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PAPER

Synthesis and biological evaluation of a potent salicylihalamide A lactam analogue†

Dan Balan,[a] Christopher J. Burns,[b,c] Nicholas G. Fisk,[d] Helmut Hügel,*[a] David C. S. Huang,[b,c] David Segal,[b,c] Charlotte White,[d] Jörg Wagler[a] and Mark A. Rizzacasa*[d]

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The first synthesis of a lactam analogue **3** of salicylihalamide A (**1**) is reported. A key step in the approach was a photochemical acylation coupling between amine **10** and dioxinone **9** to form the amide **19**. Acetylation followed by RCM with Grubbs 1st generation catalyst gave the desired *E*-lactam **23** (*E* : *Z* ratio 87 : 13) as the major compound. Conversion of macrolactam **23** into the vinyl iodide **26** followed by Cu catalysed cross coupling with the diene amide **7** gave *aza*-salicylihalamide analogue **3** in good yield. This compound demonstrated potent activity against several human leukaemia cell lines.

Introduction

Salicylihalamide A (**1**)[1] (Fig. 1) is a potent cytotoxic macrolide salicylate[2] isolated from a marine sponge of the genus *Haliclona* (~1 × 10^{-3}% w/w yield from wet sponge) collected from waters around Rottnest Island which is situated 18 km off the coast of southern Western Australia. Testing of salicylihalamide A (**1**) in the NCI 60-cell line human tumour screen gave a striking pattern of differential toxicity which did not correlate with the profiles of any known antitumor compounds in the standard database.[1] These results suggest that compound **1** has a novel mechanism of action and further biological evaluation revealed that compound **1** was the first of a new class of mammalian specific vacuolar-type (H^+)-ATPase inhibitor.[3]

Salicylihalamide A (**1**) contains a 12-membered salicylate macrolactone along with an enamide sidechain found in other salicylate metabolites such as apicularen A.[2] As would be expected, this compound elicited the interest of the synthetic community and a number of total[4] and formal syntheses[5] were reported in a short time period as well as the production of a number of potent analogues.[2,4c,f,6] The majority of SAR studies have been conducted on salicylihalamide derivatives with modified enamide sidechains and it has been shown that the presence of some form of enamide functionality is important for activity.[6a,4d,f] However, some small modifications in the macrolactone are tolerated without substantial loss of cytotoxic activity. For example, Smith and Zheng[4g] reported that the simplified analogue **2**, which is devoid of the 12-methyl and 13-hydroxyl groups, was a potent cytotoxin against solid tumour cells lines with GI_{50} values ranging from 0.27 μM (SF268: CNS glioma) to 2.12 μM (DU-145: prostate cancer) which compared well to those for the natural product **1** (GI_{50} = 0.18 μM for both SF268 and DU-145 lines). However, a smaller 6-membered

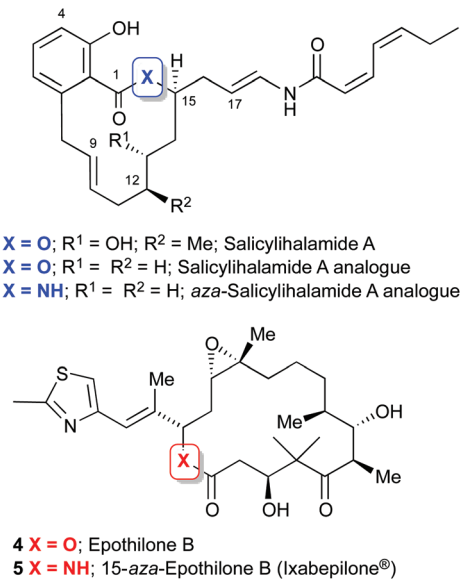


Fig. 1 Structures of salicylihalamide A, epothilone B and analogues.

[a]*School of Applied Sciences [Applied Chemistry], RMIT University, Bowen Street, Melbourne, Vic. 3001, Australia*
[b]*Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia*
[c]*Department of Medical Biology, The University of Melbourne, Parkville, Victoria, Australia*
[d]*School of Chemistry, the Bio21 Institute, The University of Melbourne, VIC 3010, Australia. E-mail: masr@unimelb.edu.au*

†Electronic supplementary information (ESI) available: Copies of the ^{1}H and ^{13}C NMR spectra of compounds **3**, **10**, **19**, **20**, **22**–**26** and crystallographic data for compounds **20** and **25** in PDF and CIF format. CCDC 886234 and 886235. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2ob26649k

benzolactone analogue[6c] of **1** displayed only modest activity (10^3 less active than **1**), showing that the 12-membered ring may be also important for activity.

We envisaged that a new modification of salicylihalamide (**1**) would be to change the 12-membered macrolactone to a macrolactam as in the *aza*-salicylihalamide A analogue **3**. This proposal was inspired by the discovery of a potent macrolactam analogue of the 15-membered macrolactone epothilone B (**4**). The semisynthetic 15-*aza*-epothilone B (**5**)[7] (ixabepilone®) is currently in use for the treatment of aggressive metastatic taxol® resistant breast cancer[8] and was found to exhibit a better therapeutic range than epothilone B (**4**). For example, 15-*aza*-epothilone B (**5**) (CCRF-CEM: leukemia IC_{50} = 2.1 nM) was ~10-fold lower in activity when compared with epothilone B (**4**) (CCRF-CEM: leukemia IC_{50} = 0.2 nM) but displayed a *50-fold decrease* in toxicity.[9] Key to the clinical success of ixabepilone (**5**) is the stability of the macrolactam. The low *in vivo* activity and toxicity of epothilone B (**4**) probably results from esterase-mediated macrolactone hydrolysis to form the inactive and toxic seco-acid.[6] Following the above precedent, the salicylate macrolactam *aza*-salicylihalamide A analogue (**3**) possesses a more stable macrocycle system and thus would be an interesting target for synthesis and biological evaluation. To our knowledge, no lactam analogues of salicylihalamide A (**1**) have been reported. We now describe an efficient first synthesis of the novel salicylihalamide A lactam analogue (**3**) and its potent *in vitro* activity against several leukemia cell lines.

