Conformational analogues of Oxamflatin as histone deacetylase inhibitors

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Conformational analogues of the hydroxamic acid Oxamflatin **1**—compounds **3a**, **3b** and **4**—have been synthesised to enable evaluation of the impact of varying the linking section on histone deacetylase inhibition. Preliminary testing indicates treatment of leukaemia cells with each of the analogues leads to significant inhibition of histone deacetylase and reduction in cell growth and proliferation.

Introduction

Histone deacetylase (HDAC) catalyses the deacetylation of ε-*N*-acetylated lysine groups on histones. Consequently, inhibition of the enzyme leads to the accumulation of acetylated histones, which is believed to result in increased gene expression associated with cell differentiation, growth arrest, and apoptosis.[1] As such, HDAC inhibitors have emerged as promising candidates for cancer therapies.

Small molecule hydroxamic acids, such as Oxamflatin **1**,[2] constitute a well-known class of HDAC inhibitors.[3] In general, potent HDAC inhibitors consist of a hydroxamic acid group, believed to be required for binding to the zinc active site of the HDAC enzyme, a six carbon linker, and a hydrophobic group (often aromatic) for surface-recognition.[3]

Recent studies within our groups have shown the methyl sulfonamide analogue of Oxamflatin, Metacept-1 **2**, is also a potent inhibitor of histone deacetylase at concentrations in the nanomolar range.[4] Based on this success, we have extended our investigations to an evaluation of the nature of the linking moiety of the compound, designing three analogues of Metacept-1, compounds **3a**, **3b** and **4** (Fig. 1). These linkers display varying degrees of conformational flexibility whilst maintaining similar separation of the hydroxamic acid and hydrophobic groups.

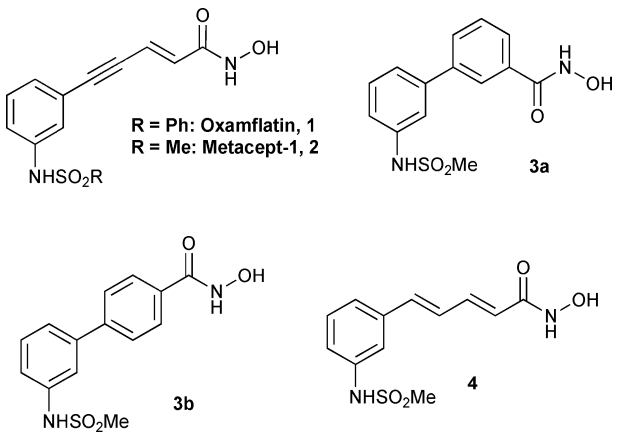
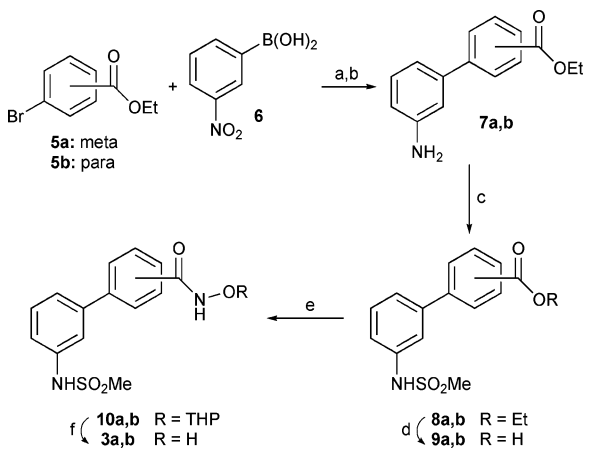


Fig. 1 Oxamflatin (**1**), Metacept-1 (**2**) and analogues of Metacept-1 (**3a**, **3b** and **4**).

Results and discussion

Synthesis of biphenyl analogues 3a and 3b

The synthesis shown in Scheme 1 was used for target compounds **3a** and **3b**. The biphenyl moiety was first constructed using a heterogeneous palladium-catalysed Suzuki coupling between 3-nitrophenylboronic acid **6** and the appropriate bromobenzoate **5a** or **5b**. In an attempt to execute a one-pot coupling–reduction procedure, the reaction mixture from the Suzuki coupling was cooled, diluted with ethyl acetate and placed under an atmosphere of hydrogen.[5] However, this often did not go to completion and addition of fresh palladium catalyst was required. The amines **7a** or **7b** were initially purified by flash chromatography before sulfonylation but it was later found that this was not necessary.



Scheme 1 *Reagents and conditions*: (a) 10% Pd/C, Na_2CO_3, EtOH, reflux, o/n; (b) H_2, 10% Pd/C, EtOH–EtOAc, rt, o/n to 48 h; (c) $MeSO_2Cl$, pyridine, CH_2Cl_2, rt, o/n; (d) NaOH (aq.), MeOH, rt, 2 h then HCl; (e) HOBt, EDC·HCl, DMF, rt, 20 to 30 min then NH_2OTHP, 50 °C, o/n; (f) HCl (aq.), MeCN–MeOH, rt, 3.5 to 4.5 h.

Sulfonylation of the amines proceeded smoothly in the presence of methanesulfonyl chloride and pyridine. Again, the sulfonamides **8a** or **8b** were initially purified by flash chromatography, but it was later found that the highly crystalline product was readily purified through recrystallisation from ethyl acetate–hexanes.

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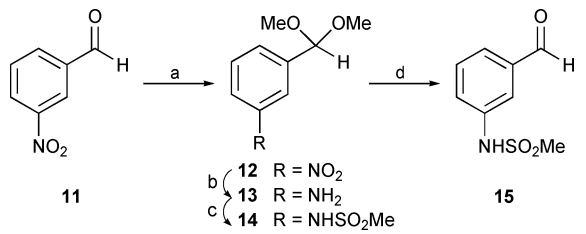
Basic hydrolysis of the esters **8a** or **8b** then yielded the carboxylic acids **9a** or **9b** which were carried through as crude material.

Conversion of the carboxylic acids **9a** or **9b** to the hydroxamic acids **3a** or **3b** was attempted using numerous methods, such as *via* the acid chloride,[6] the benzyl hydroxamate,[7] and through coupling hydroxylamine hydrochloride to the carboxylic acid.[8] The most successful of these methods involved synthesis of the THP-protected hydroxamates **10a** or **10b** under peptide coupling conditions;[9] the coupling step proceeded smoothly and in good yield and the subsequent, acid-mediated deprotection gave, directly, microanalytically pure hydroxamic acids **3a** or **3b**. Yields over six steps were 62% for compound **3a** and 48% for compound **3b**.

