Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 2877–2881

Synthetic analogues of the manzamenones and plakoridines which inhibit DNA polymerase

Jeremy R. Doncaster,[a] Laura L. Etchells,[a] Neil M. Kershaw,[a] Ryoichi Nakamura,[b] Hazel Ryan,[a] Ryo Takeuchi,[b] Kengo Sakaguchi,[b] Ali Sardarian[a,†] and Roger C. Whitehead[a,*]

[a]*The School of Chemistry, The University of Manchester, Oxford Road, Manchester M13 9PL, UK*
[b]*Department of Applied Biological Science, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan*

Received 7 February 2006; revised 1 March 2006; accepted 2 March 2006
Available online 24 March 2006

Abstract—An array of novel analogues of the marine oxylipins, the manzamenones and plakoridines, have been prepared in divergent fashion using an approach modelled on a biogenetic theory. Many of the target compounds show potent inhibition of DNA polymerases α and β and human terminal deoxynucleotidyl transferase (TdT).

Marine sponges of the genus *Plakortis* are a rich source of structurally diverse natural products with a variety of bioactivities including anti-cancer, anti-bacterial and anti-fungal properties.[1] Some time ago, we became interested in a family of oxidized fatty acid derivatives (oxylipins) isolated from Japanese *Plakortis* sponges by the research group of Jun'ichi Kobayashi. Members of this family of compounds, which include untenone A (**3**),[2] the manzamenones (e.g., manzamenone A (**5**)),[3a–c] plakevulin A (**6**)[4a,b] and the plakoridines (**9**, **10**)[3c,5] are characterized by the common structural features of at least one fully saturated C_{16} alkyl chain and at least one methoxycarbonyl group. It was originally observed by Kobayashi that many members of the family possessed structures which could be derived biosynthetically from (*E*)/(*Z*)-methyl-3,6-dioxo-4-docosenoate **1/2**. Prompted by this observation, we have described a plausible biosynthetic pathway which interrelates **1** and/or **2** with the various oxylipins (Scheme 1) and we have also provided synthetic chemical evidence in support of our proposal.[6a–e]

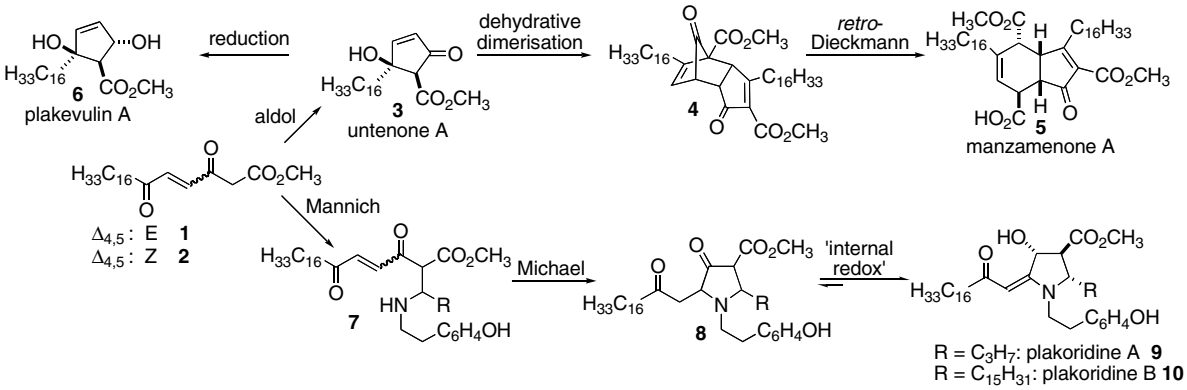
Untenone A and manzamenone A are efficient inhibitors of DNA polymerases α and β (pol α and pol β) as well as human terminal deoxynucleotidyl transferase (TdT) (IC_{50} values ranging between 1.9 and 57 μM).[7] The biological potency of **3** and **5** has provided stimulus for investigations into the synthesis and evaluation of other compounds belonging to this structural class. Herein, we describe the preparation of novel analogues of the manzamenones and the plakoridines using a divergent synthetic approach modelled on our biogenetic theory, together with the results of bioassays of the target compounds against pol α, pol β and human TdT.