The synthetic approach to **3** is detailed in Scheme 1. We envisaged that the key enamide could be introduced by a Cu catalysed cross coupling[10] between the vinyliodide intermediate **6** and the known amide **7**.[4b,11] The critical macrolactam ring could be secured by ring closing metathesis (RCM)[12] of diene **8**, hopefully with a degree of geometric control. The required diene precursor, salicylate **8** could be obtained *via* a photochemical acylation[13] of dioxinone **9** with optically pure amine **10**.

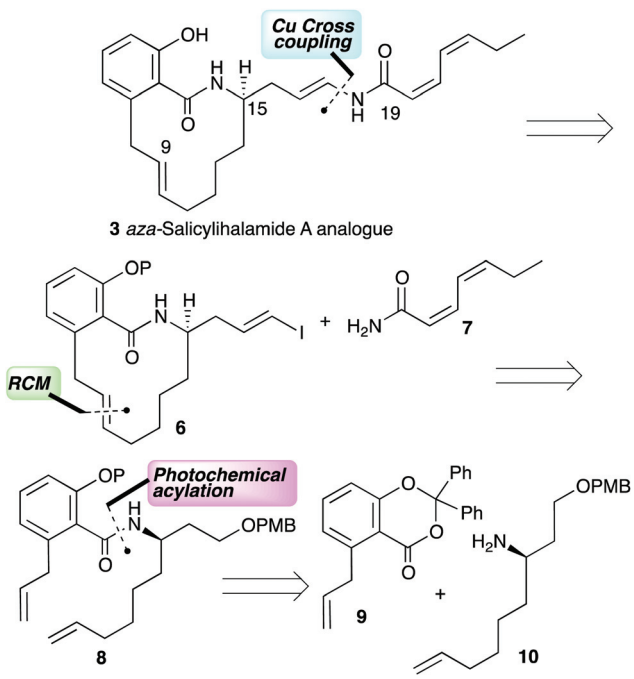
Results and discussion

Synthesis of analogue 3

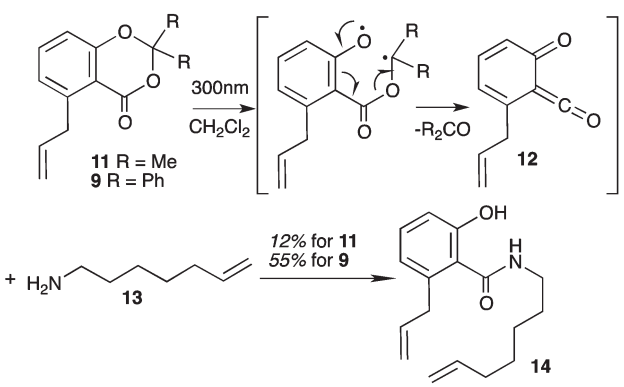
Our initial studies focused on amide formation in a simplified system devoid of the C15 sidechain. We trialled a number of traditional amine-acid coupling approaches to construct the amide bond with very limited success so we then elected to investigate the elegant photochemical method as reported by De Brabander.[13] This reaction can be utilised to form hindered *ortho*-substituted salicylate amides under essentially neutral conditions. As shown in Scheme 2, photolysis (300 nm) of a CH_2Cl_2 solution of the benzo-1,3-dioxin-4-one **11**[4b] affords the quinoneketene **12**, which was captured by the simple amine **13**[14] nucleophile to give the salicylate amide **14** in low yield (12%).

When the 2,2-diphenyl 1,3-dioxin-4-one **9**[13] was photolysed in the presence of amine **13**, the yield of **14** rose to 55%. These results add support to the original proposal[13] that the quinoneketene **12** is most likely formed by homolytic C–O bond fission as shown since an intermediate diphenylmethyl radical would be more stable than the dialkyl analogue.

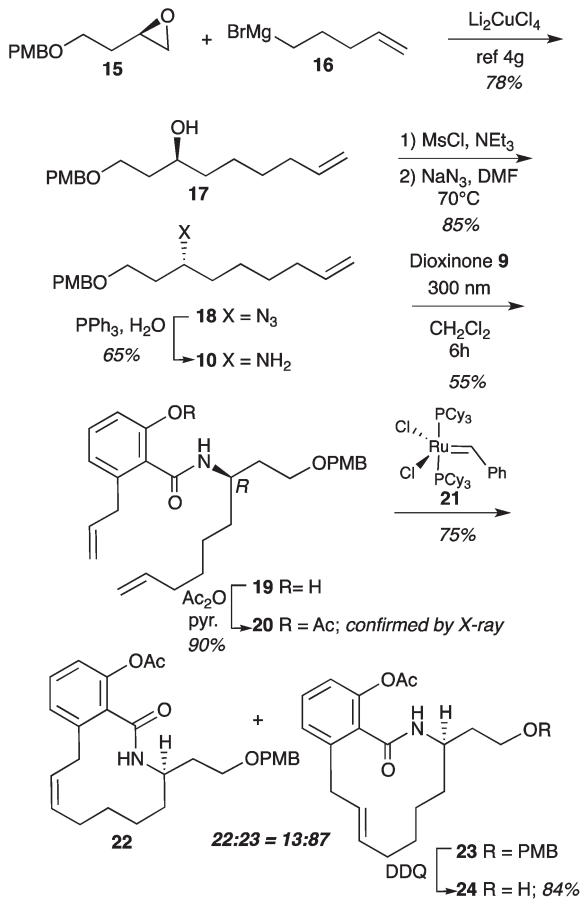
The synthesis of the chiral amine required for the production of **3** began with ring opening of the enantiopure epoxide **15**[15] and the cuprate derived from **16** as described by Smith and Zhang[4g] to afford the alcohol (−)-**17** in good yield (Scheme 3). Mesylation of **17** followed by azide displacement gave the azide **18** which underwent Staudinger reduction to give the amine **10** in pure form after aqueous acid extraction. Irradiation of a solution of dioxinone **9**[13] and amine **10** in CH_2Cl_2 at 300 nm gave the amide **19** in a similar yield to that for the simpler system. This reaction also gave similar yields on a 12 g scale demonstrating that this step is easily scalable. For the RCM reaction, preliminary experiments indicated that Grubbs 1st generation catalyst **21**[16] gave the best yields and $E:Z$ selectivity and this was also observed for the RCM based syntheses of the macrolactone in salicylihalamide A (**1**).[4f] For the formation of the macrolactam, the highest yield and selectivity was obtained when the phenol was protected as the acetate. Acetylation of phenol **19** gave crystalline acetate **20** and X-ray analysis confirmed



