Tetrahedron xxx (2018) 1–5

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis and anticancer activity of the proposed structure of caldoramide, an *N*-peptidyltetramate from the cyanobacterium *Caldora penicillata*

Anja Wunder, Matthias Rothemund, Rainer Schobert*

Department of Chemistry, University Bayreuth, Universitaetsstr. 30, D-95440 Bayreuth, Germany

ARTICLE INFO

Article history:
Received 27 February 2018
Received in revised form
29 March 2018
Accepted 3 April 2018
Available online xxx

Keywords:
Caldoramide
Tetramic acids
Anticancer drugs
Oligopeptides

ABSTRACT

The structure proposed in the literature for caldoramide, a formal pentapeptide metabolite of the marine cyanobacterium *Caldora penicillata*, was synthesised in 12 steps and 16% yield (longest linear sequence from isoleucine) following the strategy of a stepwise consecutive extension of an *N*-oligopeptidyl chain on a tetramate anchor. The synthetic product differed from the natural isolate in optical rotation, some NMR data, and cytotoxicities. Its antiproliferative effect on three human cancer cell lines was distinctly lower than that of related dolastatin 10 and belamide A.

1. Introduction

Caldoramide was isolated, together with the known highly potent anticancer compound dolastatin 10, from the tropical marine cyanobacterium *Caldora penicillata* collected at the Florida keys (Fig. 1).[1] Interestingly, geographical dispersed collections of *C. penicillata* all produced these metabolites which may thus be used as chemotaxonomic markers for the identification of this cyanobacterium.[2] The isolating group assigned caldoramide the structural formula 1 on grounds of 2D-NMR spectra and oxidative breakdown reactions to give individual amino acids. Although only moderately cytotoxic, natural caldoramide shares with dolastatin 10 **2** a selectivity for cancer cells positive in oncogenic KRAS and HIF-α transcription factors.[1] Herein, we report the first synthesis as well as additional anticancer activities of the proposed structure **1** of caldoramide.

2. Results and discussion

Unlike other syntheses of similar polypeptides, e.g. those of belamide A[3] or dolastatin 10[4] which connect oligopeptide fragments, ours builds up the tetrapeptidyl residue by four individual condensation steps on a preformed tetramate core. Thus we first converted L-phenylalanine **3** via its methylester **4** to the known methyl 5-benzyltetramate **6** by our established domino addition−intramolecular Wittig olefination reaction employing the cumulated phosphorus ylide Ph_3PCCO **5**.[5] The *N*-acylation of **6** with an *N*-Boc-protected isoleucine imidazolylamide **10** was possible under basic conditions at −40 °C to give **12** in good yield after *N*-deprotection of carbamate **11** with TFA (Scheme 1).

Isoleucine amide **10** was itself accessible in three steps and 67% yield from L-isoleucine **7** via *N*-Boc protection, and *N*-methylation of the resulting carbamate **8** with NaH/MeI to give *N*-methyl-isoleucine **9** which was finally treated with 1,1′-carbonyldiimidazole (Staab's reagent).

During the synthesis we realised that the coupling of N-methylated amino acids could not be efficiently achieved using standard solid-phase protocols. Activation of the mostly carbamate protected (e.g. Fmoc, Boc) *N*-methylamino acids leads to racemisation or *N*-carboxyanhydride formation and therefore low coupling yields.[6] This necessitated workarounds, e.g. for the attachment of an L-*N*-methylvaline residue to *N*-methylated α-amino imide **12**. First, L-valine was converted with *p*-nitrobenzenesulfonylchloride to *N*-*p*-nosyl-L-valine **13** which should allow a selective downstream *N*-monomethylation as previously demonstrated by us for threonine.[5a,7] Following a general protocol by Chaume et al.[8] amino acid **13** was converted to the corresponding acid chloride with 1-chloro-

* Corresponding author.
E-mail address: Rainer.Schobert@uni-bayreuth.de (R. Schobert).

https://doi.org/10.1016/j.tet.2018.04.004
0040-4020/

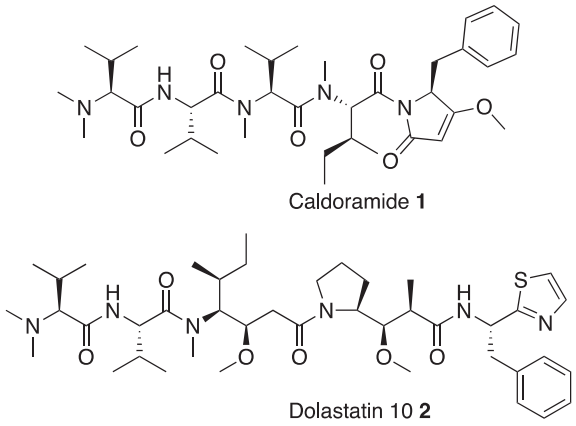
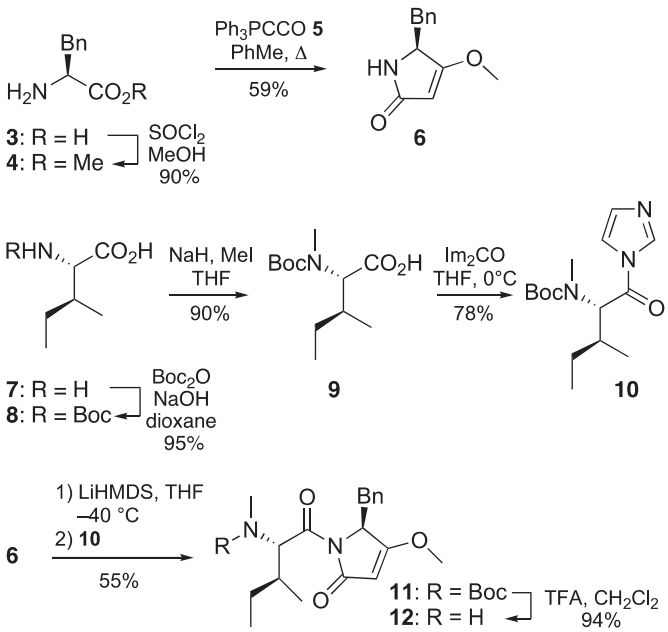


