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# Synthesis of mycalazol and mycalazal analogs with potent antiproliferating activities*

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*Abstract*: Four 2,5-disubstituted pyrrole and two 2-disubstituted thiophene analogs of the natural products mycalazol 5 and mycalazal 2 have been prepared using the Stille coupling reaction. All four analogs displayed potent antiproliferating activity against several human cancer cell lines with $IC_{50}$ values in the nanomolar range.

*Keywords*: antiproliferating activity; 2,5-disubstituted pyrroles; mycalazol 5; mycalazal 2; natural products; Stille coupling.

## INTRODUCTION

Natural products are an important source for the development of new anticancer drugs [1]. Seldom is a natural product useful directly as a drug, but more often natural products are important as lead compounds for the development of a new anticancer drug. In those cases, structural–activity relationship (SAR) studies become necessary. However, before SAR studies can be performed, the synthesis of new analogs of the natural product of interest must be conducted [2]. Analogs are synthesized in order to enhance the biological activity, but also for achieving selectivity toward, for example, cancer cells. The new analogs should ideally exhibit improved pharmacokinetic as well as pharmacodynamic properties [2].

In 1997, Salvá and co-workers [3] reported the identification of 14 structurally related 2,5-disubstituted pyrroles from the northeastern Atlantic sponge *Mycale micracanthoxea*. The compounds exhibited interesting cytotoxic activity against several cancer cell lines. The presence of a saturated or an unsaturated carbon chain attached to the pyrrole ring characterizes these natural products. Recently, we reported the first total synthesis of mycalazol 5 (**1**) and mycalazal 2 (**2**) (Fig. 1) [4]. As part of our ongoing efforts on the synthesis and biological evaluation of polyunsaturated natural products [5] as potential new anticancer agents, we herein report the synthesis and antiproliferating activity of four new analogs of these naturally occurring 2,5-disubstituted pyrroles. The synthesis and biological evaluation of one 2-acyl thiophene analog and one 2-alkyl thiophene analog are also presented.

Recently, it was reported by Zhou, Nagle, and co-workers that 2,5-disubstituted pyrroles, such as **4** and **5**, isolated from a *Mycale* sponge inhibited hypoxia-inducible factor-1 (HIF-1α) in a human breast cancer cell assay [6]. Identification of new inhibitors of HIF-1α is an attractive approach for the development of new anticancer agents [7–9]. However, limited information on the SAR of analogs of the mycalazol and mycalazal class of natural products has so far been published. Nabbs and Abell reported the synthesis and cytotoxic properties data of three saturated analogs of mycalazol 11 (**3**) [10]. Hence,

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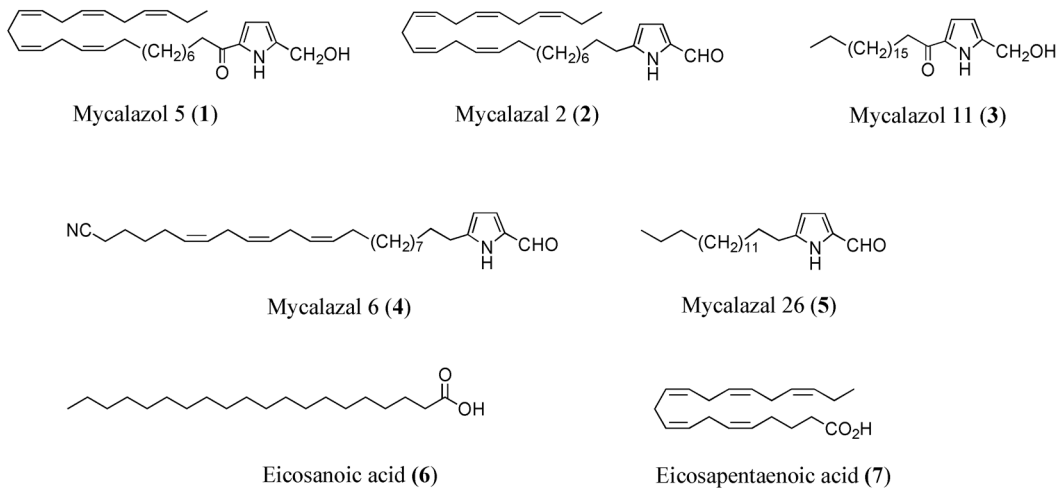


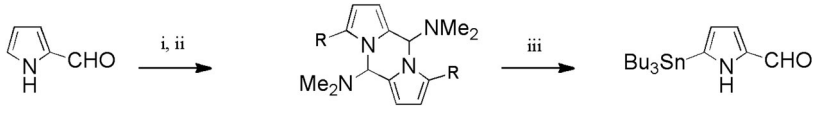

**Fig. 1** Structures of mycalazol 5 (**1**), mycalazal 2 (**2**), mycalazol 11 (**3**), mycalazal 6 (**4**), mycalazal 26 (**5**), eicosanoic acid (**6**), and eicosapentaenoic acid (**7**).

based on our published synthesis of mycalazol 5 (**1**), we decided to prepare analogs with an unsaturated C-20 side chain and a saturated C-20 side chain at the 2-position of the pyrrole ring. For comparison, we also decided to prepare the 2-acyl substituted thiophene **18** and the 2-alkyl substituted thiophene derivative **19** with a saturated C-20 side chain. These six compounds, as well as the natural products **1** and **2**, were subjected to biological testing in four human cancer cell lines.