Scheme 1 Retrosynthetic analysis of aza-salicylihalamide analogue **3**.



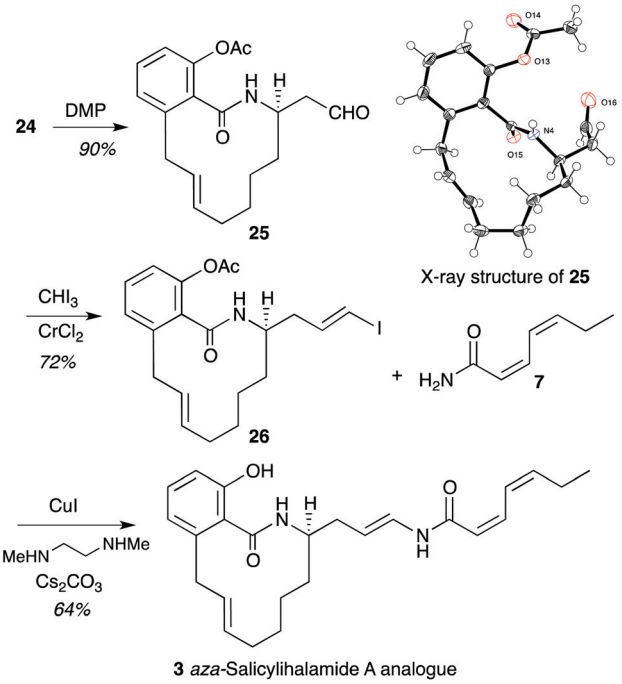
Scheme 2 Photochemical acylation studies.



Scheme 3 Synthesis of the *E*-macrolactam **23**.

the structure of this compound including the absolute configuration as *R*.[17] RCM using catalyst **21** gave the macrolactams **22** and **23** in good yield favouring the desired *E*-isomer **23** (*E* : *Z* ratio 87 : 13). Compound **23** could be separated from the small amount of *Z*-isomer **22** by flash chromatography. Again, the RCM reaction can be conducted on a multigram scale with little difference in yield and stereoselectivity. Removal of the PMB group by treatment of **23** with DDQ gave the alcohol **24**. When the methyl ether or the free phenol was used for the RCM reaction, yields and selectivities were lower and the *E* and *Z*-isomers could only be separated by HPLC.

The final steps to analogue **3** are shown in Scheme 4. Oxidation of alcohol **24** gave the crystalline aldehyde **25** in good yield (Scheme 4). This compound was also subjected to X-ray crystallographic analysis[18] which confirmed the structure (see Scheme 4). A Takai homologation[19] then afforded the *E*-vinyl iodide ready for cross coupling. The choice of acetate as protecting group in the RCM reaction was also useful in the last step. Cross coupling of iodide **26** with known dienamide **7** was conducted under the conditions developed by Buchwald[10] and utilised in a recent total synthesis of the related compound apicularen A.[20] This reaction formed the desired enamide and resulted in the concomitant cleavage of the phenolic acetate to provide the analogue (−)-**3** in 64% yield.



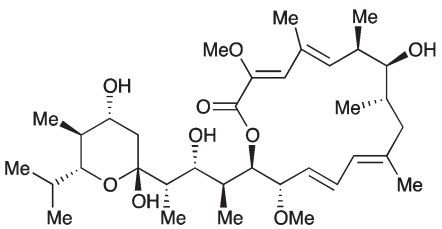
Scheme 4 Completion of the synthesis of analogue **3**.

Table 1 Growth inhibitory activity of compound **3** and bafilomycin

Cell line	Compound **3** IC_{50} (nM)	Bafilomycin A1 (**27**) IC_{50} (nM)
CCRF-CEM	371 ± 39	30 ± 1
HL-60	116 ± 14	17 ± 1
K562	506 ± 47	43 ± 15
MOLT-4	277 ± 9	23 ± 1
RPMI-8226	117 ± 4	13 ± 1

Biological assays

The analogue **3** was assayed against the NCI-60 leukaemia cell lines [*i.e.* CCRF-CEM (acute lymphoblastic leukaemia), HL-60 (acute promyelocytic leukaemia), K562 (chronic myeloid leukaemia), MOLT-4 (acute lymphoblastic leukaemia) and RPMI 8266 (myeloma)] and exhibited antiproliferative effects at sub-micromolar concentrations (Table 1). For comparison, bafilomycin A1 (**27**) was also assayed against the same cells lines since both this compound and salicylihalamide A (**1**) target vacuolar-type (H^+)-ATPase in the lysosome.[3,21] Compound **3** was very active against MOLT-4 (IC_{50} = 277 nM) and CCRF-CEM (IC_{50} = 371 nM) but was most active against RPMI 8226 and HL-60 (IC_{50} = 117 and 116 nM for each respectively).