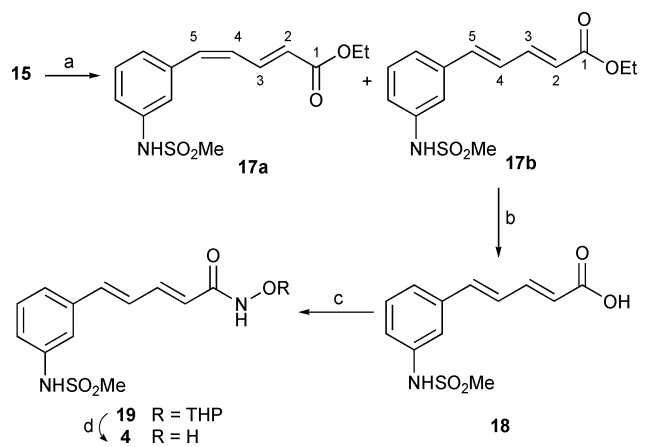
Synthesis of diene analogue 4

Target compound **4** was synthesised using an adaptation of Ohtani's procedure.[6] The aldehyde **15** was first synthesised in four steps from commercially available 3-nitrobenzaldehyde **11** in an overall yield of 63% (Scheme 2).[10,11]



Scheme 2 *Reagents and conditions*: (a) $(H_3CO)_3CH$, HCl, MeOH, rt, o/n; (b) H_2, 10% Pd/C, EtOAc, rt, o/n; (c) $MeSO_2Cl$, pyridine, CH_2Cl_2, rt, o/n; (d) Amberlyst-15, H_2O, acetone, rt, o/n.

Aldehyde **15** was then coupled to the phosphonium salt using a Wittig reaction (Scheme 3). This gave the diene as a 45 : 55 mixture of 4(*Z*) and 4(*E*) isomers **17a** and **17b**. Flash chromatography followed by recrystallisation from ethyl acetate–hexanes gave pure



Scheme 3 *Reagents and conditions*: (a) $[Ph_3PCH_2CH{=}CHCO_2Et]Br$ **16**, KO^{t}Bu, THF, 0 °C to rt, 1 h then aldehyde **15**, 0 °C to rt, 5 h; (b) NaOH (aq.), MeOH, rt, 1 h then HCl; (c) HOBt, EDC·HCl, DMF, rt, 45 min then NH_2OTHP, 50 °C, o/n; (d) HCl (aq), MeCN–MeOH, rt, 6 h.

17b and partially purified **17a**.† Assignment of the isomers was based on comparison of the coupling constants for the ^{1}H NMR spectrum of partially purified 4(*Z*) isomer **17a** ($J_{2,3} = 15.3$, $J_{3,4} = 11.6$ and $J_{4,5} = 11.6$ Hz) to those reported for similar compounds in the literature.[12,13] Overlapping resonances for H4 and H5 prevented determination of $J_{4,5}$ for the 4(*E*) isomer **17b**.

The synthesis then proceeded as for compounds **3a** and **3b**. Basic hydrolysis of ester **17b** gave the carboxylic acid **18** which was then treated with THP-hydroxylamine under peptide coupling conditions to yield the THP-hydroxamate **19**. Acid-mediated deprotection then gave the hydroxamic acid **4** in an overall yield of 11% over eight steps (17% from the aldehyde **15**).

Biological testing

Treatment of HL-60 leukaemia cells with compounds **3a**, **3b** or **4** (Fig. 2) resulted in increased expression of acetylated histone H3 at micromolar concentrations in a dose-dependent manner. Oxamflatin **1** and Metacept-1 **2** were included for comparison. The level of inhibition appeared to be similar across the three compounds **3a**, **3b** and **4** and comparable to that observed for Metacept-1.

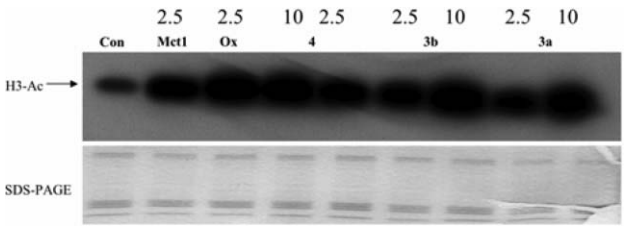


Fig. 2 HL-60 cells were treated with Oxamflatin (Ox), Metacept-1 (Mct1), **3a**, **3b**, **4** for 16 hours at the concentrations shown (all concentrations in μM). Cells were harvested and the histones extracted and separated by SDS-PAGE. Western blot analysis was performed using antibodies for acetylated histone H3. Coomassie blue staining of the SDS gel was performed to determine lane loading.

A cell growth inhibition assay performed on HL-60 cells indicated that treatment with compounds **3a**, **3b** or **4** (Fig. 3) led

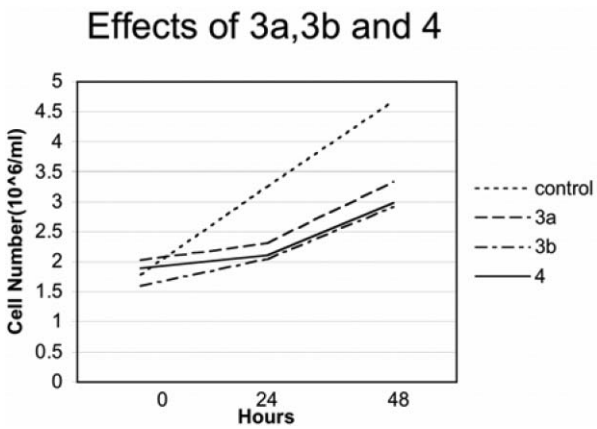


Fig. 3 HL-60 cells were treated with compounds **3a**, **3b**, **4** at concentrations of 2.5 μM. Cell viability was assessed using trypan blue staining.

† Obtained together with contaminants that did not include the 2(*E*),4(*E*) isomer as an inseparable mixture.

to a significant reduction in cell growth. The extent of inhibition is comparable across the analogues, suggesting that the varying conformations of their linkers do not impact significantly on their potencies.

The evidence of decreased cell growth and proliferation in conjunction with evidence of HDAC inhibition, supports the hypothesis that small molecule hydroxamic acids such as compounds **3a**, **3b**, and **4** mediate the reduction of cell growth and proliferation by inhibiting the histone deacetylase enzyme.