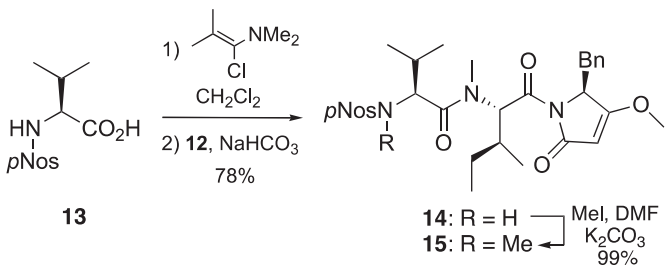
Fig. 1. Structures of caldoramide (proposed)[1] and dolastatin 10.



Scheme 1. Synthesis and *N*-acylation of anchor tetramate **6**.

(*N*,*N*,2-trimethyl)-1-propenylamine, and reacted right away with α-amino imide **12** dissolved in a suspension of $NaHCO_3$ in H_2O/CH_2Cl_2 to give *N*-dipeptidyltetramate **14** in 78% yield. The latter was then quantitatively methylated at the terminal nosylated amino group with MeI/K_2CO_3 in DMF (Scheme 2).

For the attachment of the next L-valine residue the *p*-nosyl group of compound **15** was removed by treatment with 2-methyl-5-*tert*-butylthiophenol and K_2CO_3 in DMF. The resulting crude amine was immediately reacted with *p*Nos-Val-Cl, freshly prepared once more from *N*-nosylated valine **13** and 1-chloro-(*N*,*N*,2-trimethyl)-1-propenylamine as for the conversion of **12** to **14**. The product *N*-trispeptidyltetramate **16** was de-nosylated, again with 2-methyl-5-*tert*-butylthiophenol and K_2CO_3 in DMF, to afford the corresponding primary amine. This was isolated and reacted without further purification with dimethylvaline and HATU/DIPEA to afford the proposed structure of caldoramide as a colourless amorphous solid in 64% yield after column chromatography (Scheme 3).



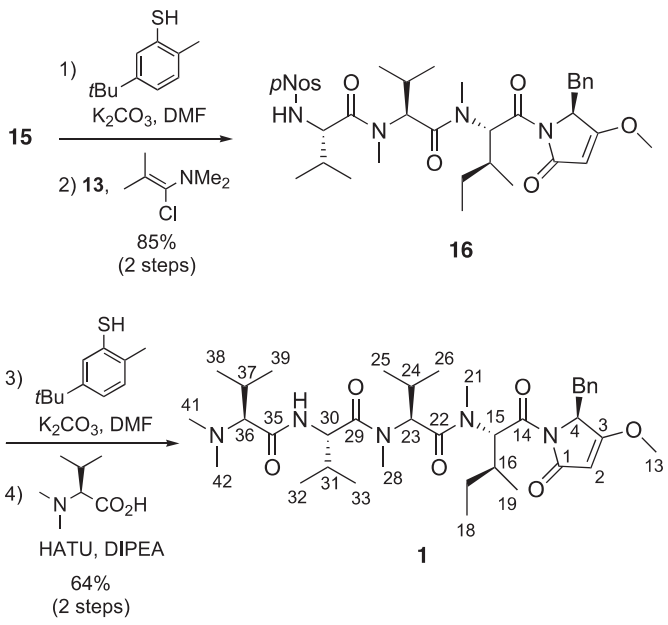
Scheme 2. *p*Nos = *p*-$NO_2(C_6H_4)SO_2$.

For a thorough comparison of its physical data with those of the natural isolate and for biological tests, ca. 10 mg of synthetic **1** were purified by HPLC. Its NMR spectra were difficult to compare to those published[1] for the natural isolate. The ^{13}C NMR shifts of the latter show a general high-field offset relative to those of synthetic **1**, superimposed by conspicuous deviations from this trend for specific carbon atoms. Discrepancies were also observed for some coupling constants in the ^1H NMR spectra, while HSQC and HMBC spectra were in acceptable consistence (*cf.* Supplementary data). The specific optical rotation of synthetic **1** was $[\alpha]^{20}_D$ −13.2 (*c* 0.50, MeOH) while the natural isolate was reported[1] to have $[\alpha]^{25}_D$ +11.1 (*c* 0.36, MeOH).

The isolating group also reported the cytotoxicities of natural caldoramide in MTT assays[9] against three isogenic human colon carcinoma HCT-116 cell lines. For the wild type cell line HCT-116^{wt} they reported an IC_{50} (48 h) value of 3.9 μM, quite unusually derived from dose-response curves not reaching zero cell viability. For synthetic **1** we found an IC_{50} (48 h) value of ca. 44 μM. We also tested it against human HT-29 colon carcinoma and MCF-7 mamma carcinoma cells. The latter were reported[3a] to be sensitive to the congeneric *N*-trispeptidyltetramate belamide A. The activities of **1** are listed in Table 1.

3. Conclusion

We reported the first synthesis of the structure proposed in the literature for the cyanobacterial metabolite caldoramide in 12 steps



Scheme 3. Completion of the synthesis of caldoramide **1**. HATU = 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium-3-oxide hexafluorophosphate; DIPEA = *N*,*N*-diisopropylethylamine.

