.; Kishan, V.; Rao, Y. M. *Curr. Drug Deliv.* **2007**, *4*, 69.
9. Hale, R. L.; Amy, T. L.; Dennis, W. S.; Michael, J. N. C. U. S. Patent 5,622,944, 1997.
10. Vaddi, H. K.; Hamad, M. O.; Chen, J.; Banks, S. L.; Crooks, P. A.; Stinchcomb, A. L. *Pharm. Res.* **2005**, *22*, 758.
11. Pillai, O.; Hamad, M. O.; Crooks, P. A.; Stinchcomb, A. L. *Pharm. Res.* **2004**, *21*, 1146.
12. Hammell, D. C.; Stolarczyk, E. I.; Klausner, M.; Hamad, M. O.; Crooks, P. A.; Stinchcomb, A. L. *J. Pharm. Sci.* **2005**, *94*, 828.
13. Valiveti, S.; Hammell, D. C.; Paudel, K. S.; Hamad, M. O.; Crooks, P. A.; Stinchcomb, A. L. *J. Controlled Release* **2005**, *102*, 509.
14. Banga, A. K. *Expert Opin. Drug Deliv.* **2009**, *6*, 343.
15. Oh, J. H.; Park, H. H.; Do, K. Y.; Han, M.; Hyun, D. H.; Kim, C. G.; Kim, C. H.; Lee, S. S.; Hwang, S. J.; Shin, S. C.; Cho, C. W. *Eur. J. Pharm. Biopharm.* **2008**, *69*, 1040.
16. Kolli, C. S.; Banga, A. K. *Pharm. Res.* **2008**, *25*, 104.
17. Lin, W.; Cormier, M.; Samiee, A.; Griffin, A.; Johnson, B.; Teng, C. L.; Hardee, G. E.; Daddona, P. E. *Pharm. Res.* **2001**, *18*, 1789.
18. Prausnitz, M. R.; Langer, R. *Nat. Biotechnol.* **2008**, *26*, 1261.
19. Henry, S.; McAllister, D. V.; Allen, M. G.; Prausnitz, M. R. *J. Pharm. Sci.* **1998**, *87*, 922.
20. Banks, S. L.; Pinninti, R. R.; Gill, H. S.; Crooks, P. A.; Prausnitz, M. R.; Stinchcomb, A. L. *Pharm. Res.* **2008**, *25*, 1677.
21. Banks, S. L.; Paudel, K. S.; Pinninti, R. R.; Gill, H. S.; Crooks, P. A.; Brogden, N. K.; Prausnitz, M. R.; Stinchcomb, A. L. *J. Pharm. Sci.* **2010**, in press.
22. Wermeling, D. P.; Banks, S. L.; Hudson, D. A.; Gill, H. S.; Gupta, J.; Prausnitz, M. R.; Stinchcomb, A. L. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 2058.
23. Riley, T.; Riggs-Sauthier, J. *Pharm. Technol.* **2008**, *32*, 88.
24. Harris, J. M.; Chess, R. B. *Nat. Rev. Drug Disc.* **2003**, *2*, 214.
25. Veronese, F. M.; Pasut, G. *Drug Discovery Today* **2005**, *10*, 1451.
26. Bonina, F. P.; Puglia, C.; Barbuzzi, T.; deCaprariis, P.; Palagiano, F.; Rimoli, M. G.; Saija, A. *J. Pharm. Sci.* **2001**, *14*, 123.
27. Thomas, J. D.; Majumdar, S.; Sloan, K. B. *Molecules* **2009**, *14*, 4231.
28. N'Da, D. D.; Breytenbach, J. C. *J. Pharm. Pharmacol.* **2009**, *61*, 721.
29. Chaphekar, S. S.; Samant, S. D. *Appl. Catal. A: General* **2003**, *242*, 11.
30. Mikolaj, M.; Thirupathi Reddy, Y.; Priyanka, G.; Crooks, P. A.; Stinchcomb, A. L. *J. Controlled Release* **2010**, in press.
