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Benzofuranquinones as inhibitors of indoleamine 2,3-dioxygenase (IDO). Synthesis and biological evaluation†

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A series of benzofuranquinones, analogues of the marine metabolite annulin A, has been synthesized and evaluated as inhibitors of human indoleamine 2,3-dioxygenase (IDO). The synthesis was carried out by copper(II)-mediated reaction of bromobenzoquinones with 1,3-dicarbonyl compounds followed by functional group interconversions. The most potent compounds were 5-methoxy-2-methylbenzofuranquinones containing a CH_2OR group at position-3 with $IC_{50} \sim 0.2$ mM. The corresponding hydroquinones were inactive. Compounds based on the benzimidazolequinone framework are also active IDO inhibitors. The quinones do not generate significant levels of oxidative stress at concentrations that inhibit IDO.

Introduction

One very attractive, but different, approach to cancer therapy is to recruit the body's own immune system to reject solid tumours, and therefore some effort has gone into trying to understand how tumours escape the host immune system.[1–3] The essential amino acid tryptophan (Trp) plays a key role in "immune escape" – T-lymphocytes are extremely sensitive to Trp shortage – and its degradation in tumours inhibits T-cell proliferation and, as a result, prevents immunological rejection of the tumour.[4,5] The first step in Trp catabolism is the oxidative cleavage of the indole 2,3-bond, catalysed by the haem-containing enzyme indoleamine 2,3-dioxygenase (IDO), and is the first and rate-limiting step in the kynurenine pathway in mammalian cells. IDO (and its relative IDO2[6]) therefore plays a major role, its function suppressing the immune response.[1,4,7–9] Hence there is increasing evidence to support the hypothesis that inhibition of IDO produces significant anticancer effects. For example, studies using siRNA to silence IDO have resulted in recovery of T-cell responses and reinstallation of antitumour immunity.[10]

IDO is an attractive target for a drug development programme since it is known to be constitutively activated in a large number of human cancers.[11] It is a monomeric catalytic haem-containing enzyme with a known structure wherein the active site is composed of two subsites with the haem pocket accommodating the indole ring of the natural substrate tryptophan,[12] and whose mechanism has been widely studied.[13–16]

There are a number of known inhibitors of IDO, encompassing many structural types as outlined in recent reviews.[17,18] The most widely studied are based on 1-methyltryptophan (1-MT), a competitive inhibitor with the natural substrate, but generally only poorly active (K_i = 62 μM).[19] Nevertheless even such a relatively poor inhibitor of IDO can increase the efficacy of chemotherapeutic agents such as paclitaxel in the inhibition of tumour growth in *in vivo* models.[7,8] Other examples of IDO inhibitors shown in Fig. 1 include arylimidazoles such as 4-phenylimidazole (IC_{50} = 48 μM),[20] a compound that binds to the haem iron of the enzyme, some simple analogues of brassinin (K_i = 98 μM), a natural product obtained from cruciferous vegetables,[21,22] *S*-benzylisothioureas (IC_{50} = 61 μM),[23] and the naturally occurring annulins (A, K_i = 0.69 μM; B, K_i = 0.12 μM; C, K_i = 0.14 μM).[24] The latter structures inspired a range of studies on naphthoquinone derivatives such as dehydro-α-lapachone (IC_{50} = 0.21 μM),[25] and a series of pyranonaphthoquinones.[26] More structurally complex natural products such as the marine metabolite exiguamine A (K_i = 0.21 μM),[27] and the marine fungal natural product plectosphaeroic acid (IC_{50} = ~2 μM) have also been identified as inhibitors of IDO.[28] In the latter case, the IDO inhibitory activity resides in the aminophenoxazinone moiety rather than the more structurally complex pyrroloindole fragment.[28]

However, it was the structures of the naturally occurring isobenzofuranquinones annulins A and C that captured our

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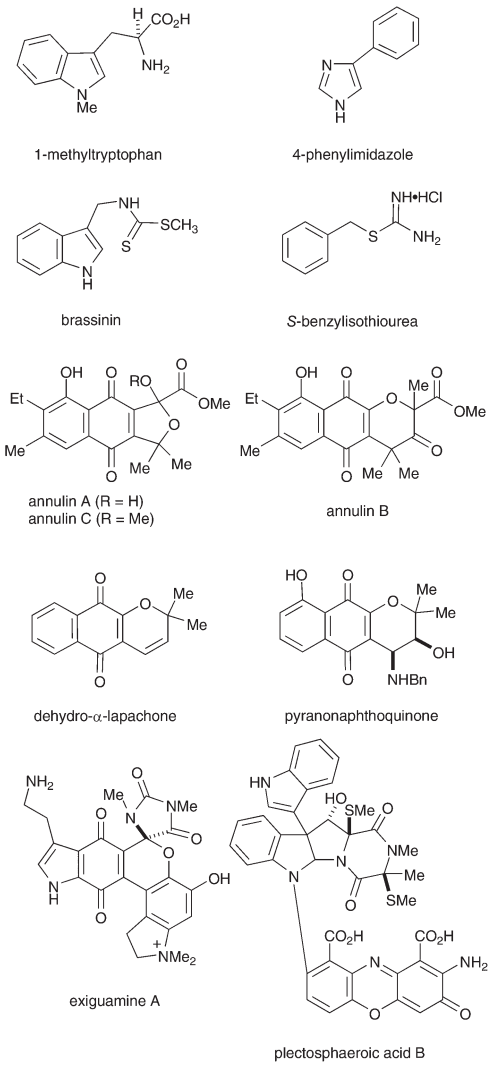


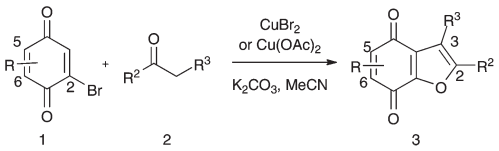
Fig. 1 Structures of known inhibitors of IDO.

attention in view of our longstanding interest in the synthesis and biologically properties of heterocyclic quinones,[29–38] and therefore we embarked on a study of the synthesis and biological evaluation of related benzofuranquinones as inhibitors of IDO.

Results and discussion

Chemistry

In a previous paper,[38] we described the synthesis of benzofuranquinones from benzofurans by way of a classical nitration, reduction sequence followed by oxidation of the 4-aminobenzofuran to the quinone. Although successful, the method was lengthy, particularly taking into account the need to synthesize the starting benzofurans, and therefore we sought a more efficient method. We have previously reported the synthesis of indolequinones *via* a regioselective copper-mediated oxidative annulation of bromoquinones **1** with enamines derived from



Scheme 1

Table 1 Preparation of benzofuranquinones from bromoquinones and 1,3-dicarbonyl compounds[a]

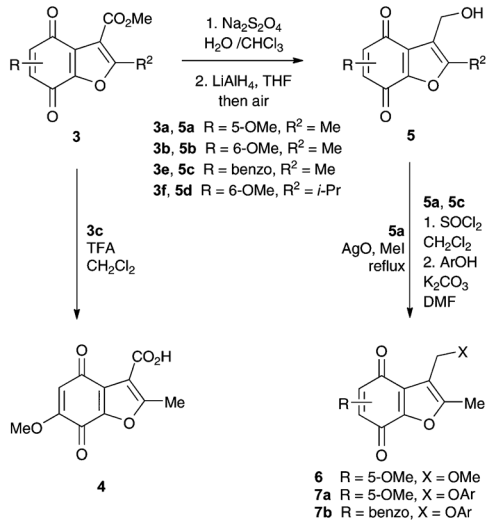
Entry	1	R	2	R^2	R^3	3	Yield/%
1	**a**	5-OMe	**a**	Me	CO_2Me	**a**	34
2	**b**	6-OMe	**a**	Me	CO_2Me	**b**	49
3	**b**	6-OMe	**b**	Me	CO_2Bu^t	**c**	21
4	**b**	6-OMe	**c**	Me	COMorph	**d**	32
5	**c**	5,6-Benzo	**a**	Me	CO_2Me	**e**	29
6	**b**	6-OMe	**d**	i-Pr	CO_2Me	**f**	46
7	**b**	6-OMe	**e**	Ph	CO_2Et	**g**	35

[a] Morph = 4-morpholino.