Please cite this article in press as: Wunder A, et al., Synthesis and anticancer activity of the proposed structure of caldoramide, an *N*-peptidyltetramate from the cyanobacterium *Caldora penicillata*, Tetrahedron (2018), https://doi.org/10.1016/j.tet.2018.04.004

Table 1
Inhibitory concentrations IC_{50} (48 h)[a] in μM of synthetic compound **1** when applied to MCF-7^{topo} mamma, HT-29 colon and HCT-116^{wt} colon carcinoma cells.

cell line/compound	MCF-7^{topo}	HCT-116^{wt}	HT-29
1	33.9 ± 1.3	43.8 ± 3.7	77.5 ± 1.3

[a] Values are derived from concentration-response curves obtained by measuring the percentual absorbance of viable cells relative to untreated controls (100%) after 48 h exposure in MTT assays. Values represent means of four independent runs ± SD.

(longest linear sequence from isoleucine) and 16% yield. Our strategy of a stepwise consecutive extension of an *N*-oligopeptidyl chain on a tetramate anchor proved feasible. The cytotoxic activities of synthetic **1** against selected human cancer cell lines were relatively low when compared to those reported for the closely related methyl *N*-(Me_2N-Val–MeN-Val–MeN-Phe)-5-benzyltetramate (belamide A) and for the natural isolate of caldoramide. After all, we cannot confirm with certainty the identity of synthetic product **1** and the natural caldoramide isolate due to some discrepancies in their NMR data, specific optical rotations, and cytotoxicities.

4. Experimental

4.1. General

Melting points (uncorrected): Büchi melting point apparatus M-565. IR: Perkin-Elmer Spectrum 100 FT-IR spectrophotometer with ATR sampling unit. NMR: ^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX 500 and Avance 300 spectrometers; chemical shifts are given in parts per million (δ) using the residual solvent peak as an internal standard. Mass spectra: Finnigan MAT 8500 (EI, 70 eV). HRMS: UPLC/Orbitrap MS system in ESI mode. Optical rotations: Perkin-Elmer Polarimeter 241 ($\lambda = 589$ nm); $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. For column chromatography Merck silica gel 60 (230–400 mesh) was used. Analytical TLC was carried out using Merck silica gel 60 F_{254s} foil plates. Analytical HPLC was performed on a Beckman System Gold Programmable Solvent Module 126 using Phenomenex Kinetex® C-18-HPLC column, length 250 × 4.6 mm, pore size 100 Å, particle size 5 μm; detection by a Beckman Instruments Diode Array Detection Module 168. Preparative HPLC was performed on an Amersham Biosciences System Programmable Solvent Module P-900 using Phenomenex Gemini-NX C-18-HPLC column, length 250 × 10.00 mm, pore size 110 Å, particle size 5 μm, detection by an Amersham Biosciences UV-900 Module. Starting compounds were bought from the usual sources and used without further purification.

4.2. Syntheses and characterisation

4.2.1. L-*N*-Boc-isoleucine imidazole-1-ylamide (***10***)

An ice-cold solution of *N*-Boc-*N*-methyl-L-isoleucine **9** (80 mg, 0.33 mmol, 1.0 equiv) in THF was treated with 1,1′-carbonyldiimidazole (63 mg, 0.39 mmol, 1.2 equiv) in several portions over 5 min and stirred for another 2.5 h. The mixture was diluted with diethyl ether, washed with water and brine, and dried over Na_2SO_4. Removal of the volatiles left 75 mg (78%) of crude **10** as a colourless oil which was submitted to the next step without further purification. ν_{max} 3123, 2970, 2933, 2881, 1734, 1684, 1531, 1473, 1390, 1368, 1308, 1275, 1233, 1214, 1153, 1118, 1094, 1062, 933, 871, 808, 748 cm^{-1}; δ_H (500 MHz, $CDCl_3$) mixture of rotamers; major rotamer: 0.89–1.00 (6H, m, C*H*$_3$CH, C*H*$_3$CH$_2$), 1.07 (1H, m, CH$_3$C*H*H), 1.36–1.46 (1H, m, partly hidden, CH$_3$CH*H*), 1.46 (9H, s, (CH$_3$)$_3$C), 2.18–2.30 (1H, m, MeC*H*), 2.72 (3H, s, NCH$_3$), 5.11 (1H, d, J_{HH} 10.9 Hz, CHCO), 7.08 (1H, d, J_{HH} 0.9 Hz, NCHC*H*N), 7.69 (1H, d, J_{HH} 0.9 Hz, NC*H*CHN), 8.43 (1H, s, NCHN); δ_C (125 MHz, $CDCl_3$) 10.7 (*C*H$_3$CH$_2$), 15.8 (*C*H$_3$CH), 23.9 (CH$_3$*C*H$_2$), 28.4 ((*C*H$_3$)$_3$C), 29.5 (NCH$_3$), 32.3 (Me*C*H), 61.3 (*C*HCO), 81.4 ((CH$_3$)$_3$*C*), 116.6 (N*C*HCHN), 131.2 (NCH*C*HN), 137.2 (NCHN), 155.9 (NCH$_3$*C*O), 167.7 (CH*C*ON).

