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Design, synthesis and antiproliferative activity of two new heteroannelated (−)-muricatacin mimics

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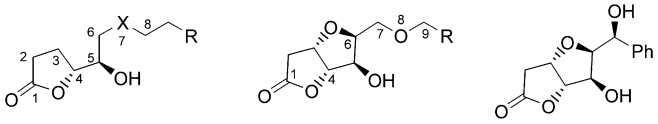
ABSTRACT

Two new (−)-muricatacin mimics bearing a furano-furanone ring and an oxygen isostere in the side chain have been designed and synthesized and their in vitro antiproliferative activity was evaluated against several human tumour cell lines. Both analogues showed an increased activity against HL-60 cells with 17- and 185-fold higher potency than (−)-muricatacin. A straightforward synthesis of (−)-muricatacin is also disclosed.

Annonaceous acetogenins, which have been isolated from a number of tropical and subtropical plants of the Annonaceae family, have attracted much attention due to their interesting biological activities.[1] (−)-Muricatacin (**1**, Scheme 1) is a simple member of the annonaceous acetogenins family. It was isolated as the major component of a pseudo-racemic mixture (ca. 25% ee based on optical rotation) from the seeds of *Annona muricata* and has received a great deal of attention due to its diverse biological profile.[2] Notably, both **1** and its enantiomer exhibit a potent antiproliferative activity towards several human tumour cell lines with SAR studies showing that activity is influenced significantly by the nature of the side chain.[3] The biological potential of muricatacin has prompted many syntheses.[4] However, development of general and flexible strategies for the preparation of new side chain analogues and more highly functionalized mimics remains a goal. A number of muricatacin analogues has also been synthesized,[3,5,6] but only a few were evaluated for their antitumour activity.[3,6] Previous studies in our laboratory showed that a mimic of (−)-muricatacin in which a methylene group from the side chain has been replaced by an ether function (compound **2**) exhibited in vitro antitumour activity against several human cancer cell lines.[7] As an extension of our previous work, we report herein on the synthesis and biological evaluation of furanolactones **3** and **4** as conformationally constrained (−)-muricatacin analogues. Compound **3** represents a heteroannelated mimic of **1** with constrained rotation around the C_4–C_5 and C_5–C_6 bonds, while the molecule **4** represents a one-carbon higher homologue of **3**. In the same time, both furanolactones **3** and **4** might also be considered as non-styryl analogues of (−)-goniofufurone (**5**), the opposite enantiomer of naturally occurring cytotoxic lactone (+)-goniofufurone.[8] Apart from the synthesis of **3** and **4**, a novel route for the preparation of (−)-muricatacin (**1**) is also disclosed in order to provide a sample of **1** that should serve as a positive control in antitumour assays.



1 X = CH_2, R = C_8H_{17}
2 X = O, R = C_8H_{17}

3 R = C_8H_{17}
4 R = C_9H_{19}

5

Scheme 1. Structures of (−)-muricatacin (**1**), (−)-goniofufurone (**5**) and (−)-muricatacin mimics **2**, **3** and **4**.

The synthesis of **1** is shown in Scheme 2. The sequence started from the known dialdose **6** that is readily available from D-xylose in five steps.[7] Wittig olefination of aldehyde **6** with the appropriate C_{11}-ylide gave the corresponding *Z*-olefine **7** as the only isolable isomer in 50% yield. Catalytic hydrogenation of **7** over PtO_2, followed by hydrolytic removal of the cyclohexylidene protective group in **8**, gave the expected lactol **9**. Oxidative cleavage of **9** with