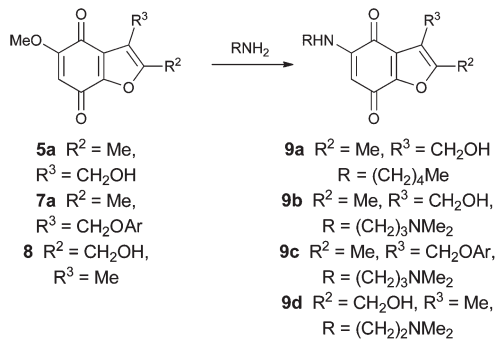
1,3-dicarbonyl compounds.[39,40] Use of the parent 1,3-dicarbonyl compound **2** in place of the enamine allowed the extension of this method to the synthesis of benzofuranquinones **3** bearing electron-withdrawing groups at the 3-position (Scheme 1, Table 1). In most cases, in contrast to the enamine annulation, copper(II) bromide was found to be a superior reagent to copper(II) acetate.

The products could be further functionalised by transformations at the 3-position; thus, the *tert*-butyl ester **3c** could be cleaved by treatment with trifluoroacetic acid to give the corresponding carboxylic acid **4**, and the methyl esters **3a**, **3b**, **3e** and **3f** could be reduced to their corresponding alcohols **5**. In some cases, the latter transformation required a two-step procedure involving reduction of the quinone using sodium dithionite, followed by ester reduction with lithium aluminium hydride, with the benzofuranquinone product **5** being obtained after re-oxidation upon stirring the quenched reaction mixture under air. Methylation of the alcohol **5a** with iodomethane and silver(I) oxide gave the corresponding methyl ether **6**. To investigate the effect of incorporating a leaving group at the 3-position, alcohols **5a** and **5c** could be converted to 2,4,6-trifluoroaryl ethers **7** by treatment with thionyl chloride, followed by reaction with 2,4,6-trifluorophenol and potassium carbonate (Scheme 2). The presence of such potential leaving groups has been found to be beneficial in the inhibition of other enzymes such as NQO1 and NQO2 (human quinone reductases QR1 and 2), and thioredoxin reductase.[34,41–45]

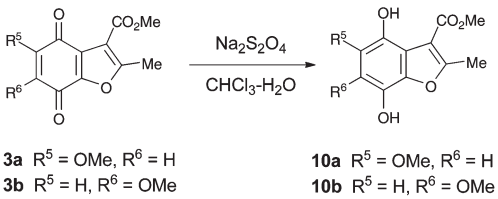
Further modification of the 5-methoxybenzofuranquinones **5a** and **7a** could be effected by substitution with primary amines, which readily undergo addition-elimination reactions to displace the methoxy group in a regioselective manner to give the 5-amino substituted benzofuranquinones **9a–c** (Scheme 3). The benzofuranquinone **8**,[38] an isomer of **5a** in which the 2-methyl and 3-hydroxymethyl groups have been



Scheme 2 [Ar = 2,4,6-F_3-C_6H_2] modification of benzofuranquinone esters.



Scheme 3 [Ar = 2,4,6-F_3-C_6H_2] conversion of 5-methoxybenzofuranquinones into 5-amino derivatives.



Scheme 4 Reduction of benzofuranquinones to corresponding hydroquinones.

exchanged, was also subjected to the same reaction, and gave the corresponding amino derivative **9d**.

For comparison, the hydroquinones **10a** and **10b**, corresponding to benzofuranquinones **3a** and **3b** were synthesised by reduction with sodium dithionite (Scheme 4), and a range of previously prepared heterocyclic quinones (see Table 3) were also studied.[33,36,38]

Biology

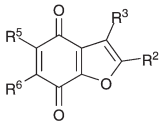
With a range of benzofuranquinones in hand, we turned our attention to their evaluation as IDO inhibitors. Human IDO, with histidine tagged N-terminus, was expressed in *E. coli* and purified using Ni^{2+} affinity chromatography as previously described.[46] Compounds were evaluated for their ability to inhibit IDO-catalyzed oxidative degradation of L-tryptophan to *N*-formylkynurenine. This was converted into kynurenine, by trichloroacetic acid cleavage of the *N*-formyl group, which was assayed by reaction with Ehrlich's reagent to produce a product with strong absorbance at 480 nm. The data that show the amount of enzyme activity remaining at four different inhibitor concentrations are shown in Table 2. For selected benzofuranquinones more detailed IC_{50} values were also determined. The known IDO inhibitor 4-phenylimidazole was used as a control.

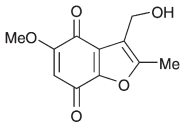
The most active inhibitors of IDO in the benzofuranquinone series are the 5- or 6-methoxy substituted quinones bearing a CH_2OH, CH_2OMe or CH_2OAr group at position-3 that show 90–97% enzyme inhibition at 10 μM (Table 2, entries 8, 9, 11–13). Incorporation of electron withdrawing ester or acid substituents at C-3 is detrimental to biological activity (entries 1, 2, 4–7), although the morpholino amide (entry 3) retains some activity. The fusion of an additional benzene ring at C-5/C-6 (entries 4, 10, 14) results in less potent compounds, as does the substitution of the 5-methoxy group by amino groups (entries 15–18). For comparison, the hydroquinones **10a** and **10b** corresponding to **3a** and **3b** were assayed, and found to be ineffective IDO inhibitors with 80–90% enzyme activity remaining at 10 μM inhibitor concentrations. These data demonstrate that the benzofuranquinones (entries 8, 9, 11–13), particularly compounds **5a** and **5b** (IC_{50} = 0.24 and 0.50 μM respectively), are potent inhibitors of IDO and are comparable with many of the known IDO inhibitors shown in Fig. 1, including the naturally occurring isobenzofuranquinones annulin A and annulin C,[24] and that much simpler benzofuranquinones are as efficient at inhibiting IDO as the larger highly substituted isobenzofuranquinone natural products.

We also examined a number of other heterocyclic quinones as IDO inhibitors, and the results are shown in Table 3, again using 4-phenylimidazole as a control.

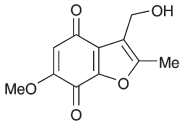
Although the range of heterocyclic quinones shown in Table 3 is limited, some conclusions can be drawn. The benzothiophenequinone **11** (Table 3, entry 1) has similar activity to its benzofuran counterpart **5a** (30% activity remaining *vs.* 36% at 1 μM concentration of inhibitor). Indolequinones (entries 2–4) are poor inhibitors of IDO; the parent indolequinone **14** has previously been reported with a K_i of 190 ± 20 nmol.[19] Whilst the indazole derivative **15** has modest activity, it is the three benzimidazolequinones **16–18** (entries 6–8) that are the most potent IDO inhibitors (IC_{50} ~ 0.2 μM). Overall the data demonstrate that benzimidazolequinones are also potent compounds, and are comparable with the benzofuranquinones, and some of the

Table 2 Inhibition of rhIDO by benzofuranquinones[a]





5a

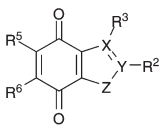


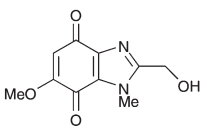
5b

Entry		R^2	R^3	R^5	R^6	IC_{50} (μM)	Percent activity remaining of IDO at 0.1 μM	1 μM	10 μM	100 μM
1	**3a**	Me	CO_2Me	OMe	H	7.2	96	81	40	5
2	**3b**	Me	CO_2Me	H	OMe	34	100	98	72	6
3	**3d**	Me	COMorph	H	OMe		80	65	10	5
4	**3e**	Me	CO_2Me	Benzo			95	95	85	50
5	**3f**	i-Pr	CO_2Me	H	OMe		100	100	70	12
6	**3g**	Ph	CO_2Et	H	OMe		90	85	40	12
7	**4**	Me	CO_2H	H	OMe		95	95	90	55
8	**5a**	Me	CH_2OH	OMe	H	0.24	83	36	3	2
9	**5b**	Me	CH_2OH	H	OMe	0.50	95	45	4	2
10	**5c**	Me	CH_2OH	Benzo			95	95	70	25
11	**5d**	i-Pr	CH_2OH	H	OMe		85	40	10	5
12	**6**	Me	CH_2OMe	OMe	H		84	26	4	5
13	**7a**	Me	CH_2OAr	OMe	H	≈1.0	100	41	8	7
14	**7b**	Me	CH_2OAr	Benzo			85	85	95	90
15	**8a**	Me	CH_2OH	$NH(CH_2)_4Me$	H		82	72	35	16
16	**8b**	Me	CH_2OH	$NH(CH_2)_3NMe_2$	H		84	57	22	12
17	**8c**	Me	CH_2OAr	$NH(CH_2)_3NMe_2$	H		86	56	22	14
18	**8d**	CH_2OH	Me	$NH(CH_2)_2NMe_2$	H		90	50	12	10
19	—	4-Phenylimidazole (control)				70				

[a] Morph = 4-morpholino; Ar = 2,4,6-F_3-C_6H_2.