4.2.2. (5*S*)-1-[(*N*-Boc,*N*-methyl)-L-isoleucinoyl]-5-benzyl-4-methoxy-2,5-dihydropyrrol-2-one (***11***)

A solution of (5*S*)-benzyl-4-methoxy-2,5-dihydropyrrol-2-one **6** (203 mg, 1.0 mmol, 1.00 equiv) in 10 mL THF was cooled to −40 °C and slowly treated with LiHMDS (1.05 mL, 1 M solution in THF, 1.05 mmol, 1.05 equiv). The yellow solution was stirred for 10 min and then treated dropwise (0.1 mL/min) with a solution of freshly prepared isoleucine amide **10** (295 mg, 1.0 mmol, 1.00 equiv) in THF (4 mL). The resulting mixture was stirred for 1 h, quenched with H_2O (0.5 mL), warmed to room temperature, and diluted with ethyl acetate. The layers were separated and the organic phase was washed with 1 M NaOH, 1 M HCl and brine, dried over Na_2SO_4 and concentrated in vacuo. The remainder was purified by column chromatography (cyclohexane/ethyl acetate, 4:1) to yield **11** (237 mg, 55%) as a waxy white solid which solidified upon prolonged storage (m.p. 77–84 °C); $[\alpha]_D^{20}$ + 150.7 (*c* 0.90, $CHCl_3$); ν_{max} 3031, 2969, 2936, 2882, 1730, 1679, 1629, 1455, 1429, 1407, 1380, 1365, 1301, 1247, 1228, 1190, 1148, 1120, 1079, 1048, 1023, 965, 872, 803, 699 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1:3.3 mixture of two rotamers: 0.82–0.95 (6H, m, C*H*$_3$CH, C*H*$_3$CH$_2$), 1.11–1.21 (1H, m, CH$_3$C*H*H), 1.42 and 1.51 (9H, s, (CH$_3$)$_3$C), 1.42–1.49 (1H, m, partly hidden, CH$_3$CH*H*), 2.08–2.19 (1H, m, C*H*CH$_3$), 3.02 and 3.03 (3H, s, NCH$_3$), 3.10 (1H, dd, J_{HH} 14.1, 2.9 Hz, Ph-C*H*H), 3.44 (1H, dd, J_{HH} 14.1, 4.9 Hz, Ph-CH*H*), 3.76 and 3.83 (3H, s, OCH$_3$), 4.80 and 4.84 (1H, s, C=CHCO), 4.91 (1H, dd, J_{HH} 4.9, 2.9 Hz, Bn-C*H*), 6.18 (1H, d, J_{HH} 11.1 Hz, NCHCON), 6.89–7.25 (5H, m, H^{ar}); δ_C (125 MHz, $CDCl_3$) 10.4 (*C*H$_3$CH$_2$), 14.7 (*C*H$_3$CH), 24.7 (CH$_3$*C*H$_2$), 28.5/28.6 ((*C*H$_3$)$_3$C), 31.1 (*C*HEt), 33.8 (NCH$_3$), 34.7/35.0 (*C*H$_2$Ph), 58.5 (CH$_3$O), 59.4 (*C*HBn), 59.8 (NCHCON), 80.2 ((CH$_3$)$_3$*C*), 95.1 (C=*C*HCO), 127.1 (C^{ar}), 128.3 (C^{ar}), 128.5 (C^{ar}), 129.6 (C^{ar}), 129.74 (C^{ar}), 134.3 (C^{ar}), 156.4 (OCON), 169.2 (=CH*C*ON), 171.6 (NCH*C*ON), 177.7 (*C*OMe). HRMS: *m/z* calcd for [M+Na, $C_{24}H_{34}N_2O_5Na^+$]: 453.23599; found: 453.23503.

4.2.3. (5*S*)-1-(*N*-methyl-L-isoleucinoyl)-5-benzyl-4-methoxy-2,5-dihydropyrrol-2-one (***12***)

A solution of carbamate **11** (0.25 g, 0.58 mmol, 1.0 equiv) in CH_2Cl_2 (8 mL) was cooled to 0 °C and slowly treated with 0.67 mL trifluoroacetic acid (0.67 mL, 8.71 mmol, 15 equiv). The solution was warmed to room temperature, stirred for 4 h, and then diluted with CH_2Cl_2, cooled in an ice-bath and quenched with sat. aqueous $NaHCO_3$. The layers were separated, the aqueous one was extracted with CH_2Cl_2, and the combined organic phases were dried over Na_2SO_4 and concentrated in vacuo to yield **11** (0.18 g, 94%) as a colourless oil; $[\alpha]^{20}_D$ + 204.2 (*c* 1.0, $CHCl_3$); ν_{max} 3336, 3031, 2964, 1724, 1674, 1627, 1455, 1379, 1351, 1304, 1262, 1245, 1188, 1115, 963, 799, 732, 700 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.85 (3H, t, J_{HH} 7.3 Hz, C*H*$_3$CH$_2$), 0.99 (3H, d, J_{HH} 6.7 Hz, C*H*$_3$CH), 1.12 (1H, ddq, J_{HH} 13.3, 10.2, 7.3 Hz, CH$_3$C*H*H), 1.49 (1H, dqd, J_{HH} 13.3, 7.3, 3.1 Hz, CH$_3$CH*H*), 1.60 (1H, dqdd, J_{HH} 10.2, 6.7, 4.6, 3.1 Hz, C*H*Et), 2.44 (3H, s, NCH$_3$), 3.18 (1H, dd, J_{HH} 14.0, 2.9 Hz, PhC*H*H), 3.65 (1H, dd, J_{HH} 14.0, 5.2 Hz, PhCH*H*), 3.85 (3H, s, CH$_3$O), 4.30 (1H, d, J_{HH} 4.6 Hz, NCHCON), 4.85 (1H, s, C=CHCO), 4.86 (1H, dd, J_{HH} 5.2, 2.9 Hz, BnC*H*), 6.98–7.24 (5H, m, H^{ar}); δ_C (125 MHz, $CDCl_3$) 11.9 (*C*H$_3$CH$_2$), 16.6 (*C*H$_3$CH), 23.4 (CH$_3$*C*H$_2$), 35.2 (PhCH$_2$), 35.7 (NCH$_3$), 38.0 (CH$_3$*C*H), 58.5 (CH$_3$O), 60.3 (BnC*H*), 67.7 (NCHCON), 95.3 (=*C*HCO), 127.3 (C^{ar}), 128.2 (C^{ar}), 129.7 (C^{ar}), 134.3 (ipso-C^{ar}), 170.1 (=CH*C*ON), 176.4 (NCH*C*ON), 178.1 (*C*OMe). HRMS: *m/z* calcd for [M+H, $C_{19}H_{27}N_2O_3^+$]: 331.20162; found: 331.20115.