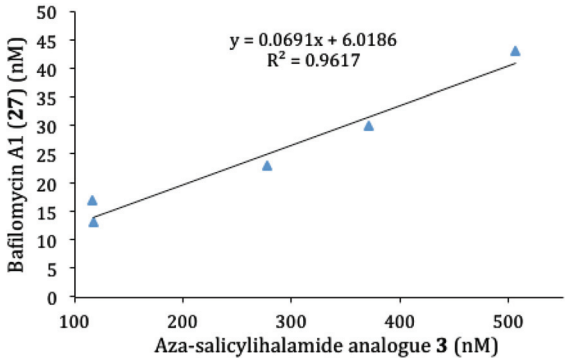


Fig. 2 Sensitivity of NCI-60 leukemia cells to bafilomycin A1 (**27**) *versus* the *aza*-salicylihalamide analogue **3**.

Bafilomycin A1 (**27**) was approximately 10 fold higher in activity than the *aza*-salicylihalamide analogue **3** across all the cell lines. In addition, the sensitivity profiles of compound **27** and analogue **3** were strongly correlated (Fig. 2) supporting a similar mode of action against vacuolar-type (H^+)-ATPase.

Conclusions

The *aza*-salicylihalamide analogue **3** was synthesised for the first time in a short sequence that is amenable to large scale. Key steps include a photochemical acylation to form the amide linkage, a *E*-selective ring closing metathesis to secure the macrolactam and a Cu mediated cross coupling to introduce the enamide and remove the phenolic acetate. Analogue **3** showed potent activity against several leukemia cell lines correlating strongly with bafilomycin A1 (**27**) and shows promise for further development as an anti-cancer compound. The preliminary biological results demonstrate that the introduction of a stable lactam linkage in the salicylihalamide structure affords a potent simplified analogue **3** of this family of compounds which compares well to other reported analogues and the natural product itself. Further tests to determine the *in vivo* activity and stability of this compound and derivatives are underway.

Experimental

General

Optical rotations were recorded in a 10 cm microcell. High resolution mass spectra (HRMS) were run using electrospray ionisation (ESI). Proton nuclear magnetic resonance (^{1}H NMR, 300 MHz) and proton decoupled carbon nuclear magnetic resonance spectra (^{13}C NMR, 75.5 MHz) were recorded for deuteriochloroform solutions with residual chloroform as internal standard unless otherwise stated. Analytical thin layer chromatography (TLC) was conducted on aluminium backed 2 mm thick silica gel GF254. Compounds were visualized with solutions of 20% w/w phosphomolybdic acid in ethanol or under UV (365 nm). Anhydrous tetrahydrofuran (THF) and diethyl ether were dried using a Glass Contour cartridge solvent dispensing system. All other commercial reagents were used as received. All air and moisture sensitive reactions were performed in glassware that was either flame dried under an atmosphere of dry argon or oven dried at 150 °C.

Amide (14)

A solution of the and amine **13**[14] (350 mg, 2.04 mmol) and the benzodioxinone **9**[13] (1.34 g, 4.08 mmol) in CH_2C1_2 (8 mL) under N_2 was placed in a Rayonet photochemical reactor equipped with 16 × 300 nm lamps and the solution was irradiated for 4 h. The solvent was removed under reduced pressure and the crude product purified by flash chromatography with 15% EtOAc/petrol as eluent to afford the amide **14** (308 mg, 55%) as a white solid. R_f (20% EtOAc/hexane) 0.39; ν_{max}: 3258, 3077, 1747, 1631, 1588, 1533. δ_H 10.69 (1H, s) 7.24 (1H, t, J = 7.6 Hz), 6.85 (1H, d, J = 8.0 Hz), 6.68 (1H, d, J = 7.2 Hz), 6.59 (1H, br s), 6.14–6.23 (1H, m), 5.74–5.84 (1H, m), 5.28 (1H, dd, J = 10.4, 1.6 Hz), 5.01 (1H, dd, J = 10.0, 2.0 Hz), 4.96 (1H, dd, J = 10.0, 2.0 Hz), 4.94 (1H, dd, J = 9.6, 1.6 Hz), 3.52 (2H, m), 3.40 (2H, q, J = 5.6 Hz), 2.05 (2H, q, J = 6.8 Hz), 1.57 (2H, m), 1.31–1.47 (4H, m); δ_C 169.7, 159.6, 138.5, 138.4, 136.0, 132.0, 122.8, 118.5, 117.7, 115.9, 114.6, 39.8, 38.6, 33.5, 29.0, 28.4, 26.4; HRMS (ESI) calc for $C_{17}H_{23}NO_2Na$ $[M + Na]^+$ 296.1621, found 296.1621.

Azide (18)