Table 3 Inhibition of rhIDO by heterocyclic quinones





16

Entry		X	Y	Z	R^2	R^3	R^5	R^6	IC_{50} (μM)	Percent activity remaining of IDO at 0.1 μM	1 μM	10 μM	100 μM	Ref.
1	**11**	C	C	S	Me	CH_2OH	OMe	H	1.0	74	32	5	4	38
2	**12**	C	C	NMe	Me	CH_2OH	OMe	H	~10	94	86	48	27	33
3	**13**	C	C	NMe	Me	CHMeOH	OMe	H	~10	99	87	46	33	33
4	**14**	C	C	NH	H	H	H	H	26[a]	99	43	13	6	
5	**15**	C	N	NMe	—	CH_2OH	OMe	H	~1.0	82	53	28	—	38
6	**16**	N	C	NMe	CH_2OH	—	H	OMe	0.25	67	24	1.1	—	36
7	**17**	N	C	NMe	CH_2OAc	—	H	OMe	0.20	69	32	13	9	36
8	**18**	N	C	NMe	CH_2OAr[b]	—	H	OMe	0.18	66	30	11	8	36
9	—	4-Phenylimidazole (control)							70					

[a] K_i reported as 190 ± 20 nmol.[19] [b] Ar = 4-nitrophenyl.

known inhibitors shown in Fig. 1 in their ability to inhibit IDO.

To determine if these quinones were themselves cytotoxic to cancer cells we treated BxPc-3 human pancreatic cancer cells with benzofuranquinone **5a** and heterocyclic quinone **16**. BxPc-3 cells were chosen since they have moderate levels of quinone reductase (NQO1) activity and have a large cytoplasmic to nuclear ratio which makes them ideal for examining damage to microtubules and actin cytoskeleton. Quinones **5a** (Table 2) and **16** (Table 3) were selected because they

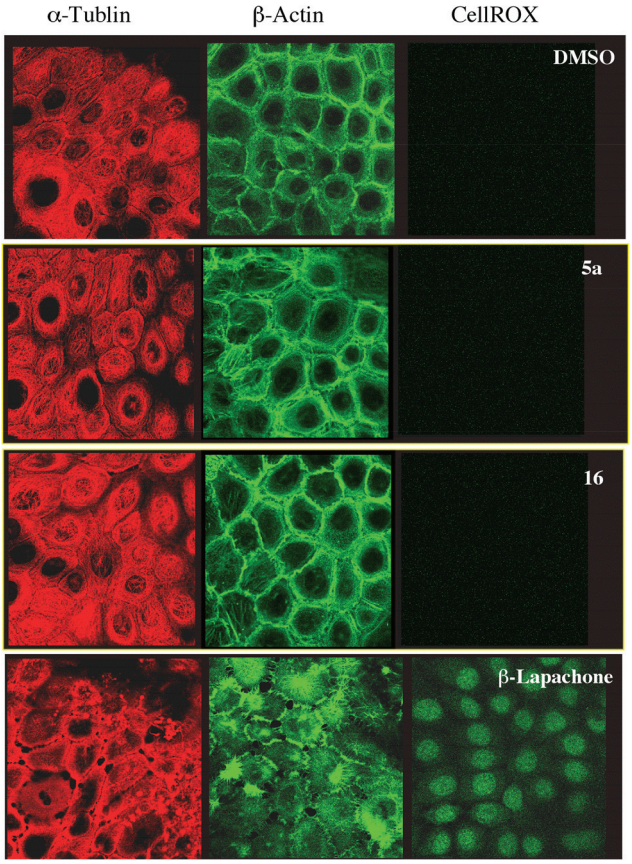


Fig. 2 Immunostaining of microtubules and actin cytoskeleton following treatment with quinones. BxPc-3 human pancreatic cancer cells were treated with quinones **5a** and **16** (10 μM × 4 h) and then analysed by immunostaining and confocal microscopy for quinone-induced damage to microtubules and actin cytoskeleton. Cells were also examined for intracellular oxidative stress (CellROX) following treatment with quinones (10 μM × 4 h). Treatment with β-lapachone (2.5 μM × 4 h) was included as a positive control for quinone induced oxidative stress.

represent two different classes of potent quinone IDO inhibitors. Exposure of BxPc-3 cells to **5a** or **16** did not result in visible damage to microtubules or actin cytoskeleton nor did they generate detectable levels of oxidative stress as determined by confocal microscopy (Fig. 2). Treatment, however, with β-lapachone a quinone which undergoes NQO1-dependent redox cycling, did generate high levels of oxidative stress and significant damage to microtubules and the actin cytoskeleton as shown in Fig. 2. In agreement with these data quinones **5a** and **16** did not induce significant levels of cytotoxicity while treatment with β-lapachone did result in pronounced cell death (Fig. 3). These data confirm that at concentrations that inhibit IDO these quinones do not generate significant levels of oxidative stress.

Conclusions

In summary, we have prepared a range of benzofuranquinones by copper(II)-mediated reaction of bromobenzoquinones with

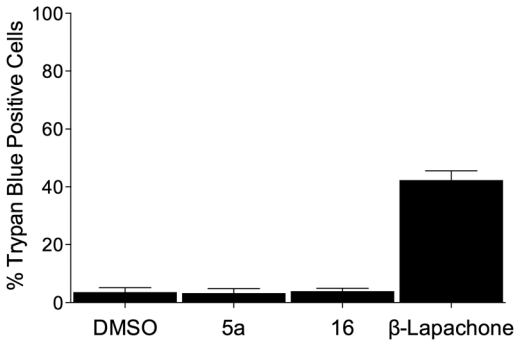


Fig. 3 Quinone-induced cytotoxicity in BxPc-3 cells. Quinones **5a** and **16** were examined for their ability to induce cytotoxicity in BxPc-3 human pancreatic cancer cells. For these studies, cells were treated quinones (10 μM) for 4 h after which cytotoxicity was assayed using trypan blue exclusion. β-Lapachone (2.5 μM × 4 h) was included as a positive control. Results are expressed as mean ± standard deviation of three determinations.

1,3-dicarbonyl compounds. The compounds were evaluated for their ability to inhibit IDO, and the study identified two compounds (**5a** and **5b** with IC_{50} = 0.24 and 0.50 μM respectively), that are potent inhibitors of IDO comparable to the naturally occurring annulins. In contrast, hydroquinones were much less active, and whereas indolequinones were essentially inactive, benzimidazolequinones showed comparable activity to the benzofurans. Importantly, the quinones do not do not generate significant levels of oxidative stress at concentrations that inhibit IDO.

Experimental section

Chemistry: general experimental details

Commercially available reagents were used throughout without purification unless otherwise stated. All anhydrous solvents were used as supplied, except tetrahydrofuran and dichloromethane that were freshly distilled according to standard procedures. Reactions were routinely carried out under an argon atmosphere unless otherwise stated, and all glassware was flame-dried before use. Light petroleum refers to the fraction with bp 40–60 °C. Ether refers to diethyl ether.

Analytical thin layer chromatography was carried out on aluminium backed plates coated with silica gel, and visualised under UV light at 254 and/or 360 nm and/or by chemical staining. Flash chromatography was carried out using silica gel, with the eluent specified.

Infrared spectra were recorded using an FT-IR spectrometer over the range 4000–600 cm^{-1}. NMR spectra were recorded at 400 or 500 MHz (^1H frequency, 100 or 125 MHz ^{13}C frequency). Chemical shifts are quoted in parts per million (ppm), and are referenced to residual H in the deuterated solvent as the internal standard. Coupling constants, *J*, are quoted in Hz. In the ^{13}C NMR spectra, signals corresponding to CH, CH_2, or CH_3 groups are assigned from the DEPT spectra. Mass spectra were recorded on a time-of-flight mass spectrometer

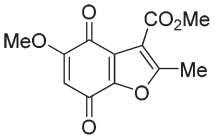
using electrospray ionisation (ESI), or an EI magnetic sector instrument.

