Bioorganic & Medicinal Chemistry Letters xxx (2014) xxx-xxx

Contents lists available at ScienceDirect



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Bioorganic & Medicinal Chemistry Letters

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

Anti-cancer activity of carbamate derivatives of melampomagnolide B

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ARTICLE INFO

Article history: Received 10 April 2014 Revised 15 May 2014 Accepted 16 May 2014 Available online xxxx

Keywords: Parthenolide Melampomagnolide B Anti-cancer activity Carbamates Heterocyclic amines

ABSTRACT

Melampomagnolide B (MMB) is a natural sesquiterpene structurally related to parthenolide (PTL). We have shown that MMB exhibits anti-leukemic properties similar to PTL. Unlike PTL, the presence of a primary hydroxyl group in the MMB molecule allows the opportunity for examining the biological activity of a variety of conjugated analogs of MMB. We have now synthesized a series of carbamate analogs of MMB and evaluated these derivatives for anti-cancer activity against a panel of sixty human cancer cell lines. Analogs **6a** and **6e** exhibited promising anti-leukemic activity against human leukemia cell line CCRF-CEM with GI_{50} values of 680 and 620 nM, respectively. Analog **6a** also showed GI_{50} values of 1.98 and 1.38 μ M respectively, against RPMI-8226 and SR leukemia cell lines and GI_{50} values of 460 and 570 nM against MDA-MB-435 melanoma and MDA-MB-468 breast cancer cell lines, respectively. Analog **6e** had GI_{50} values of 650 and 900 nM against HOP-92 non-small cell lung and RXF 393 renal cancer cell lines.

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Parthenolide (PTL; **1**, Fig. 1), a sesquiterpene lactone isolated from the medicinal herb Feverfew (*Tanacetum parthenium*), has been widely reported in the literature as an anticancer agent that is effective against both hematological and solid tumors.^{1,2} PTL and its analogs promote apoptosis by inhibiting the activity of the NF-κB transcription factor complex, and thereby downregulates anti-apoptotic genes under NF-κB control.³ Recent studies also demonstrate that PTL induces robust apoptosis of primary acute myeloid leukemia (AML) stem cells in culture.^{4,5} AML is a clonal malignancy of the hematopoietic system characterized by accumulation of immature cell populations in the bone marrow or peripheral blood,⁶ and is the most common type of leukemia in adults but has the lowest survival rate of all leukemias.⁷

More recently, we have shown that PTL and PTL analogs also selectively induce almost complete glutathione depletion and severe cell death in CD34+ AML cells,⁸ but exhibit significantly less toxicity in normal CD34+ cells. PTL analogs perturb glutathione homeostasis by a multifactorial mechanism, including inhibition of key glutathione metabolic enzymes (GCLC and GPX1), and direct depletion of glutathione. Thus, primitive leukemia cells are uniquely sensitive to agents that target aberrant glutathione metabolism, an intrinsic property of primary human AML cells.

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http://dx.doi.org/10.1016/j.bmcl.2014.05.059 0960-894X/© 2014 Published by Elsevier Ltd. PTL is a major source for several novel anti-leukemic compounds arising from our research program over the past decade. The two best examples are dimethylaminoparthenolide (DMAPT; **2**, Fig. 1) and melampomagnolide B (MMB; **3**, Fig. 1). MMB is a melampolide originally isolated from *Magnolia grandiflora*.⁹ MMB can be synthesized from commercially available PTL via $SeO_2/tBuOOH$ oxidation.^{10,11} Both of the above compounds **2** and **3** have been identified as new antileukemic sesquiterpenes with properties similar to PTL.^{11,12} DMAPT is currently in phase 1 clinical studies for evaluation as a treatment for acute myeloid leukemia cell (AML).¹²

More importantly, from a drug design point of view, MMB is a more intriguing molecule than either PTL or DMAPT because of the presence of the primary hydroxyl group at C-14, which can be structurally modified to improve potency, water solubility, bioavailability and tissue targeting of the molecule.

In the current study, we have prepared a series of novel carbamate analogs of MMB. These compounds were initially designed as



Figure 1. Structures of PTL (1), DMAPT fumarate (2) and MMB (3).

potential prodrugs of MMB. However, we have found that on examining the anticancer activity of these compounds, several of the molecules exhibited significant growth inhibition properties in a panel of sixty human cancer cell lines. Two of these compounds exhibited GI_{50} values of $\leq 10 \mu$ M against the majority of the human cancer cell lines in the panel.