## RESULTS AND DISCUSSION

### Synthesis

The synthesis of analogs **13**, **14**, **15**, and **16** started with the preparation of 5-(*tri*-*n*-butylstannyl)pyrrole-2-carboxaldehyde (**10**) from commercial available pyrrole 2-carboxaldehyde **8** [11] via the known compound **9a** [12] (Scheme 1). First, an aqueous solution of dimethyl amine was reacted with pyrrole-2-carboxaldehyde (**8**) that yielded *aza*fulvene **9a** in a quantitative yield. Compound **9a** was then treated with 2.3 equiv of *n*-butyl lithium followed by reaction with excess *n*-tributyltin chloride. This afforded the known *aza*fulvene derivative **9b**. Then aqueous hydrolysis of **9b** afforded 5-(*tri*-*n*-butylstannyl)pyrrole-2-carboxaldehyde (**10**).



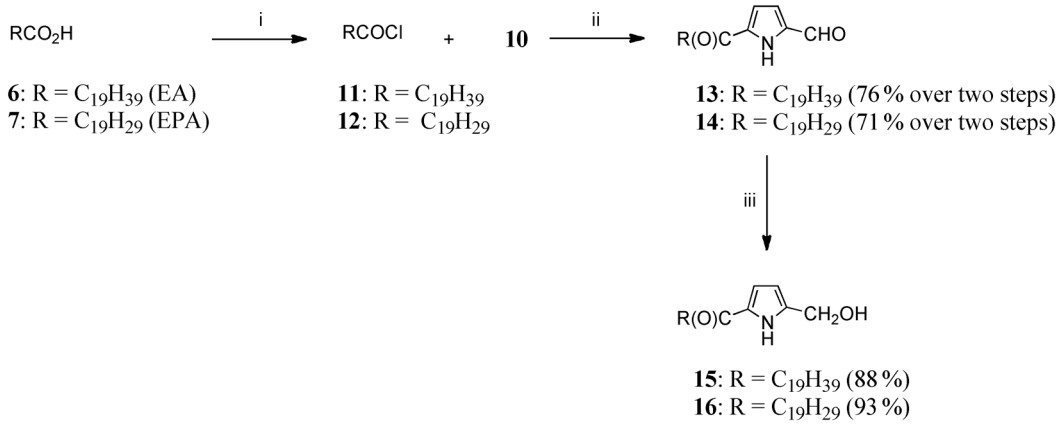

**8** **R = H: 9a** **10**
**R = SnBu$_3$: 9b**

i) $HNMe_2$, $H_2O$, 25 °C ii) a) *n*-BuLi, THF b) *n*-$Bu_3SnCl$, THF, -78 - 25 °C
iii) 0.4 M NaOAc, $H_2O$, THF, Δ (57 % from **8**).

**Scheme 1** Synthesis of stannyl pyrrole 10.

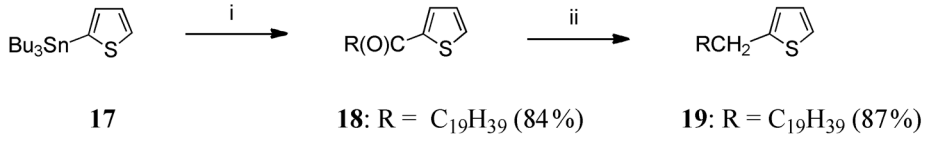
The commercially available eicosanoic acid (EA, **6**) and eicosapentaenoic acid (EPA, **7**) were converted into their respective acid chlorides **11** and **12** by reaction with oxalyl chloride in methylene chloride. The crude acid chlorides underwent Stille cross-coupling reaction [13] with the stannyl pyrrole **10** affording compounds **13** and **14** in 76 and 71 % yields, respectively (Scheme 2). Chemioselective reduction of the formyl group in **13** and **14** with $Zn(BH_4)_2$ [14] in THF at 0–25 °C afforded **15** and **16** in 88 and 93 % yields, respectively.




i) $(COCl)_2$, $CH_2Cl_2$ ii) $Pd(PPh_3)_4$, THF, Δ iii) $Zn(BH_4)_2$, THF, 0-25 °C.

