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Full Paper

The Synthesis and Biological Evaluation of Anithiactin A/Thiasporine C and Analogues

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The synthesis of anithiactin A has been achieved in four steps. Several closely related analogues were synthesised and their biological activity against colon and breast cancer cell lines evaluated. Anithiactin A was found not to be cytotoxic even at a high concentration (100 μM); however, two 4-substituted phenyl thiazoles were found to be moderately cytotoxic at 10 μM. Based on these results, 4-substitution on the phenyl group appears to be critical for cytotoxicity. However, the exact electronic and structural requirements are unclear.

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Introduction

The structural diversity and vast biological activity of natural products is especially evident when examining clinically used drugs. Approximately 50 % of all clinically used drugs are either natural products, analogues thereof, or directly inspired by natural products.[1] Marine actinomycetes account for ~7000 of the natural compounds listed in the Dictionary of Natural Products. These actinomycetes are a rich source of bioactive secondary metabolites with a wide range of properties including antibiotic, antitumour, and immunosuppressive.[2]

Recently, anithiactin A (**1**) was isolated from *streptomyces* sp., which was found in a sample of intertidal mudflat sediment collected on the southern coast of the Korean peninsula.[3] At approximately the same time, the same molecule was isolated from the marine derived *actinomycetospora chlora* SNC-032 and named thiasporine C.[4] Anithiactin A (thiasporine C) contains a 2-phenylthiazole core which has previously been shown to be a useful scaffold in the design of compounds with anti-cancer properties.[5] In particular, methoxybenzoyl phenylthiazoles have been shown to possess low nanomolar activity with selectivity for both melanoma and prostate cancer cell lines (**2**, Fig. 1)[6] and preliminary investigations on their mode of action suggests they operate via inhibition of tubulin polymerisation.[6] While anithiactin A was found to possess moderate activity against acetylcholinesterase,[3,7] no significant biological activity was found against four non-small-cell lung cancer cell lines.[4] Notably, no data has been provided for their activity against other cancer types.

In light of previous work establishing the cytotoxicity of the 2-phenylthiazoles motif against cancer cells,[6] and with only one reported synthesis of anithiactin A,[3] we sought to develop a synthesis of anithiactin A which could also allow ready access to analogues. While the previous synthesis provided rapid access to anithiactin A, a potential limitation lies in the limited number of commercially available primary aryl amides. We believe greater pre-existing structural diversity could be found using commercially available aromatic carboxylic acids.

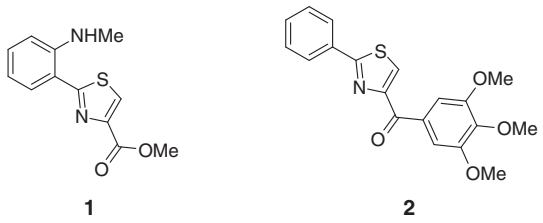


Fig. 1. Anithiactin A (**1**) and the potent cytotoxic thiazole **2**.

Our general approach to the key 2-phenyl thiazole motif is outlined in Fig. 2. Disconnection through the thiazole ring unveiled the amide **4** which could be further disconnected to cystine methyl ester (**6**) and benzoic acid (**5**) (Fig. 2). The advantage of using cystine is that the disulfide functional group provides protection of the reactive thiol functional group while maintaining complete atom economy. An additional benefit to this approach is the plethora of commercially available aromatic carboxylic acids which could provide rapid access to analogues for a medicinal chemistry program.