The synthesis of compounds **11–13** and **15–18** has been described previously.[33,36,38]

General procedure for the synthesis of benzofuran-4,7-diones

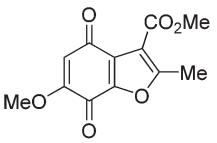
A solution of 1,3-dicarbonyl compound (1.0 equiv.) in acetonitrile (5–10 mL mmol^{-1}) was added to a mixture of bromoquinone (1.0 equiv.), copper(II) bromide or copper(II) acetate monohydrate (1.5–2.0 equiv.) and potassium carbonate (3.0 equiv.). The resulting mixture was stirred at reflux for the indicated time, cooled to room temperature, diluted with dichloromethane (20 mL mmol^{-1}), filtered through Celite and concentrated *in vacuo*. Column chromatography of the residue gave the benzofuran-4,7-dione.

Methyl 4,7-dihydro-5-methoxy-2-methyl-4,7-dioxobenzofuran-3-carboxylate 3a.



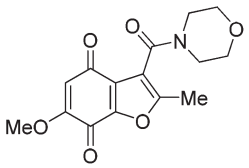
Prepared by the general procedure from 2-bromo-5-methoxybenzoquinone[39] (0.200 g, 0.92 mmol), methyl acetoacetate (0.099 mL, 0.92 mmol), copper(II) acetate monohydrate (0.299 g, 1.38 mmol) and potassium carbonate (0.381 g, 2.76 mmol) stirred at reflux in acetonitrile (10 mL) for 1.5 h. Column chromatography eluting with ethyl acetate and light petroleum (1 : 2) gave the *title compound* as a yellow solid (78.5 mg, 34%); mp 203–205 °C; (found: M + Na$^+$, 273.0365. $C_{12}H_{10}O_6$ + Na$^+$ requires: 273.0375); ν_{max} ($CHCl_3$)/cm^{-1} 1718, 1701, 1661, 1610, 1551, 1456, 1248, 1187, 1026, 992; λ_{max} (acetonitrile)/nm 232 (log ε 4.17), 267 (log ε 3.87), 302 (log ε 3.92), 404 (log ε 3.08); δ_H (400 MHz; $CDCl_3$) 5.80 (1 H, s, H-6), 3.92 (3 H, s, OMe), 3.87 (3 H, s, OMe), 2.68 (3 H, s, 2-Me); δ_C (100 MHz; $CDCl_3$) 175.4, 174.7, 164.0, 162.4, 160.6, 150.2, 123.6, 112.5, 104.6 (CH), 57.0 (Me), 52.2 (Me), 14.0 (Me); *m*/*z* (ESI) 523 (2M + Na$^+$, 100%), 273 (M + Na$^+$, 65%).

Methyl 4,7-dihydro-6-methoxy-2-methyl-4,7-dioxobenzofuran-3-carboxylate 3b.



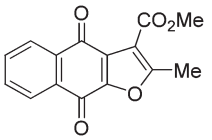
Prepared by the general procedure from 2-bromo-6-methoxybenzoquinone[39] (0.200 g, 0.92 mmol), methyl acetoacetate (0.099 mL, 0.92 mmol), copper(II) acetate monohydrate (0.299 g, 1.38 mmol) and potassium carbonate (0.381 g, 2.76 mmol) stirred at reflux in acetonitrile (10 mL) for 1.5 h. Column chromatography eluting with ethyl acetate and light petroleum (1 : 2) gave the *title compound* as a yellow solid (114 mg, 49%); mp 191–193 °C; (found: M + Na$^+$, 273.0369. $C_{12}H_{10}O_6$ + Na$^+$ requires: 273.0375); ν_{max} ($CHCl_3$)/cm^{-1} 3011, 1690, 1662, 1611, 1458, 1313, 1248, 1094, 848; λ_{max} (acetonitrile)/nm 232 (log ε 4.11), 259 (log ε 3.84), 303 (log ε 4.12), 402 (log ε 2.90); δ_H (400 MHz; $CDCl_3$) 5.85 (1 H, s, H-5), 3.94 (3 H, s, OMe), 3.86 (3 H, s, OMe), 2.68 (3 H, s, 2-Me); δ_C (100 MHz; $CDCl_3$) 179.9, 169.4, 165.2, 162.3, 158.1, 148.6, 127.0, 113.1, 108.2 (CH), 56.9 (Me), 52.3 (Me), 14.3 (Me); *m*/*z* (ESI) 273 (M + Na$^+$, 100%).

6-Methoxy-2-methyl-3-(morpholine-4-carbonyl)benzofuran-4,7-dione 3d.



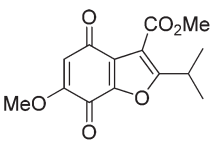
Prepared by the general procedure from 2-bromo-6-methoxybenzoquinone (0.217 g, 1.0 mmol), acetoacetylmorpholine (0.171 g, 1.0 mmol), copper(II) bromide (0.448 g, 2.0 mmol) and potassium carbonate (0.414 g, 3.0 mmol) stirred at reflux in acetonitrile (10 mL) for 5 h. Column chromatography eluting with ethyl acetate and light petroleum (1 : 1) gave the *title compound* as an orange solid (0.098 g, 32%), mp 168–170 °C; (found: M + Na$^+$, 328.0785. $C_{15}H_{15}NO_6Na$ requires: 328.0792); ν_{max} ($CHCl_3$)/cm^{-1} 3011, 1689, 1657, 1638; δ_H (400 MHz; $CDCl_3$) 5.83 (1 H, s, 5-H), 3.89 (3 H, s, OMe), 3.88–3.72 (5 H, m, CH_2), 3.58–3.52 (1 H, m, CH_2), 3.42–3.36 (1 H, m, CH_2), 3.32–3.26 (1 H, s, CH_2), 2.51 (3 H, s, 2-Me); δ_C (75 MHz; $CDCl_3$) 181.2, 168.8, 161.3, 159.7, 159.4, 147.8, 127.1, 115.3, 106.9 (CH), 66.6 (CH_2), 66.5 (CH_2), 57.0 (Me), 47.3 (CH_2), 42.4 (CH_2), 13.1 (Me); *m*/*z* (EI) 328 (M + Na$^+$, 100%).

Methyl 4,9-dihydro-2-methyl-4,9-dioxonaphtho[2,3-*b*]furan-3-carboxylate 3e.



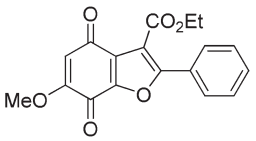
Prepared by the general procedure from 2-bromo-1,4-naphthoquinone (0.474 g, 2.0 mmol), methyl acetoacetate (0.232 g, 2.0 mmol), copper(II) bromide (0.670 g, 3.0 mmol) and potassium carbonate (0.828 g, 6.0 mmol) stirred at reflux in acetonitrile (20 mL) for 16 h. Column chromatography eluting with ethyl acetate and dichloromethane (1 : 49) gave the *title compound* as a yellow solid (0.158 g, 29%), mp 163–165 °C; (found: M + Na$^+$, 293.0409. $C_{15}H_{10}O_5Na$ requires: 293.0420); ν_{max} ($CHCl_3$)/cm^{-1} 3606, 3011, 1602; δ_H (400 MHz; $CDCl_3$) 8.23–8.21 (2 H, m, ArH), 7.80–7.77 (2 H, m, ArH), 4.01 (3 H, s, OMe), 2.75 (3 H, s, 2-Me); δ_C (75 MHz; $CDCl_3$) 178.8, 173.5, 164.8, 162.6, 151.4, 134.2 (CH), 133.7 (CH), 133.6, 131.5, 128.1, 127.4 (CH), 126.5 (CH), 113.3, 52.4 (Me), 14.2 (Me); *m*/*z* (EI) 293 (M + Na$^+$, 82%), 563 (2M + Na$^+$, 100).

Methyl 4,7-dihydro-2-isopropyl-6-methoxy-4,7-dioxobenzofuran-3-carboxylate 3f.