To a solution of alcohol **17** (2.4 g, 8.6 mmol) in Et_2O (60 mL) at 0 °C was added triethylamine (4.23 mL, 30.2 mmol) and methanesulfonyl chloride (2.33 mL, 30.2 mmol). After 15 min of stirring at 0 °C the reaction mixture was warmed to room temperature and the reaction was left to proceed for further 2 h. Then the resulting mixture was quenched with water, the organic layer was separated and the aqueous solution was extracted with Et_2O (3 × 50 mL). The combined organic extracts were washed successively with 50 mL H_2O and 50 mL sat. aq. NaCl, dried over Na_2SO_4 and concentrated affording the crude mesylate (2.88 g, 94%) directly used for the next step without further purification. To a solution of above mesylate (2.80 g, 7.86 mmol) in DMF (30 mL) was added NaN_3 (1.12 g, 17.3 mmol) and the mixture was stirred at 72 °C for 20 h. After cooling to room temperature the reaction mixture was quenched with water and extracted with diethyl ether (3 × 50 mL). The combined organic extracts were washed with water and brine and dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude product, which was purified by flash chromatography with 10% Et_2O/hexane as eluent to give azide **18** (2.14 g, 90%) as a colourless oil $[\alpha]_D^{26}$ − 27.8 (*c* 1.12, CH_2Cl_2); R_f (10% Et_2O/hexane) 0.58; IR ν_{max}: 2933, 2858, 2096, 1612, 1512, 1245, 1089, 1034, 910, 819 cm^{-1}; δ_H 7.30 (2H, d, J = 8.5 Hz), 6.92 (2H, d, J = 8.5 Hz), 5.84 (1H, ddt, J = 17.1, 10.2, 6.6 Hz), 5.03 (2H, m), 4.48 (2H, ABq, J = 11.6 Hz), 3.83 (3H, s), 3.59 (2H, m), 3.54 (1H, m) 2.10 (2H, m), 1.79 (2H, m) 1.50 (6H, m); δ_C 158.9, 138.3, 130.0, 129.0, 114.3, 113.5, 72.5, 66.2, 59.8, 54.9, 34.4, 34.2, 33.3, 28.3, 25.2; HRMS (ESI) calculated for $C_{17}H_{25}N_3O_2Na$ $[M + Na]^+$ 326.18390, found 326.18396.

Amine (10)

To a solution of azide **18** (4.8 g, 15.8 mmol) and triphenylphosphine (16.9 g, 63.3 mmol) in THF (100 mL) was added water

(3.13 mL, 174 mmol) and the mixture was stirred for 3 h at 70 °C. The reaction mixture was cooled to room temperature and extracted into 0.5% aq. HCl. The aqueous phase was successively washed with Et_2O (3 × 40 mL) and 5% aq. NaOH and then extracted with CH_2Cl_2 (3 × 40 mL). The combined organic extracts were dried over $MgSO_4$, filtered and then concentrated under reduced pressure to give pure amine **10** (4.08 g, 65%) as an oil. $[\alpha]_D^{26}$ +3.13 (c 1.280, CH_2Cl_2); R_f (10% MeOH/CH_2Cl_2) 0.55; IR ν_{max}: 3369, 2927, 2854, 1612, 1586, 1512, 1245, 1092, 1034, 908, 819, 721 cm^{-1}; δ_H 7.25 (2H, d, J = 8.6 Hz), 6.86 (2H, d, 8.6 Hz), 5.79 (1H, ddt, J = 17.1, 10.2, 6.6 Hz), 4.99 (1H, ddt, J = 17.1, 1.9, 1.6 Hz), 4.92 (1H, ddt, J = 10.2, 1.1, 1.1 Hz), 4.42 (2H, br s), 3.78 (3H, s), 3.54 (2H, m), 2.86 (1H, m), 2.04 (2H, m), 1.79 (2H, m), 1.50 (2H, m), 1.38 (4H, m); δ_C 159.3, 139.1, 130.8, 129.4, 114.5, 114.0, 72.8, 68.0, 55.4, 49.3, 38.4, 37.9, 33.9, 29.2, 25.8; HRMS (ESI) calculated for $C_{17}H_{28}NO_2$ $[M + H]^+$ 278.21146, found 278.21155.

Amide (19)

A solution of the amine **10** (116.4 mg, 0.42 mmol) and benzodioxinone **9**[13] (215.5 mg, 0.63 mmol) in CH_2Cl_2 (10 mL) under N_2 was placed in a Rayonet photochemical reactor equipped with 16 × 300 nm lamps and the solution was irradiated for 6 h. The solvent was evaporated under reduced pressure and the crude product was purified by flash chromatography with 20% EtOAc/hexane as eluent to afford amide **19** (151.4 mg, 55%) as a colorless oil. R_f (30% EtOAc/hexane) 0.55; $[\alpha]_D^{23}$ −1.6 (c 0.790, CH_2Cl_2); IR ν_{max}: 3280, 3240, 2932, 2857, 1631, 1517, 1462, 1245, 1091, 909, 817, 761 cm^{-1}; δ_H 10.65 (1H, s), 7.25 (1H, t, J = 7.9 Hz), 7.19 (2H, d, J = 8.6 Hz), 6.87 (1H, dd, J = 8.2, 1.1 Hz), 6.81 (2H, d, J = 8.6 Hz), 6.66 (1H, dd, J = 7.5, 1.1 Hz), 6.61 (1H, d, J = 8.5 Hz), 6.05 (1H, ddt J = 17.2, 10.2, 5.1 Hz), 5.79 (1H, ddt, J = 17.1, 10.2, 6.6 Hz), 5.20 (1H, ddt, J = 10.2, 1.7, 1.7 Hz), 5.02 (1H, ddt, J = 17.2, 1.9, 1.6 Hz), 4.94 (1H, ddt, J = 10.1, 1.2, 0.9 Hz), 4.90 (1H, ddt, J = 17.3, 1.8, 1.5 Hz), 4.40 (2H, s), 4.28 (1H, m), 3.78 (3H, s), 3.55 (2H, m), 3.43 (2H, m), 2.05 (2H, dt, J = 6.9, 6.9 Hz), 1.93 (1H, m), 1.72 (1H, m), 1.54 (2H, m), 1.39 (4H, m); δ_C 169.2, 159.3, 159.2, 138.6, 138.0, 136.4, 131.8, 130.0, 129.4, 122.6, 118.8, 117.6, 115.8, 114.6, 113.7, 72.9, 67.0, 55.2, 48.3, 38.4, 34.4, 34.2, 33.5, 28.6, 25.4; HRMS (ESI) calculated for $C_{27}H_{35}NO_4Na$ $[M + Na]^+$ 460.24583, found 460.24586.