Results and Discussion

In a forward sense, the synthesis began with treatment of N-Boc anthranilic acid with sodium hydride followed by methyl

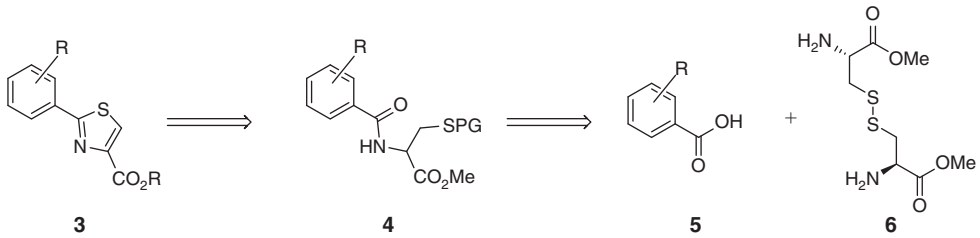
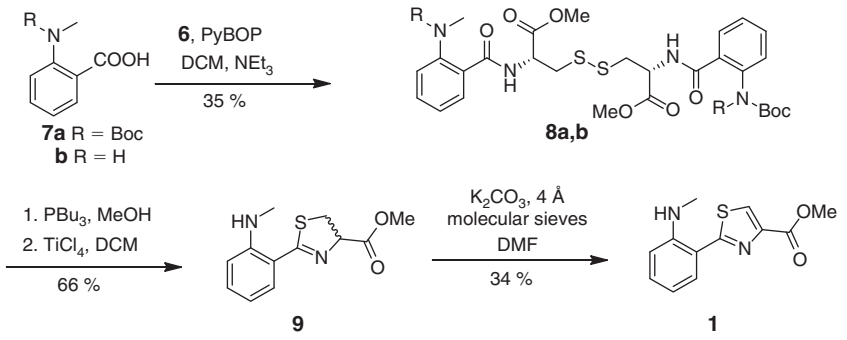


Fig. 2. General strategy to access 2-phenyl thiazoles.



Scheme 1.

iodide to provide the commercially available compound **7a** (Scheme 1). Treatment of the carboxylic acid with standard 1-ethyl-3-(3′-(dimethylamino)propyl)carbodiimide (EDCI)/1-hydroxybenzotriazole (HOBt) coupling protocols failed to deliver the amide **8a**; however, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) was found to afford the *bis*-amide **8a** in 35 % yield. Protection of the amine with the Boc group was found to be unnecessary with a similar yield obtained for the coupling of the free amine **7b** with cystine. Reduction of the disulfide bond by treatment with tributylphosphine in methanol,[8] provided the corresponding thiol, which was used in the subsequent reaction without further purification. Titanium(IV) mediated cyclodehydration of the thiol forged the thiazoline ring and concomitantly removed the Boc group to deliver **9** in 66 % yield.[9]† Subsequent oxidation under literature conditions provided anithiactin A (**1**) in 34 % yield, which was spectroscopically identical to that reported for the material isolated from the natural sources.[3,4]

With a synthetic procedure in place to access the 2-phenyl thiazole core, a small series of electronically diverse aromatic carboxylic acids (**5a**–**d**) were subjected to the reaction conditions outlined above with the following exception. The formation of amides **10a**–**d** was conducted using standard EDCI/HOBt coupling conditions as this cleanly provided the corresponding compounds without the need for chromatographic purification. Following this sequence the unsubstituted phenyl 2-thiazole **12a**, the electron poor *ortho* and *para*-nitrophenyl 2-thiazoles **12b** and **12c** and the electron rich 3,5-dimethoxyphenyl 2-thiazole **12d** were all obtained in moderate yield (Scheme 2). Iron mediated reduction of **12b** and **12c** provided the anilines **13b** and **13c**, respectively, in good yield (Scheme 3).[10]

Compounds **1**, **12a**, **b**, **d**, and **13b** were found to be non-toxic up to concentrations of 100 μM in the colon cancer cell line HT29, the breast cancer cell line MCF7, the triple negative breast cancer cell line MDA-MB-231, and the non-transformed mesenchymal stem cell line RCB2157. However, 4-substituted phenyl thiazoles **12c** and **13c** did have cytotoxic effects in all cell types and at all-time points tested in a concentration- and time-dependent manner. The levels of cytotoxicity at 10 μM in the MCF7 breast cancer cells and the HT29 colon cancer cells are shown in Table 1.

