Design and Cytotoxic Evaluation of New Annonaceous Acetogenin Analogues

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Acetogeninas anonáceas análogas foram preparadas a partir de 5-iodofurano-2-carbaldeído e ácido undec-10-inoico ou undec-10-inol pela reação de Sonogashira, seguida da reação de Grignard e hidrólise catalizada por mercúrio. A citocidade foi avaliada por ensaios MTT contra células HL e no Instituto Nacional de Câncer (Alemanha).

Analogues of annonaceous acetogenins were built up from 5-iodofuran-2-carbaldehyde and undec-10-ynoic acid or undec-10-ynoyl by a Sonogashira reaction, followed by a Grignard reaction and a mercury catalysed hydratation. The cytotoxicity was evaluated with MTT assay ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric assay for measuring cellular proliferation) against HL cells and at the National Cancer Institute (NCI).

Keywords: Sonogashira reaction, Grignard reaction, cytotoxicity

Introduction

The tropical plant family of the Annonaceae contains pharmacologically active natural products of the group of alkaloids and acetogenins. These plants are often used in traditional medicine. The annonaceous acetogenins are an interesting target for the development of new anti cancer drugs. Compounds like asitrocin exhibit high selectivity against several cancer cell lines.1,2 On the other hand, the total syntheses reported up to know are long and expensive,1,7,8 so we tried a simpler approach towards some analogues. The mechanism of action of the annonaceous acetogenins is closed to the cellular mitochondria, following inhibition of the mitochondrial complex I, leading to a lower production of ATP in the tumor cell and subsequent apoptosis.

Results and Discussion

Chemistry

In continuation of our work on the synthesis of analogues of annonaceous acetogenin analogues1 we describe here a new approach towards the acetogenin skeleton. Commercially available 5-iodofuran-2-carbaldehyde (Aldrich) (1) was reacted in a Sonogashira reaction with undec-10-yn-1-ol to give the aldehyde 3. This aldehyde 3 was converted in a Grignard reaction with dodecyl magnesium bromide to the racemic diol 4. Subsequent regioselective hydratation4 of the triple bond under mercury catalysis led to the corresponding ketone 5.

In a second series, undec-10-ynoic acid was esterified via the acid chloride with methanol to give the methyl ester 2a and with lactic acid ethyl ester to give the double ester 2b. These esters 2a and 2b were reacted with 5-iodofuran-2-carbaldehyde (1) under Sonogashira conditions to give the esters 6a and 6b. In a Grignard reaction with one equivalent of dodecyl-magnesium bromide 6a and 6b were converted to compounds 7a and 7b.

Antimicrobial and cytotoxicity screening

The resulting compounds were tested against several bacteria and fungi in an agar diffusion assay, but did not show any significant activities.

The cytotoxicity of 3, 4, 6b and 7b was evaluated in MTT assay against a HL 60 cell line using the method of Mosman.5 The results were compared to common alkylating drug cisplatin. Compounds 4 and 7a were also tested at the NCI against 60 cancer cell lines, but showed only weak cytotoxicity and no cellular selectivity.6
**Scheme 1.** Annonacea acetogenin asitrocin.

\[
\begin{align*}
\text{asitrocin} & \quad \text{MgBr} \\
1 & \quad \text{a} \quad [69\%] \\
2 & \quad \text{b} \quad [48\%] \\
3 & \quad \text{c} \quad [28\%]
\end{align*}
\]

**Scheme 2.** a: EDMA, Pd((PPh₃)₂Cl₂, CuI; b: THF; c: HgO, H₂O, HOAc.

\[
\begin{align*}
\text{MgBr} & \quad \text{b} \\
1 & \quad \text{a} \quad [95\%] \\
2a & \quad \text{a} \quad [90\%] \\
2b & \quad \text{c} \quad [62-78\%]
\end{align*}
\]

**Scheme 3.** a: C₂Cl₂O₂, EDMA, methanol. b: EDMA, Pd((PPh₃)₂Cl₂, CuI. c: THF.
Conclusions

In this paper we report the synthesis and cytotoxic evaluation of new annonaceous acetogenine analogues containing a furan ring instead of the naturally occurring tetrahydrofuran ring. The cytotoxic activity of the resulting compounds is only in µM range and so much than that the natural products with a cytotoxicity in nM range.

