Synthesis of antitumour (1*H*-1,2,3-triazol-4-yl)-4-hydroxycyclohexa-2,5-dien-1-ones by copper-catalysed Huisgen cycloadditions†

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4-Ethynyl-4-hydroxycyclohexa-2,5-dien-1-one **5** undergoes cycloaddition reactions with a range of substituted azides in the presence of copper salts to form 1,4-disubstituted triazoles **8–11** bearing the 4-hydroxycyclohexa-2,5-dien-1-one (quinol) pharmacophore; one example of an isomeric 1,5-disubstituted triazole **12** was formed from **5** and benzyl azide in the presence of a ruthenium catalyst. Compounds were screened for growth-inhibitory activity against five cancer cell lines of colon, breast and lung origin, but were overall less potent than the benzothiazolyl- and indolyl-substituted quinols **2** and **3**.

Introduction

Our interest in exploring the oxidation of simple phenols (*eg* **1**) as a source of novel antitumour agents has been vindicated in the identification of the 4-substituted 4-hydroxycyclohexa-2,5-dien-1-one moiety ('quinol') as an unexploited pharmacophore in drug discovery. Thus, oxidation of **1** with [(diacetoxy)iodo]benzene (DAIB) in aqueous acetonitrile in the presence of TEMPO yielded the prototypic benzothiazole-substituted quinol **2** (52%) (Fig. 1), which displayed potent and selective antitumour activities against colon, renal, and breast cancer cell lines *in vitro*.[2] We have identified the redox homeostasis-controlling protein thioredoxin (Trx)[3] as being an important molecular target.[3] Subsequently, we have identified a second, but more potent, series of indolyl-quinols exemplified by the 6-fluoro-1-(phenylsulfonyl)indolylquinol **3**, which maintains the selectivity fingerprint against colon, renal and breast cell lines *in vitro*[4] but preferentially targets the protein thioredoxin reductase (TrxR).[5] Significantly, microarray analyses of untreated human colorectal HCT 116 cells and those exposed to **2** (1 μmol L^{-1}) determined that, in a panel of over 10K cancer-related genes, expression of only TrxR was upregulated > 3-fold.[3] As expected for agents inhibiting the thioredoxin-signalling pathway,[6] compound **2** also displays anti-angiogenic properties in proliferating endothelial cells;[7] variant **3** also perturbs signalling events modulated by downstream effectors of the tumour angiogenesis process triggered by cellular hypoxia (*eg* Hif-1 and VEGf).[8]

In earlier work, we established a preliminary SAR for substituents at the 4-position in the quinols series regarding their potency as inhibitors of cancer cell growth *in vitro* (Fig. 2). Hydrogen is preferred at position R rather than acyl or alkyl.[2] At position R^1 a 6/5 heterocyclic (*e.g.* benzothiazole in **2** or indole in **3**) is preferred over a 6/6 heterobicyclic (*e.g.* quinoline) or a monocyclic system (*e.g.* benzene or pyridine).[2] In this paper, we report an extension of these studies in which we have examined the consequences of attaching a bulky aryl or alkyl group to a 1,2,3-triazole substituent at R^1 (compounds **8**) in anticipation that these modifications might display the desirable properties of quinols **2** and **3**.

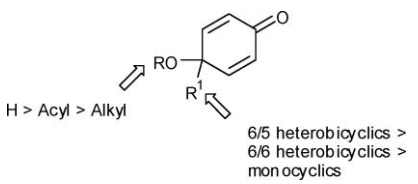

Fig. 2 Summary of SAR in antitumour quinols .

We have shown previously that quinol **3** can be prepared efficiently in a Sonogashira coupling between the phenylsulfonyl-substituted fluoroiodoaniline **4** and the 4-alkynylquinol **5**.[9] The

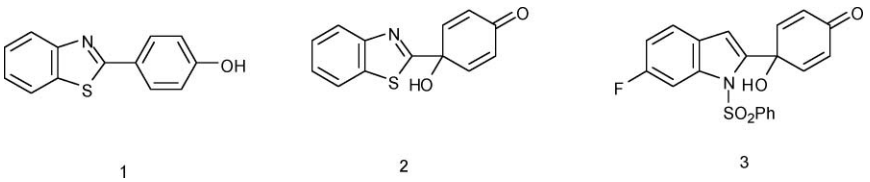

Fig. 1 Structures of 2-(4-hydroxyphenyl)benzothiazole **1** and antitumour 4-substituted-4-hydroxycyclohexa-2,5-dien-1-ones ('quinols') **2** and **3**.