Carbamate analogs of MMB were prepared by reaction of the pnitrophenyloxycarbonyl ester of MMB¹³ with a variety of primary and secondary heterocyclic amines containing pyrrolidine, morpholine, piperidine, imidazole, triazole and pyridine moieties, to afford carbamate products **6a-6g**¹⁴ with generally improved water-solubility (Scheme 1, Table 1) compared to MMB. The key *p*-nitrophenyloxycarbonyl ester of MMB was prepared by the reaction of MMB with *p*-nitrophenylchloroformate in the presence of triethylamine. All conjugation reactions were carried out at ambient temperature in dichloromethane. We have reported previously that the reaction of sesquiterpenes containing an exocyclic double bond attached to the 13-position of the 5-membered lactone ring with primary and secondary amines leads to the facile formation of Michael addition products.¹⁵ However, under the reaction conditions employed in Scheme 1, the rate of O-carbamoylation appears to be much faster than the rate of C-13 Michael addition, and only in a few cases, with amines such as 2-morpholinoethylamine, 2piperidinoethylamine and 3-aminopropylimidazole, were Michael addition byproducts observed (usually in low yields of 5-10%), due likely to the high nucleophilicity of these amines.

All compounds were purified by column chromatography (silica gel; methanol/dichloromethane) to afford pure compounds in 50–75% yield. The synthesized compounds were fully characterized by ¹H NMR, ¹³C NMR and high resolution mass spectral analysis.¹⁴

The above carbamate analogs were evaluated for growth inhibition properties against a panel of 60 human cancer cell lines derived from nine human cancer cell types, grouped into disease sub-panels that represent leukemia, lung, colon, central nervous system (CNS), melanoma, renal, ovary, breast, and prostate cancer cells. Growth inhibitory (GI₅₀) effects were measured as a function of the variation of optical density as a percentage of control.^{16,17} Initial screening assays were carried out at a single concentration of 10 µM. Five analogs, 6a-6e, were identified as hits based on their ability to inhibit by 60% the growth of at least 8 of the 60 tumor cell lines in the panel. These five analogs were then evaluated in 5-dose assays over the concentration range 10^{-4} - $10^{-8} \mu$ M, and their GI₅₀ values against the tumor cell lines in the panel determined (Table 2). Two analogs, 6a and 6e, were identified as lead compounds and generally exhibited improved growth inhibition against all human tumor cell lines when compared to PTL (1) and DMAPT (2), with the exception of the leukemia cell line subpanel; in these cell lines, the GI₅₀ values for DMAPT compared very favorably with those for both 6a and 6e. Compound 6a exhibited potency against leukemia cell line CCRF-CEM, melanoma cell line MDA-MB-435 and breast cancer cell line MDA-MB-468 in the nanomolar range with GI₅₀ values of 680, 460 and 570 nM, respectively. Compound 6e was found to possess potent anti-leukemic activity against leukemia cell line CCRF-CEM, non-small cell

Table 1

Structures, reaction conditions, yields, and melting points for carbamate analogs of melampomagnolide B



lung cancer cell line HOP-92 and renal cancer cell line RXF 393 with GI₅₀ values of 620, 650 and 900 nM, respectively, (Table 2).

Compounds **6a** and **6e** also exhibited significant growth inhibition against the following sub-panels of human cancer cell lines: non-small cell lung cancer (GI_{50} values 0.65–1.45 μ M); colon cancer (GI_{50} values 1.12–2.06 μ M); melanoma (GI_{50} values



Scheme 1. Synthesis of carbamoylated MMB analogs 6a-6g: Reagents and conditions: (a) CH₂Cl₂, triethylamine, rt, 24 h; (b) CH₂Cl₂, heterocyclic amines, rt, 5–12 h.