In summary, we describe here a shorter and more efficient way towards the annonceous acetogenine skeleton. The resulting compounds might be an interesting starting material for the synthesis of enantiomeric pure tetrahydrofuran analogues.

Experimental

IR-Spectra: Jasco FT-IR Paragon; MS: Hewlett Packard MS-Engine, electron ionisation (EI) 70 eV, chemical ionisation (CI) with CH₄ (300 eV); NMR (400 MHz): Jeol GSX 400 (1H: 400 MHz, 13C: 100 MHz); GLC-MS: Shimadzu GC 17 A; flash column chromatography (FCC): silica gel 60 (230-400 mesh, E. Merck, Darmstadt).

5-(11-Hydroxy-undec-1-ynyl)-furan-2-carbaldehyde (3)

2.2 g (10.0 mmol) 5-iodofuran-2-carbaldehyde, 1.7 g (10.0 mmol) undec-10-yn-1-ol, 200 mg CuI and 800 mg PdCl₂(PPh₃)₂ were dissolved in 20 ml EDMA and stirred for 12 h at room temperature. The solvent was evaporated and the residue was dissolved in 20 mL 5% aqueous Na₂S₂O₃ solution. The solution was extracted with diethyl ether (3×25 mL). The combined organic layers were dried over Na₂SO₄, the solvent was evaporated and the residue was purified by FCC (n-hexane/ethyl acetate 5:1) to give 1.8 g (69%) of 3 as a brown solid. IR(KBr disc) ν max/cm⁻¹: 3405, 3337, 3101, 2920, 2835, 2223, 1677, 1510, 1271, 819, 763. EI-MS m/z (rel. int.): 262 (M⁺, 20), 190 (25), 136 (100). HRMS: Calc.: 262.1569. Found: 262.1578.1H NMR (400 MHz, CDCl₃) δ (J, Hz) 1.38 (m, 10 H, 5 CH₂), 1.59 (m, 4 H, 2 CH₂), 2.46 (t, J 7.6, 2 H, CH₂), 3.65 (t, J 7.2, 2 H, CH₂O), 6.60 (d, J 3.6, 1 H, aromat. CH), 7.20 (d, J 3.6, 1 H, aromat. CH), 9.58 (s, 1 H, CHO). 13C NMR (400 MHz, CDCl₃) δ 21.1 (CH₂), 25.7 (CH₂), 28.0 (CH₂), 28.8 (CH₂), 29.0 (CH₂), 29.3 (CH₂), 29.4 (CH₂), 32.8 (CH₂), 63.0 (CH₂), 70.4 (quat. C), 99.0 (quat. C), 115.9 (2 aromat. CH), 142.6 (quat. C), 151.9 (quat. C), 177.1 (CHO).

1-[5-(11-Hydroxy-undec-1-ynyl)-furan-2-yl]tridecan-1-ol (4)