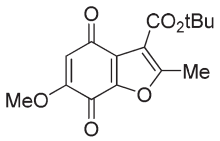
Prepared by the general procedure from 2-bromo-6-methoxybenzoquinone (0.434 g, 2.0 mmol), methyl 4-methyl-3-oxopentanoate (0.288 g, 2.0 mmol), copper(II) bromide (0.670 g, 3.0 mmol) and potassium carbonate (0.828 g, 6.0 mmol) stirred at reflux in acetonitrile (20 mL) for 4 h. Column chromatography eluting with ethyl acetate and dichloromethane (1 : 49) gave the *title compound* as a bright yellow solid (0.256 g, 46%), mp 163–165 °C; (found: M + Na^+, 301.0693. $C_{14}H_{14}O_6Na$ requires: 301.0683); ν_{max} ($CHCl_3$)/cm^{-1} 3606, 3048, 1690, 1661, 1603; δ_H (400 MHz; $CDCl_3$) 5.86 (1 H, s, 5-H), 3.96 (3 H, s, OMe), 3.88 (3 H, s, OMe), 3.66 (1 H, sept, *J* 7.0, CH), 1.38 (6 H, d, *J* 7.0, Me); δ_C (75 MHz; $CDCl_3$) 180.2, 172.1, 169.3, 162.5, 158.4, 148.4, 127.1, 111.4, 108.0 (CH), 56.9 (Me), 52.4 (Me), 27.9 (CH), 20.5 (Me); *m*/*z* (EI) 301 (100%).

Ethyl 4,7-dihydro-6-methoxy-4,7-dioxo-2-phenylbenzofuran-3-carboxylate 3g.



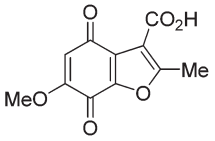
Prepared by the general procedure from 2-bromo-6-methoxybenzoquinone (0.217 g, 1.0 mmol), ethyl benzoylacetate (0.192 g, 1.0 mmol), copper(II) bromide (0.448 g, 2.0 mmol) and potassium carbonate (0.414 g, 3.0 mmol) stirred at reflux in acetonitrile (10 mL) for 4 h. Column chromatography eluting with ethyl acetate and light petroleum (1 : 3) gave the *title compound* as an orange solid (0.114 g, 35%), mp 154–156 °C; (found: M + Na^+, 349.0658. $C_{18}H_{14}O_6Na$ requires: 349.0688); ν_{max} ($CHCl_3$)/cm^{-1} 3012, 2985, 1730, 1689, 1662, 1606; δ_H (400 MHz; $CDCl_3$) 7.89 (2 H, m, ArH), 7.51 (3 H, m, ArH), 5.90 (1 H, s, 5-H), 4.49 (2 H, q, *J* 7.2, CH_2), 3.92 (3 H, s, OMe), 1.41 (3 H, t, *J* 7.2, Me); δ_C (75 MHz; $CDCl_3$) 180.3, 169.0, 162.8, 159.3, 158.7, 147.8, 131.1 (CH), 128.9 (CH), 128.6, 127.5 (CH), 113.0, 107.4 (CH), 62.4 (CH_2), 57.0 (Me), 13.9 (Me); one carbon unobserved; *m*/*z* (EI) 349 (M + Na^+, 100%).

***tert*-Butyl 4,7-dihydro-6-methoxy-2-methyl-4,7-dioxobenzofuran-3-carboxylate 3c.**



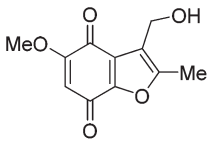
Prepared by the general procedure from 2-bromo-6-methoxybenzoquinone (0.217 g, 1.0 mmol), *tert*-butyl acetoacetate (0.158 g, 1.0 mmol), copper(II) bromide (0.448 g, 2.0 mmol) and potassium carbonate (0.414 g, 3.0 mmol) stirred at reflux in acetonitrile (10 mL) for 5 h. Column chromatography eluting with ethyl acetate and light petroleum (1 : 4) gave the *title compound* as a yellow solid (0.060 g, 21%), mp 152–154 °C; (found: M + Na^+, 315.0832. $C_{15}H_{15}O_6Na$ requires: 315.0839); ν_{max} ($CHCl_3$)/cm^{-1} 3011, 1721, 1688, 1663, 1610; δ_H (400 MHz; $CDCl_3$) 5.85 (1 H, s, 5-H), 3.88 (3 H, s, OMe), 2.67 (3 H, s, 2-Me), 1.64 (9 H, s, *t*Bu); δ_C (75 MHz; $CDCl_3$) 179.9, 169.5, 164.4, 160.9, 158.1, 148.4, 127.2, 115.1, 108.2 (CH), 82.7, 56.8 (Me), 28.3 (Me), 14.2 (Me); *m*/*z* (EI) 607 (2M + Na^+, 68%), 315 (100).

4,7-Dihydro-6-methoxy-2-methyl-4,7-dioxobenzofuran-3-carboxylic acid 4.



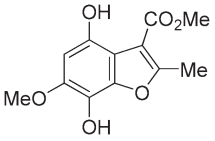
Trifluoroacetic acid (0.4 mL) was added to a stirred solution of **3c** (0.100 g, 0.34 mmol) in dichloromethane (4 mL), and the resulting mixture was stirred at room temperature for 18 h, washed with water (3 × 5 mL), dried (Na_2SO_4), filtered and concentrated. Flash column chromatography eluting with ethyl acetate gave the *title compound* as an orange solid (0.059 g, 73%), mp 165–167 °C; (found: M + Na^+, 259.0218. $C_{11}H_8O_6Na$ requires: 259.0213); ν_{max} ($CHCl_3$)/cm^{-1} 3044, 2699, 1738, 1698, 1636, 1574, 1324; δ_H (400 MHz; $CDCl_3$) 6.00 (1 H, s, 5-H), 3.98 (3 H, s, OMe), 2.86 (3 H, s, 2-Me); δ_C (75 MHz; $CDCl_3$) 185.8, 169.5, 168.0, 160.7, 147.3, 125.3, 112.9, 105.9 (CH), 57.7 (Me), 14.5 (Me); one carbon unobserved; *m*/*z* (EI) 259 (M + Na^+, 100%).

4,7-Dihydro-3-hydroxymethyl-5-methoxy-2-methylbenzofuran-4,7-dione 5a.



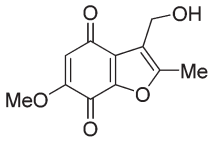
A solution of **3a** (75.0 mg, 0.30 mmol) in THF (3 mL) was added at 0 °C to a suspension of lithium aluminium hydride (57.0 mg, 1.50 mmol) in THF (6 mL) under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 1.5 h, and then acetone (0.72 mL) and a solution of ferric chloride (1 M; 0.36 mL) in hydrochloric acid (1 M; 0.36 mL) were added. The mixture was stirred at room temperature for 5 min and then extracted with dichloromethane (3 × 5 mL). The combined organic extracts were washed with water (10 mL), brine (10 mL), dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. The residue was purified by column chromatography using light petroleum–EtOAc (2 : 1) to give the *title compound* (19.8 mg, 30%) as an orange solid; mp 183 °C; (found: M + Na^+, 245.0424. $C_{11}H_{10}O_5$ + Na^+ requires: 245.0420); ν_{max} ($CHCl_3$)/cm^{-1} 2360, 1666, 1602, 1554, 1187; δ_H (400 MHz; $CDCl_3$) 5.79 (1 H, s, H-6), 4.62 (2 H, s, CH_2), 3.87 (3 H, s, OMe), 2.42 (3 H, s, 2-Me); δ_C (100 MHz; $CDCl_3$) 179.2, 175.3, 159.7, 154.9, 150.3, 125.8, 119.7, 105.9 (CH), 56.9 (Me), 54.8 (CH_2), 12.0 (Me); *m*/*z* (ESI) 245 (M + Na^+, 42%).

Methyl 4,7-dihydroxy-6-methoxy-2-methylbenzofuran-3-carboxylate 10b.



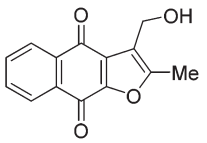
To a solution of **3b** (439 mg, 1.75 mmol) in chloroform (3.7 mL) was added a solution of sodium dithionite (1.84 g, 10.5 mmol) in water (7.3 mL). After being stirred at room temperature for 3 h, the precipitate formed was filtered and dried *in vacuo* to yield the *title compound* as a colourless solid (351 mg, 79%); mp 183–184 °C; (found: M + Na^+, 275.0525. $C_{12}H_{12}O_6$ + Na^+ requires: 275.0526); δ_H (400 MHz; DMSO-d_6) 9.78 (1 H, s, OH), 8.70 (1 H, s, OH), 6.41 (1 H, s, H-5), 3.94 (3 H, s, OMe), 3.78 (3 H, s, OMe), 2.66 (3 H, s, 2-Me); δ_C (100 MHz; DMSO-d_6) 167.5, 162.0, 146.5, 143.7, 141.4, 124.3, 108.1, 107.1, 96.7 (CH), 56.5 (Me), 53.0 (Me), 14.6 (Me); *m*/*z* (ESI) 275 (M + Na^+, 47%), 253 (M + H^+, 46%).