Acetate (20)

To a solution of phenol **19** (0.909 g, 2.07 mmol) and 4-(dimethylamino) pyridine (DMAP, 378 mg) in CH_2Cl_2 (10 mL) was added pyridine (0.84 mL, 10.3 mmol) and freshly distilled acetic anhydride (0.29 mL, 3 mmol). After stirring for 12 h at room temperature, the mixture was diluted with Et_2O (20 mL), and aqueous $NaHCO_3$ (40 mL) was added and the mixture stirred at rt for 10 min. The organic layer was separated and the aqueous layer was extracted with Et_2O (3 × 40 mL). The combined organic layers were washed successively with $CuSO_4$ solution, water, $NaHCO_3$ solution and sat. aq. NaCl, dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography with 30%, EtOAc/CH_2Cl_2 as eluent to give acetate **20** as a colorless crystalline solid (915 mg, 90%). A single-crystal X-ray diffraction analysis confirmed the identity of this compound (see ESI† and CCDC-886235). mp 55 °C; R_f (40%, EtOAc/CH_2Cl_2) 0.52; $[\alpha]_D^{23}$ +2.4 (c 0.91, CH_2Cl_2); IR ν_{max}: 3296, 3074, 2933, 2858, 1764, 1641, 1610, 1512, 1456, 1246, 1198, 1092, 1018, 911, 820, 733 cm^{-1}; δ_H 7.33 (1H, t, J = 7.9 Hz), 7.19 (2H, d, J = 8.6 Hz), 7.12 (1H, d, J = 7.9 Hz), 6.95 (1H, d, J = 7.9 Hz), 6.82 (2H, d, J = 8.6 Hz), 6.07 (1H, d, J = 8.6 Hz), 5.93 (1H, ddt, J = 17.0, 10.2, 6.5 Hz), 5.80 (1H, ddt, J = 17.1, 10.2, 6.6, Hz), 5.07 (1H, ddt, J = 10.2, 1.5, 1.3 Hz), 4.95 (1H, ddt, J = 17.0, 1.7, 1.5 Hz), 5.00 (1H, ddt, J = 17.0, 1.9, 1.5 Hz), 4.94 (1H, ddt, J = 10.0, 1.9, 1.1 Hz), 4.41 (2H, ABq, J = 11.5 Hz), 4.23 (1H, m), 3.79 (3H, s), 3.56 (2H, m), 3.33 (2H, d, J = 6.4 Hz), 2.27 (3H, s), 2.05 (2H, m), 1.82 (1H, m), 1.64 (1H, m), 1.43 (2H, m), 1.32 (4H, m); δ_H 170.0, 165.5, 159.1, 147.0, 138.8, 138.6, 136.6, 131.0, 130.1, 129.6, 129.3, 127.3, 120.5, 116.5, 114.5, 113.7, 72.8, 66.8, 55.2, 47.8, 37.0, 34.2, 34.0, 33.6, 28.8, 25.4, 21.0; HRMS (ESI) calculated for $C_{29}H_{37}NO_5Na$ $[M + Na]^+$ 502.25639, found 502.25646.

E- and *Z*-Macrolactams (23) and (22)

Grubbs 1st generation catalyst $(Cy_3P)_2Cl_2Ru$ = CHPh **21** (66.9 mg, 0.081 mmol) in degassed CH_2Cl_2 was added to a solution of **20** (390 mg, 0.814 mmol) in CH_2Cl_2 (8 mL) and the resulting solution was heated to reflux for 24 h. The solution was cooled and evaporated until *ca.* 30 mL remained and then filtered through a short plug of silica gel eluting with EtOAc. The filtered solution was stirred with activated charcoal (2 g) for 12 h at room temperature, and the charcoal was removed by filtration and the filtrate was concentrated under reduced pressure. Purification of the crude product on silica gel with EtOAc–hexane 1 : 20 → 1 : 4 → 1 : 1 as eluent afforded the (*E*)-macrolactam **23** (258 mg, 66%) and (*Z*)-macrolactam **22** (41 mg, 10%; contaminated with ~5% of the *E*-isomer) as colourless solids.

(*E*)-macrolactam **23**: mp 117 °C; R_f (50% EtOAc/hexane) 0.44; $[\alpha]_D^{23}$ −98.3 (c 0.720, CH_2Cl_2); IR ν_{max}: 3293, 2926, 2855, 1765, 1644, 1512, 1455, 1246, 1200, 1089, 1020, 804, 733 cm^{-1}; δ_H 7.30 (1H, t, J = 7.8 Hz), 7.26 (2H, d, J = 8.6 Hz), 7.11 (1H, d, J = 7.5 Hz), 6.92 (1H, d, J = 8.1 Hz), 6.88 (2H, d, J = 8.6 Hz), 5.51 (1H, d, J = 8.6 Hz), 5.50 (1H, m), 5.26 (1H, dddd, J = 14.9, 10.3, 3.3, 1.3 Hz), 4.46 (2H, ABq, J = 11.6 Hz), 4.11 (1H, m), 3.92 (1H, dd, 14.2, 3.8 Hz), 3.73 (3H, s), 3.58 (2H, app. t, J = 7.3 Hz), 3.21 (1H, dd, J = 14.2, 2.5 Hz), 2.16 (3H, s), 1.72 (4H, m), 1.41 (4H, m), 1.11 (2H, m); δ_C 170.8, 165.4, 159.2, 147.3, 140.6, 133.1, 130.7, 130.4, 129.9, 129.4, 128.5, 128.0, 120.8, 113.8, 72.8, 67.3, 55.2, 45.0, 37.9, 36.7, 32.8, 32.6, 24.7, 20.9, 20.7; HRMS (ESI) calculated for $C_{27}H_{33}NO_5Na$ $[M + Na]^+$ 474.22509, found 474.22504.