Please cite this article in press as: Janganati, V.; et al. Bioorg. Med. Chem. Lett. (2014), http://dx.doi.org/10.1016/j.bmcl.2014.05.059

Table 2

Growth inhibition (GI₅₀; μ M)^b data for PTL (1), DMAPT (2) and carbamoylated MMB analogs **6a–6e** against a panel of human cancer cell lines

Panel/cell line	1 ª GI ₅₀	2 ^a GI ₅₀	6a GI ₅₀	6b GI ₅₀	6c GI ₅₀	6d GI ₅₀	6e GI ₅₀
Leukemia							
CCRF-CEM	7.94	1.99	0.68	2.49	2.65	3.03	0.62
HL-60(TB)	5.01	1.58	2.04	4.15	ND	3.59	ND
K-562	19.9	2.51	3.45	3.26	ND	3.37	ND
MOLT-4	15.8	3.16	2.05	3.48	5.54	5.00	2.32
RPMI-8226	7.94	2.51	1.98	8.71	8.20	5.72	2.57
SR	ND ^c	ND	1.38	10.2	4.10	3.65	2.36
Non-small cell lung cancer							
HOP-92	12.5	10.0	1.45	2.25	2.25	2.30	0.65
NCI-H522	5.01	2.51	1.25	1.78	1.64	2.13	1.26
Colon cancer							
COLO 205	15.8	31.6	2.06	4.78	3.54	8.89	1.79
HCT-116	10.0	5.01	1.41	3.18	1.89	2.87	1.13
SW-620	15.8	3.98	1.46	3.46	3.13	3.48	1.12
CNS cancer							
SF-539	19.9	2.51	1.98	15.0	5.77	14.4	1.76
SNB-75	50.1	ND	6.13	18.0	3.78	19.5	1.71
Melanoma							
I OX IMVI	7 94	10.0	2.23	7 88	4 96	4 84	1 95
MALME-3M	12.5	ND	1 90	4 18	7.52	6.12	2.32
M14	ND	15.8	2.84	9.65	5.58	7.97	1.59
MDA-MB-435	ND	7.94	0.46	6.56	6.01	5.89	2.24
Ovarian cancer							
IGROV1	199	199	2 31	4 09	144	3 4 9	3 66
OVCAR-3	19.9	12.5	1.69	8.41	ND	6.20	ND
Panal cancor							
ACHN	ND	15.8	1 79	3.80	2 75	3 74	1 75
CAKI-1	10.0	12.5	2.03	6.86	2.75	431	1.75
RXF 393	12.5	15.8	1 20	4.08	2.00	3.00	0.90
TK-10	ND	3 16	2.60	3 1 1	3 78	3 93	2.51
D		5.1.0	2.00	5111	5170	5.05	2101
Prostate cancer	ND	5.01	2.44	7.40	4.50	2.74	2.40
DU-145	ND	5.01	2.44	7.42	4.59	3.74	3.49
Breast cancer							
MCF7	15.8	5.01	1.62	3.91	2.85	3.54	1.33
BT-549	ND	5.01	2.69	4.75	2.60	4.99	1.47
T-47D	ND	39.8	3.07	4.86	6.19	6.32	2.23
MDA-MB-468	ND	ND	0.57	2.30	3.22	3.26	1.29

GI₅₀ values <1 µM are bolded.

^a GI₅₀ values obtained from NCI database.

 $^{b}\,$ GI_{50}, concentration of analog ($\mu M)$ that halves cellular growth.

^c ND not determined.

0.46–2.84 μ M); renal cancer (GI₅₀ values 0.90–2.60 μ M); and breast cancer (GI₅₀ values 0.57–3.07 μ M) (Table 2).

We have determined the hydrolytic stability of the above five carbamate derivatives in human plasma and have shown that compounds **6b–6e** have half-lives in the range 100–180 min, while compound **6a** has a much longer half-life of 8 h in human plasma. Thus, we consider compounds **6b–6d** to be anticancer agents that are also metabolized by plasma esterases to the active parent compound MMB, while compound **6a** would be considered a more potent anticancer agent than MMB that is likely not metabolized to MMB in vivo.

The above results are interesting for a number of reasons: first, the antileukemic activities of **6a** and **6e** against the sub-panel of human leukemia cells indicates that these carbamate analogs of MMB are more potent than the parent compound. Second, the potent growth inhibition of human solid tumor cell lines by **6a** and **6e** is the first report of such activities for MMB analogs.

Third, these interesting results indicate that structural modification of the MMB molecule through appropriate carbamoylation of the primary hydroxyl group can lead to an improvement in the anticancer properties of MMB.



Figure 2. Cytotoxic mechanism of action of MMB analogs.

