Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 505–508

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

THE SYNTHESIS AND P388 CYTOTOXICITY OF MYCALAZOL 11 AND RELATED 5-ACYL-2-HYDROXYMETHYLPYRROLES

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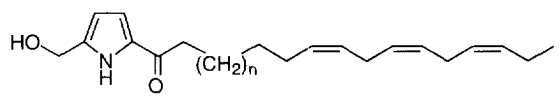
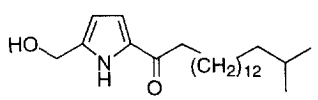
Received 9 November 1998; accepted 28 December 1998

Abstract: We report a general method for the synthesis of mycalazol 11 and some related 5-acyl-2-hydroxymethylpyrroles using a Stille coupling of 5-(tri-*n*-butylstannyl)pyrrole-2-carboxaldehyde with an acid chloride as the key step. The newly prepared 5-acyl-2-hydroxymethylpyrroles **5–7**, together with the 5-carboxamido-2-hydroxymethylpyrrole **10**, have been assayed for in vitro cytotoxicity against the P388 murine leukemia cell line.

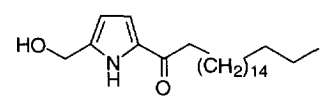
A series of pyrrole-based metabolites, which were isolated from the north-eastern Atlantic sponge *Mycale micracanthoxea*,[1] has been reported to show significant in vitro cytotoxicity against five different cell lines.[2] The authors of this work characterised twelve 5-acyl-2-hydroxymethylpyrroles (mycalazols 1–12) and two 5-alkylpyrrole-2-carboxaldehydes (mycalazals 1–2) that differ in the length and degree of unsaturation of their side chain (see Figure 1). The mycalazols 1–12 are the first reported examples of 5-acyl-2-hydroxymethylpyrrole-based natural products. However, an examination of the literature revealed that a number of 5-alkylpyrrole-2-carboxaldehydes related to the mycalazals 1–2 has been isolated previously from marine sources.[3] A series of 3-alkylpyrrole-2-carboxaldehydes has also been reported,[4] although it has since been suggested that they might also be 2,5-disubstituted pyrroles.[3b]



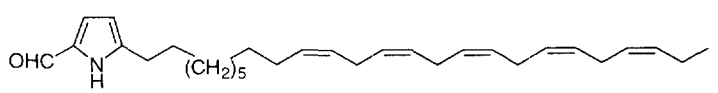
n = 7 **Mycalazol 2**
n = 9 **Mycalazol 8**



Mycalazol 7



Mycalazol 11



Mycalazal 2

Figure 1. Representative examples of the pyrrole-based metabolites

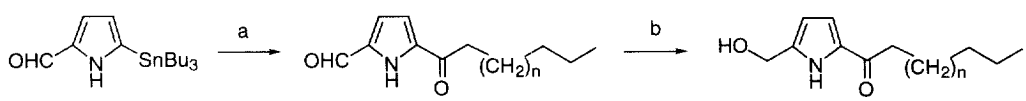
0960-894X/99/$ - see front matter
PII: S0960-894X(99)00021-9

The mycalazals 1–2 are, nevertheless, the first reported 5-alkylpyrrole-2-carboxaldehydes to possess an extended conjugated unsaturated side chain. No cytotoxicity data has been reported on the 5-alkylpyrrole-2-carboxaldehydes from the earlier studies.

A continuation of our studies on the use of substituted 2-hydroxymethylpyrroles[5] as both mechanism-based inhibitors of serine proteases[6] and also as intermediates in the synthesis of porphyrins,[7] led us to develop a new and efficient synthesis of 5-acylpyrrole-2-carboxaldehydes based on a Stille coupling[8] reaction of a stannylpyrrole. We have synthesised the 5-acylpyrrole-2-carboxaldehydes **2–4** in good yields using this method, and without the need to protect either the α-formyl group or the pyrrole nitrogen. The synthesised compounds were then reduced to the corresponding 5-acyl-2-hydroxymethylpyrroles **5–7**. The in vitro cytoxicity of the product hydroxymethylpyrroles **5–7**, along with a related 5-carboxamido-2-hydroxymethylpyrrole **10**, against the P388 murine leukemia cell line is reported.