(*Z*)-Macrolactam **22**: mp 119–120 °C; R_f (50% EtOAc/hexane) 0.43; $[\alpha]_D^{27}$ −8.82 (CH_2Cl_2); IR ν_{max}: 3289, 2928, 2855, 1764, 1642, 1512, 1455, 1245, 1200, 1094, 1023, 818, 733 cm^{-1}; δ_H 7.35 (1H, t, J = 7.8 Hz), 7.24 (2H, d, J = 8.6 Hz), 7.20 (1H, d, J = 7.8 Hz), 6.93 (1H, d, J = 8.1 Hz), 6.87 (2H, d, J = 8.5 Hz), 5.80 (1H, d, J = 8.1 Hz), 5.46 (1H, app. t, J = 10.3 Hz), 5.18 (1H, app. t, J = 10.3 H), 4.44 (2H, ABq, J = 11.9 Hz), 4.28 (1H, m), 3.81 (3H, s), 3.70 (1H, dd, J = 14.9, 4.8 Hz), 3.52 (2H, m, H17), 3.22 (1H, br d, J = 14.9 Hz), 2.18

(3H, s), 1.94 (2H, m), 1.73 (2H, m), 1.42 (2H, m), 1.02 (2H, m); δ_C 170.4, 166.1, 159.5, 147.1, 140.6, 131.3, 130.7, 130.5, 130.3, 130.2, 129.5, 128.1, 120.5, 114.1, 73.0, 67.4, 55.6, 47.6, 33.1, 31.8, 30.3, 26.6, 24.5, 21.2, 19.0; HRMS (ESI) calculated for $C_{27}H_{33}NO_5Na$ $[M + Na]^+$ 474.22509, found 474.22515.

Alcohol (24)

To a solution of PMB ether **23** (120 mg, 0.266 mmol) in CH_2Cl_2 (40 mL) at 0 °C was added pH 7 buffer (6 mL) followed by DDQ (302 mg, 1.33 mmol). After being stirred at 0 °C for 5 min and at room temperature for 2 h, the reaction was quenched with saturate aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$, and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and then concentrated under reduced pressure. The residue was purified by flash chromatography with 30% EtOAc/hexane as eluent to afford alcohol **24** (81 mg, 84%) as a white solid. mp 156–157 °C; R_f (40% EtOAc/hexane) 0.47; $[\alpha]_D^{23}$ −169.5 (c 0.710, CH_2Cl_2); IR ν_{max}: 3369, 3264, 2944, 2926, 2862, 1759, 1548, 1456, 1215, 966, 750 cm^{-1}; δ_H 7.33 (1H, t, J = 7.9 Hz), 7.13 (1H, d, J = 7.5 Hz), 6.94 (1H, d, J = 8.2), 5.47 (1H, d, J = 8.7 Hz), 5.48 (1H, m), 5.27 (1H, m), 4.15 (1H, m), 3.89 (1H, dd, J = 14.0, 3.3 Hz), 3.72 (2H, m), 3.20 (1H, app. dd, J = 14.0, 2.3 Hz), 2.26 (3H, s), 1.81 (2H, m), 1.61 (2H, m), 1.48 (2H, m), 1.33 (2H, m), 1.13 (2H, m); δ_C 170.9, 167.6, 147.2, 140.4, 133.3, 130.3, 130.1, 128.5, 128.1, 120.9, 58.3, 43.6, 40.0, 37.9, 33.8, 32.5, 24.4, 20.9, 20.8; HRMS (ESI) calculated for $C_{19}H_{25}NO_4Na$ $[M + Na]^+$ 354.16758, found 354.16763.

Aldehyde (25)

Dess–Martin periodinane (1.53 g, 3.625 mmol) was added to a cooled (0 °C) solution of alcohol **24** (300 mg, 0.906 mmol) in CH_2Cl_2 (50 mL) and the resultant mixture was stirred at rt for 45 min. The white suspension was then cooled to 0 °C and saturated aqueous $Na_2S_2O_3$ (100 mL) and saturated aqueous solution of $NaHCO_3$ (100 mL) were added and the resulting mixture stirred for 30 min until it became clear. The mixture was diluted with CH_2Cl_2 (50 mL), the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with a 40% aqueous solution of sodium thiosulfate (200 mL) and brine, dried over $MgSO_4$ and concentrated. Flash chromatography provided aldehyde **25** (250.5 mg, 90%) as a white solid, which was recrystallized from EtOAc affording colourless crystals suitable for X-ray crystallographic analysis. A single-crystal X-ray diffraction analysis confirmed the identity of this compound (see ESI† and CCDC-886234) mp 168 °C; R_f (80% EtOAc/hexane) 0.78; $[\alpha]_D^{23}$ −112.5 (*c* 1.03, CH_2Cl_2); IR ν_{max}: 3254, 2930, 2852, 2737, 1763, 1724, 1634, 1546, 1458, 1202, 1019, 973, 756 cm^{-1}; δ_H 9.81 (1H, t, J = 2.1 Hz), 7.28 (1H, t, J = 7.8 Hz), 7.08 (1H d, J = 7.4 Hz), 6.90 (1H, d, J = 8.1 Hz), 5.74 (1H, d, J = 7.8 Hz), 5.47 (1H, dddd, J = 14.9, 10.1, 4.5, 1.6 Hz), 5.26 (1H, dddd, J = 14.9, 10.1, 3.4, 1.3 Hz), 4.44 (1H, m), 3.82 (1H, dd, J = 14.1, 3.9 Hz), 3.21 (1H, dq, J = 14.1, 2.7 Hz), 2.57, (2H, m), 2.29 (3H, s), 2.18 (1H, m, H14), 1.69 (1H, m), 1.56 (2H, m), 1.43 (2H, m), 1.11 (2H, m); δ_C 200.5, 170.9, 165.7, 147.2, 140.3, 132.7, 130.2, 130.1, 128.4, 128.3, 120.8, 50.1, 43.0, 37.8, 32.9, 32.7, 24.6, 21.0, 20.8; HRMS (ESI) calculated for $C_{19}H_{23}NO_4Na$ $[M + Na]^+$ 352.15193, found 352.15199.