From this small set of analogues it appears that substitution in the 2-position of the phenyl ring is detrimental to activity, probably due to excessive twisting of the thiazole out of the plane of the phenyl group. Substitution of the phenyl ring in the 4-position is clearly optimal for activity, but the electronic and precise structural requirements are unclear at this stage. The synthesis of more analogues will be required to unravel the structure–activity relationship.

Conclusions

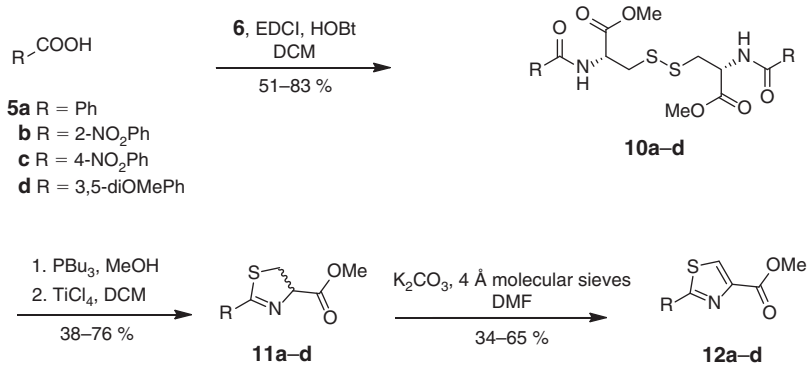
A robust synthesis of anithiactin A has been achieved in four steps, this synthetic procedure was then applied to the synthesis of five analogues. The work suggests that substituents at the 4-position of the phenyl ring is important for cytotoxicity. Work on creating a series of structurally diverse analogues at this position is underway and will be reported in due course.

Experimental

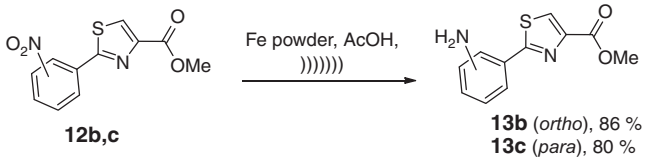
General

Thin-layer chromatography (TLC) was performed on ALUGRAM aluminium-backed UV_{254} silica gel 60 (0.20 mm) plates. Compounds were visualised with either *p*-anisaldehyde or 20 % w/w phosphomolybdic acid in ethanol. Column chromatography was performed using silica gel 60. Infrared spectra were recorded on a Bruker Optics Alpha ATR FT-IR spectrometer. High-resolution mass-spectra (HRMS) were recorded on a

†As previously noted,[8] under these reaction conditions racemisation can be minimised through conducting the reaction at lower temperatures. Since this stereocentre is destroyed in the subsequent oxidation we did not monitor for racemisation.



Scheme 2.



Scheme 3.

Table 1. Compounds 12c and 13c are cytotoxic towards HT29 colon cancer cells and MCF7 breast cancer cells. The cells were treated with the compounds at 10 μM for 24–72 h and an MTT assay conducted to assess the quantity of remaining viable cells. Cell viability is expressed as a percentage of vehicle-only treated control cells and the standard deviation is shown

Cell type	Treatment time	Cell viability ± s.d.	
		12c	**13c**
HT29	24 h	76.7 ± 1.5	82.3 ± 1.6
	48 h	52.5 ± 3.8	73.1 ± 3.2
	72 h	52.5 ± 3.0	54.7 ± 4.8
MCF7	24 h	40.2 ± 1.9	74.3 ± 2.1
	48 h	37.8 ± 1.4	57.3 ± 2.0
	72 h	35.5 ± 2.2	50.9 ± 3.9