1.7 g (6.5 mmol) of 3 were dissolved in 10 mL dry THF and 13 mL of 1 mol L⁻¹ (13 mmol) dodecylmagnesium bromide solution in n-hexane were added dropwise. The mixture was stirred for 10 h, was diluted with 20 mL saturated NH₄Cl solution and was extracted with diethyl ether (3×30 mL). The combined organic layers were dried over Na₂SO₄, the solvent was evaporated and the residue was purified by FCC (n-hexane/ethyl acetate 5:1) to give 1.34 g (48%) of 4 as a brown oil. CI-MS: m/z (rel. int.): 460 (M⁺, 6), 443 (100), 304 (34), 259 (38). HR-MS: Calc.: 460.3553. Found: 460.3538. 1H NMR (400 MHz, CDCl₃) δ (J, Hz) 0.88 (t, J 6.7, 3 H, CH₃), 1.28 (m, 20 H, 10 CH₂), 1.43 (m, 4 H, 2 CH₂), 1.59 (m, 6 H, 3 CH₂), 1.82 (m, 2 H, CH₂), 2.43 (t, J 7.7, 2 H, CH₂), 3.64 (t, J 6.4, 2 H, CH₂O), 4.62 (t, J 6.4, 1 H, CH), 6.18 (d, J 3.2, 1 H, aromat. CH), 6.40 (d, J 3.2, 1 H, aromat. CH). 1C NMR (400 MHz, CDCl₃): δ 14.2 (CH₃), 22.8 (CH₂), 25.6 (CH₂), 25.7 (CH₂), 25.8 (CH₂), 28.4 (CH₂), 28.8 (CH₂), 29.0 (CH₂), 29.3 (CH₂), 29.4 (CH₂), 29.4 (CH₂), 29.5 (CH₂), 29.6 (CH₂), 29.6 (CH₂), 29.7 (CH₂), 29.8 (CH₂), 32.0 (CH₂), 32.8 (CH₂), 35.6 (CH₂), 63.1 (CH₂O), 67.9 (CH), 71.0 (quat. C), 94.9 (quat. C), 106.8 (aromat. CH), 114.4 (aromat. CH), 136.8 (quat. C), 157.2 (quat. C).

11-Hydroxy-1-[5-(1-hydroxy-tridecyl)-furan-2-y1]-undecan-1-one (5)

200 mg (0.4 mmol) of 4 were dissolved in 20 mL methanol, 10 mL 5% aqueous H₂SO₄ and 200 mg (0.9 mmol) yellow HgO were added. The solution was stirred for 12 h at room temperature. The methanol was evaporated and residue was quenched with 20 mL saturated Na₂CO₃ solution and extracted with diethyl ether (3×30 mL). The combined organic layers were dried over Na₂SO₄ and the residue was purified by FCC (n-hexane/ethyl acetate 5:1) to give 50 mg (28%) of 5
as a brown oil. HR-MS: Calc.: 450.3709. Found: 450.3708. 1H NMR (400 MHz, CDCl3) δ (J, Hz) 0.88 (t, J 6.8, 3 H, CH3), 1.29 (m, 28 H, 14 CH2), 1.56 (m, 4 H, 2 CH2), 1.71 (m, 4 H, 2 CH2), 1.86 (m, 2 H, CH2), 2.78 (t, J 8.8, 2 H, CH2), 3.64 (t, J 6.6, 2 H, CH2O), 4.74 (t, J 6.5, 1 H, CH), 6.38 (dd, J 0.6, J 3.6, 1 H, aromat. CH), 7.13 (J J 3.6, 1 H, aromat. CH), 13C NMR (400 MHz, CDCl3): δ 14.1 (CH3), 22.7 (CH2), 24.5 (CH2), 25.3 (CH2), 25.7 (CH2), 29.2 (CH2), 29.3 (3 x CH2), 29.4 (2 x CH2), 29.5 (CH2), 29.5 (CH2), 29.6 (CH2), 29.7 (CH2), 29.8 (CH2), 31.9 (CH2), 32.7 (CH2), 35.7 (CH2), 38.4 (CH2), 63.1 (CH2O), 68.0 (CH), 108.0 (CH2), 115.9 (2 aromat. CH), 142.6 (quat. C), 151.9 (quat. C), 157.2 (quat. C), 171.0 (CO), 173.2 (CO).

11-([5-(1-Hydroxy-tridecyl)-furan-2-yl]-undec-10-ynoic acid methyl ester (6a)

The compound was prepared as described for 3 from 1.9 g (10 mmol) methyl undecynoate and 2.2 g (10 mmol) 5-iodofuran carbaldehyde to give 2.6 g (90%) of 6a as a brown solid. CI-MS m/z (rel. int.): 291 (M++1, 6), 529 (100). HR-MS: Calc.: 290.1553. Found: 290.1553. 1H NMR (400 MHz, CDCl3): δ (J, Hz) 1.29 (m, 6 H, 3 CH2), 1.61 (m, 4 H, 2 CH2), 2.31 (t, J 7.0, 2 H, CH2), 2.45 (t, J 7.0, 2 H, CH2), 3.67 (s, 1 H, OCH2), 6.60 (d, J 4.0, 1 H, aromat. CH), 7.20 (dd, J 4.0, J 0.4, 1 H, aromat. CH), 9.58 (d, J 0.4, 1 H, CHO). 13C NMR (400 MHz, CDCl3): δ 19.5, 22.70 (CH2), 24.9 (CH2), 28.0 (CH2), 28.8 (CH2), 28.9 (CH2), 29.1 (CH2), 34.1 (CH2), 51.5 (OCH2), 70.4 (quat. C), 98.9 (quat. C), 115.9 (2 aromat. CH), 142.6 (quat. C), 151.9 (quat. C), 174.4 (CO).