Please cite this article in press as: Wunder A, et al., Synthesis and anticancer activity of the proposed structure of caldoramide, an *N*-peptidyltetramate from the cyanobacterium *Caldora penicillata*, Tetrahedron (2018), https://doi.org/10.1016/j.tet.2018.04.004

4.2.4. pNos-L-Val-(NMe-L-Ile)-(5-benzyl-4-methoxy-2,5-dihydropyrrol-2-one) (**14**)

In a Schlenk tube *N*-*p*-nosyl-L-valine **13** (98 mg, 0.32 mmol, 1.3 equiv) was suspended in 2.5 mL of dry CH_2Cl_2, cooled to 0 °C and then treated with 1-chloro-*N*,*N*,2-trimethyl-1-propenylamine (43 μL, 0.32 mmol, 1.3 equiv). The reaction was stirred at 0 °C for 1 h while being monitored by tlc (cyclohexane/ethyl acetate, 3:1). To this end an aliquot was quenched with CH_3OH and the resulting methyl ester taken as a measure of consumption of the starting material. Eventually, a suspension of amine **12** (82 mg, 0.25 mmol, 1.0 equiv) in CH_2Cl_2 (2.5 mL) and 10% aqueous $NaHCO_3$ (2.5 mL) was added to the so prepared solution of acid chloride and the resulting mixture was stirred for 1 h. The layers were separated, the aqueous one was extracted three times with ethyl acetate, and the combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. After column chromatography (cyclohexane/diethyl ether, 3:1 to 4:1) **14** (118 mg, 78%) was obtained as a colourless solid of m. p. 90–95 °C; $[\alpha]_D^{20}$ +118.0 (*c* 1.0, $CHCl_3$); ν_{max} 2964, 2931, 1733, 1677, 1628, 1530, 1456, 1378, 1369, 1350, 1306, 1171, 737, 616 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 78:22 mixture of rotamers; major rotamer: 0.56–0.63 (2H, m, CH_3CH_2), 0.66 (3H, t, J_{HH} 7.6 Hz, CH_3CH_2), 0.77 (3H, d, J_{HH} 6.4 Hz, CH_3CH^{ile}), 0.80 and 1.11 (3H each, d, J_{HH} 6.7 Hz, $(CH_3)_2CH$), 1.86–1.95 (2H, m, *CH*Et, $(CH_3)_2CH$), 2.97 (3H, s, NCH_3), 3.11 (1H, dd, J_{HH} 14.0, 3.2 Hz, PhC*H*H), 3.29 (1H, dd, J_{HH} 14.0, 5.5 Hz, PhCH*H*), 3.77 (3H, s, CH_3O), 4.01 (1H, m, SNCH), 4.78 (1H, dd, J_{HH} 5.5, 3.2 Hz, BnC*H*), 4.84 (1H, s, C=CHCO), 5.96 (1H, br d, NH), 6.17 (1H, d, J_{HH} 10.7 Hz, NCHCON), 6.92–6.99 (2H, m, *meta*-H^{ar}), 7.16–7.23 (3H, m, H^{ar}), 8.01–8.06 (2H, m, $CHCNO_2$), 8.31–8.35 (2H, m, 2H, CHCS); δ_C (125 MHz, $CDCl_3$) 10.8 (CH_3CH_2), 14.5 (CH_3CH), 16.0 and 20.2 ($(CH_3)_2CH$), 24.9 (CH_3CH_2), 31.0 (Me_2CH), 31.3 (CH_3N), 33.9 (*CH*Et), 35.6 ($PhCH_2$), 58.6 (CH_3O), 58.8 (SNCH), 59.4 (NCHCON), 59.9 (BnCH), 94.8 (=CHCO), 124.3 (C^{ar}), 127.2 (C^{ar}), 128.4 (C^{ar}), 128.6 (C^{ar}), 129.5 (C^{ar}), 134.7 (ipso-C^{ar}), 145.9 and 150.0 (ipso-C^{ar}), 169.1 (=CHCON), 171.2 (MeNCO), 171.3 (NCHCON), 178.4 (COMe). HRMS: *m/z* calcd for [M+Na, $C_{30}H_{38}N_4O_8NaS^+$]: 637.23026; found: 637.23010.

4.2.5. pNos-NMe-L-Val-(NMe-L-Ile)-(5-benzyl-4-methoxy-2,5-dihydropyrrol-2-one) (**15**)