Conclusions

Three conformational analogues of Oxamflatin, **3a**, **3b** and **4**, have been successfully synthesised. The synthetic strategy employed for analogues **3a** and **3b** is both high-yielding and scaleable, and, as such, should prove useful for the synthesis of further derivatives of these compounds. Initial biological testing indicates that the analogues all inhibit HDAC at levels comparable to Metacept-1, while also inhibiting cell growth. The results also suggest that the differing linker conformations of analogues **3a**, **3b** and **4** do not have a significant impact on biological activity. Further testing to more accurately assess the differences in the potencies of the analogues is currently underway.

Experimental

Chemistry

Proton NMR (^{1}H NMR) were recorded at 300 MHz on a Bruker AM 300 spectrometer or at 400 MHz on a Bruker Advance DRX 400 spectrometer. Chemical shifts were recorded on the δ scale in parts per million (ppm). Spectra were recorded in $CDCl_3$ using residual $CHCl_3$ (7.26 ppm) as an internal reference, or in DMSO-d_6 using residual DMSO (2.54 ppm) as an internal reference. Carbon NMR (^{13}C NMR) were recorded at 75 MHz on a Bruker AM 300 spectrometer or at 100 MHz on a Bruker Advance DRX 400 spectrometer. Spectra were recorded in $CDCl_3$ using $CDCl_3$ (77.2 ppm) as an internal reference, or in DMSO-d_6 using DMSO-d_6 (39.5 ppm) as an internal reference. COSY, HSQC and HMBC spectra were used to aid assignment of some NMR spectra. Melting points were recorded on an Electrothermal melting point apparatus. IR spectra were recorded on a Perkin Elmer 1600 series Fourier Transform spectrometer as nujol mulls, $CDCl_3$ solutions, or neat films. Mass spectra (ESI) were recorded on a Micromass Platform QMS spectrometer. High resolution mass spectra (HRMS) were recorded on a Bruker BioApex 47e FTMS. Elemental microanalyses were performed by the University of Otago, Dunedin, New Zealand. Silica gel used for flash chromatography was 40–63 μm (230–400 mesh) silica gel 60 (Merck no. 9385). Analytical thin layer chromatography (TLC) was performed with Merck TLC aluminium sheets coated with silica gel containing F_{254} fluorescent indicator and visualised under UV light. Dichloromethane was distilled from P_2O_5 and tetrahydrofuran (THF) distilled from sodium–benzophenone ketal prior to use. Dimethylformamide (DMF) was dried over 4 Å molecular sieves. 3-Nitrophenylboronic acid was purchased from Boron Molecular, all other reagents were purchased from the Aldrich Chemical Company.

3′-Amino-biphenyl-3-carboxylic acid ethyl ester 7a. To a solution of 3-nitrophenylboronic acid **6** (3.0 g, 18 mmol) and ethyl 3-bromobenzoate **5a** (3.1 mL, 19 mmol) in 18 mL ethanol was added sodium carbonate (2.2 g, 20 mmol) and palladium on charcoal (960 mg, 10% wt Pd, 0.90 mmol) at room temperature and under an atmosphere of nitrogen. The resulting suspension was stirred and heated at reflux for 28 hours before being cooled to room temperature, diluted with 35 mL ethyl acetate and placed under an atmosphere of hydrogen. After stirring under hydrogen overnight, the suspension was filtered through a pad of Celite® before being diluted with 25 mL water. The aqueous layer was then separated and the organic layer washed twice with water, dried ($MgSO_4$), filtered, and the filtrate concentrated *in vacuo* to yield a yellow oil. Analysis of the ^{1}H NMR spectrum revealed incomplete hydrogenation, therefore the oil was dissolved in a mixture of 18 mL ethanol and 35 mL ethyl acetate, palladium on charcoal (960 mg, 10% wt Pd, 0.90 mmol) once again added, and the resulting suspension placed under an atmosphere of hydrogen. After stirring under hydrogen overnight, the suspension was worked up as above to yield a yellow oil. Flash chromatography (25% ethyl acetate–hexanes) yielded the title compound **7a** (3.4 g, 78%) as a pale yellow oil.

IR (neat film) ν = 3463m, 3373m, 2981m, 1714s cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.41 (t, J = 7.1 Hz, 3H, ethyl CH_3), 4.41 (q, J = 7.1 Hz, 2H, ethyl CH_2), 6.71 (ddd, J = 8.0, 2.3, 1.0 Hz, 1H, H4′), 6.94 (m, 1H, H2′), 7.02 (ddd, J = 7.7, 1.7, 1.0 Hz, 1H, H6′), 7.25 (apparent t, J = 7.9 Hz, 1H, H5′), 7.48 (td, J = 7.8, 0.4 Hz, 1H, H5), 7.75 (ddd, J = 7.8, 1.9, 1.3 Hz, 1H, H4), 8.01 (dt, J = 7.8, 1.3 Hz, 1H, H6), 8.25 (m, 1H, H2). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 14.6 (ethyl CH_3), 61.2 (ethyl CH_2), 114.1 (C2′), 114.7 (C4′), 117.8 (C6′), 128.4 (C2), 128.5 (C4), 128.9 (C5), 130.0 (C5′), 131.2 (C3), 131.6 (C6), 141.6 (C1′), 141.8 (C1) 147.1 (C3′), 168.9 (C=O). ESI-MS m/z 296.1 [M + Na^+ + MeOH], 274.1 [M + H^+ + MeOH], 264.1 [M + Na^+], 242.1 [M + H^+]. HRMS Calc. for $[C_{15}H_{16}NO_2]^+$ m/z 242.1181. Found 242.1174. Microanalysis Calc. for $C_{15}H_{15}NO_2$: C 74.67, H 6.27, N 5.81. Found: C 74.48, H 6.38, N 5.69%.

3′-Methanesulfonylamino-biphenyl-3-carboxylic acid ethyl ester 8a. To a solution of the aromatic amine **7a** (3.4 g, 14 mmol) in 50 mL dichloromethane was added pyridine (2.4 mL, 31 mmol) followed by methanesulfonyl chloride (2.3 mL, 31 mmol) at room temperature and under an atmosphere of argon. The resulting orange solution was stirred overnight after which time it was washed with 60 mL each of water, 1 M aqueous HCl, and water. The organic phase was then dried ($MgSO_4$), filtered, and the filtrate concentrated *in vacuo* to yield an orange solid. Flash chromatography (30% ethyl acetate–hexanes) yielded the title compound **8a** (4.0 g, 90%) as a white solid.