The above MMB analogs, like PTL and DMAPT, are inhibitors of the NF κ B pathway, activators of the nuclear kinase JNK, and selectively deplete glutathione levels in hematopoietic cancer stem cells, leading to an increase in reactive oxygen species (ROS) and subsequent apoptosis (Fig. 2).^{4,8,11} We have recently shown that hematopoietic cancer stem cells have lower levels of reduced glutathione (GSH) and increased levels of oxidized glutathione (GSSG) when compared to normal stem cells, and are thus more vulnerable to agents such PTL and MMB and its analogs that induce oxidative stress through generation of ROS.¹¹ Specifically, PTL and MMB analogs inhibit several crucial enzymes in the glutathione pathway (i.e., GCLC and GPX1) leading to severe depletion of cellular glutathione and resulting in oxidative stress and apoptosis.

In summary, we have reported on a series of novel carbamate derivatives of MMB derived from heterocyclic and heteroaromatic amines. Among these derivatives, compounds **6a** and **6e** have been identified as potent anticancer agents with growth inhibition activities in the nanomolar range against a variety of hematological and solid tumor cell lines. Analogs **6a** and **6e** exhibit promising antileukemic activity against human leukemia cell line CCRF-CEM with GI₅₀ values of 680 and 620 nM, respectively. Compound **6a** also exhibits GI₅₀ values of 460 and 570 nM against MDA-MB-435 melanoma and MDA-MB-468 breast cancer cell lines, respectively, and **6e** has GI₅₀ values of 650 and 900 nM against HOP-92 non-small cell lung and RXF 393 renal cancer cell lines, respectively. Further structure–activity relationship studies will focus on the structural optimization of these interesting lead analogs and on the molecular basis for their mechanism of action.

Acknowledgments

We are grateful to the NIH/National Cancer Institute (Grant # R01 CA158275) for supporting this research, to the Arkansas Research Alliance for an Arkansas Scholar award and to the NCI drug screening program.

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- Synthetic procedure and analytical data for the p-nitrophenyloxycarbonyl ester of 13. MMB (5): To the reaction mixture of MMB (100 mg, 0.378 mmol) and triethylamine (45.8 mg, 0.454 mmol) in dichloromethane (2 mL), pnitrophenylchloroformate (76.3 mg, 0.378 mmol) was added at 0 °C. The reaction mixture was stirred for 24 h at ambient temperature. When the reaction was completed (monitored by TLC), water was added to the reaction mixture and the aqueous mixture was extracted with dichloromethane. The organic layer was washed with water, followed by brine solution, dried over anhydrous Na2SO4 and concentrated to afford the crude product. The crude product was purified by column chromatography (silica gel, 2% methanol in dichloromethane) to afford compound **5** as a pale yellow solid. ¹H NMR (CDCl₃, 400 MHz): δ 8.26 (d, J = 9.6 Hz, 2H), 7.37 (d, J = 9.8 Hz, 2H), 6.25 (s, 1H), 5.83 (t, J = 8.4 Hz, 1H), 5.56 (s, 1H), 4.81(d, J = 12.8 Hz, 1H), 4.72 (d, J = 12.4 Hz, 1H), 3.86 (m, 1H), 2.85 (m, 2H), 2.56 (m, 7H), 1.77 (m, 2H), 1.55 (s, 1H), 1.16 (t, I = 13.2 Hz, 1H). ¹³C NMR (CDCl₃, 100 MHz): δ 169.1, 155.2, 152.2, 145.4, 138.6, 133.6, 132.8, 125.3, 121.5, 120.3, 80.8, 71.5, 63.1, 59.8, 42.7, 36.4, 25.6, 24.9, 23.9, 17.9 ppm.
- 14. General synthetic procedure and analytical data for carbamate derivatives of MMB: To the *p*-nitrophenyloxycarbonyl ester derivative of MMB (5) (70 mg, 0.16 mmol) in dichloromethane (2 mL), the appropriate amine (0.16 mmol) was added at 0 °C. The reaction mixture was stirred for 18 h at ambient temperature. When the reaction was completed (monitored by TLC), water was added to the reaction mixture and the aqueous mixture was extracted with dichloromethane. The organic layer was washed with water, followed by brine solution, dried over anhydrous Na₂SO₄ and concentrated to afford the crude product. The crude product was purified by column chromatography (silica gel, 5% methanol in dichloromethane) to afford the carbamate analogs (6a-g) as white solids. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7. 7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methyl-4,4-difluoropiperidine-1-carboxylate (**6a**): ¹H NMR (CDCl₃), 400 MHz): δ 6.27 (d, J = 2.8 Hz, 1H), 5.67 (t, J = 8.4 Hz, 1H), 5.56 (s, 1H), 4.69 (d, J = 12.4 Hz, 1H), 4.52 (d, J = 12 Hz, 1H), 3.87 (t, J = 0.6 Hz, 1H), 3.60 (br s, 4H), 2.87 (t, J = 9.2 Hz, 2H), 2.50-2.16 (m, 6H), 1.96 (br s, 4H), 1.71 (t, J = 10 Hz, 1H), 1.55 (s, 3H), 1.14 (t, J = 10 Hz), 1.5 (t, J = 10 Hz), 1. $\int = 12 \text{ Hz}, 1\text{ H}, \frac{13}{20} \text{ NMR (CDCl_3, 100 \text{ MHz}); } \delta 169.4, 154.8, 138.7, 135.3, 129.9, 121.5, 120.5 (t, <math>J_{CF}$ = 5.3 Hz, 1C), 81.1, 67.7, 63.4, 60.0, 42.7, 41.0, 36.7, 34.0, 25.8, 24.4, 23.9, 18.1 ppm. HRMS (ESI) m/z calcd for C₂₁H₂₈F₂NO₅ (M+H)⁴