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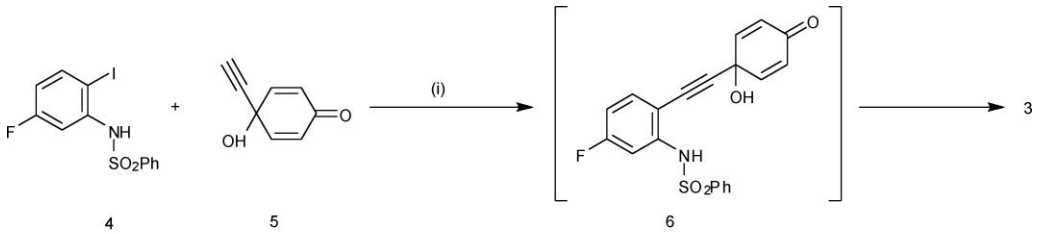


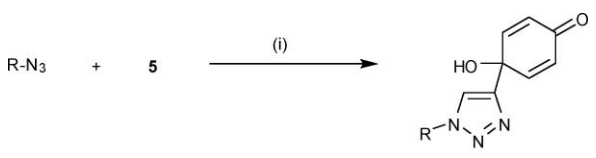
[a]Reagents: (i) $Pd(PPh_3)_4$, CuI, *i*-Pr_2NH, DMAC, H_2O.

Scheme 1 Synthesis of compound **3** by a Sonogashira reaction[a].

(presumed) intermediate alkynyl-ol **6** cyclises spontaneously to **3** under the reaction conditions (Scheme 1). We were interested to explore if the acetylene fragment of **5** was receptive to other heterocycle-forming cyclizations, such as the generation of 1,2,3-triazoles *via* the copper-catalysed Huisgen cycloaddition with azides (a 'click' synthesis) which has found numerous applications in a range of chemical/biological scenarios.[10] In the current case, outcomes of the proposed syntheses are non-obvious for two reasons: the alkyne group in the quinol substrates is attached to a quaternary carbon bearing an alcohol moiety; and the cyclohexadiene double bonds are potentially available for competing [3+2] cycloadditions with an azide reagent.

Results and discussion

A range of aryl azides (**7a–i**), prepared by diazotisation of various anilines followed by treatment with sodium azide, were for the most part used in the cycloaddition reactions without further purification. The general 'click' procedure (Method A) involved mixing the appropriate azide and alkyne-ol **5** in a mixture of *t*-BuOH and water containing 1 M sodium ascorbate solution and $CuSO_4 \cdot 5H_2O$ at 25 °C (Scheme 2). Cycloadditions proceeded efficiently in the majority of cases, furnishing analytically pure triazoles **8a–i** on addition of water to the reaction mixture (with the exception of **8i**, which required further chromatographic purification). 4-Azidopyridine **7j**, prepared from 4-chloropyridine and sodium azide, similarly gave the triazole **8j**, but 4-nitrophenyl azide failed to participate in the cycloaddition reaction under the standard conditions, as did 4-azido-2-hydroxybenzoic acid and 2-(4-azidophenyl)-6-methyl-benzothiazole.



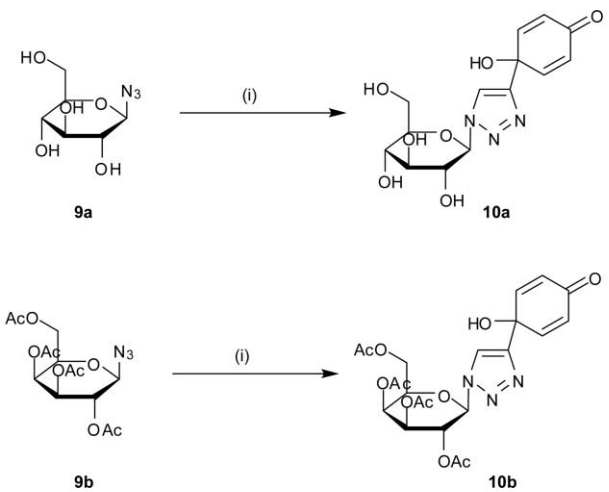
	R		R
a	Ph	**b**	4-BrC_6H_4
c	4-FC_6H_4	**d**	3-$CF_3C_6H_4$
e	4-$CF_3C_6H_4$	**f**	4-$MeOC_6H_4$
g	4-AcC_6H_4	**h**	4-*n*-BuC_6H_4
i	Benzothiazol-5-yl	**j**	Pyridin-4-yl
k	*n*-$C_{10}H_{21}$	**l**	Bn
m	$C_6H_5(CH_2)_2CH_2$	**n**	4-$MeOC_6H_4CH_2$

[a] Reagents: (i) $CuSO_4.5H_2O$, sodium ascorbate, *t*-BuOH, water, 16 h.

Scheme 2 Synthesis of triazoles **8a–n**[a].

Owing to the potential hazards of purifying aryl azides, particularly those of low molecular weight, we investigated a one-pot route to triazole **8a**, avoiding the isolation of phenyl azide **7a** by generating it *in situ* from phenyl iodide.[11] However, this was an inefficient process leading to a low yield (7%) of product.