4,7-Dihydro-3-hydroxymethyl-6-methoxy-2-methylbenzofuran-4,7-dione 5b.



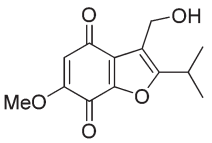
A solution of **10b** (96.8 mg, 0.38 mmol) in THF (3 mL) was added at 0 °C to a suspension of lithium aluminium hydride (75.0 mg, 1.98 mmol) in THF (6 mL). The reaction mixture was stirred at room temperature for 1.5 h, cooled to 0 °C and quenched by addition of water (0.15 mL), sodium hydroxide (1 M; 0.15 mL) and silica gel (150 mg). The granular precipitate was filtered off through a pad of Celite. The filtrate was dried (Na_2SO_4) and concentrated *in vacuo* to give the *title compound* as an orange solid (44.7 mg, 52%); mp 142–144 °C; (found: M + Na^+, 245.0424. $C_{11}H_{10}O_5$ + Na^+ requires: 245.0420); ν_{max} ($CHCl_3$)/cm^{-1} 3479, 3011, 1686, 1647, 1588, 1321, 1095; λ_{max} (acetonitrile)/nm 222 (log ε 4.30), 267 (log ε 4.06), 318 (log ε 3.99), 433 (log ε 3.05); δ_H (400 MHz; $CDCl_3$) 5.83 (1 H, s, H-5), 4.60 (2 H, s, CH_2), 3.88 (3 H, s, OMe), 2.43 (3 H, s, 2-Me); δ_C (100 MHz; $CDCl_3$) 184.6, 168.5, 160.1, 156.7, 148.2, 129.4, 121.0, 106.3 (CH), 57.1 (Me), 55.0 (CH_2), 12.3 (Me); *m*/*z* (ESI) 245 (M + Na^+, 42%).

4,9-Dihydro-3-hydroxymethyl-2-methylnaphtho[2,3-*b*]furan-4,9-dione 5c.



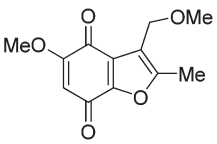
To a solution of **3e** (0.143 g, 0.53 mmol) in THF (10 mL) was added lithium aluminium hydride (0.101 g, 2.65 mmol). The resulting mixture was stirred at room temperature for 1.5 h, then quenched by addition of ethyl acetate (1 mL), water (1 mL) and silica gel (1 g). The mixture was stirred under air for 30 min, filtered and concentrated. Column chromatography of the residue eluting with ethyl acetate and dichloromethane (1 : 49) gave the *title compound* as a yellow solid (0.048 g, 38%), mp 166–168 °C; (found: M + Na^+, 265.0481. $C_{14}H_{10}O_4Na$ requires: 265.0471); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 3011, 1661, 1601; δ_H (400 MHz; $CDCl_3$) 8.25–8.19 (2 H, m, ArH), 7.81–7.77 (2 H, m, ArH), 4.71 (2 H, s, CH_2), 3.95 (1 H, br s, OH), 2.51 (3 H, s, 2-Me); δ_C (75 MHz; $CDCl_3$) 183.3, 173.0, 156.0, 151.6, 134.4 (CH), 133.8 (CH), 132.8, 132.5, 130.2, 127.1 (CH), 127.0 (CH), 120.7, 55.0 (CH_2), 12.3 (Me); *m*/*z* (EI) 265 (100%).

4,7-Dihydro-3-hydroxymethyl-2-isopropyl-6-methoxybenzofuran-4,7-dione 5d.



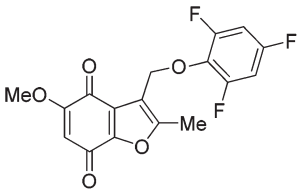
To a solution of **3f** (0.244 g, 0.88 mmol) in chloroform (5 mL) was added a solution of sodium dithionite (0.764 g, 4.39 mmol) in water (5 mL), and the mixture was stirred vigorously for 1 h. The aqueous phase was extracted with dichloromethane (3 × 5 mL), and the combined organic phases were dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in THF (10 mL) and lithium aluminium hydride (0.167 g, 4.39 mmol) was added. The resulting mixture was stirred at room temperature for 1.5 h, then quenched by addition of ethyl acetate (1 mL), water (1 mL) and silica gel (1 g). The mixture was stirred under air for 30 min, filtered and concentrated. Column chromatography of the residue eluting with ethyl acetate and dichloromethane (1 : 19) gave the *title compound* as an orange solid (0.096 g, 44%), mp 128–130 °C; (found: M + Na^+, 273.0711. $C_{13}H_{14}O_5Na$ requires: 273.0739); ν_{max} ($CHCl_3$)/cm^{-1} 3691, 3606, 1687, 1646, 1603; δ_H (400 MHz; $CDCl_3$) 5.85 (1 H, s 5-H), 4.64 (2 H, br s, CH_2), 3.95 (1 H, br s, OH), 3.91 (3 H, s, OMe), 3.20 (1 H, sept, *J* 7.0, CH), 1.38 (6 H, d, *J* 7.0, Me); δ_C (75 MHz; $CDCl_3$) 184.8, 168.4, 164.8, 160.2, 148.2, 129.5, 119.3, 106.3 (CH), 57.1 (Me), 54.7 (CH_2), 27.0 (CH), 21.0 (Me); *m*/*z* (EI) 273 (M + Na^+, 98%), 413 (100).

4,7-Dihydro-5-methoxy-3-methoxymethyl-2-methylbenzofuran-4,7-dione 6.



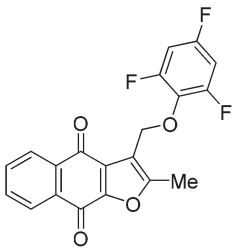
A mixture of **5a** (15.0 mg, 0.067 mmol), silver(I) oxide (23.4 mg, 0.10 mmol) and iodomethane (1 mL) was heated to reflux for 22 h and then filtered through Celite. The filter cake was washed with dichloromethane (5 mL) and the filtrate and washings were combined and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with light petroleum–ethyl acetate (1 : 1) to give the *title compound* as an orange solid (8.2 mg, 51%); mp 164–165 °C; (found: M + Na^+, 259.0549. $C_{12}H_{12}O_5$ + Na^+ requires: 259.0582); ν_{max} ($CHCl_3$)/cm^{-1} 1691, 1658, 1533, 1390, 1121, 1020, 842; λ_{max} (acetonitrile)/nm 225 (log ε 4.07), 259 (log ε 3.92), 310 (log ε 3.65), 426 (log ε 3.02); δ_H (400 MHz; $CDCl_3$) 5.75 (1 H, s, H-6), 4.54 (2 H, s, CH_2), 3.84 (3 H, s, OMe), 3.41 (3 H, s, OMe), 2.46 (3 H, s, 2-Me); δ_C (100 MHz; $CDCl_3$) 177.7, 175.5, 159.9, 157.6, 149.9, 125.2, 116.5, 105.4 (CH), 63.3 (CH_2), 58.5 (Me), 56.8 (Me), 12.3 (Me); *m*/*z* (ESI) 259 (M + Na^+, 100%).

4,7-Dihydro-3-(2,4,6-trifluorophenoxy)methyl-5-methoxy-2-methylbenzofuran-4,7-dione 7a.