Bruker microTOF$_Q$ mass spectrometer using an electrospray ionisation (ESI) source in either the positive or negative modes. ^{1}H NMR spectra were recorded at either 400 MHz on a Varian 400-MR NMR system or at 500 MHz on a Varian 500 MHz AR premium shielded spectrometer. All spectra were recorded from samples in $CDCl_3$ at 25°C in 5 mm NMR tubes. Chemical shifts are reported relative to the residual chloroform singlet at 7.26 ppm. Resonances were assigned as follows: chemical shift (multiplicity, coupling constant(s), number of protons, assigned proton(s)). Multiplicity abbreviations are reported by the conventions: s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets), t (triplet), td (triplet of doublets), q (quartet), qd (quartet of doublets), and m (multiplet). Proton decoupled ^{13}C NMR spectra were recorded at either 100 MHz on a Varian 400-MR NMR system or at 125 MHz on a Varian 500 MHz AR premium shielded spectrometer under the same conditions as the ^{1}H NMR spectra. Chemical shifts have been reported relative to the $CDCl_3$ triplet at 77.16 ppm. Dichloromethane (DCM) was dried using a PURE SOLV MD-6 solvent purification system. All other solvents and reagents were used as received

*Amide **8a***

A solution of **7a** (177 mg, 1.17 mmol), pyBOP (582 mg, 1.17 mmol), and Et_3N (179 μL, 1.28 mmol) in DCM (5 mL) was stirred for 15 min to which was added L-cystine dimethyl ester dihydrochloride (200 mg, 0.586 mmol). The mixture was stirred overnight under an inert atmosphere. The mixture was concentrated under vacuum, and taken up in EtOAc (10 mL). The organic layer was washed with H_2O (10 mL), $NaHCO_3$ (10 mL), brine (10 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum. The product was purified using column chromatography with EtOAc–petroleum eluent, which afforded the title compound as a white amorphous solid (300 mg, 35 % yield). δ_H (400 MHz, $CDCl_3$) 7.70 (d, *J* 7.6, 1H), 7.45 (td, *J* 7.7, 1.6, 1H), 7.40 – 7.07 (m, 4H), 5.02 (s, 1H), 3.76 (s, 3H), 3.29 (m, *2*H), 3.19 (s, 3H), 1.33 (s, 9H). δ_C (100 MHz, $CDCl_3$) 170.5, 141.4, 131.7, 129.5, 128.1, 127.3, 80.8, 52.8, 51.9, 40.5, 29.7, 28.2. HRMS (ESI) *m/z* 757.2507; calcd for $C_{34}H_{46}N_4O_{10}S_2Na$ [M + Na^+] 757.2548. ν_{max}/cm^{-1} 3337, 2960, 1743, 1664, 1152, 730.

General Procedure for EDCI/HOBt Amide Coupling

A suspension of the benzoic acid (1.17 mmol) and L-cystine dimethyl ester dihydrochloride (341 mg, 0.586 mmol) in anhydrous DCM (5 mL) was cooled to 0°C to which was added Et_3N (179 μL, 1.28 mmol), HOBt (17 mg, 0.117 mmol), and EDCI (181 mg, 1.17 mmol). The resulting suspension was warmed to room temperature and stirred overnight, eventually turning into a clear solution. The mixture was diluted with EtOAc (10 mL) and washed successively with HCl (1 M, 10 mL), saturated $NaHCO_3$ (10 mL), and brine (10 mL). The organic phases were dried over $MgSO_4$, filtered, and concentrated under vacuum to afford the corresponding amide.