A solution of sulfonamide **14** (166 mg, 0.27 mmol, 1.0 equiv) in DMF (2.7 mL) was treated with K_2CO_3 (75 mg, 0.54 mmol, 2.0 equiv) and CH_3I (34 μL, 0.54 mmol, 2.0 equiv). The resulting mixture was stirred at room temperature for 1 h, quenched with H_2O and diluted with diethyl ether. The layers are separated and the aqueous phase was extracted with diethyl ether. The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. Purification by column chromatography (cyclohexane/ethyl acetate, 2:1) afforded **15** (168 mg, 99%) as a colourless solid of m. p. 183–187 °C; $[\alpha]_D^{20}$ +79.0 (*c* 0.52, $CHCl_3$); ν_{max} 2972, 2932, 2882, 1729, 1677, 1643, 1625, 1529, 1453, 1384, 1340, 1304, 1272, 1240, 1192, 1159, 1134, 1120, 1106, 1084, 968, 956, 936, 864, 852, 819, 797, 737, 720, 699, 602, 567 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 95:5 mixture of rotamers; major rotamer: 0.81 (3H, d, J_{HH} 6.6 Hz, $(CH_3)_2CH$), 0.86 (3H, t, J_{HH} 7.4 Hz, CH_3CH_2), 0.91 (3H, d, J_{HH} 6.4 Hz, overlapped, $(CH_3)_2CH$), 0.92 (3H, d, J_{HH} 6.7, overlapped, CH_3CH), 1.12 (1H, ddq, J_{HH} 13.7, 8.0, 7.4 Hz, CH_3CHH), 1.31 (1H, dqd, J_{HH} 13.7, 7.4, 2.9 Hz, CH_3CHH), 2.13 (1H, ddqd, J_{HH} 11, 8.0, 6.7, 2.9 Hz, *CH*Et), 2.32 (1H, dqq, J_{HH} 10.7, 6.7, 6.4 Hz, $(CH_3)_2CH$), 3.04 (3H, s, $SNCH_3$), 3.12 (1H, dd, J_{HH} 14.0, 3.4 Hz, PhC*H*H), 3.30 (1H, dd, J_{HH} 14.0, 5.2 Hz, PhCH*H*), 3.36 (3H, s, NCH_3), 3.74 (3H, s, OCH_3), 4.68 (1H, d, J_{HH} 11 Hz, SNCH), 4.82 (1H, s, C=CHCO), 4.84 (1H, dd, J_{HH} 5.2, 3.4 Hz, BnC*H*), 6.44 (1H, d, J_{HH} 10.7 Hz, NCHCON), 7.02–7.08 (2H, m, H^{phenyl}), 7.17–7.26 (3H, m, H^{phenyl}), 7.97–8.02 (2H, m, H^{ar}), 8.33–8.38 (2H, m, H^{ar}); δ_C (125 MHz, $CDCl_3$) 10.6 (CH_3CH_2), 14.5 (CH_3CH), 18.9 and 19.8 ($(CH_3)_2CH$), 25.5 (CH_3CH_2), 28.8 (Me_2CH), 30.4 ($SNCH_3$), 32.3 (CH_3N), 34.5 (*CH*Et), 35.7 ($PhCH_2$), 58.5 (CH_3O), 58.8 (NCHCON), 59.9 (BnCH), 60.4 (SNCH), 94.9 (=CHCO), 124.4 (C^{ar}), 127.2 (C^{ar}), 128.3 (C^{ar}), 128.4 (C^{ar}), 129.7 (C^{ar}), 134.9 (ipso-C^{ar}), 145.8/150.0 (ipso-C^{ar}), 169.3 (=CHCON), 171.8 (MeNCO), 172.7 (NCHCON), 178.3 (COMe). HRMS: *m/z* calcd for [M+H, $C_{31}H_{40}N_4O_8NaS^+$]: 651.24591; found: 651.24377.

4.2.6. pNos-L-Val-(NMe-L-Val)-(NMe-L-Ile)-(5-benzyl-4-methoxy-2,5-dihydropyrrol-2-one) (**16**)

A solution of **15** (45 mg, 72 μmol, 1.0 equiv) in DMF (0.7 mL) was treated with K_2CO_3 (59 mg, 429 μmol, 6.0 equiv) and 2-methyl-5-*tert*-butylthiophenol (32 mg, 179 μmol, 2.5 equiv), stirred for an hour and quenched with 1 M HCl and brine. The aqueous layer was repeatedly extracted with diethyl ether until no thiophenol could be detected in the extract by tlc (cyclohexane/ethyl acetate, 1:1). The pH value of the aqueous layer was adjusted to 9 with Na_2CO_3 and 2 mL of CH_2Cl_2 were added. This solution was directly used for the subsequent coupling. In a separate Schlenk tube *N*-*p*-nosyl-L-valine **13** (69 mg, 215 μmol, 3.0 equiv) was suspended in 1 mL of dry CH_2Cl_2, cooled to 0 °C, and treated with 1-chloro-*N*,*N*,2-trimethyl-1-propenylamine (34 μL, 215 μmol, 3.0 equiv). The mixture was stirred at 0 °C for 1 h while being monitored by tlc as described for the synthesis of **14**. After consumption of all starting material the previously prepared solution of the de-nosylated amine derived from **15** was added via syringe, the mixture was stirred for 30 min, the layers were separated, and the aqueous one was extracted with CH_2Cl_2 (3×). The combined organic phases were washed with 1 M NaOH, dried over Na_2SO_4 and concentrated in vacuo. The remainder was purified by column chromatography (cyclohexane/ethyl acetate, 1:1) to yield **16** (44 mg, 85%) as an amorphous white solid of m. p. 120–128 °C; $[\alpha]_D^{20}$ +61.7 (*c* 0.94, $CHCl_3$); ν_{max} 3200, 3106, 3034, 2965, 2931, 2876, 1728, 1626, 1531, 1456, 1379, 1349, 1305, 1246, 1193, 1170, 1089, 965, 854, 738, 719, 700, 686, 671, 615, 565, 554 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.17 (3H, d, J_{HH} 6.7 Hz, $(CH_3)_2CH^{val1}$), 0.80 (3H, d, J_{HH} 6.5 Hz, $(CH_3)_2CH^{val1}$), 0.82 (3H, d, J_{HH} 6.8 Hz, $(CH_3)_2CH^{val2}$), 0.83 (3H, t, J_{HH} 7.3 Hz, overlapped, CH_3CH_2) 0.88 (3H, d, J_{HH} 6.7 Hz, CH_3CH), 1.06 (1H, ddq, J_{HH} 13.5, 9.0, 7.3 Hz, CH_3CHH), 1.12 (3H, d, J = 6.8 Hz, $(CH_3)_2CH^{val2}$) 1.20 (1H, dqd, J_{HH} 13.5, 7.3, 3.2 Hz, CH_3CHH), 1.88 (1H, sptd, J_{HH} 6.8, 3.1, Me_2CH^{val2}), 2.08 (1H, ddqd, J_{HH} 10.8, 9.0, 7.0, 3.2 Hz, *CH*Et), 2.22 (1H, dqq, J_{HH} 10.8, 6.7, 6.5 Hz, Me_2CH^{val1}), 2.88 (3H, s, CH_3N^{val1}), 3.09 (1H, dd, J_{HH} 14.0, 3.4 Hz, PhC*H*H), 3.15 (3H, s, CH_3N^{ile}), 3.31 (1H, dd, J_{HH} 14.0, 4.9 Hz, PhCH*H*), 3.75 (3H, s, CH_3O), 4.01 (1H, dd, J_{HH} 9.2, 3.1 Hz, SNCH), 4.81 (1H, s, C=CHCO), 4.83 (1H, dd, J_{HH} 4.9, 3.4 Hz, BnH), 5.02 (1H, d, J_{HH} 10.8 Hz, $NCHCO^{val1}$), 5.86 (1H, d, J_{HH} 9.2 Hz, NH), 6.44 (1H, d, J_{HH} 10.8 Hz, $NCHCO^{ile}$), 6.96–7.00 (2H, m, ortho-Ph), 7.18–7.23 (3H, m, Ph), 8.00–8.05 (2H, m, nosyl-H), 8.29–8.34 (2H, m, nosyl-H); δ_C (125 MHz, $CDCl_3$) 10.6 (CH_3CH_2), 14.5 (CH_3CH), 15.8 ($(CH_3)_2CH^{val2}$), 17.8 and 19.2 ($(CH_3)_2CH^{val1}$), 20.2 ($(CH_3)_2CH^{val2}$), 25.2 (CH_3CH_2), 27.0 (Me_2CH^{val1}), 30.3 (CH_3N^{val1}), 30.8 (Me_2CH^{val2}), 31.8 (CH_3N^{ile}), 34.4 (*CH*Et), 35.6 ($PhCH_2$), 58.48*, 58.49*, 58.54*, 58.6 ($NCHCO^{ile}$), 59.8 (BnCH), 94.9 (=CHCO), 124.5 (C^{ar}), 127.2 (C^{ar}), 128.4 (C^{ar}), 128.6 (C^{ar}), 129.6 (C^{ar}), 134.7 (ipso-C^{ar}), 145.9/150.2 (ipso-C^{ar}), 169.2 (=CHCON), 170.8 (CO^{val2}), 170.9 (CO^{val1}), 172.1 (NCHCON), 178.3 (COMe); *assignment interchangeable: CH_3O/$NCHCO^{val1}$/SNCH. HRMS: *m/z* calcd for [M+Na, $C_{36}H_{49}N_5O_9NaS^+$]: 750.31432; found: 750.31347.