Our synthesis (Scheme 1) of the 5-acyl-2-hydroxymethylpyrroles **5–7** began with the preparation of 5-(tri-*n*-butylstannyl)pyrrole-2-carboxaldehyde **1** from pyrrole-2-carboxaldehyde.[9] This was then coupled with an appropriate (fatty) acid chloride[10] using bis(triphenylphosphine)palladium(II) chloride in refluxing toluene[11] to give the 5-acylpyrrole-2-carboxaldehydes **2–4** in good yields (77%, 91%, and 92%, respectively). The acylation occurred exclusively at the 5-position under these conditions, with no *N*-acylated pyrrole side-products being detected by ^{1}H NMR. The synthesis was then completed by simply reducing the formyl group with zinc borohydride[5a] to give the desired 5-acyl-2-hydroxymethylpyrroles **5 – 7** in high yields (93%, 84%, and 93%, respectively).[12] The ^{1}H NMR data obtained for **7** were identical to that reported for the natural product, mycalazol 11.[1] It is important to note that our synthetic methodology allows for the efficient coupling of a stannylpyrrole with an acyl group without the need for protection of the pyrrole nitrogen. Previously reported examples of palladium-catalysed cross-coupling reactions of a stannylpyrrole and an acyl, aryl, heteroaryl or alkyl halide have employed methyl,[13] *tert*-butoxycarbonyl (BOC)[13c,14] or tosyl[15] protection of the pyrrole nitrogen. It should also be noted that a synthesis of **2** in 54% yield has been reported using a two-step, one-pot lithiation sequence of the 6-dimethylamino-1-azafulvene dimer.[16]

The related 5-carboxamido-2-hydroxymethylpyrrole **10** was also prepared for testing against the P388 murine leukemia cell line. This compound enabled a comparison of the activity of derivatives bearing an amide side chain (compound **10**) and a ketone (compounds **5–7**). The synthesis of **10** simply required the coupling of the hydrolysis product of ethyl 5-formylpyrrole-2-carboxylate **8**[17] with

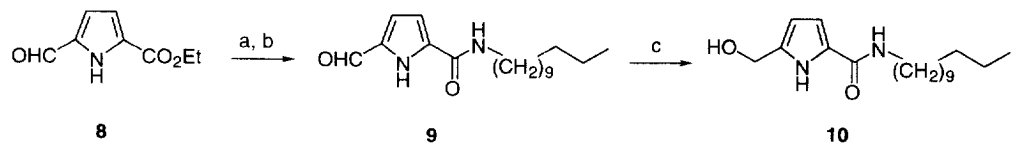


1

n = 1 **2**
n = 7 **3**
n = 14 **4**

n = 1 **5**
n = 7 **6**
n = 14 **7**

Scheme 1. *Reagents and conditions*: (a) $CH_3(CH_2)_4COCl$ **2** or $CH_3(CH_2)_{10}COCl$ **3** or $CH_3(CH_2)_{17}COCl$ **4**, $(Ph_3P)_2PdCl_2$, $PhCH_3$, reflux, 90 min then KF, CH_3CN, rt, 1 h; (b) $Zn(BH_4)_2$, diethyl ether, 0 °C.



8 9 10

Scheme 2. *Reagents and conditions*: (a) KOH, H_2O; (b) EDCI, HOBT, dodecylamine, CH_2Cl_2, rt, 16 h; (c) $Zn(BH_4)_2$, diethyl ether, 0 °C.

dodecylamine using standard EDCI-coupling methodology[18] to give **9**, followed by zinc borohydride reduction of the formyl group to give **10** in 68% yield overall for the 3 steps (Scheme 2).

Compounds **5–7** and **10** were assayed for in vitro cytotoxicity against the P388 cell line and the resulting ID_{50} values are summarised in Table 1. These preliminary results suggest that an increased chain length leads to greater activity (c.f. **5** with **6** and **7**), although it should be noted that compounds **6** and **7** have comparable activity. The cytotoxicity of the synthetic sample of Mycalazol 11 (**7**), in our assay, was ten-fold lower than that reported[1] for the natural product.[19] Our results would also suggest that an acyl side chain leads to compounds with an increased activity relative to those containing a carboxamido side chain, with **6** having 2.5-fold greater activity compared to **10**, despite similar chain lengths.

Table 1. Cytotoxicity data (ID_{50}, μg/mL) of the hydroxymethylpyrroles **5–7**, **10**

Compound	**5**	**6**	**7**	**10**
ID_{50} (μg/mL)	78	21	24[19]	52

In conclusion, we present a new and general synthesis of 5-acylpyrrole-2-carboxaldehydes using a Stille coupling reaction of 5-(tri-*n*-butylstannyl)pyrrole-2-carboxaldehyde that proceeds in good yield and without the need to protect either the α-formyl group or the pyrrole nitrogen. We have used this methodology to prepare a series of 5-acyl-2-hydroxymethylpyrroles, including the previously reported natural product mycalazol 11, and together with a 5-carboxamido-2-hydroxymethylpyrrole, assayed their in vitro cytotoxicity against the P388 cell line. Ongoing work is centred on the synthesis and assay of further examples of these compounds, incorporating either an acyl chain with varying degrees of unsaturation, or a polypeptide chain, in order to further explore their biological activity.

Acknowledgments: We thank Dr Murray Munro, Dr John Blunt and Ms Gill Ellis for assistance with the P388 cytotoxicity assays, Mr Bruce Clarke for running the mass spectra and finally the financial support of the New Zealand Marsden fund.

References and Notes

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