Compound **10a** was obtained as a white amorphous solid (60 % yield). δ_H (400 MHz, $CDCl_3$) 7.82 (d, *J* 7.1, 2H), 7.51 (ddd, *J* 8.5, 8.5, 2.3), 7.42 (t, *J* 7.5, 2H), 7.10 (d, *J* 7.2, 1H), 5.08 (m, 1H), 3.78 (s, 3H), 3.35 (d, *J* 5.1, 2H). δ_C (100 MHz, $CDCl_3$) 170.9, 167.1, 133.4, 132.0, 128.6, 127.2, 52.9, 52.3, 40.9. HRMS (ESI) *m/z* 499.0926; calcd for $C_{22}H_{24}N_2O_6S_2Na$ [M + Na^+] 499.0968. ν_{max}/cm^{-1} 3320, 3067, 2919, 1740, 1579, 1266, 1016, 702, 418.

Compound **10b** was obtained as a white amorphous solid (51 % yield). δ_H (400 MHz, $CDCl_3$) 8.02 (m, 1H), 7.71–7.61 (m, 1H), 7.62–7.54 (m, 2H), 6.90 (d, *J* 7.3, 1H), 5.07 (m, 1H), 3.82 (s, 3H), 3.40 (d, *J* 4.9, 2H).δ_C (100 MHz, $CDCl_3$) 170.4, 166.1, 146.4, 133.8, 132.0, 130.7, 128.8, 124.5, 53.0, 52.3, 40.4. HRMS (ESI) *m/z* 589.0658; calcd for $C_{22}H_{22}N_4O_{10}S_2Na$ [M + Na^+] 589.0670. ν_{max}/cm^{-1} 3263, 2925, 1740, 1528, 1347, 732.

Compound **10c** was obtained as a white amorphous solid (56 % yield). δ_H (400 MHz, $CDCl_3$) 8.27 (d, *J* 8.9, 2H), 7.98 (d, *J* 8.9, 2H), 7.18 (d, *J* 7.1, 1H), 5.15–4.97 (m, 1H), 3.82 (s, 3H), 3.37 (dd, *J* 5.0, 2.3, 2H).δ_C (100 MHz, $CDCl_3$) 170.5, 165.2, 149.9, 138.9, 128.4, 123.9, 53.1, 52.5, 40.5. HRMS (ESI) *m/z* 589.0620; calcd for $C_{22}H_{22}N_4O_{10}S_2Na$ [M + Na^+] 589.067. ν_{max}/cm^{-1} 3284, 2924, 1739, 1520, 1342, 719.

Compound **10d** was obtained as a white amorphous solid (83 % yield). δ_H (400 MHz, $CDCl_3$) 7.03 (d, *J* 7.3, 1H), 6.95 (d, *J* 2.3, 2H), 6.59 (t, *J* 2.3, 1H), 5.04 (m, 1H) 3.81 (s, 6H), 3.79 (s, 3H), 3.34 (d, *J* 5.1, 2H). δ_C (100 MHz, $CDCl_3$) 170.8, 166.9, 160.9, 135.6, 105.1, 104.1, 55.6, 52.9, 52.3. HRMS (ESI) *m/z* 619.1373; calcd for $C_{26}H_{32}N_2O_{10}S_2Na$ [M + Na^+] 619.1391. ν_{max}/cm^{-1} 3291, 2953, 1744, 1593, 1206, 1158, 421.

General Procedure for Disulfide Reduction and Cyclodehydration

To a solution of the disulfide (0.811 mmol) in MeOH (5 mL) was added *n*-tributylphosphine (0.444 mmol) and the reaction was monitored by TLC. Upon consumption of the starting material the mixture was concentrated under vacuum and filtered through a plug of silica to remove any tributylphosphine oxide. The filtrate was concentrated under vacuum and subsequently taken up in DCM (5 mL), to which was added $TiCl_4$ (2.43 mmol, 1 M in DCM) dropwise over 5 min at 0°C. The mixture was stirred at room temperature for 4 h and then quenched with saturated NH_4Cl(aq). The aqueous phase was extracted with EtOAc (3 × 5 mL) and the combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum. Purification by column chromatography eluting with EtOAc/petroleum ether afforded the corresponding thiazoline.