Mp 124–125 °C. IR (nujol mull) ν = 3246m, 1709s cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.41 (t, J = 7.1 Hz, 3H, ethyl CH_3), 3.07 (s, 3H, SO_2CH_3), 4.41 (q, J = 7.1 Hz, 2H, ethyl CH_2), 7.23 (s, 1H, NH), 7.27–7.50 (m, 5H, H5, H2′, H4′, H5′ and H6′), 7.75 (ddd, J = 7.8, 1.9, 1.2 Hz, 1H, H6), 8.04 (dt, J = 7.8, 1.4 Hz, 1H, H4), 8.25 (m, 1H, H2). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 14.5 (ethyl CH_3), 39.6 (SO_2CH_3), 61.4 (ethyl CH_2), 119.7, 120.0, 124.3, 138.4 (C2), 129.0 (C4), 129.2, 130.4, 131.3, 131.7, 137.7, 140.6, 142.1 (C1), 166.8 (C=O). ESI-MS m/z 374.2 [M + Na^+ + MeOH],

342.1 [M + Na^+]. HRMS Calc. for $[C_{16}H_{18}NO_4S]^+$ *m/z* 320.0957. Found 320.0956.

3′-Methanesulfonylamino-biphenyl-3-carboxylic acid 9a. To a suspension of the ester **8a** (4.0 g, 13 mmol) in 40 mL methanol was added sodium hydroxide (48 mL, 1 M aqueous solution, 48 mmol). The resulting solution was stirred at room temperature for 2 hours after which time it was acidified with 1 M aqueous HCl. The resulting white suspension was then dissolved in ethyl acetate, the organic phase separated, and the aqueous phase extracted with ethyl acetate (2 × 150 mL). The organic extracts were then combined, dried ($MgSO_4$), filtered, and the filtrate concentrated *in vacuo* to yield the title compound **9a** (3.5 g 96%) as a white solid. The crude material was used without further purification.

Mp 191–194 °C. IR (nujol mull) ν = 3255m, 1689s cm^{-1}. ^1H NMR (300 MHz, DMSO) δ = 3.08 (s, 3H, SO_2CH_3), 7.31 (m, 1H), 7.51 (m, 3H), 7.65 (m, 1H, H5), 7.91 (d, J = 7.0 Hz, 1H, H6), 8.00 (d, J = 7.8 Hz, 1H, H4), 8.19 (s, 1H, H2), 9.86 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO) δ = 39.4 (SO_2CH_3), 118.0, 119.1, 122.3, 127.3 (C2), 128.6 (C4), 129.4 (C5), 130.1, 131.0 (C6), 131.6, 139.1, 140.1, 140.4, 167.2 (C=O). ESI-MS *m/z* 314.0 [M + Na]$^+$, 346.1 [M + MeOH + Na]$^+$. HRMS Calc. for $[C_{14}H_{13}NO_4SNa]^+$ *m/z* 314.0463. Found 314.0461.

3′-Methanesulfonylamino-biphenyl-3-hydroxamic acid tetrahydro-2*H*-pyran-2-yl ester 10a. To a solution of carboxylic acid **9a** (1.0 g, 3.4 mmol) in 40 mL of DMF was added HOBt (700 mg, 5.2 mmol) and EDC·HCl (800 mg, 4.2 mmol) under an atmosphere of nitrogen. The resulting solution was stirred at room temperature for 20 minutes before *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (600 mg, 5.1 mmol) was added. The solution was then heated at 50 °C for 22 hours before being cooled to room temperature and diluted with 250 mL water. The solution was extracted with dichloromethane (3 × 100 mL) and the organic extracts combined, washed with water, saturated aqueous $NaHCO_3$ and then water, dried (Na_2SO_4), filtered, and the filtrate concentrated *in vacuo* to yield a yellow liquid. Flash chromatography (80% ethyl acetate–hexanes) yielded the title compound **10a** (1.3 g, 97%) as a sticky, white foam.

IR (nujol mull) ν = 3184w, 1651s cm^{-1}. ^1H NMR (300 MHz, DMSO) δ = 1.69 (m, 6H, pyran H3, pyran H4 and pyran H5), 3.07 (s, 3H, SO_2CH_3), 3.58 (m, 1H, pyran H6), 4.08 (m, 1H, pyran H6), 5.07 (s, 1H, pyran H2), 7.30 (m, 1H), 7.50 (m, 3H), 7.62 (t, J = 7.8 Hz, 1H, H5), 7.82 (dm, J = 7.8 Hz, 2H, H4 and H6), 8.03 (m, 1H, H2). ^{13}C NMR (75 MHz, DMSO) δ = 18.3 and 25.0 and 27.9 (pyran C3, pyran C4 and pyran C5), 39.3 (SO_2CH_3), 61.4 (pyran C6), 101.1 (pyran C2), 118.2, 119.2, 122.5, 125.4 (C2), 126.5 (C4), 129.2 (C5), 129.7 (C6), 130.0, 133.0, 139.1, 139.9, 140.6, 164.0 (C=O). HRMS Calc. for $[C_{19}H_{22}N_2O_5SNa]^+$ *m/z* 413.1147. Found *m/z* 413.1136.

3′-Methanesulfonylamino-biphenyl-3-hydroxamic acid 3a. To a solution of protected hydroxamate **10a** (1.2 g, 3.1 mmol) in 50 mL of a 1 : 1 mixture of acetonitrile and methanol was added HCl (1.0 M aqueous solution, 6.9 mL, 6.9 mmol) at room temperature. After stirring for 3.5 hours the solution was concentrated *in vacuo* to yield the title compound **3a** (900 mg, 95%) as a white solid.

Mp 174–175 °C. IR (nujol mull) ν = 3242m, 3118w, 1625s cm^{-1}. ^1H NMR (300 MHz, DMSO) δ = 3.08 (s, 3H, SO_2CH_3), 7.31 (m, 1H), 7.52 (m, 3H), 7.60 (t, J = 7.8 Hz, 1H, H5), 7.81 (m, 2H, H4 and H6), 8.04 (t, J = 1.6 Hz, 1H, H2), 9.13 (br s, 1H, OH), 9.88 (s, 1H, SO_2NH), 11.38 (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO) δ = 39.4 (SO_2CH_3), 118.2, 119.2, 122.5, 125.2 (C2), 126.2 (C6), 129.2 (C5), 129.3 (C4), 130.0, 133.5, 139.1, 139.9, 140.7, 164.0 (C=O). ESI-MS *m/z* 307.1 [M + H]$^+$, 329.0 [M + Na]$^+$, 339.1 [M + MeOH + H]$^+$. HRMS Calc. for $[C_{14}H_{14}N_2O_4SNa]^+$ *m/z* 329.0572. Found 329.0570. Microanalysis Calc. for $C_{14}H_{14}N_2O_4S$: C 54.89, H 4.61, N 9.14. Found C 54.96, H 4.73, N 9.11%.