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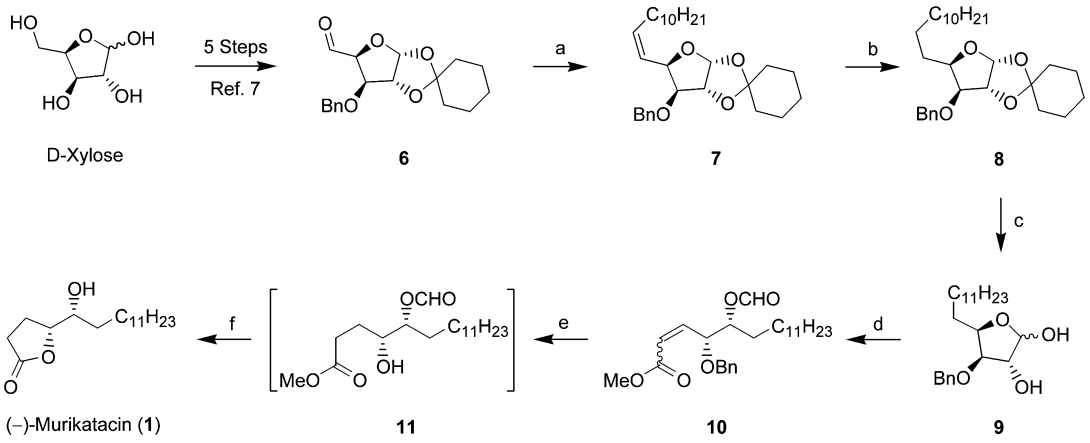
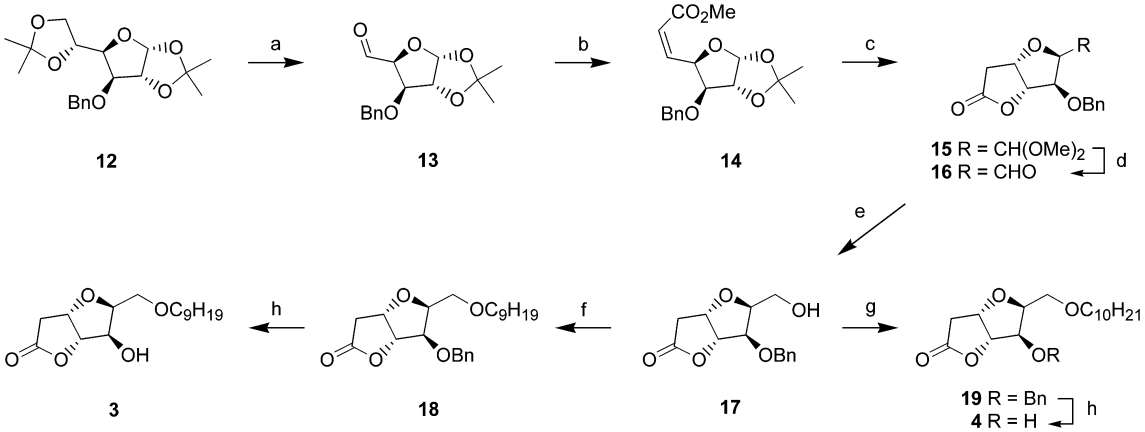


Scheme 2. Reagents and conditions: (a) $[Ph_3PC_{11}H_{23}]^+Br^-$, *n*-BuLi, THF, −78 °C → rt, then rt for 46 h, 50%; (b) H_2-PtO_2, MeOH, rt, 16 h, 90%; (c) 70% AcOH, reflux, 18 h, 57%; (d) aq $NaIO_4$, silica gel, Et_2O, rt, 24.5 h, then Ph_3P:$CHCO_2Me$, rt, 3 h, 68%; (e) H_2-Pd/C, MeOH, rt, 3 h; (f) 2:1 TFA/H_2O, rt, 1.5 h, 92% (from **10**).

sodium periodate on silica gel followed by Wittig olefination of the resulting aldehyde with a stabilized C_2-ylide ($Ph_3P = CHCO_2Me$) afforded the unsaturated ester **10** as a mixture of the corresponding *Z*- and *E*-isomers. The mixture was not separated, but was subsequently subjected to catalytic hydrogenation over 10% Pd/C to afford the saturated ester **11**, which was isolated in pure form after the usual work-up and used in the next step without any further purification. Subsequent treatment of crude **11** with aq trifluoroacetic acid gave (−)-muricatacin (**1**) in 92% yield (from **10**). A synthetic sample of **1** {mp 68–69 °C, $[\alpha]_D = -22.0$ (*c* 1.3, $CHCl_3$) literature[5d] mp 68–70 °C, $[\alpha]_D = -19.0$ (*c* 1.8, $CHCl_3$)} exhibited ^1H and ^{13}C spectral data identical to those previously reported in the literature.[5d]

The synthesis of analogues **3** and **4** is shown in Scheme 3. The sequence commenced with the formation of the protected aldehydo-lactone **16** from the known[9] D-Glucose derivative **12** by a slight modification of a literature method.[10] The terminal isopropylidene domain in **12** was directly converted to an aldehyde group in a single step using 1.25 M equivalents of periodic acid in dry ethyl acetate.[11] The resulting crude aldehyde **13** was used in the next step without any purification. The *Z*-selective Wittig reaction with the stabilized C_2-ylide ($Ph_3P = CHCO_2Me$), that was performed in dry methanol at low temperature,[12] afforded predominantly the *Z*-enoate **14** (73% from **12**), accompanied with a minor amount (10%) of the corresponding *E*-isomer (not shown in the reaction scheme). The IR, ^1H and ^{13}C NMR data are consistent with structure **14**. An acid-catalyzed methanolysis of **14**, in the presence of catalytic amount of sulphuric acid gave the known[10] furano-lactone **15** in 79% yield. The ^1H and ^{13}C NMR spectral data of thus prepared sample **15** were in full agreement with the reported data. Hydrolytic removal of the dimethyl acetal protection in **15** followed by a subsequent $NaBH_4$ reduction of the resulting aldehyde **16** gave the corresponding primary alcohol **17**. This procedure provided the key intermediate **17** in 32.3% overall yield with respect to the starting compound **12**. Alcohol **17** readily reacted with an excess of nonyl bromide and silver oxide in ether, in the presence of a catalytic amount of silver triflate, to give the expected 7-*O*-nonyl derivative **18** in 33% yield. Hydrogenolytic removal of the benzyl ether protective group in **18** furnished **3**[13] in 82% yield. Finally, by using the former two-step sequence, and the decyl bromide as an alkylation agent, compound **17** was converted to the target **4**.[14]