The ultimate aim of our research was to prepare a range of analogues of the manzamenones (general structure **13**) and the plakoridines (general structure **14**) using an approach modelled on our biogenetic theory (Scheme 2).

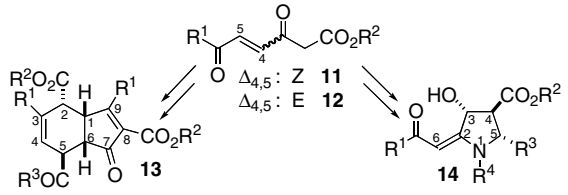
Our first goal towards this end was the synthesis of (*E*)- and (*Z*)-enediones of type **11/12**, which was accomplished in expedient fashion from the methyl ester of 2-furan acetic acid **15** (Scheme 3). Thus, Friedel–Crafts acylation of **15** with the appropriate acid chloride followed by ketone reduction using the Huang-Minlon modification of the Wolff–Kishner conditions[8a,b] gave 5-alkyl-furan-2-yl-acetic acids **16** in moderate yields. Subsequent esterification under either acid-catalysed conditions or in the presence of DCCI gave ester derivatives **17**.

Keywords: Oxylipins; Natural products; DNA polymerase; Biomimetic synthesis.
* Corresponding author. Tel.: +44 161 275 7856; fax: +44 161 275 4939; e-mail: roger.whitehead@manchester.ac.uk
† Present address: The Department of Chemistry, The College of Sciences, The University of Shiraz, Shiraz 71454, Iran.

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.03.005



Scheme 1.



Scheme 2. Compound **13**: R^1 = linear alkyl; R^2 = linear or branched alkyl; R^3 = hydroxy, alkoxy or alkylamino. Compound **14**: R^1 and R^2 = linear alkyl; R^3 = linear alkyl or aryl; R^4 = terminally substituted alkyl.

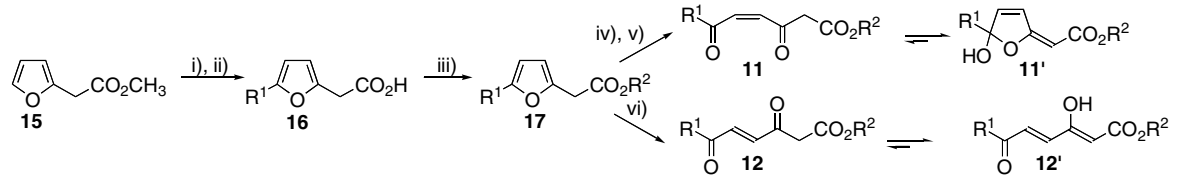
Conditions were then developed for the controlled oxidation of the furan ring of **17** to give either **11** or **12**.[6e] Treatment of **17** with bromine in methanol,[9] followed by hydrolysis of the resulting bis-acetals under mildly acidic conditions,[10] gave (*Z*)-enediones **11** which exist predominantly in solution as the cyclic hemi-ketal tautomers **11′**. Alternatively, exposure of **17** dissolved in a mixture of acetone and water (5:1) to one equivalent of bromine at −20 °C gave (*E*)-enediones **12**,[11] which exist in solution almost exclusively as enol tautomers **12′**.[6e]

Mildly basic treatment of hemiketals **11′** resulted in facile aldol cyclisation to give untenone analogues **18** from which the target array of manzamenone analogues **13** could be prepared (Scheme 4).