3′-Amino-biphenyl-4-carboxylic acid ethyl ester 7b. To a solution of 3-nitrophenylboronic acid **6** (1.0 g, 6.0 mmol) and ethyl 4-bromobenzoate **5b** (1.0 mL, 6.1 mmol) in 6.0 mL ethanol was added sodium carbonate (730 mg, 6.9 mmol) and palladium on charcoal (230 mg, 10% wt Pd, 0.30 mmol) at room temperature and under an atmosphere of nitrogen. The resulting suspension was stirred and heated at reflux for 22 hours before being cooled to room temperature, filtered and concentrated *in vacuo* to yield a white solid. The solid was then taken up in 18 mL of ethyl acetate, fresh palladium on charcoal added (320 mg, 10% wt Pd, 0.30 mmol), and the resulting suspension placed under an atmosphere of hydrogen. After stirring under hydrogen overnight, the suspension was diluted with ethanol before being filtered through a pad of Celite®. The filtrate was then washed three times with water, dried ($MgSO_4$), filtered, and the filtrate concentrated *in vacuo* to yield the crude title compound **7b** a yellow solid (1.4 g, >90% conversion). The crude material was used without further purification.

^1H NMR (300 MHz, $CDCl_3$) δ = 1.41 (t, J = 7.1 Hz, 3H, ethyl CH_3), 3.78 (broad s, 2H, NH_2), 4.40 (q, J = 7.1 Hz, 2H, ethyl CH_2), 6.72 (dm, J = 8.0 Hz, 1H), 6.98 (m, 2H), 7.25 (m, 1H), 7.62 (d, J = 8.2 Hz, 2H, H2 and H6), 8.09 (d, J = 8.2 Hz, 2H, H3 and H5).

3′-Methanesulfonylamino-biphenyl-4-carboxylic acid ethyl ester 8b. To a solution of aromatic amine **7b** (1.2 g, 5.0 mmol) in 50 mL dichloromethane was added pyridine (0.80 mL, 11 mmol) followed by methanesulfonyl chloride (0.85 mL, 11 mmol) at room temperature and under an atmosphere of argon. The resulting orange solution was stirred overnight after which time it was washed with 20 mL each of water, 1 M aqueous HCl, and water. The organic phase was then dried ($MgSO_4$), filtered, and the filtrate concentrated *in vacuo* to yield an orange solid. Recrystallisation (ethyl acetate–hexanes) afforded the title compound **8b** (1.1g, 67% over three steps) as a pink, crystalline solid.

Mp 103–104 °C. IR (nujol mull) ν = 3250m, 1698s cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$) δ = 1.40 (t, J = 7.1 Hz, 3H, ethyl CH_3), 3.03 (s, 3H, SO_2CH_3), 4.39 (q, J = 7.1 Hz, 2H, ethyl CH_2), 7.26 (m, 1H), 7.42 (m, 3H), 7.58 (d, J = 8.5 Hz, 2H, H2 and H6), 8.07 (d, J = 8.5 Hz, 2H, H3 and H5). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 14.5 (ethyl CH_3), 39.6 (SO_2CH_3), 61.4 (ethyl CH_2), 119.7, 120.3, 124.4, 127.2 (C2 and C6), 129.9, 130.3 (C3 and C5), 130.4, 137.8, 141.9, 144.6, 166.7 (C=O). ESI-MS *m/z* 320.2 [M + H]$^+$, 352.3 [M + MeOH + H]$^+$, 374.2 [M + MeOH + Na]$^+$. HRMS Calc. for $[C_{16}H_{17}NO_4SNa]^+$ *m/z* 342.0776. Found 342.0776.

3′-Methanesulfonylamino-biphenyl-4-carboxylic acid 9b. To a suspension of ester **8b** (820 mg, 2.6 mmol) in 8.0 mL methanol was added sodium hydroxide (1.0 M aqueous solution, 10 mL, 10 mmol). The yellow solution was stirred at room temperature for 2 hours before being acidified with 1 M aqueous HCl. The resulting

white suspension was then dissolved in 50 mL ethyl acetate, the organic phase separated, and the aqueous phase extracted with ethyl acetate (2 × 50 mL). The organic extracts were then combined, dried ($MgSO_4$), filtered, and the filtrate concentrated *in vacuo* to yield the title compound **9b** (660 mg, 87%) as a white solid. The crude material was used without further purification.

Mp 159–161 °C. IR (nujol mull) $\nu = 3254$m, 1686s cm^{-1}.

^{1}H NMR (300 MHz, DMSO) $\delta = 3.08$ (s, 3H, SO_2CH_3), 7.31 (m, 1H), 7.52 (m, 3H), 7.78 (dm, $J = 8.6$ Hz, 2H, H2 and H6), 8.07 (dm, $J = 8.6$ Hz, 2H, H3 and H5). ^{13}C NMR (75 MHz, DMSO) $\delta = 39.4$ (SO_2CH_3), 118.1, 119.5, 122.5, 126.8 (C2 and C6), 130.0, 130.1 (C3, C5 and phenyl CH), 139.1, 140.3, 143.9, 167.1 (C=O). HRMS Calc. for $[C_{14}H_{13}NO_4SNa]^+$ m/z 314.0463. Found 314.0454.

3′-Methanesulfonylamino-biphenyl-4-hydroxamic acid tetrahydro-2*H*-pyran-2-yl ester 10b. To carboxylic acid **9b** (380 mg, 1.3 mmol), HOBt (265 mg, 2.0 mmol) and EDC·HCl (310 mg, 1.6 mmol) was added 20 mL DMF under an atmosphere of nitrogen. The resulting solution was stirred at room temperature for 30 minutes before *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (230 mg, 2.0 mmol) was added. The solution was then heated at 50 °C for 21 hours before being cooled to room temperature and diluted with 90 mL water. The solution was extracted with dichloromethane (3 × 50 mL) and the organic extracts combined, washed with water, saturated aqueous $NaHCO_3$ and then water, dried (Na_2SO_4), filtered, and the filtrate concentrated *in vacuo* to yield a yellow oil. Flash chromatography (75% ethyl acetate–hexanes) yielded the title compound **10b** (470 mg, 93%) as a white solid.