Compounds **2**, **3** and **4** were evaluated for their in vitro antitumour activity against the following human cell lines: myelogenous leukaemia (K562), promyelocytic leukaemia (HL-60), T cell leukaemia (Jurkat), cervix carcinoma (HeLa) and normal foetal lung fibroblasts (MRC-5). Cell growth inhibition was evaluated after 72-h cells treatment by using the MTT assay. The (−)-muricatacin (**1**) was used as a reference compound. The results are shown in Table 1.



Scheme 3. Reagents and conditions: (a) H_5IO_6, EtOAc, rt, 1.5 h; (b) Ph_3P:$CHCO_2Me$, MeOH, 0 °C for 0.5 h, then rt for 1.5 h, 73% from **12**; (c) i—H_2SO_4, MeOH, reflux, 2 h, ii—$NaHCO_3$, MeOH, 35 °C, 1 h, 79%; (d) 9:1 TFA/H_2O, rt, 0 °C for 0.5 h, then rt for 0.5 h; (e) $NaBH_4$, MeOH, rt, 1.5 h, 56% from **15**; (f) $C_9H_{19}Br$, Ag_2O, AgOTf, Et_2O, reflux, 28 h, 33%; (g) $C_{10}H_{21}Br$, Ag_2O, AgOTf, Et_2O, reflux, 32 h, 54%; (h) H_2-Pd/C, MeOH, rt, 18 h for **18**, 82% of **3**, 21 h for **19**, 80% of **4**.

Table 1
Antiproliferative activities of **1**, **2**, **3** and **4**

Compound	IC_{50} (μM)[a]				
	K562	HL-60	Jurkat	HeLa	MRC-5
1	0.04	25.85	>100	0.17	>100
2	0.13	0.15	3.01	21.45	>100
3	8.61	1.53	6.64	9.59	>100
4	1.25	0.14	>100	0.30	>100

[a] IC_{50} is the concentration of compound required to inhibit the cell growth by 50% compared to an untreated control. Values are means of three independent experiments done in quadriplicates. Coefficients of variation were <10% (range: 0.13–9.89%).

All three (−)-muricatacin mimics (**2**–**4**) retained the selectivity between the normal human cells (MRC-5) and tumour cells (K562, HL-60, Jurkat and HeLa). Among **2**–**4**, only the analogue **4** exhibited the same growth inhibition pattern as (−)-muricatacin (**1**) and represents a bioisostere of **1**. As (−)-muricatacin itself, compound **4** was inactive against T cell leukaemia (Jurkat), but showed a potent antiproliferative activity towards K562, HL-60 and HeLa malignant cells. On the contrary to **1** and **4**, both analogues **2** and **3** exhibited significant antiproliferative activities towards the all tested cell lines, including a notable activity against the Jurkat cells. (−)-Muricatacin (**1**) was the most potent compound in the K562 cell line, as it exhibited 1–2 order of magnitude higher antiproliferative effect when compared to **2**, **3** and **4**. The most pronounced antiproliferative activity of analogues **2–4** was recorded against the HL-60 cell line. Compounds **2** and **4** exhibited 172- and 185-fold, stronger activities when compared to the parent compound **1**. The analogue **3** also showed a remarkable antiproliferative activity towards this cell line, being almost 17-fold, more potent than (−)-muricatacin. A sub-micromolar activity of **4** was also recorded against the HeLa malignant cells, comparable to that observed for the reference compound **1**.

According to the above biological results, replacement of the side chain methylene group in (−)-muricatacin with an oxygen isostere increases the activity of the analogue (compound **2**) against the HL-60, and Jurkat cell lines, but significantly decreases its antiproliferative activity in K562 and HeLa cells. However, a comparison of biological data of **2** and **3** revealed that introduction of a THF ring decreases the activity of annelated (−)-muricatacin mimic **3** in K562, HL-60 and Jurkat cell lines, but increases its activity against the HeLa cells. Finally, one-carbon homologation of the side chain in **3** increases the activity of resulting homologue **4** against the K562, HL-60 and HeLa cell lines. The respective IC_{50} values of **4** were found to be 7-, 10- and 32-fold lower then those recorded for analogue **3**. These results are in good agreement with previous findings that the length of the side chain is crucial for antitumour activity of muricatacin analogues.[3] However, the mechanism of action is still not understood. Since it has been proposed that acetogenins of Annonaceae act as an inhibitor of complex I in the mitochondria1 respiratory system,[1] it is possible that muricatacin and analogues act via an identical mechanism. The observed differences in the antiproliferative potencies in respect to the cancer cell lines used may then be explained by a specificity difference in the hosts' mitochondrial complex I (NADH-ubiquinone oxidoreductase), but more work is needed before any conclusion can be made.