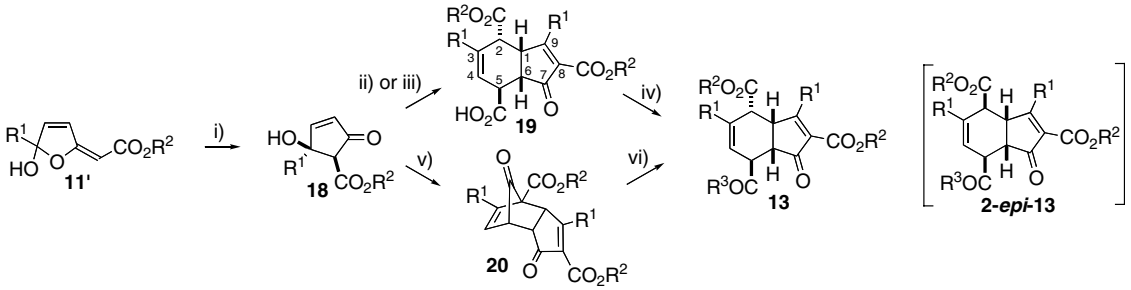
Our preferred approach to the synthesis of compounds **13** involved initial preparation of manzamenone A analogues **19** using previously described procedures.[6a–d] Subsequent derivatisation of the free carboxyl group of **19** using standard coupling procedures then provided the target compounds **13**. An intrinsically more attractive approach to compounds **13** which closely mirrors the proposed biogenesis of the manzamenones involves retro-Dieckmann reaction of tri-cyclic adducts **20** mediated by different nucleophiles R^3H. Herein, we report for the first time that dehydrative dimerisation of cyclopentenones **18** to give adducts **20** can be achieved using trifluoroacetic anhydride (TFAA) as dehydrating agent: the success of this reagent being a consequence of the absence of nucleophilic entities in the reaction mixtures which would otherwise quench the reactive species **20**. Disappointingly, exposure of **20** to a range of O and N centred nucleophiles gave manzamenone analogues **13** in generally poor yields with formation of alternative diastereoisomers (believed to be **2-*epi*-13**), being competitive with the desired outcome. Formation of the latter was found most significant following incomplete removal of trifluoroacetic acid (TFA) from samples of **20** prior to nucleophilic quenching and is believed to be a consequence of reduced facial selectivity in protonation of the intermediate enols/enolates formed during the retro-Dieckmann reactions.

The expediency of the synthetic route to manzamenone analogues of type **13** has allowed investigations into the effect of core structural changes on the enzyme inhibitory activity of this class of compounds. For example, cyclopropyl compound **23** was synthesized in three steps, and in moderate yield, from 43-*O*-methylmanzamenone A (**21**) (Scheme 5):[6d] a more direct approach to **23** utilising dimethylsulfoxonium methylide (Corey's reagent[12]) for incorporation of the cyclopropane ring was, unfortunately, unsuccessful.

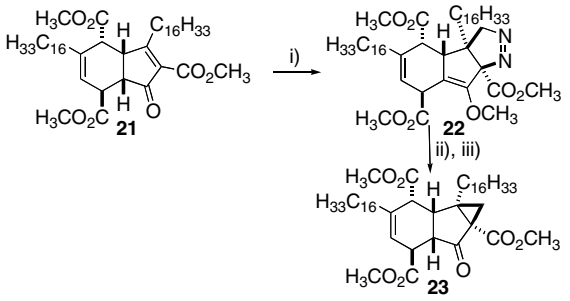
A selection of our target compounds of type **13** were assayed against pol α, pol β and human TdT using the



Scheme 3. Reagents and conditions; (i) RCOCl, $SnCl_4$, CH_2Cl_2, −5 °C, 84–98%; (ii) H_2NNH_2, NaOH, $HOCH_2CH_2OH$, Δ, 50–96%; (iii) R^2OH, Amberlite® IR 120 (H), Δ or R^2OH, DCCI, CH_2Cl_2, 0 °C, 75–98%; (iv) Br_2, CH_3OH, Na_2CO_3, 67–96%; (v) 0.005 M H_2SO_4, H_2O, dioxane, rt; (vi) Br_2, acetone, H_2O, −20 °C to −10 °C, 56–61%.



Scheme 4. Reagents and conditions; (i) $NaHCO_3$ (aq), dioxane, rt, 1 h, 65–70%; (ii) 40–70 °C, neat, 1–6 day, 48–94%; (iii) scandium trisdodecylsulfate (0.1 equiv.), H_2O, 25 °C, 1–24 h, 70–80%; (iv) R^3H, DCCI, CH_2Cl_2, 41–57%; (v) TFAA, $CDCl_3$, rt, 24 h; (vi) for ester derivatives: R^3H, rt, 24 h, 15–63% from **18**; for amide derivatives: R^3H, CH_2Cl_2, rt, 24 h, 10–15% from **18**.