Mp 203–204 °C. IR (nujol mull) $\nu = 3326$m, 3201m, 1658s cm^{-1}. ^{1}H NMR (300 MHz, DMSO) $\delta = 1.69$ (m, 6H, pyran H3, pyran H4 and pyran H5), 3.08 (s, 3H, SO_2CH_3), 3.58 (m, 1H, pyran H6), 4.11 (m, 1H, pyran H6), 5.06 (s, 1H, pyran H2), 7.30 (m, 1H), 7.51 (m, 3H), 7.70 (dm, $J = 8.6$ Hz, 2H, H2 and H6), 7.92 (dm, $J = 8.6$ Hz, 2H, H3 and H5). ^{13}C NMR (75 MHz, DMSO) $\delta =$ 18.3 and 24.7 and 27.9 (pyran C3, pyran C4 and pyran C5), 39.4 (SO_2CH_3), 61.4 (pyran C6), 101.0 (pyran C2), 118.1, 119.3, 122.4, 126.6 (C2 and C6), 127.9 (C3 and C5), 130.0, 131.4, 139.1, 140.3, 142.7, 163.9 (C=O). ESI-MS m/z 391.2 $[M + H]^+$, 413.2 $[M + Na]^+$. HRMS Calc. for $[C_{19}H_{22}N_2O_5SNa]^+$ m/z 413.1147. Found 413.1142.

3′-Methanesulfonylamino-biphenyl-4-hydroxamic acid 3b. To a solution of protected hydroxamate **10b** (410 mg, 1.0 mmol) in 100 mL of a 1 : 1 mixture of acetonitrile and methanol was added HCl (1.0 M aqueous solution, 2.4 mL, 2.4 mmol) at room temperature. After stirring for 2.5 hours the solution was concentrated *in vacuo* to yield a white solid (310 mg). Analysis of the ^{1}H NMR spectrum revealed the reaction was incomplete so a portion of the crude material (240 mg) was re-dissolved in 50 mL of a 1 : 1 mixture of acetonitrile and methanol and HCl (1.0 M aqueous solution, 2.0 mL, 2.0 mmol) was once again added. After stirring for a further 2 hours the solution was concentrated *in vacuo* to yield the title compound **3b** (210 mg, 88% extrapolated yield) as a white solid.

Mp 166–168 °C. IR (nujol mull) $\nu = 3296$m, 3220w, 1641 cm^{-1}. ^{1}H NMR (300 MHz, DMSO) $\delta = 3.08$ (s, 3H, SO_2CH_3), 7.29 (m, 1H), 7.49 (m, 3H), 7.73 (d, $J = 8.4$ Hz, 2H, H2 and H6), 7.90 (d, $J = 8.4$ Hz, 2H, H3 and H5), 9.07 (s, 1H, OH), 9.87 (s, 1H, SO_2NH), 11.29 (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO) $\delta = 39.4$ (SO_2CH_3), 118.0, 119.3, 122.4, 126.6 (C2 and C6), 127.6 (C3 and C5), 130.0, 131.9, 139.1, 140.4, 142.3, 163.9 (C=O). ESI-MS m/z 307.0 $[M + H]^+$. HRMS Calc. for $[C_{14}H_{15}N_2O_4S]^+$ m/z 307.0753. Found 307.0748. Microanalysis Calc. for $C_{14}H_{14}N_2O_4S$: C 54.89, H 4.61, N 9.14. Found C 54.49, H 4.72, N 9.02%.

3-Nitrobenzaldehyde dimethyl acetal 12. To a solution of 3-nitrobenzaldehyde **11** (3.0 g, 20 mmol) in 30 mL methanol was added trimethylorthoformate (2.4 mL, 22 mmol), and concentrated HCl (6 drops, catalytic), at room temperature and under an atmosphere of nitrogen. The yellow solution was stirred at room temperature overnight before being neutralised with potassium carbonate, diluted with hexanes, and filtered. The filtrate was then concentrated *in vacuo* to yield the crude title compound **12** a cloudy yellow liquid.

^{1}H NMR (300 MHz, $CDCl_3$)[14] $\delta = 3.33$ (s, 6H, OCH_3), 5.46 (s, 1H, PhCH), 7.53 (t, $J = 7.8$ Hz, H5), 7.77 (dm, $J = 7.8$ Hz, H6), 8.16 (m, 1H, H4), 8.31 (m, 1H, H2).

3-Aminobenzaldehyde dimethyl acetal 13. To a solution of nitro compound **12** (4.0 g crude, approx. 20 mmol) in 50 mL ethyl acetate was added palladium on charcoal (10%, 1.0 g, 0.95 mmol). The suspension was stirred under an atmosphere of hydrogen for 22 hours before being filtered through Celite®. The filtrate was then concentrated *in vacuo* to yield the crude title compound **13** as a pink liquid.

^{1}H NMR (300 MHz, $CDCl_3$)[14] $\delta = 3.32$ (s, 6H, OCH_3), 3.68 (brs, 2H, NH_2), 5.29 (s, 1H, PhCH), 6.63 (ddd, $J = 7.9$, 2.4, 0.8 Hz, 1H, H4), 6.78 (m, 1H, H2), 6.82 (dm, $J = 7.6$ Hz, 1H, H6), 7.13 (m, 1H, H5).

3-Methanesulfonylaminobenzaldehyde 15. To a solution of amine **13** (4.0 g crude, approx. 20 mmol) in 65 mL dichloromethane was added pyridine (3.2 mL, 40 mmol) followed by methanesulfonyl chloride (3.1 mL, 40 mmol) at room temperature and under an atmosphere of nitrogen. The yellow solution was stirred overnight before being washed with water, followed by dilute aqueous HCl and then water. The organic layer was dried ($MgSO_4$), filtered, and the filtrate concentrated *in vacuo* to yield crude 3-methanesulfonylaminobenzaldehyde dimethyl acetal **14**.

To a solution of acetal **14** (4.0 g, approx. 20 mmol) in 85 mL acetone was added Amberlyst-15 (930 mg) and a few drops of water. The suspension was stirred overnight after which time it was concentrated *in vacuo* to yield a pink solid. The solid was washed with dichloromethane and filtered to yield the title compound **15** (2.5 g, 63% over 4 steps) as a beige solid.