Compound **9** was obtained as a white amorphous solid (66 % yield). δ_H (400 MHz, $CDCl_3$) 7.49 (dd, *J* 7.9, 1.6, 1H), 7.33 (ddd, *J* 8.6, 7.1, 1.6, 1H), 6.73 (d, *J* 8.4, 1H), 6.70–6.59 (m, 1H), 5.33 (t, *J* 8.8, 1H), 3.83 (s, 3H), 3.52 (m, 2H), 2.93 (s, 3H). HRMS (ESI) *m/z* 273.0653; calcd for $C_{12}H_{14}N_2O_2SNa$ [M + Na^+] 273.0668. δ_C (100 MHz, $CDCl_3$) 172.0, 171.5, 148.9, 132.9, 132.8, 114.9, 110.7, 78.3, 52.7, 33.6, 29.8.

Compound **11a** was obtained as a white amorphous solid (76 % yield). δ_H (400 MHz, $CDCl_3$) 7.87 (m, 1H), 7.48 (ddd, *J* 7.3, 7.3, 1.7, 1H), 7.41 (m, 1H), 5.29 (t, *J* 9.1, 1H), 3.83 (s, 3H), 3.76–3.59 m, 1H). δ_C (100 MHz, $CDCl_3$) 163.1, 161.5, 148.5, 147.4, 132.8, 132.1, 131.1, 129.3, 127.7, 124.7, 52.6. HRMS (ESI) *m/z* 244.0394; calcd for $C_{11}H_{11}NO_2SNa$ [M + Na^+] 244.0403. ν_{max}/cm^{-1} 2950, 1737, 1446, 1227, 1199, 931, 688.

Compound **11b** was obtained as a white amorphous solid (41 % yield). δ_H (400 MHz, $CDCl_3$) 7.96 (d, *J* 7.8, 1H), 7.67–7.62 (m, 2H), 7.62–7.56 (m, 1H), 5.28 (t, *J* 9.2, 1H), 3.84 (s, 3H), 3.90–3.71 (m, 2H). δ_C (100 MHz, $CDCl_3$) 170.4, 168.0, 132.9, 131.2, 130.5, 128.4, 124.5, 78.4, 52.9, 36.9. HRMS (ESI) *m/z* 289.0239; calcd for $C_{11}H_{10}N_2O_4SNa$ [M + Na^+] 289.0253. ν_{max}/cm^{-1} 2952, 1736, 1527, 1347, 1201, 945, 709.

Compound **11c** was obtained as a white amorphous solid (43 % yield). δ_H (400 MHz, $CDCl_3$) 8.27 (d, *J* 8.9, 2H), 8.04 (d, *J* 8.9, 2H), 5.35 (t, *J* 9.3, 1H), 3.86 (s, 3H), 3.95–3.61 (m, 1H). δ_C (100 MHz, $CDCl_3$) 170.6, 169.4, 149.6, 137.9, 129.6, 123.7, 78.4, 53.0, 35.9. HRMS (ESI) *m/z* 289.0233; calcd for $C_{11}H_{10}N_2O_4SNa$ [M + Na^+] 289.0253. ν_{max}/cm^{-1} 2956, 1738, 1521, 1347, 1203, 850, 690.

Compound **11d** was obtained as a white amorphous solid (38 % yield). δ_H (400 MHz, $CDCl_3$) 7.01 (s, 1H), 6.56 (s, 1H), 5.27 (t, *J* 9.1, 1H), 3.82 (s, 3H), 3.81 (s, 6H), 3.73–3.58 (m, 2H). δ_C (100 MHz, $CDCl_3$) 171.1, 160.7, 134.2, 106.5, 104.5, 78.0, 55.6, 52.8, 35.4. HRMS (ESI) *m/z* 304.0592; calcd for $C_{15}H_{15}NOSNa$ [M + Na^+] 304.0614. ν_{max}/cm^{-1} 2950, 1737, 1583, 1424, 1153, 1061, 684.