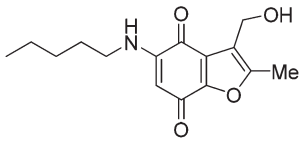
Thionyl chloride (0.64 mL, 8.87 mmol) was added at 0 °C to a solution of **5a** (37.5 mg, 0.17 mmol) in dichloromethane (2 mL). After being stirred at room temperature for 2 h, the solvent was removed *in vacuo* and the resulting residue was purified by column chromatography, eluting with light petroleum–ethyl acetate (2 : 1) to give 3-chloromethyl-5-methoxy-2-methylbenzofuran-4,7-dione as a yellow solid (23.5 mg, 58%). To the chloride (23.5 mg, 0.097 mmol) in anhydrous DMF (2 mL) was added sodium hydride (60% dispersion in mineral oils; 4.68 mg, 0.195 mmol) followed by 2,4,6-trifluorophenol (31.0 mg, 0.211 mmol) and the reaction mixture was stirred at room temperature for 5 h. Water (3 mL) was then added and the reaction mixture was extracted with ether (3 × 5 mL) and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was purified by column chromatography, eluting with light petroleum–ethyl acetate (2 : 1) to give the *title compound* as a yellow solid (20.1 mg, 58%); mp 124–125 °C; (found: M + Na^+, 375.0459. $C_{17}H_{11}F_3O_5$ + Na^+ requires: 375.0451); ν_{max} ($CHCl_3$)/cm^{-1} 2927, 2855, 1692, 1660, 1509, 1454, 1390, 1120, 1042, 842; δ_H (400 MHz; $CDCl_3$) 6.66 (2 H, t, *J* 8.0, ArH), 5.76 (1 H, s, H-6), 5.20 (2 H, s, CH_2), 3.84 (3 H, s, OMe), 2.46 (3 H, s, Me); δ_C (100 MHz; $CDCl_3$) 177.3, 175.4, 159.9, 159.0, 157.6 (dt, *J* 246, 15, CF), 156.3 (ddd, *J* 250, 14, 7, CF), 149.9, 147.3, 124.7, 114.8, 105.5 (CH), 100.8 (ddd, J_{CF} 27, 19, 8, CH), 64.7 (CH_2), 56.8 (Me), 12.0 (Me); *m*/*z* (ESI) 375 (M + Na^+, 100%).

4,9-Dihydro-2-methyl-3-(2,4,6-trifluorophenoxy)methylnaphtho-[2,3-*b*]furan-4,9-dione 7b.



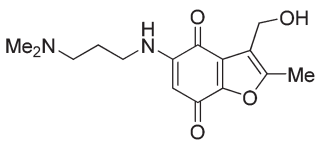
Thionyl chloride (0.105 mL, 1.45 mmol) was added to a stirred solution of **5c** (0.035 g, 0.14 mmol) in dichloromethane (2 mL), and the mixture was stirred at room temperature for 6 h, concentrated and the excess thionyl chloride was azeotroped with dichloromethane (2 mL). The residue was dissolved in DMF (2 mL), and 2,4,6-trifluorophenol (0.107 g, 0.72 mmol) and potassium carbonate (0.100 g, 0.72 mmol) were added sequentially. The mixture was stirred at room temperature for 16 h, diluted with water (20 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic phases were washed with water (10 mL), dried ($MgSO_4$), filtered and concentrated. Column chromatography of the residue eluting with dichloromethane and light petroleum (2 : 1) gave the *title compound* as a yellow solid (0.038 g, 70%), mp 174–176 °C; (found: M + Na^+, 395.0513. $C_{20}H_{11}F_3O_4Na$ requires: 395.0502); ν_{max} ($CHCl_3$)/cm^{-1} 3045, 3007, 1674, 1601, 1551, 1509; δ_H (400 MHz; $CDCl_3$) 8.24–8.15 (2 H, m, ArH), 7.78–7.75 (2 H, m, ArH), 6.70 (2 H, t, *J* 8.4, ArH), 5.36 (2 H, s, CH_2), 2.56 (3 H, s, Me); δ_C (75 MHz; $CDCl_3$) 181.4, 173.3, 160.4, 157.6 (dt, *J* 247, 15, CF), 156.4 (ddd, *J* 250, 15, 7, CF), 151.1, 133.9 (CH), 133.8 (CH), 133.2, 132.3, 131.5 (td, *J* 15, 5, C), 128.9, 126.9 (CH), 126.8 (CH), 115.7, 100.8 (td, *J* 27, 7, CH), 65.0 (CH_2), 12.4 (Me); *m*/*z* (EI) 395 (M + Na^+, 58%), 413 (100).

4,7-Dihydro-3-hydroxymethyl-2-methyl-5-(pentylamino)benzofuran-4,7-dione 8a.



Pentylamine (0.15 mL, 1.31 mmol) was added to a solution of **5a** (29.1 mg, 0.13 mmol) in anhydrous acetonitrile (1.5 mL) and the resulting mixture was stirred at room temperature for 45 min. The solvent was removed *in vacuo* and the crude product was purified by column chromatography on silica gel, previously inactivated with triethylamine, eluting with light petroleum–ethyl acetate (1 : 1) to give the *title compound* as a purple solid (23.4 mg, 64%); mp 102 °C (dichloromethane–light petroleum); (found: M + Na^+, 300.1199. $C_{15}H_{19}NO_4$ + Na^+ requires: 300.1212); ν_{max} ($CHCl_3$)/cm^{-1} 3491, 3007, 2961, 2863, 1671, 1633, 1591, 1467, 1390, 1190; λ_{max} (acetonitrile)/nm 214 (log ε 4.76), 245 (log ε 4.56), 341 (log ε 4.17), 518 (log ε 3.82); δ_H (400 MHz; $CDCl_3$) 5.84 (1 H, s br, NH), 5.28 (1 H, s, H-6), 4.59 (2 H, s, CH_2), 3.14 (2 H, q, *J* 6.9, CH_2), 2.38 (3 H, s, Me), 1.72–1.65 (2 H, quin, *J* 6.9, CH_2), 1.39–1.35 (4 H, m, 2 × CH_2), 0.93 (3 H, t, *J* 6.9, Me); δ_C (100 MHz; $CDCl_3$) 180.7, 175.1, 153.2, 152.9, 147.9, 123.5, 118.9, 95.8 (CH), 55.0 (CH_2), 43.0 (CH_2), 29.1 (CH_2), 27.8 (CH_2), 22.3 (CH_2), 13.9 (Me), 11.8 (Me); *m*/*z* (ESI) 300 (M + Na^+, 100%).

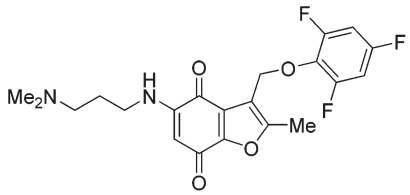
4,7-Dihydro-5-(3-(dimethylamino)propylamino)-3-(hydroxymethyl)-2-methylbenzofuran-4,7-dione 8b.



N,*N*-Dimethylpropane-1,3-diamine (0.16 mL, 1.28 mmol) was added to a solution of dione **5a** (28.5 mg, 0.13 mmol) in anhydrous acetonitrile (1.5 mL) and the resulting mixture was stirred at room temperature for 1 h. The solvent was removed *in vacuo* and the residue purified by column chromatography, eluting with methanol–dichloromethane (0–20%) to give the *title compound* as a purple solid (20.5 mg, 55%); mp 127–128 °C (dichloromethane–light petroleum); (found: M + H^+, 293.1495. $C_{15}H_{20}N_2O_4$ + H^+ requires: 293.1496); ν_{max}

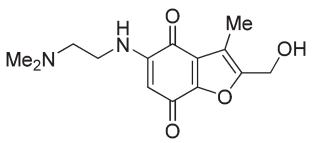
($CHCl_3$)/cm^{-1} 3053, 2360, 2341, 1733, 1716, 1698, 1192, 1007; λ_{max} (acetonitrile)/nm 215 (log ε 4.29), 246 (log ε 4.02), 343 (log ε 3.64), 315 (log ε 3.31); δ_H (400 MHz; $CDCl_3$) 7.83 (1 H, s br, NH), 5.23 (1 H, s, H-6), 4.59 (2 H, s, CH_2), 3.26 (2 H, q, *J* 6.0, CH_2), 2.53 (2 H, t, *J* 6.0, CH_2), 2.37 (3 H, s, Me), 2.34 (6 H, s, Me), 1.86 (2 H, quin, *J* 6.0, CH_2); δ_C (100 MHz; $CDCl_3$) 180.8, 175.1, 153.4, 152.6, 148.8, 123.5, 118.9, 95.2 (CH), 57.9 (CH_2), 55.0 (CH_2), 45.0 (Me), 42.9 (CH_2), 24.2 (CH_2), 11.8 (Me); *m*/*z* (ESI) 293 ($M + H^+$, 100%).

4,7-Dihydro-3-(2,4,6-trifluorophenoxy)methyl-5-(3-(dimethylamino)propylamino)-2-methylbenzofuran-4,7-dione 8c.