Scheme 5. Reagents and conditions; (i) CH_2N_2, Et_2O, rt, 94%; (ii) neat, 125 °C, 4 h, 57%; (iii) HCl, Et_2O, rt, 24 h, 56%.

methods previously described by Mizushina et al.[13a–c] The results of the assays are presented in Table 1. Regarding the structural features which influence the extent of inhibition of the polymerases, a number of conclusions can be reached: (i) lengthy alkyl chains and the presence of a conjugated enone system are both prerequisites for potent inhibition; (ii) a free carboxylic acid group attached to C5 is not a requirement for good activity and ester moieties at this position are well tolerated; (iii) inversion of relative configuration at C2 has little effect on the extent of enzyme inhibition. With regard to inhibition of human TdT, all structural alterations resulted in a reduction of potency compared with manzamenone A and, in particular, lengthy pendant alkyl chains as well as a free carboxylic acid group at C5 seemed to be important for good levels of enzyme inhibition.

The second phase of our research has involved the preparation and bioassay of novel analogues of the plakoridines with general structure **14**. These compounds have been prepared from enols **12′** in one simple synthetic operation, using an approach modelled on the biogenetic theory.[6e] Thus, storage of $CDCl_3$ solutions of **12′** and imines **26**[14] at rt for prolonged periods of time gave plakoridine analogues **14**, via intermediate diketones **27**, in acceptable yields (Scheme 6).

Small quantities of the diastereoisomers **3-*epi*-14** were also generated in these reactions which could be separated from the major product by careful chromatography.

The results of bioassays of the novel plakoridine analogues against DNA polymerases α/β and human TdT are presented in Table 2. All of the pyrrolidine derivatives are good to moderate inhibitors of the polymerases, although generally less active than the manzamenone analogues. Potency is sensitive to even a small reduction in the length of the alkyl chain R^1 and is insensitive to inversion of the stereochemistry at C3 as well as variation of the ring substituents R^3 and R^4: the only exception to the latter generalization being the analogue derived from tryptamine (entry 6) which showed good potency and slight selectivity for DNA polymerase β. None of the analogues displayed significant inhibition of human TdT.

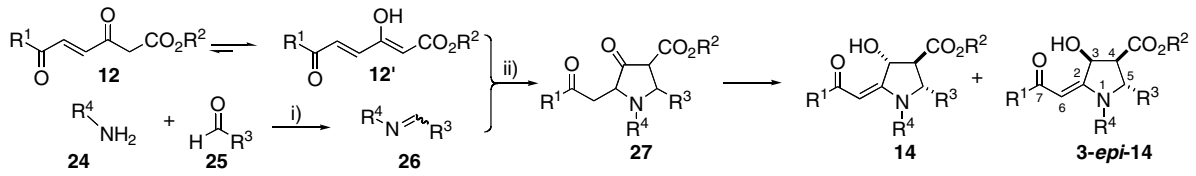
Table 1. Inhibition of pol α, pol β and human TdT by manzamenone analogues

Compound	R^1	R^2	R^3	IC_{50} (μM)		
				Pol α	Pol β	TdT
1	$C_{16}H_{33}$	CH_3	OH	1.9	3.2	2.5
2	C_2H_5	CH_3	OH	>50[c]	>50[c]	>50[c]
3	$C_{12}H_{25}$	CH_3	OH	1.9	2.3	8.6
4	$C_{16}H_{33}$	^{i}Pr	OH	3.3	4.4	20
5	$C_{16}H_{33}$	CH_3	$HN(CH_2)_2Ph$	6.9	8.7	>12.5[c]
6	$C_{16}H_{33}$	CH_3	OC_2H_5	2.3	1.9	39.7
7[a]	$C_{16}H_{33}$	CH_3	OC_2H_5	3.5	2.4	48.4
8[b]	$C_{16}H_{33}$	CH_3	OCH_3	>12.5[c]	>12.5[c]	>12.5[c]

[a] Inverted stereochemistry at C2.