Vinyl iodide (26)

To a slurry of chromium(II) chloride (112.95 mg, 0.919 mmol) in dry THF (5 mL) under a N_2 atmosphere was added *via* cannula a solution of aldehyde **25** (43.2 mg, 0.13 mmol) and iodoform (155.1 mg, 0.39 mmol) in dry THF (4 mL) and the initial grey suspension turned almost immediately reddish-brown. After stirring at room temperature for 8 h, the reaction mixture was diluted with water and extracted in Et_2O (3 × 20 mL). The combined organic layers were washed with aqueous solution of $Na_2S_2O_3$, brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The product was purified by chromatography with 30% EtOAc/hexane as eluent to afford the (*E*)-iodoalkene **26** (38 mg, 72%) as a white solid. mp 154–156 °C; R_f (80% Et_2O/hexane) 0.72; $[\alpha]_D^{23}$ −96.4 (c 0.805, CH_2Cl_2); IR ν_{max}: 3255, 2924, 1760, 1633, 1546, 1456, 1207, 1019, 966, 873, 708 cm^{-1}; δ_H 7.32 (1H, t, J = 7.9 Hz), 7.16 (1H, d, J = 7.6 Hz), 6.91 (1H, dd, J = 8.08, 0.93 Hz), 6.37 (1H, dtd, J = 14.3, 7.3, 7.1 Hz), 6.09 (1H, app. dt, J = 14.3, 1.2 Hz), 5.92 (1H, d, J = 8.1 Hz), 5.42 (1H, app. t, J = 10.0 Hz), 5.13 (1H, app. t, J = 10.5 Hz), 4.10 (1H, m), 3.61 (1H, dd, 15.1, 4.7 Hz), 3.17 (1H, br dd, J = 14.9, 2.5 Hz), 2.38 (1H, m), 2.29 (3H, m), 2.17 (2H, m), 1.94 (1H, m), 1.83 (1H, m), 1.48 (1H, m), 1.37 (2H, m), 1.16 (2H, m); δ_C 169.8, 165.8, 146.6, 142.0, 139.9, 130.5, 130.2, 129.76, 129.71, 127.4, 119.9, 77.2, 48.8, 39.0, 31.2, 29.8, 26.0, 24.0, 21.2, 18.2; HRMS (ESI) calculated for $C_{20}H_{24}INO_3Na$ $[M + Na]^+$ 476.06931, found 476.06950.

Aza-salicylihalamide analogue (3)

A degassed solution of iodoalkene **26** (34 mg, 0.075 mmol), Cs_2CO_3 (132 mg, 0.375 mmol), CuI (29 mg, 0.151 mmol), *N*,*N*′-dimethylethylenediamine (41 μL, 0.38 mmol) and dienamide **7**[4b] (49 mg, 0.38 mmol) in dry DMF (2 mL) was stirred under nitrogen at rt for 2 h. The reaction mixture was then diluted with water and the aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$ and concentrated. Flash chromatography with 40% EtOAc/hexane as eluent provided the *aza*-salicylihalamide analogue **3** (19.7 mg, 64%) as a colourless solid. mp 151–152 °C; R_f (55% EtOAc/hexane) 0.41; $[\alpha]_D^{23}$ −13.4 (*c* 0.21, CH_2Cl_2); IR ν_{max}: 3348, 2931, 2471, 2243, 2214, 2141, 2071, 1633, 1595, 1461, 1121, 1090, 972, 821 cm^{-1}; δ_H (d_4-methanol) 7.32 (1H, dt, J = 10.8, 1.2 Hz), 7.08 (1H, t, J = 7.9 Hz), 6.87 (1H, dd, J = 11.6, 1.1 Hz), 6.80 (1H, d, J = 14.4 Hz), 6.73 (1H, d, J = 7.9 Hz), 6.6 (1H, d, J = 7.7 Hz), 5.83 (1H, dddd, J = 10.8, 7.7, 7.5, 1.3 Hz), 5.70 (1H, d, J = 11.6 Hz), 5.41 (2H, m), 5.26 (1H, m), 3.97 (1H, m), 3.76 (1H, dd, J = 14.3, 4.1 Hz), 3.13 (1H, br dd, J = 14.3, 2.3 Hz) 2.31 (2H, dquin, J = 7.5, 1.5 Hz), 2.25 (2H, m), 2.16 (1H, m), 1.69 (1H, m), 1.56 (2H, m), 1.42 (2H, m), 1.20 (2H, m), 1.03 (3H, t, J = 7.5 Hz); δ_C (d_4-methanol) 170.0, 165.9, 155.9, 142.6, 141.0, 137.7, 133.4, 131.0, 130.0, 126.3, 125.7, 125.5, 122.5, 120.6, 115.2, 112.1, 49.6, 39.3, 37.0, 33.9, 32.9, 26.3, 22.2, 21.6, 14.5;

HRMS (ESI) calculated for $C_{25}H_{32}N_2O_3Na$ $[M + Na]^+$ 431.23051, found 431.23053.

Biological assays

Serial dilutions of compound **3** were incubated with leukemia cell lines for 72 h and proliferation was assessed using the Cell Titre Glo assay (Promega). Data was analysed using nonlinear regression algorithms in Prism Graphpad software to calculate IC_{50} values. Data shown represents the mean $IC_{50} \pm$ SEM of n = 6 (*aza*-salicylihalamide analogue) and n = 3 (bafilomycin A1) independent experiments.

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