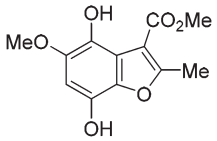
To a solution of **7a** (20.1 mg, 0.057 mmol) in anhydrous acetonitrile (1 mL) was added *N*,*N*-dimethylpropane-1,3-diamine (0.071 mL, 0.57 mmol) and the resulting mixture was stirred at room temperature for 1 h. The solvent was removed *in vacuo* and the crude product was purified by column chromatography, eluting with methanol–dichloromethane (0–10%) to give the *title compound* as a purple solid (8.4 mg, 35%); mp 60–62 °C; (found: $M + H^+$, 423.1546. $C_{21}H_{21}F_3N_2O_4 + H^+$ requires: 423.1526); ν_{max} ($CHCl_3$)/cm^{-1} 3693, 3639, 2360, 2338, 1658, 1631, 1601, 1120; λ_{max} (acetonitrile)/nm 212 (log ε 4.05), 246 (log ε 3.74), 336 (log ε 3.39), 507 (log ε 3.03); δ_H (400 MHz; $CDCl_3$) 7.50 (1 H, s br, N-H), 6.65 (2 H, t, *J* 8.4, ArH), 5.23 (1 H, s, H-6), 5.17 (2 H, s, CH_2), 3.22 (2 H, q, *J* 6.0, CH_2), 2.45 (2 H, t, *J* 6.0, CH_2), 2.38 (3 H, s, Me), 2.28 (6 H, s, Me), 1.82 (2 H, quin, *J* 6.0, CH_2); δ_C (125 MHz; $CDCl_3$) 178.9, 175.1, 157.5 (dt, J_{CF} 245, 14, CF), 156.4 (ddd, J_{CF} 249, 15, 8, CF), 156.9, 152.9, 149.0, 131.3 (dt, J_{CF} 15, 5, C), 122.4, 113.8, 100.7 (ddd, J_{CF} 26, 20, 7, CH), 94.9 (CH), 65.0 (CH_2), 57.9 (CH_2), 45.2 (2Me), 42.8 (CH_2), 24.6 (CH_2), 11.7 (Me); *m*/*z* (ESI) 423 ($M + H^+$, 100%).

4,7-Dihydro-5-(2-(dimethylamino)ethylamino)-2-(hydroxymethyl)-3-methylbenzofuran-4,7-dione 8d.



To a solution of 4,7-dihydro-2-(hydroxymethyl)-5-methoxy-3-methylbenzofuran-4,7-dione[38] (50.0 mg, 0.225 mmol) in anhydrous acetonitrile (3 mL) was added *N*,*N*-dimethylethane-1,2-diamine (0.24 mL, 2.25 mmol). After being stirred for 3 h at room temperature, the solvent was removed *in vacuo* and the crude product purified by column chromatography, eluting with dichloromethane to dichloromethane–methanol (9 : 1) to give the *title compound* as a purple solid (62.5 mg, quantitative yield); mp 146–147 °C (dichloromethane–*n*-hexane); (found: $M + H^+$, 279.1341. $C_{14}H_{18}N_2O_4 + H^+$ requires: 279.1339); ν_{max} ($CHCl_3$)/cm^{-1} 3352, 1687, 1632, 1589, 1381, 1006; λ_{max} (acetonitrile)/nm 214 (log ε 4.44), 246 (log ε 4.22), 341 (log ε 3.78), 507.2 (log ε 3.54); δ_H (400 MHz; $CDCl_3$) 6.48 (1 H, s br, NH), 5.24 (1 H, s, H-6), 4.63 (2 H, s, CH_2), 3.15 (2 H, q, *J* 5.7, CH_2), 2.61 (2 H, t, *J* 5.7, CH_2), 2.30 (6 H, s, Me), 2.26 (3 H, s, Me); δ_C (100 MHz; $CDCl_3$) 179.1, 175.3, 153.9, 153.2, 148.5, 123.3, 117.3, 96.1 (CH), 56.4 (CH_2), 54.8 (CH_2), 45.1 (CH_3), 39.9 (CH_2), 8.5 (Me); *m*/*z* (ESI) 279 ($M + H^+$, 100%).

Methyl 4,7-dihydroxy-6-methoxy-2-methylbenzofuran-3-carboxylate 10a.



To a solution of **3a** (200 mg, 0.80 mmol) in chloroform (4 mL) was added a solution of sodium dithionite (835 mg, 4.80 mmol) in water (8 mL) and the reaction mixture was stirred at room temperature for 3 h. The precipitate formed was filtered and dried *in vacuo* to give the *title compound* as a colourless solid (161 mg, 80%); mp 238–239 °C; (found: $M + Na^+$, 275.0527. $C_{12}H_{12}O_6Na^+$ requires: 275.0526); δ_H (400 MHz; DMSO-d_6) 9.64 (1 H, s, OH), 9.52 (1 H, s, OH), 6.52 (1 H, s, H-6), 3.94 (3 H, s, OMe), 3.72 (3 H, s, OMe), 2.67 (3 H, s, Me); δ_C (100 MHz, DMSO-d_6) 167.5, 163.3, 142.6, 136.9, 133.8, 131.1, 114.8, 108.5, 100.8 (CH), 57.0 (Me), 53.0 (Me), 14.9 (Me); *m*/*z* (ESI) 527 ($2M + Na^+$, 100%), 275 ($M + Na^+$, 40).

Biology

IDO assay. IDO inhibitors were assayed as previously described.[47] Briefly, the oxidative cleavage of L-tryptophan catalyzed by rhIDO, expressed in *E. coli* and purified using Ni^{2+} affinity chromatography as described,[46] was measured as described previously by Takikawa *et al.*,[48] and modified by Austin *et al.*[46] Reactions (0.25 mL) were performed in 50 mM potassium phosphate buffer pH 7.4, containing 20 mM ascorbic acid, 10 μM methylene blue, 0.4 mg mL^{-1} catalase and 4 μg mL^{-1} rhIDO. DMSO or inhibitor dissolved in DMSO was added for 10 min then reactions were initiated by the addition of 400 μM L-tryptophan. After 30 min at 37 °C, reactions were terminated by the addition of 100 μL of 30% (w/v) trichloroacetic acid. Samples were heated to 65 °C for 15 min and then centrifuged at 13k rpm for 5 min. Supernatant (100 μL) was transferred to a 96-well plate and 100 μL of 4-dimethylaminobenzaldehyde (Ehrlich's reagent, 2% in acetic acid) was added to each well. After 2 min, the absorbance was determined using a microplate reader at 490 nm. Results were quantified against a standard curve generated using authentic kynurenine. Final results were calculated as percent of DMSO treated controls. IC_{50} values were calculated using GraphPad Prism 4.0 using 6–8 inhibitor concentrations (0.03–100 μM) from three determinations.

Analysis of quinone-induced toxicity

BxPc-3 human pancreatic cancer cells were obtained from ATCC and grown on glass coverslips in RPMI1640

supplemented with 10% (v/v) fetal bovine serum, 100 units mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. To determine if quinones induce toxicity by damaging microtubules or the actin cytoskeleton, BxPx-3 cells were treated with quinones **5a** and **16** (10 μM) in 2 mL of complete medium for 4 h. The redox cycling quinone β-lapachone (2.5 μM × 4 h) was included as a positive control. Immediately following treatment with quinones cells were fixed in 3.5% (v/v) formaldehyde in phosphate buffered saline for 12 min followed by 0.15% (v/v) Triton X-100 diluted in phosphate buffered saline for 10 min. Cells were then blocked in RPMI1640 supplemented with 10% (v/v) fetal bovine serum for 1 h at room temperature. Immunostaining for microtubules was performed using anti-α-tubulin antibodies (Abcam) diluted 1 : 1000 for 1 h followed by Alexafluor 594-labeled goat anti-mouse IgG secondary antibody (1 : 1000) for 30 min. Staining for F-actin was performed using Alexa488 conjugated phalloidin (Invitrogen) diluted to 200 units mL^{-1} for 30 min at room temperature. To detect the presence of intracellular oxidative stress cells were treated with quinones as described above and the fluorogenic probe CellROX green (Invitrogen, 20 μL per coverslip) was added for the final 30 min of quinone exposure. Following oxidation by superoxide CellROX green, which is a dihydroethidium analogue, intercalates into DNA and then can be observed in the nucleus following excitation at 488 nm. Imaging was performed using a Nikon C1 confocal system at 900× magnification.

Cytotoxicity assay. BxPc-3 cells (5×10^5) were seeded onto 100 mm plates in complete medium and allowed to attach for 16 h. Cells were then treated with quinones for 4 h after which the cells were gently scrapped off and collected by centrifugation at 800 rpm for 5 min. Cells were resuspended in phosphate buffered saline containing 0.2% (v/v) trypan blue for 15 min. The percentage of trypan blue positive cells was determined using a hemocytometer.

Acknowledgements

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