31. Analytical data and yields of the all the synthesized compounds: (**2a**): ^{1}H NMR ($CDCl_3$): δ 1.43 (s, 9H), 2.48 (t, 2H), 3.57 (s, 3H), 3.56 (t, 2H), 3.61–3.65 (6H), 3.69 (t, 2H); ^{13}C NMR ($CDCl_3$): δ 28.5, 33.5, 59.1, 66.2, 70.1, 70.2, 70.4, 71.5, 81.9, 172.9; MS (ESI) *m*/*z*: 249 (MH^+); Yield: 92%: (**2b**): ^{1}H NMR ($CDCl_3$): δ 1.39 (s, 9H), 2.43 (t, 2H), 3.55–3.73 (m, 10H), 4.72 (s, 2H), 7.32–7.43 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 28.6, 33.3, 66.7, 70.0, 70.1, 70.3, 72.8, 82.0, 127.3, 127.4, 128.6, 137.5, 173.2; MS (ESI) *m*/*z*: 325 (MH^+); Yield: 97%: (**3a**): ^{1}H NMR ($CDCl_3$): δ 2.61 (t, 2H), 3.38 (s, 3H), 3.59 (t, 2H), 3.63–3.66 (m, 6H), 3.76 (t, 2H), 10.82 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 34.6, 59.5, 66.2, 70.1, 70.2, 70.4, 70.6, 177.2; MS (ESI) *m*/*z*: 193 (MH^+); Yield: 94%: (**3a**^{1}): ^{1}H NMR ($CDCl_3$): δ 2.58 (t, 2H), 3.56–3.76 (m, 10H), 4.54 (s, 2H), 7.24–7.33 (m, 5H), 11.18 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 33.9, 66.5, 70.0, 70.2, 70.4, 73.1, 127.5, 127.6, 128.5, 137.3, 177.3; MS (ESI) *m*/*z*: 269 (MH^+); Yield: 95%: (**3b**): ^{1}H NMR ($CDCl_3$): δ 2.56 (t, 2H), 3.56–3.62 (m, 6H), 3.63–3.76 (m, 4H), 7.12 (br s, 2H); ^{13}C NMR ($CDCl_3$): δ 34.6, 61.3, 66.4, 70.1, 70.3, 70.4, 177.3; MS (ESI) *m*/*z*: 179 (MH^+); Yield: 98%: (**4a**): ^{1}H NMR ($CDCl_3$): δ 0.15–0.18 (m, 2H), 0.55–0.58 (m, 2H), 0.87 (m, 1H), 1.57–1.67 (m, 3H), 1.86 (m, 1H), 2.13 (m, 1H), 2.28–2.42 (m, 4H), 2.87 (t, 2H), 2.96–3.20 (m, 2H), 3.38 (s, 3H), 3.55–3.66 (m, 8H), 3.87, (t, 2H), 4.68 (s, 1H), 6.67 (d, *J* = 8.4 Hz, 1H), 6.82 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 3.98, 4.22, 9.44, 23.07, 30.71, 31.05, 31.26, 34.92, 34.96, 36.12, 43.51, 50.66, 59.15, 59.28, 61.82, 69.73, 63.75, 66.42, 66.45, 69.06, 70.05, 70.25, 70.33, 70.34, 70.43, 70.53, 71.62, 72.53, 90.52, 119.33, 122.84, 129.98, 130.21, 132.24, 147.46, 169.01, 207.63; MS (ESI) *m*/*z*: 516 (MH^+); Yield: 86%: (**4b**): ^{1}H NMR ($CDCl_3$): δ 0.12–0.16 (m, 2H), 0.51–0.57 (m, 2H), 0.88 (m, 1H), 1.55–1.66 (m, 3H), 1.85 (m, 1H), 2.16 (m, 1H), 2.25–2.40 (m, 4H), 2.85 (t, 2H), 2.99–3.21 (m, 2H), 3.58–3.67 (m, 8H), 3.72, (t, 2H), 4.67 (s, 1H), 6.65 (d, *J* = 8.1 Hz, 1H), 6.83 (d, *J* = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 3.93, 4.19, 9.43, 23.04, 30.72, 31.03, 31.25, 34.90, 34.98, 36.13, 43.53, 50.65, 59.17, 61.70, 61.81, 69.72, 63.77, 66.40, 66.47, 69.05, 70.03, 70.24, 70.32, 70.35, 70.45,