[b] Cyclopropyl compound **23**.

[c] Poor solubility at higher concentrations did not allow accurate assessment of IC_{50}.



Scheme 6. Reagents and conditions; (i) H_2O, rt, 3 h, 90–97%; (ii) $CDCl_3$, rt, 4–28 d, 26–40%.

Table 2. Inhibition of pol α, pol β and human TdT by plakoridine analogues

Compound	R^1	R^2	R^3	R^4	IC_{50} (μM)		
					Pol α	Pol β	TdT
1	$C_{16}H_{33}$	CH_3	C_2H_5	$Ph(CH_2)_2$	8.9	8.7	>25[b]
2	$C_{16}H_{33}$	CH_3	C_6H_5	$Ph(CH_2)_2$	7.9	8.8	>50[b]
3	$C_{12}H_{25}$	CH_3	C_2H_5	$Ph(CH_2)_2$	25	22	>50[b]
4	$C_{12}H_{25}$	CH_3	C_2H_5	$PhCH_2$	19.9	30	>50[b]
5[a]	$C_{12}H_{25}$	CH_3	C_2H_5	$PhCH_2$	19.7	27.1	>50[b]
6	$C_{12}H_{25}$	CH_3	C_6H_5	3-indolyl$(CH_2)_2$	21	10	>50[b]

[a] Inverted stereochemistry at C3.
[b] Poor solubility at higher concentrations did not allow accurate assessment of IC_{50}.

Table 3. Inhibition of pol α and pol β by enedione **1**, untenone A (**3**) and plakevulin A (**6**)

	IC_{50}(μM)	
	Pol α	Pol β
(*E*)-Enedione **1**	3.7	8.8
Untenone A (**3**)	4.3	57
Plakevulin A (**6**)	66	179

The results reported here for the array of novel analogues of the manzamenones and plakoridines indicate that the more potent inhibitors of DNA polymerases α and β share three common structural features: at least one long saturated alkyl chain, an α,β-unsaturated carbonyl moiety and at least one carboxyl group. Untenone A (**3**) also possesses these three features and is a potent inhibitor of pol α,[7] whereas plakevulin A (**6**) lacks an α,β-unsaturated carbonyl moiety and is only a moderate inhibitor of pol α and a poor inhibitor of pol β.[4b] Prompted by these observations, we have assayed (*E*)-methyl-3,6-dioxo-4-docosenoate (**1**), the putative biosynthetic precursor of the natural plakoridines, against the polymerases. This compound also possesses the three key structural features and, pleasingly, was found to be a potent inhibitor of both pol α and pol β, showing a slight selectivity for inhibition of the former (Table 3).

In conclusion, we have prepared a range of novel analogues of the marine oxylipins, the manzamenones and the plakoridines, using an approach modelled on a biogenetic theory. We have found that many of the analogues show strong inhibition of DNA polymerases α and β and human TdT with the plakoridine analogues being generally less potent than the manzamenones. The data indicate that three structural features are key for good inhibition of the polymerases: at least one long saturated alkyl chain, an α,β-unsaturated carbonyl moiety and at least one carboxyl group. The results of previous SAR studies of untenone A analogues are in accord with this observation.

Acknowledgments

We acknowledge, with thanks, the EPSRC for funding (J.R.D., L.L.E., N.M.K., H.R. and A.S.) and we are also very grateful to Professor Gareth Morris of The University of Manchester for invaluable advice concerning the NMR analysis of several of our target compounds. We also thank Dr. Masaharu Takemura of the Functional Genomics Institute at Mie University for the generous donation of DNA polymerase α.