**Scheme 2** Synthesis of mycalazol 5 and mycalazal 11 analogs.

The 2-acyl thiophene analog **18** was prepared in 84 % yield from the commercially available 2-(*tri-n*-butylstannyl)pyrrole **17** and acid chloride **11** again using the Stille coupling. Reduction of the carbonyl group in **18** under standard hydrogenation conditions (Pd/C, $H_2$, EtOH) in the presence of catalytic amounts of sulfuric acid afforded thiophene **19** in 87 % yield (Scheme 3). Spectral data were in agreement with the assigned structures. The preparation of mycalazol 5 (**1**) and mycalazal 2 (**2**) has previously been described [4].




i) **5a**, $Pd(PPh_3)_4$, THF, Δ ii) Pd/C, $H_2$, EtOH, $H_2SO_4$

**Scheme 3** Synthesis of thiophene analogs **18** and **19**.

## Biological results

Mycalazol 5 (**1**), mycalazal 2 (**2**) and the four pyrrole derivatives **13**, **14**, **15**, and **16** and the two thiophene analogs **18** and **19** were applied to four human cancer cell lines for the determination of their in vitro antiproliferating activity [15]. The results are summarized in Table 1. Mycalazol 5 (**1**) and mycalazal 2 (**2**) were the two most potent compounds in all four cell lines. In general, **1** was slightly

more active than **2** exhibiting $IC_{50}$ values in the ranges 2.1–14.9 nM and 4.1–23.3 nM, respectively. This is in accord with the results reported by Salvá and co-workers [3]. Analogs **13** and **14** were less active than **1** and **2**. Interestingly, the presence of both an acyl-group and a formyl-group in the 2- and 5-positions does not enhance the antiproliferating activity. The $IC_{50}$ values ranged from 11.3 to 22.9 nM for **14** and 13.0 to 31.1 nM for **13**. Only a slight increase in activity was observed in all cell lines with a polyunsaturated C-20 side chain in compound **16** compared to the saturated derivative **15**. Moreover, both compounds were less active than both mycalazol 5 (**1**) and mycalazal 2 (**2**). The $IC_{50}$ values ranged from 27.7 to 38.5 nM for **15** and 20.5 to 34.7 nM for **16**. A further reduction was observed for the 2-acyl substituted thiophene **18** with $IC_{50}$ values from 45.5 to 58.7 nM. The 2-alkyl thiophene **19** was inactive in all cancer cell assays.

**Table 1** Antiproliferating activity data of mycalazol 5 (**1**), mycalazal 2 (**2**) and analogs.

| Comp. | SKOV[a] | OVCAR[a] | WM35[a] | WM239[a] | VERO[a] |
|---|---|---|---|---|---|
| **1** | 14.9 (±2.1) | 9.4 (±1.8) | 2.9 (±0.9) | 2.1 (±0.9) | 2.1 (±0.9) |
| **2** | 23.3 (±3.0) | 18.0 (±2.1) | 4.1 (±1.0) | 4.3 (±0.8) | 4.3 (±0.8) |
| **13** | 31.1 (±2.3) | 24.1 (±1.5) | 13.0 (±1.3) | 15.9 (±1.9) | 15.9 (±1.9) |
| **14** | 22.9 (±1.9) | 21.7 (±2.8) | 11.3 (±1.9) | 12.1 (±2.1) | 12.1 (±2.1) |
| **15** | 38.1 (±2.1) | 38.5 (±2.2) | 36.4 (±2.1) | 27.7 (±2.2) | 27.7 (±2.2) |
| **16** | 34.3 (±3.3) | 32.7 (±4.1) | 24.1 (±2.6) | 20.5 (±2.9) | 20.5 (±2.9) |
| **18** | 49.1 (±2.1) | 58.7 (±3.3) | 45.5 (±3.3) | 58.0 (±2.9) | 58.0 (±2.9) |
| **19** | >100 | >100 | >100 | >100 | >100 |

[a]Values (nM) are means of three experiments in each cell assay; standard deviation is given in parentheses.

The biological results reported herein indicated that the NH-pyrrole ring is important for the observed antiproliferating activity against all four human cancer cell lines. Moreover, both mycalazol 5 (**1**) and mycalazal 2 (**2**) as well as all new analogs except **19**, exhibited potent activity in the non-cancerous VERO cell line. These results, in combination with the results from the cancer cell lines, showed that the compounds exhibited low selectivity. Hence, other analogs of the mycalazols and mycalzals should be prepared with antiproliferating activity toward only the cancer cell lines.

## CONCLUSION

The Stille cross-coupling reaction was employed for efficient syntheses of mycalazol and mycalazal analogs without protection of the NH-pyrrole. Moreover, the conservation of the all-*Z*-configuration of the methylene-interrupted double bonds was observed for natural products 1 and 2, as well as analogs **14** and **16**. In vitro antiproliferating activity against four human cancer cell lines was determined. All four 2,5-disubstiuted pyrrole analogs revealed significant activity in both ovarian and melanoma human cancer cell lines. The most active analog was **14**. For the thiophene analogs, only compound **18** exhibited antiproliferating activity. However, all analogs were less active than the natural products mycalazol 5 (**1**) and mycalazal 2 (**2**) and low selectivity was observed. Since inhibitors of HIF-1$\alpha$ continue to be of interest as potential remedies against various cancers, further structural–activity studies will focus on the synthesis and biological evaluation of additional analogs of mycalazol 5 (**1**). These efforts will be reported in due time.

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