70.51, 72.52, 90.54, 119.31, 122.82, 129.99, 130.23, 132.25, 147.48, 169.03, 207.61; MS (ESI) *m*/*z*: 502 (MH^+); Yield: 85%: (**6a**): ^1H NMR ($CDCl_3$): δ 2.20 (s, 1H), 3.43 (t, 2H), 3.50–3.57 (m, 6H), 3.65 (t, 2H), 3.82 (t, 2H), 7.60–7.64 (m, 2H), 7.74–7.78 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 38.3, 61.1, 65.5, 70.3, 70.5, 70.6, 123.5, 123.7, 132.1, 132.3, 167.6; MS (ESI) *m*/*z*: 280 (MH^+); Yield: 69.7%: (**6b**): ^1H NMR ($CDCl_3$): δ 3.34 (s, 3H), 3.41 (t, 2H), 3.52–3.59 (m, 6H), 3.63 (t, 2H), 3.81 (t, 2H), 7.59–7.62 (m, 2H), 7.73–7.76 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 38.2, 59.5, 65.2, 70.2, 70.4, 70.5, 71.6, 123.6, 123.7, 132.0, 132.1, 167.8; MS (ESI) *m*/*z*: 294 (MH^+); Yield: 78%: (**7a**): ^1H NMR ($CDCl_3$): δ 2.61 (br s, 3H), 2.72 (t, 2H), 3.37–3.58 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 41.4, 61.5, 70.0, 70.2, 70.4, 72.6; MS (ESI) *m*/*z*: 150 (MH^+); Yield: 76%: (**7b**): ^1H NMR ($CDCl_3$): δ 1.69 (br s, 2H), 2.73 (t, 2H), 3.26 (s, 3H), 3.38–55 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 41.7, 59.5, 70.1, 70.3, 70.5, 71.3, 72.8; MS (ESI) *m*/*z*: 164 (MH^+); Yield: 83%: (**8**): ^1H NMR ($CDCl_3$): δ 0.11–0.17 (m, 2H), 0.53–0.59 (m, 2H), 0.90 (m, 1H), 1.58–1.64 (m, 3H), 1.88 (m, 1H), 2.18 (m, 1H), 2.36–2.44 (m, 4H), 2.60 (m, 2H), 3.0–3.19 (m, 2H), 4.78 (s, 1H), 6.73 (d, J = 8.1 Hz, 1H), 7.00 (d, J = 8.4 Hz, 1H), 7.53 (dd, J = 8.6 Hz, 2H), 8.29 (dd, J = 9.0, 1.5 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 4.08, 4.39, 9.43, 23.18, 30.72, 31.45, 34.91, 36.19, 44.2, 50.68, 59.23, 61.83, 63.75, 72.58, 90.58, 119.38, 122.59, 129.89, 130.33, 132.35, 147.45, 148.78, 207.33; MS (ESI) *m*/*z*: 507 (MH^+); Yield: 85%: (**9a**): ^1H NMR ($CDCl_3$): δ 0.14–0.18 (m, 2H), 0.53–0.59 (m, 2H), 0.88 (m, 1H), 1.57–1.66 (m, 3H), 1.87 (m, 1H), 2.10 (m, 1H), 2.28–2.47 (m, 4H), 2.68 (m, 2H), 3.02–3.23 (m, 2H), 3.45–3.48 (m, 2H), 3.62–3.68 (m, 10H), 4.72 (s, 1H), 6.05 (br s, NH), 6.57 (d, J = 8.4 Hz, 1H), 6.94 (d, J = 8.4 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 4.05, 4.37, 9.42, 23.21, 30.72, 31.53, 36.30, 38.89, 41.34, 43.53, 50.63, 59.29, 61.84, 61.99, 69.06, 69.98, 70.21, 70.45, 70.51, 72.71, 90.71, 119.43, 122.89, 128.83, 130.13, 132.28, 147.38, 154.02, 207.98; MS (ESI) *m*/*z*: 517 (MH^+); Yield: 73%: (**9b**): ^1H NMR ($CDCl_3$): δ 0.10–0.11 (m, 2H), 0.49–0.52 (m, 2H), 0.86 (m, 1H), 1.54–1.65 (m, 3H), 1.86 (m, 1H), 2.11 (m, 1H), 2.26–2.46 (m, 4H), 2.69 (m, 2H), 3.01–3.22 (m, 2H), 3.35 (s, 3H), 3.38–3.41 (m, 2H), 3.54–3.62 (m, 10H), 4.66 (s, 1H), 5.95 (br s, NH), 6.62 (d, J = 8.1 Hz, 1H), 6.86 (d, J = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 4.02, 4.35, 9.40, 23.23, 30.73, 31.52, 36.31, 38.87, 40.92, 43.51, 50.62, 59.27, 59.34, 61.97, 69.065 69.97, 70.22, 70.43, 70.53, 71.61, 72.72, 90.73, 119.42, 122.87, 128.81, 130.11, 132.26, 147.37, 153.43, 207.96; MS (ESI) *m*/*z*: 531 (MH^+); Yield: 78%.

32. Mikolaj, M.; Stinchcomb, A. L. *Pharm. Res.* **2010**, in press.