IR (nujol mull) $\nu = 3147$m, 1675s cm^{-1}. ^{1}H NMR (300 MHz, DMSO) $\delta = 3.09$ (s, 3H, SO_2CH_3), 7.55 (ddd, $J = 8.0$, 2.3, 1.5 Hz, 1H, H4), 7.61 (m, 1H, H5), 7.69 (dt, $J = 7.3$, 1.5 Hz, 1H, H6), 7.76 (m, 1H, H2), 10.01 (s, 1H, OCH), 10.11 (brs, 1H, NH). ^{13}C NMR (75 MHz, DMSO) $\delta = 39.5$ (SO_2CH_3), 118.8 (C2), 125.3 and 125.4 (C4 and C6), 130.2 (C5), 137.2 (C1), 139.3 (C3), 192.8 (C=O). ESI-MS m/z 198.0 $[M - H]^-$. HRMS Calc. for $[C_8H_8NO_3S]^-$ m/z 198.0230. Found 198.0219.

(*trans*-3-Ethoxycarbonylallyl)phosphonium bromide 16. To a solution of triphenylphosphine (16 g, 61 mmol) in 40 mL toluene was added ethyl 4-bromocrotonate (75%, 11 mL, 60 mmol). The resulting suspension was stirred at room temperature for 4 hours

before being filtered and the residue washed with acetonitrile to yield the title compound **16** (15 g, 54%) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$)[15] $\delta = 1.21$ (t, $J = 7.1$ Hz, 3H, ethyl CH_3), 4.10 (q, $J = 7.1$ Hz, 2H, ethyl CH_2), 5.27 (ddm, $J = 16.4$, 7.5 Hz, 2H, H1), 6.48 (dd, $J = 15.4$, 4.8 Hz, 1H, H3), 6.69 (m, 1H, H2), 7.64–7.71 (m, 6H, phenyl), 7.76–7.91 (m, 9H, phenyl).

(2*E*)(4*E*)-5-(3-Methanesulfonylaminophenyl)pentadienoic acid ethyl ester 17b. To a chilled suspension of (*trans*-3-ethoxycarbonylallyl)phosphonium bromide **16** (7.2 g, 16 mmol) in 100 mL THF was added potassium *tert*-butoxide (1.7 g, 15 mmol) under an atmosphere of nitrogen. The resulting orange suspension was stirred at room temperature for an hour before being re-cooled to 0 °C. Aldehyde **15** (1.3 g, 6.0 mmol) in 30 mL THF was then added and the suspension was stirred at 0 °C for 10 minutes before being allowed to warm to room temperature. After stirring at room temperature for 5 hours the mixture was diluted with 100 mL 1 M aqueous HCl and extracted with ethyl acetate (2 × 80 mL, 1 × 50 mL). The organic extracts were combined, washed with brine, dried ($MgSO_4$), filtered, and the filtrate concentrated *in vacuo* to yield an orange oil. Flash chromatography (40% ethyl acetate–hexanes) followed by recrystallisation (ethyl acetate–hexanes) yielded the title compound **17b** (630 mg, 36%) as a white solid together with the partially purified (2*E*)(4*Z*) isomer **17a** as an oil.

Mp 152–154 °C. IR (nujol mull) $\nu = 3275$m, 2854m, 1712s cm^{-1}. ^{1}H NMR (2*E*)(4*E*) isomer: (300 MHz, $CDCl_3$) $\delta = 1.32$ (t, $J = 7.1$ Hz, 3H, ethyl CH_3), 3.03 (s, 3H, SO_2CH_3), 4.24 (q, $J = 7.1$ Hz, 2H, ethyl CH_2), 6.02 (d, $J = 15.2$ Hz, 1H, H2), 6.87 (m, 2H, H4 and H5), 7.17 (m, 1H, phenyl), 7.28 (m, 1H, phenyl), 7.35 (m, 2H, phenyl), 7.42 (ddd, $J = 15.2$, 7.5, 2.8 Hz, 1H, H3). ^{1}H NMR partially purified (2*E*)(4*Z*) isomer: (300 MHz, $CDCl_3$) $\delta = 1.28$ (t, $J = 7.1$ Hz, 3H, ethyl CH_3), 3.06 (s, 3H, SO_2CH_3), 4.20 (q, $J = 7.1$ Hz, 2H, ethyl CH_2), 6.06 (dm, $J = 15.3$ Hz, 1H, H2), 6.39 (t, $J = 11.6$ Hz, 1H, H4), 6.77 (d, $J = 11.6$ Hz, 1H, H5), 7.09–7.38 (m, phenyl), 7.72 (dd, $J = 15.3$, 11.6 Hz, 1H, H3). ^{13}C NMR (75 MHz, DMSO) $\delta = 14.1$ (ethyl CH_3), 39.4 (SO_2CH_3), 59.8 (ethyl CH_2), 118.5 and 120.5 (phenyl CH), 121.4 (C2), 122.5 (phenyl CH) 126.9 (C4), 129.7 (phenyl CH) 136.9 and 139.0 (4° C) 140.0 (C5), 144.4 (C3), 166.1 (C1). ESI-MS m/z 294.2 $[M - H]^-$. HRMS Calc. for $[C_{14}H_{16}NO_4S]^-$ m/z 294.0800. Found 294.0798. Microanalysis Calc. for $C_{14}H_{17}NO_4S{\cdot}0.2H_2O$: C 56.25, H 5.87, N 4.69. Found C 56.21, H 5.80, N 4.65%.

(2*E*)(4*E*)-5-(3-Methanesulfonylaminophenyl)pentadienoic acid 18. To a suspension of ester **17b** (1.0 g, 3.4 mmol) in 11 mL methanol was added sodium hydroxide (13 mL, 1 M aqueous solution, 13 mmol). The resulting yellow solution was stirred at room temperature for 1 hour before being acidified with 1 M aqueous HCl. The resulting suspension was then extracted with ethyl acetate (3 × 50 mL) and the extracts combined, dried ($MgSO_4$), filtered, and the filtrate concentrated *in vacuo* to yield the crude title compound **18** (890 mg, 89%) as a pale yellow solid.