11-([5-(1-Hydroxy-tridecyl)-furan-2-yl]-undec-10-ynoic acid methyl ester (6b)

The compound was prepared as described for 3 from 0.5 g (1.8 mmol) 2b and 0.4 g (1.8 mmol) 5-iodofuran carbaldehyde to give 610 mg (90%) of 6b as a brown solid. CI-MS m/z (rel. int.): 377 (M++1, 12), 259 (100). HR-MS: Calc.: 376.1886. Found: 376.1875. 1H NMR (400 MHz, CDCl3): δ (J, Hz) 1.27 (t, J 7.5, 3 H, CH3), 1.34 (m, 6 H, 3 CH2), 1.43 (m, 2 H, CH2), 1.48 (d, J 7.2, 3 H, CH2), 1.61 (m, 4 H, 2 CH2), 2.38 (m, 2 H, CH2), 2.45 (t, J 6.6, 2 H, CH2), 4.20 (q, J 7.5, 2 H, CH2O), 5.06 (q, J 7.2, 1 H, CH), 6.60 (d, J 3.9, 1 H, aromat. CH), 7.20 (d, J 3.9, 1 H, aromat. CH), 9.58 (s, 1 H, CHO). 13C NMR (400 MHz, CDCl3): δ 14.0 (CH3), 16.9 (CH2), 19.4 (CH2), 24.7 (CH2), 27.9 (CH2), 28.7 (CH2), 28.8 (CH2), 28.9 (CH2), 29.0 (CH2), 33.9 (CH2), 61.2 (CH2O), 68.4 (CH), 70.3 (quat. C), 98.8 (quat. C), 142.5 (quat. C), 151.8 (quat. C), 115.8 (2 aromat. CH), 170.9 (CO), 173.1 (CO), 177.0 (CHO).

11-([5-(1-Hydroxy-tridecyl)-furan-2-yl]-undec-10-ynoic acid 1-ethoxycarbonyl-ethyl ester (7a)

The compound was prepared as described for 4 from 263 mg (0.7 mmol) of 6b and 2 mL 1 mol L-1 (2.0 mmol) dodecyl magnesium bromide solution in n-hexane to give 300 mg (78%) of 7b as a yellow oil. HR-MS: Calc.: 546.3920. Found: 546.3915. CI-MS m/z (rel. int.): 546 (M+, 6), 529 (100), 304 (54), 259 (64). 1H NMR (400 MHz, CDCl3): δ (J, Hz) 0.81 (t, J 7.0, 3 H, CH3), 1.19 (m, 20 H, 10 CH2), 1.33 (m, 6 H, 3 CH2), 1.53 (m, 6 H, 3 CH2), 1.75 (m, 2 H, CH2), 2.23 (t, J 8.7, 2 H, CH2), 2.35 (t, J 7.7, 2 H, CH2), 3.59 (s, 3 H, OCH3), 4.54 (t, J 6.7, 1 H, CH), 6.11 (d, J 3.0, 1 H, aromat. CH), 6.33 (d, J 3.0, 1 H, aromat. CH). 13C NMR (400 MHz, CDCl3): δ 14.09 (CH3), 22.7 – 35.5 (19 CH2), 51.4 (CH), 67.9 (OCH2), 74.5 (quat. C), 94.8 (quat. C), 106.7 (aromat. CH), 114.3 (aromat. CH), 136.8 (quat. C), 157.3 (quat. C), 174.3 (CO).

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References


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