412.1930, found 412.1933. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methyl-(2-(pyrrolidin-1-yl)ethyl)carbamate (6b): ¹H NMR (CDCl₂. 400 MHz): δ 6.22 (d, J = 3.2 Hz, 1H), 5.65 (t, J = 7 Hz, 1H), 5.55 (s, 1H), 4.62 (d, J = 11.6 Hz, 1H), 4.47 (d, J = 12.4 Hz, 1H), 3.82 (t, J = 9.6 Hz, 1H), 3.35 (s, 2 H), 2.90–2.83 (m, 2H), 2.70 (s, 4H), 2.42 (d, J = 9.6 Hz, 2H), 2.38–2.13 (m, 7H) 1.84 (s, 4H), 1.66 (t, J = 12 Hz, 1H), 1.52 (s, 3H), 1.13 (t, J = 11.6 Hz, 1H). ¹³C NMR (CDCl₃, 100 MHz): δ 169.3, 156.1, 138.6, 135.3, 129.8, 120.1, 80.9, 67.0, 63.1, z calcd for $C_{22}H_{33}N_2O_5$ (M+H)⁺ 405.2384, found 405.2390. z calcd for $C_{22}H_{33}N_2O_5$ (M+H)^{*} 405.2384, found 405.2390 ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10bdecahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methyl-(2-(pyridin-2yl)ethyl)carbamate (6c): ¹H NMR (CDCl₃, 400 MHz): δ 8.50 (s, 1H), 7.62 (t, J = 7.2 Hz, 1H), 7.15 (d, J = 7.6 Hz, 2H), 6.19 (s, 1H), 5.64 (t, J = 8 Hz, 1H), 5.56-5.56 (m, 2H), 4.62 (d, J = 12.4 Hz, 1H), 4.44 (d, J = 12.8 Hz, 1H), 3.84 (t, J = 9.2 Hz, 11H) 3.60 (d, *J* = 6 Hz, 2H), 2.99–2.82 (m, 4H), 2.41–2.12 (m, 5H), 1.75 (s, 1H), 1.64 (d, *J* = 10.4 Hz, 1H), 1.52 (s, 3H), 1.07 (t, *J* = 13.6 Hz, 1H). ¹³C NMR (CDCl₃, 100 MHz): 8 169.3, 159.1, 156.0, 149.1, 138.6, 136.6, 135.5, 129.9, 123.4, 121.6, 120.2, 81.0, 67.0, 63.2, 59.8, 42.5, 40.1, 37.1, 36.6, 25.8, 24.4, 23.7, 17.9 ppm. HRMS (ESI) m/z calcd for C23H29N2O5 (M+H)⁺ 413.2071, found 413.2073. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10bdecahydrooxireno[2',3':9,10]-cyclodeca[1,2-b]furan-5-yl)-methyl(2-morpholino ethyl)carbamate (**6d**): ¹H NMR (CDCl₃, 400 MHz): δ 6.11(d, J = 3.6 Hz, 1H), 5.58 (t, J = 8 Hz, 1H), 5.43 (d, J = 2.8 Hz, 1H), 5.06 (s, 1H), 4.53 (d, J = 12.4 Hz, 1H), 4.34 (d, J = 12 Hz, 1H), 3.74 (t, J = 8.8 Hz, 1H), 3.56 (s, 4 H), 3.16 (t, J = 5.2 Hz, 2H) 2.78–2.72 (m, 2H) 2.32 (5, 8H) 2.21–2.00 (m, 4H) 1.55 (L) = 10.4 Hz, 1H), 1.41 (s, 3H), 1.01 (t, J = 12 Hz, 1H). ¹³C NMR (CDCl₃, 100 MHz): δ 169.2, 155.9, 138.7, 135.4, 130.2, 120.0, 80.9, 67.0, 66.7, 63.1, 59.8, 57.2, 53.1, 42.5, 37.0, 36.5, 25.7, 24.4, 23.7, 17.8 ppm. HRMS (ESI) *m*/*z* calcd for C₂₂H₃₃N₂O₆ (M+H)⁺ 421.2333, found 421.2331. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methyl-(5-(methylthio)-1H-1,2,4-triazol-3-yl)carbamate (**6e**): ^{1}H NMR (CDCl₃, 400 MHz): δ 6.22 (s, 2H), 5.82 (t, J = 8 Hz, 1H), 5.50 (s, 1H), 4.90 (d, J = 12.4 Hz, 1H), 4.81(d, J = 12.4 Hz, 1H), 3.85 (t, J = 9.6 Hz, 1H), 2.95 (s, 1H), 2.85 (d, J = 9.2 Hz, 1H), 2.48–2.15 (m, 8H), 1.70–1.53 (m, 6H), 1.13 (t, J = 12.4 Hz, 1H) ppm. ¹³C NMR (CDCl₃, 100 MHz): δ 169.1, 163.1, 157.4, 149.9, 138.5, 133.3, 132.7, 120.2, 80.7, 70.1, 62.9, 59.7, 42.4, 36.2, 25.6, 24.3, 23.7, 17.8, 13.5 ppm. HRMS (ESI) *m*/*z* calcd for C₁₉H₂₅N₄O₅S (M+H)⁺ 421.1540, 421.1524.((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxofound 1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5yl)methyl-(3-(1H-imidazol-1-yl)-propyl)carbamate (6f): ¹H NMR (CDCl₃, 400 MHz): δ 7.56 (s, 1H), 7.09 (s, 1H), 6.95 (s, 1H), 6.26 (d, J = 3.6 Hz, 1H), 5.69 (t, J = 8 Hz, 1H), 5.56 (d, J = 3.2 Hz, 1H), 4.85 (s, 1H), 4.64 (d, J = 12.4 Hz, 5.09 (t, J = 8 Hz, 1H), 5.50 (d, J = 3.2 Hz, 1H), 4.83 (s, 1H), 4.04 (d, J = 12.4 Hz, 1H), 4.51 (d, J = 12.4 Hz, 1H), 4.04 (t, J = 7.2 Hz, 2H), 3.88 (t, J = 9.2 Hz, 1H), 3.21 (d, J = 6 Hz, 2H), 2.93–2.85 (m, 2H), 2.47–2.16 (m, 7H), 2.03 (t, J = 6.4 Hz, 1H) 1.70 (t, J = 10.8 Hz, 1H), 1.55 (s, 3H), 1.15 (t, J = 12.4 Hz, 1H). ¹³C NMR (CDCl₃, 100 MHz): δ 169.5, 156.3, 138.9, 137.1, 135.3, 130.4, 129.6, 120.2, 118.8, 81.1, (ESI) m/z calcd for $C_{22}H_{30}N_3O_5$ (M+H)* 416.2180, found 416.2183. ((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10bdecahydrooxireno[2',3':9,10]-cyclodeca[1,2-b]furan-5-yl)methyl-(3- $\begin{array}{l} \mbox{(acting a loss in log_2, j. 3), for (states 1), 2, 5), and (states 1), for (states 1), and (stat$ 2H), 2.46–2.11 (m, 12H), 1.69 (t, J = 12 Hz 3H), 1.52 (s, 3H), 2.10 (H), 2.10 (H), 2.11 (t, J = 12 Hz, 1H), 13 C NMR (CDCl₃, 100 MHz): δ 169.5, 156.3, 138.9, 135.7, 129.9, 120.4, 81.2, 67.0, 66.9, 63.4, 60.1, 57.4, 53.6, 42.7, 36.8, 25.9, 25.5, 24.6, 23.9, 18.1 ppm. HRMS (ESI) m/z calcd for C₂₃H₃₅N₂O₆ (M+H)⁺ 435.2490, found 435.2482 15. Neelakantan, S.; Shama, N.; Guzman, M. L.; Jordan, C. T.; Crooks, P. A. Bioorg.

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