Mp 192–196 °C. IR (nujol mull) $\nu = 3265$m, 2854m, 1672s cm^{-1}. ^{1}H NMR (300 MHz, DMSO) $\delta = 3.05$ (s, 3H, SO_2CH_3), 6.08 (d, $J = 15.2$ Hz, 1H, H2), 7.07 (m, 2H, H4 and H5), 7.21 (m, 1H, phenyl), 7.38 (m, 4H, H3 and phenyl), 9.80 (brs, 1H, NH), 12.29 (brs, 1H, OH). ^{13}C NMR (75 MHz, DMSO) $\delta = 39.4$ (SO_2CH_3), 118.5 and 120.4 (phenyl CH), 122.4 and 122.7 (C2 and phenyl CH), 127.2 (C4), 129.8 (phenyl CH), 137.1 and 138.9 (4° C), 139.3 (C5), 144.0 (C3), 167.5 (C1). ESI-MS m/z 266.1 $[M - H]^-$. HRMS Calc. for $[C_{12}H_{12}NO_4S]^-$ m/z 266.0487. Found 266.0481.

(2*E*)(4*E*)-5-(3-Methanesulfonylaminophenyl) pentadienohydroxamic acid tetrahydro-2*H*-pyran-2-yl ester 19. To carboxylic acid **18** (660 mg, 2.5 mmol), HOBt (500 mg, 3.7 mmol) and EDC·HCl (570 mg, 3.0 mmol) was added 38 mL DMF under an atmosphere of nitrogen. The resulting solution was stirred at room temperature for 45 minutes before *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (230 mg, 2.0 mmol) was added. The solution was then heated at 50 °C for 24 hours before being cooled to room temperature and diluted with 180 mL water. The solution was extracted with dichloromethane (3 × 100 mL) and the organic extracts combined, washed with water, saturated aqueous $NaHCO_3$ and then water, dried (Na_2SO_4), filtered, and the filtrate concentrated *in vacuo* to yield a yellow oil. Flash chromatography (80% ethyl acetate–hexanes) yielded the title compound **19** (520 mg, 57%) as a white solid.

IR (nujol mull) $\nu = 3176$m, 1652m cm^{-1}. ^{1}H NMR (300 MHz, DMSO) $\delta = 1.57$–1.73 (m, 6H, pyran H3, pyran H4 and pyran H5), 3.04 (s, 3H, SO_2CH_3), 3.56 (m, 1H, pyran H6), 4.05 (m, 1H, pyran H6), 4.92 (s, 1H, pyran H2), 6.11 (d, $J = 14.7$ Hz, 1H, H2), 7.06 (m, 2H, H4 and H5), 7.20 (m, 1H, phenyl), 7.33 (m, 4H, H3 and phenyl). ^{13}C NMR (75 MHz, DMSO) $\delta = 18.3$ and 24.6 and 27.8 (pyran C3, pyran C4 and pyran C5), 39.3 (SO_2CH_3), 61.4 (pyran C6), 101.1 (pyran C2), 118.4 and 120.1 (phenyl CH), 122.3 (C2 and phenyl CH), 127.4 (C4), 129.7 (phenyl), 137.2 (4° C), 138.1 (C5), 138.8 (4° C), 139.7 (C3), 162.7 (C1). ESI-MS m/z 389.1 $[M + Na]^+$, 421.2 $[M + MeOH + Na]^+$. Microanalysis Calc. for $C_{17}H_{22}N_2O_5S{\cdot}H_2O$: C 53.11, H 6.29, N 7.29. Found C 53.12, H 6.30, N 6.98%.

(2*E*)(4*E*)-5-(3-Methanesulfonylaminophenyl) pentadienohydroxamic acid 4. To a solution of protected hydroxamate **19** (400 mg, 1.1 mmol) in 18 mL of a 1 : 1 mixture of acetonitrile and methanol was added HCl (1.0 M aqueous solution, 2.4 mL, 2.4 mmol) at room temperature. After stirring for 6 hours the solution was concentrated *in vacuo* to yield the title compound **4** (290 mg, 93%) as a brown foam.

IR (powder) $\nu = 1642$m cm^{-1}. ^{1}H NMR (400 MHz, DMSO) $\delta = 3.04$ (s, 3H, SO_2CH_3), 6.07 (d, $J = 15.0$ Hz, 1H, H2), 7.03 (m, 2H, H4 and H5), 7.19 (m, 1H, phenyl), 7.26 (dd, $J = 15.0$, 9.6 Hz, 1H, H3), 7.38 (m, 3H, phenyl), 9.78 (s, 1H, SO_2NH), 10.75 (brs, 1H, CONH). ^{13}C NMR (75 MHz, DMSO) $\delta = 39.4$ (SO_2CH_3), 118.3 and 120.0 and 122.3 (phenyl CH), 122.8 (C2), 127.6 (C4), 129.7 (phenyl CH), 137.3 (4° C), 137.4 (C5), 138.5 (C3), 138.8 (4° C), 162.8 (C1). ESI-MS m/z 283.3 $[M + H]^+$, 305.2 $[M + Na]^+$. HRMS Calc. for $[C_{12}H_{15}N_2O_4S]^+$ m/z 283.0753. Found 283.0748.

Biology. Western blot analysis

Histones were isolated by acid extraction. Cells (5×10^6) treated with or without agents (Oxamflatin **1**, Metacept-1 **2**, **3a**, **3b**, **4**) were harvested and washed with PBS. Cells were lysed in ice-cold lysis buffer [10 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, and 1.5 mM phenylmethylsulfonyl fluoride], and 5 M H_2SO_4 was added. After incubation on ice for 1 h, the suspension was centrifuged, and the supernatant was harvested, mixed with acetone at a ratio of 9 : 1, and incubated at −20 °C overnight. After centrifugation, the pellet was washed with 70%

ethanol, air dried, and the acid-soluble histone fraction dissolved in H_2O. A BCA protein assay was then used for quantitation (Pierce), and histones were electrophoresed through a 15% SDS-PAGE gel and transferred to PVDF membrane. Membranes were incubated with anti-acetylated histone H3 (Upstate Biotechnology), followed by horseradish peroxidase-conjugated secondary antibody. Immunoreactive bands were visualised by enhanced chemiluminescence.

Cell growth assay

HL-60 cells were cultured in RPMI1640 c(Gibco BRL) containing 10% heat inactivated foetal calf serum and kept in a 5% CO_2 incubator at 37 °C. Agents (**3a**, **3b**, **4**) were added to plates at concentrations of 2.5 μM. Cell viability was assessed using trypan blue staining. After culture, cells were harvested and stained with 0.4% trypan blue solution. Stained cells were counted immediately using conventional microscopy. Stained black cells were considered as non-viable cells, and unstained bright cells as viable.

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