General Procedure for Thiazoline Oxidation

To a solution of thiazoline (0.139 mmol) dissolved in DMF (3 mL) was added 4 Å molecular sieves (200 wt-%) and anhydrous K_2CO_3 (0.419 mmol) and the suspension stirred at 80°C for 4 h. The mixture was cooled to room temperature and partitioned between H_2O (5 mL) and EtOAc (5 mL) and the aqueous layer was extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under vacuum. Purification by column chromatography yielded the corresponding thiazole.

Compound **1** was obtained was a white amorphous solid (34 % yield). δ_H (400 MHz, $CDCl_3$) 8.4 (s, 2H), 8.0 (s, 1H), 7.6 (dd, *J* 7.8, 1.5, 1H), 7.3 (ddd, *J* 8.6, 7.1, 1.5, 1H), 6.8 (dd, *J* 8.4, 1.0, 1H), 6.7 (td, *J* 7.6, 1.1, 1H), 4.0 (s, 4H), 3.0 (s, 5H).

Compound **12a** was obtained as a white amorphous solid (56 % yield). δ_H (400 MHz, $CDCl_3$) 8.17 (s, 1H), 8.00 (dd, *J* 6.6, 3.1, 2H), 7.53–7.38 (m, 3H), 3.98 (s, 3H). δ_C (100 MHz, $CDCl_3$) 169.0, 161.9, 147.7, 132.7, 130.7, 129.0, 127.3, 126.9, 78.8, 52.5.

Compound **12b** was obtained a yellow amorphous solid (65 % yield). δ_H (400 MHz, $CDCl_3$) 8.18 (s, 1H), 8.00 (dd, *J* 6.7, 2.9, 2H), 7.49–7.42 (m, 3H), 3.98 (s, 3H). δ_C (100 MHz, $CDCl_3$) 163.1, 161.6, 147.5, 132.7, 132.1, 131.1, 129.3, 127.7, 124.7, 77.2, 52.6. HRMS (ESI) *m/z* 287.0087; calcd for $C_{11}H_8N_2O_4SNa$ [M + Na^+] 287.0097. ν_{max}/cm^{-1} 3071, 2921, 2851, 1718, 1680, 1528, 1289, 1211, 704, 545.

Compound **12c** was obtained as a yellow amorphous solid (60 % yield). δ_H (400 MHz, $CDCl_3$) 8.32 (dd, *J* 8.9, 2.1, 2.1, 2H), 8.30 (s, 1H), 8.18 (dd, *J* 8.9, 2.1, 2.1, 2H), 4.00 (s, 3H). δ_C (100 MHz, $CDCl_3$) 165.9, 161.5, 148.9, 148.5, 138.1, 128.8, 127.7, 124.4, 52.7. HRMS (ESI) *m/z* 287.0075; calcd for $C_{11}H_8N_2O_4SNa$ [M + Na^+] 287.0097. ν_{max}/cm^{-1} 3090, 2923, 2851, 1718, 1522, 1343, 1209, 851, 752, 638.

Compound **12d** was obtained as a white amorphous solid (64 % yield). δ_H (400 MHz, $CDCl_3$) 8.17 (s, 1H), 7.14 (d, *J* 2.3, 2H), 6.56 (s, 1H), 3.98 (s, 4H), 3.87 (s, 8H). δ_C (100 MHz, $CDCl_3$) 168.9, 161.9, 161.1, 147.5, 134.5, 127.5, 104.9, 103.1, 55.6, 52.5. HRMS (ESI) *m/z* 302.0429; calcd for $C_{13}H_{13}NO_4SNa$ [M + Na^+] 302.0457. ν_{max}/cm^{-1} 3112, 2952, 2836, 1734, 1708, 1594, 1444, 1222, 747, 672.