References and notes

1. Rahm, F.; Hayes, P. Y.; Kitching, W. *Heterocycles* **2004**, *64*, 523.
2. Ishibashi, M.; Takeuchi, S.; Kobayashi, J. *Tetrahedron Lett.* **1993**, *34*, 3749.
3. (a) Tsukamoto, S.; Takeuchi, S.; Ishibashi, M.; Kobayashi, J. *J. Org. Chem.* **1992**, *57*, 5255; (b) Kobayashi, J.; Tsukamoto, S.; Takeuchi, S.; Ishibashi, M. *Tetrahedron* **1993**, *49*, 5955; (c) Takeuchi, S.; Kikuchi, T.; Tsukamoto, S.; Ishibashi, M.; Kobayashi, J. *Tetrahedron* **1995**, *51*, 5979.
4. (a) Tsuda, M.; Endo, T.; Perpelescu, M.; Yoshida, S.; Watanabe, K.; Fromont, J.; Mikami, Y.; Kobayashi, J. *Tetrahedron* **2003**, *59*, 1137; (b) Saito, F.; Takeuchi, R.; Kamino, T.; Kuramochi, K.; Sugawara, F.; Sakaguchi, K.; Kobayashi, S.; Tsuda, M.; Kobayashi, J. *Tetrahedron Lett.* **2004**, *45*, 8069.
5. Takeuchi, S.; Ishibashi, M.; Kobayashi, J. *J. Org. Chem* **1994**, *59*, 3712.
6. (a) Al-Busafi, S.; Drew, M. G. B.; Sanders, T.; Whitehead, R. C. *Tetrahedron Lett.* **1998**, *39*, 1647; (b) Al-Busafi, S.; Whitehead, R. C. *Tetrahedron Lett.* **2000**, *41*, 3467; (c) Al-Busafi, S.; Doncaster, J. R.; Drew, M. G. B.; Regan, A. C.; Whitehead, R. C. *J. Chem. Soc., Perkin Trans. 1* **2002**, 476; (d) Doncaster, J. R.; Ryan, H.; Whitehead, R. C. *Synlett* **2003**, 651; (e) Etchells, L. L.; Sardarian, A.; Whitehead, R. C. *Tetrahedron Lett.* **2005**, *46*, 2803.
7. Saito, F.; Takeuchi, R.; Kamino, T.; Kuramochi, K.; Sugawara, F.; Sakaguchi, K.; Kobayashi, S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1975.

8. (a) Huang-Minlon *J. Am. Chem. Soc.* **1946**, *68*, 2487; (b) Huang-Minlon *J. Am. Chem. Soc.* **1949**, *71*, 3301.
9. Levisalles, J. *Bull. Soc. Chim. Fr.* **1957**, 997.
10. Williams, P. D.; LeGoff, E. *J. Org. Chem.* **1981**, *46*, 4143.
11. Jurczak, J.; Pikul, S. *Tetrahedron Lett.* **1985**, *26*, 3039.
12. Golobolov, Y. G.; Nesmeyanov, A. N.; Lysenko, V. P.; Boldeskul, I. E. *Tetrahedron* **1987**, *43*, 2609.
13. (a) Mizushina, Y.; Tanaka, N.; Yagi, H.; Kurosawa, T.; Onoue, M.; Seto, H.; Horie, T.; Aoyagi, N.; Yamaoka, M.; Matsukage, A.; Yoshida, S.; Sakaguchi, K. *Biochim. Biophys. Acta* **1996**, *1308*, 256; (b) Mizushina, Y.; Yagi, H.; Tanaka, N.; Kurosawa, T.; Seto, H.; Katsumi, K.; Onoue, M.; Ishida, H.; Iseki, A.; Nara, T.; Morohashi, K.; Horie, T.; Onomura, Y.; Narusawa, M.; Aoyagi, N.; Takami, K.; Yamaoka, M.; Inoue, Y.; Matsukage, A.; Yoshida, S.; Sakaguchi, K. *J. Antibiot.* **1996**, *49*, 491; (c) Mizushina, Y.; Yoshida, S.; Matsukage, A.; Sakaguchi, K. *Biochim. Biophys. Acta* **1997**, *1336*, 509.
14. Simion, A.; Simion, C.; Kanda, T.; Nagashima, S.; Mitoma, Y.; Yamada, T.; Mimura, K.; Tashiro, M. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2071.