In summary, two novel heteroannelated (−)-muricatacin mimics bearing a furano-furanone ring and an oxygen isostere in the side chain (**3** and **4**) have been designed and synthesized. Their in vitro antitumour activities were evaluated and compared to those recorded for the parent compound **1** (synthesized in this work), and to those recorded for analogue **2** (previously synthesized in our laboratory).[7] Analogue **4** exhibited the same growth inhibition pattern as (−)-muricatacin (**1**) itself, being completely inactive against the Jurkat cell line, but highly active towards the K562, HL-60 and HeLa malignant cells. However, compounds **2** and **3** demonstrated high antiproliferative activity against the all tested malignant cell lines. Both analogues **3** and **4** showed selective increase of activity against HL-60 cells with 17- and 185-fold higher potency than (−)-muricatacin, respectively. Remarkably, neither of the lactones **1**–**4** exhibited any activity towards the normal foetal lung MRC-5 cells. Based upon these biological results, we believe that the analogues **2–4** may serve as important leads in the synthesis of more potent and selective antitumour agents derived from the natural product **1**. Further optimization of the structures, including the preparation of novel muricatacin and goniofufurone mimics is currently underway, and results will be reported in due course.

Acknowledgments

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13. Selected data for **3**: mp 53 °C (from CH_2Cl_2–hexane); $[\alpha]_D^{23} = -35.0$ (*c* 0.5, in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 6.7$ Hz, CH_3), 1.11–1.39 (m, 12H, 6× CH_2), 1.59 (m, 2H, CH_2), 2.65 (dd, 1H, $J_{2a,2b} = 18.8$, $J_{2a,3} = 1.1$ Hz, H-2a), 2.76 (dd, 1H, $J_{2a,2b} = 18.8$, $J_{2b,3} = 5.4$ Hz, H-2b), 3.51 (m, 2H, 2× H-9), 3.88 (m, 2H, 2× H-7), 4.11 (m, 1H, H-6), 4.22 (br s, 1H, exchangeable with D_2O, OH), 4.53 (d, 1H, $J_{4,5} = 3.2$ Hz, H-5), 4.86 (d, 1H, $J_{3,4} = 4.2$ Hz, H-4), 5.02 (td, 1H, $J_{2a,3} = 1.1$, $J_{2b,3} = 5.4$, $J_{3,4} = 4.2$ Hz, H-3); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.0 (CH_3), 22.6, 25.9, 29.6, 29.2, 29.3, 29.4 and 31.8 (7× CH_2), 36.0 (C-2), 69.5 (C-7), 72.6 (C-9), 76.0 (C-5), 76.8 (C-3), 78.6 (C-6), 88.2 (C-4), 175.4 (C-1); LRMS (ESI): *m/z* 339 (M^++K), 323 (M^++Na), 301 (M^++H).

14. Selected data for **4**: mp 59–60 °C (from CH_2Cl_2–hexane); $[\alpha]_D^{23} = -29.1$ (*c* 0.97, in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 6.6$ Hz, CH_3), 1.07–1.68 (m, 16H, 8× CH_2), 2.67 (d, 1H, $J_{2a,2b} = 18.7$ Hz, H-2a), 2.77 (dd, 1H, $J_{2a,2b} = 18.7$, $J_{2b,3} = 5.4$ Hz, H-2b), 3.54 (m, 2H, 2× H-9), 3.86 (m, 2H, 2× H-7), 4.11 (m, 1H, H-6), 4.27 (br s, 1H, exchangeable with D_2O, OH), 4.54 (d, 1H, $J_{5,6} = 3.0$ Hz, H-5), 4.88 (d, 1H, $J_{3,4} = 4.0$ Hz, H-4), 5.03 (dd, 1H, $J_{2,3} = 5.4$, $J_{3,4} = 4.2$ Hz, H-3); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.0 (CH_3), 22.6, 25.9, 29.2, 29.3, 29.4, 29.5 and 31.8 (8× CH_2), 36.1 (C-2), 69.6 (C-7), 72.6 (C-9), 76.1 (C-5), 76.9 (C-3), 78.6 (C-6), 88.2 (C-4), 175.3 (C-1); LRMS (CI): *m/z* 315 (M^++H).