4.2.7. Caldoramide (proposed structure **1**)

Analogously to **15,** compound **16** (57 mg, 79 μmol, 1.0 equiv) was de-nosylated with K_2CO_3 (65 mg, 473 μmol, 6.0 equiv) and 2-methyl-5-*tert*-butylthiophenol (35 μL, 189 μmol, 2.5 equiv) in DMF (0.78 mL) to give the corresponding primary amine.

A solution of it in DMF (0.4 mL) and DIPEA (66 μL, 379 μmol, 4.8 equiv) was added to a Schlenk tube containing dimethyl-L-valine

Please cite this article in press as: Wunder A, et al., Synthesis and anticancer activity of the proposed structure of caldoramide, an *N*-peptidyltetramate from the cyanobacterium *Caldora penicillata*, Tetrahedron (2018), https://doi.org/10.1016/j.tet.2018.04.004

(14 mg, 95 μmol, 1.2 equiv) and HATU (36 mg, 95 μmol, 1.2 equiv), dissolved in 0.3 mL DMF, cooled to 0 °C, and having pre-reacted for 20 min. The resulting mixture was stirred for 2 h at room temperature, diluted with ethyl acetate, washed with brine, dried over Na_2SO_4 and concentrated in vacuo. After purification by column chromatography (ethyl acetate/EtOH, 96:4) caldoramide **1** (proposed structure) was obtained as a colourless, amorphous solid (34 mg, 64%) of m. p. 177–179 °C; $[\alpha]_D^{20}$ −13.2 (*c* 0.50, CH_3OH) [lit[1]: $[\alpha]_D^{20}$ +11.1 (*c* 0.36, CH_3OH)]; ν_{max} 3318, 2964, 2932, 1733, 1627, 1497, 1455, 1380, 1304, 1259, 1247, 1193, 1093, 1112, 1033, 1026, 965, 909, 807, 799, 729, 699, 670, 645 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.78 (3H, d, J_{HH} 6.7 Hz, 25/26-H), 0.86 (3H, t, J_{HH} 7.3 Hz, 18-H), 0.91 (3H, d, J_{HH} 6.7 Hz, 19-H), 0.91 (3H, d, J_{HH} 6.7 Hz, 25/26-H), 0.94 (3H, d, J_{HH} 6.7 Hz, 38/39-H), 0.97 (3H, d, J_{HH} 6.7 Hz, 32/33-H), 1.01 (3H, d, J_{HH} 6.7 Hz, 32/33-H), 1.02 (3H, d, J_{HH} 6.7 Hz, 38/39-H), 1.13 (1H, ddq, J_{HH} 13.9, 8.7, 7.3 Hz, 17-H^a), 1.26–1.30 (1H, m, 17-H^b, overlapped), 1.99 (1H, sptd, J_{HH} 6.7, 5.9 Hz, 31-H), 2.09 (1H, sptd, J_{HH} 6.7, 6.1 Hz, 37-H, overlapped), 2.11 (1H, m, 16-H, overlapped), 2.27 (6H, s, 41/42-H), 2.41 (1H, dspt, J_{HH} 11.0, 6.7 Hz, 24-H), 2.49 (1H, d, J_{HH} 6.1 Hz, 36-H), 3.11, (1H, dd, J_{HH} 14.0, 3.4 Hz, 6-H^a), 3.13 (3H, s, 28-H), 3.24 (3H, s, 21-H), 3.33 (1H, dd, J_{HH} 14.0, 5.2 Hz, 6-H^b), 3.75 (3H, s, 13-H), 4.83 (1H, s, 2-H), 4.84 (1H, dd, J_{HH} 8.9, 5.9 Hz, 30-H), 4.86 (1H, dd, J_{HH} 5.2, 3.4 Hz, H-4), 5.30 (1H, d, J_{HH} 11.0 Hz, 23-H), 6.48 (1H, d, J_{HH} 10.7 Hz, 15-H), 6.99 (1H, d, J_{HH} 8.9 Hz, 30-NH), 7.00–7.04 (2H, m, H^{ar}), 7.22 (3H, m, H^{ar}); δ_C (125 MHz, $CDCl_3$) 10.7 (C-18), 14.6 (C-19), 17.8 (C-38/39), 17.9 (C-32/33), 18.4 (C-25/26), 19.7 (C-25/26), 20.0 (C-32/33), 20.3 (C-38/39), 25.2 (C-17), 27.3 (C-24), 27.8 (C-37), 30.7 (C-28), 31.0 (C-31), 31.9 (C-21), 34.4 (C-16), 35.7 (C-6), 43.1 (C-41/42), 53.8 (C-30), 58.4 (C-23), 58.5 (C-13), 58.7 (C-15), 59.8 (C-4), 76.6 (C-36), 94.9 (C-2), 127.2 (para-C^{ar}), 128.4 (meta-C^{ar}), 129.7 (ortho-C^{ar}), 134.8 (ipso-C^{ar}), 169.2 (C-1), 171.3 (C-22), 171.9 (C-35), 172.3 (C-14), 172.9 (C-29), 178.2 (C-3). HRMS: *m/z* calcd for [M+H, $C_{37}H_{60}N_5O_6^+$]: 670.45186; found: 670.45381.