General Procedure for Nitro Reduction

To a solution of the nitro aromatic compound (0.174 mmol) in a mixture of glacial acetic acid (0.60 mL), ethanol (0.6 mL), and water (0.3 mL) was added reduced iron powder (0.870 mmol). The resulting suspension was exposed to ultrasonic irradiation for 1 h at 30°C and monitored by TLC. The reaction mixture was filtered and the iron residue was washed with EtOAc (2 × 5 mL). The reaction mixture was partitioned between 2 M KOH (5 mL) and the basic layer further extracted with EtOAc (3 × 5 mL). The combined organic extracts were washed with brine (10 mL), dried over $MgSO_4$, and concentrated under reduced pressure. Purification by column chromatography using EtOAC/petroleum ether as eluent yielded the title compound.

Compound **13b** was obtained as a brown amorphous solid (86 % yield). δ_H (400 MHz, $CDCl_3$) 8.07 (s, 1H), 7.61 (dd, *J* 7.9,

1.4, 1H), 7.22 (ddd, *J* 8.3, 7.2, 1.5, 1H), 6.84 (dd, *J* 8.2, 0.8, 1H), 6.77–6.73 (m, 1H), 3.95 (s, 2H). δ_C (100 MHz, $CDCl_3$) 169.6, 161.7, 146.4, 145.1, 131.4, 129.2, 125.3, 117.5, 52.3. HRMS (ESI) *m/z* 257.0354; calcd for $C_{11}H_{10}N_2O_2SNa$ [M + Na^+] 257.0355. ν_{max}/cm^{-1} 3433, 3316, 2954, 2921, 1719, 1614, 1486, 1219, 754.

Compound **13c** was obtained as a brown amorphous solid (80 % yield). δ_H (500 MHz, $CDCl_3$) 8.06 (s, 1H), 7.81 (d, *J* 8.5, 2H), 6.70 (d, *J* 8.4, 2H), 3.97 (s, 3H). δ_C (125 MHz, $CDCl_3$) 172.2, 164.8, 151.6, 149.8, 131.2, 128.5, 126.0, 117.4, 55.0. HRMS (ESI) *m/z* 257.0341; calcd for $C_{11}H_{10}N_2O_2SNa$ [M + Na^+] 257.0355. ν_{max}/cm^{-1} 3479, 3323, 2952, 2923, 1717, 1618, 1467, 1211, 775.

Cell Lines and Cell Culture

MDA-MB-231 and MCF7 breast cancer cells were obtained from Prof. R. Rosengren. HT29 colon cancer cells were purchased from CellBank Australia and the immortalised bone marrow-derived mesenchymal stem cell line RCB2157 (MSC) was from the Riken BioResource Center through the National Bio-Resource Project of the MEXT, Japan. Cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 5 or 10 % (for the MSCs) heat inactivated fetal bovine serum (FBS), 100 units mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin. Cells were incubated at 37°C in humidified conditions with 5 % CO_2.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) Assay

The quantity of cells remaining 24, 48 and 72 h after treatment of the cells with the compounds or DMSO only (vehicle control) was compared using the MTT assay, which assesses mitochondrial and endoplasmic reticulum dehydrogenase activity (Mosmann reference, see below). MTT was made up to 5 mg mL^{-1} in phosphate buffered saline (PBS), added to cells at a final concentration of 0.5 mg mL^{-1}, and incubated at 37°C for 3 h. The formazan crystals were dissolved with fresh DMSO and the resulting absorbance was read at 560 nm.[11]

DMSO at 10 % was used as a positive control and resulted in ~70–75 % cell death in each assay. DMSO was also used as a vehicle control, added at the volume used for the highest concentration of each compound. No significant toxicity was found.

Statistical Analysis

The results are presented as mean ± standard deviation (s.d.) of three independent experiments. Absorbance values were normalized to the vehicle only control (DMSO) for each experiment.

Supplementary Material

^{1}H NMR and ^{13}C NMR spectra of all compounds are available on the Journal's website.

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