4.3. Cell cultures and growth inhibition (MTT) assay

Cell cultures: The human colon adenocarcinoma cell line HT-29 (DSMZ no.: ACC 299), the human colon carcinoma cell line HCT116 (DSMZ no.: ACC 581) and the human breast adenocarcinoma cell line MCF-7 (DSMZ no.: ACC 115) were grown in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% Antibiotic-Antimycotic solution (all from Gibco). The cells were maintained in a moisture-saturated atmosphere (5% CO_2, 95% humidity) at 37 °C. The BCRP overexpressing, multi-resistant MCF-7^{topo} clone was generated over a course of 90 days via addition of increasing amounts of topotecan (up to 500 nM) into the culture medium of MCF-7 breast cancer cells.

MTT assay[9]*:* MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium hydrobromide; ABCR] was used to identify the metabolic activity of vital cells which are capable of reducing it to a violet formazan. MCF-7^{topo}, HT-29, and HCT-116^{wt} cells (5×10^4/mL) were seeded (100 μL/well) and cultured for 24 h in 96-well microplates. Incubation of cells following treatment with the test compound (final concentrations ranging from 50 nM to 200 μM) was continued for 48 h. Solvent controls with DMF were treated identically. Then, the plates were centrifuged (300 g, 4 °C) for 5 min and the medium was discarded by a swift turn of the microplates. MTT in phosphate-buffered saline (0.05%) was added. After 2 h the precipitate of formazan was dissolved in DMSO containing 10% sodium dodecylsulfate and 0.6% AcOH. The microplates were centrifuged as before and swiftly turned to discard the MTT solution before adding the solvent mixture. The microplates were incubated for 1 h at 37 °C and absorbances at 570 nm and 630 nm (background) were measured with a Tecan F-200 plate reader. All experiments were carried out in quadruplicate, and the percentage of the viable cells was calculated with Graphpad Prism 7 as the mean ± standard deviation relative to the control (100%).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.tet.2018.04.004.

References

1. Gunasekera SP, Imperial L, Garst C, et al. *J Nat Prod*. 2016;79:1867–1871.
2. Engene N, Tronholm A, Salvador-Reyes LA, Luesch H, Paul VJ. *J Phycol*. 2015;51:670–681.
3. (a) Simmons TL, McPhail KL, Ortega-Barria E, Mooberry S, Gerwick WH. *Tetrahedron Lett*. 2006;47:3387–3390;
(b) Lan H-O, Ye J-L, Wang A-E, Ruan Y-P, Huang P-Q. *Chem Eur J*. 2011;17:958–968.
4. Pettit GR, Srirangam JK, Singh SB, et al. *JCS Perkin Trans*. 1996;1:859–863.
5. (a) Kempf K, Raja A, Sasse F, Schobert R. *J Org Chem*. 2013;78:2455–2461;
(b) Löffler J, Schobert R. *J Chem Soc Perkin Trans*. 1996;1:2799–2802.
6. (a) Frérot E, Coste J, Poncet J, Jouin P. *Tetrahedron Lett*. 1992;33:2815–2816;
(b) Teixidó M, Albericio F, Giralt E. *J Pept Res*. 2005;65:153–166.
7. Loke I, Park N, Kempf K, Jagusch C, Schobert R, Laschat S. *Tetrahedron*. 2012;68:697–704.
8. Chaume G, Barbeau O, Lesot P, Brigaud T. *J Org Chem*. 2010;75:4135–4145.
9. Mosmann T. *J Immunol Meth*. 1983;65:55–63.

Please cite this article in press as: Wunder A, et al., Synthesis and anticancer activity of the proposed structure of caldoramide, an *N*-peptidyltetramate from the cyanobacterium *Caldora penicillata*, Tetrahedron (2018), https://doi.org/10.1016/j.tet.2018.04.004