Examples of alkyl and aralkyl azides **7k–n** (prepared from the corresponding bromides and sodium azide) were also effective substrates for the cycloadditions (Scheme 2), leading to triazoles **8k–n** in excellent yields, although 6-azidohexanoic acid failed to give the desired triazole. To further expand the broad applicability of the click reaction in the quinols series, azido sugars **9a,b** (which are highly-modified alkyl azides) were employed in the cycloaddition with alkyne-ol **5**, although the highly polar pyranyl-substituted triazole **10a** (Scheme 3), which contains primary, secondary and tertiary alcoholic functionalities, was hygroscopic and unsuitable for biological evaluation. Different reaction conditions, a mixture of Cu(I) iodide in chloroform containing 2,6-lutidine (Method B), were required to facilitate cycloaddition of arylsulfonyl azides and alkynyl-ol **5**. The arylsulfonyl-substituted triazoles **11a,b** (Fig. 3) were obtained in moderate yields.



[a] Reagents: (i) $CuSO_4.5H_2O$, sodium ascorbate, *t*-BuOH, water, 16 h.

Scheme 3 Synthesis of triazoles **10a,b**[a].

While the 1,4-disubstituted triazoles **8** are readily straightforward to obtain and isolate, we were also interested in the preparation of an isomeric 1,5-disubstituted analogue. Recent reports

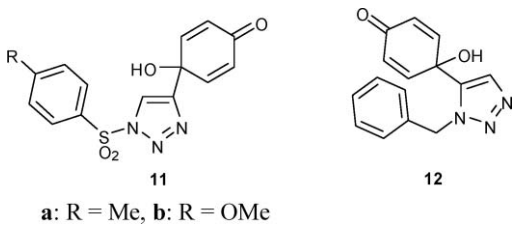
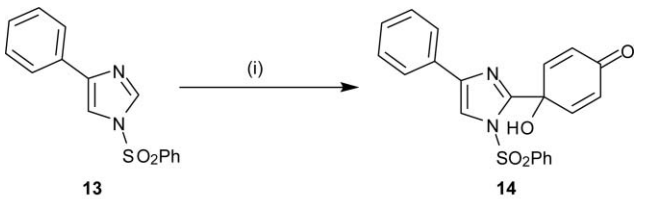


Fig. 3 Structures of **11** and **12**.



Reagents: (i) *n*-BuLi, THF, -78 ºC, then 4,4-dimethoxycyclohexa-2,5-dienone, followed by NH_4Cl (aq).

Scheme 4 Synthesis of imidazole **14**.

described the preparation of such triazoles from azides and alkynes using a ruthenium catalyst $Cp^*RuCl(PPh_3)_2$.[12] The variation in the regioselectivity from the copper-catalysed reaction is due to mechanistic differences—a copper acetylide is formed leading to 1,4-disubstituted triazoles, whereas a ruthenium acetylide is not[12b]—and the solvent of choice is benzene, but work-up is less straightforward. However, benzyl azide and alkyne **5** were successfully combined to yield the desired triazole **12** (Fig. 3), albeit in poor yield (<10%).

The substituted imidazole **14**, which is a modified analogue of **3** that also contains structural similarities to the triazoles, was prepared by the standard lithiation route[2] from precursor **13** shown in Scheme 4.

Biological results and discussion

The growth-inhibitory activities of new compounds were evaluated against a panel of two human colon cancer cell lines (HCT 116 and HT 29), two mammary cell lines (MCF-7 and MDA 468) and the generally chemoresistant lung adenocarcinoma line (A549). Results of MTT assays following 72 h drug exposure are collated in Table 1. The dramatic differences in potency between the phenol **1** and the highly growth-inhibitory benchmark quinols **2** and **3** have been noted previously.[4] The HCT 116 cell line was most sensitive to compounds of the series **8**; the least sensitive was the A549 line, which is generally more resistant to compounds of the quinols class.[2,4,9] Against HCT 116 cells, GI_{50} values were in the sub-micromolar range, with the exception of the pyrido-analog **8j**, which was approximately 10-fold less active. Against the breast cell lines there were no notable potency differences of compounds **8** against MCF-7 (ER^+) and MDA 468 (ER^-). Disappointingly, the acetylated pyranyltriazole-substituted quinol **10b** and the arylsulfonyltriazolyl-quinols **11a,b** were generally about 100-fold less growth-inhibitory than compounds in series **8**. There were no notable differences between the potencies of the 1,4-(**8l**) and 1,5-(**12**) triazole regioisomers across the cell panel. The two most active compounds amongst the new quinols in these MTT assays were the decyl-substituted triazole **8k** and the phenylsulfonyl-imidazole **14**, but neither displayed the nanomolar growth-inhibitory potency of **2** and **3** against the HCT 116 cell line (Table 1).

Table 1 *In vitro* Growth-inhibitory potencies (GI_{50} values in μm)[a] of triazolyl- and imidazolyl-substituted 4-hydroxycyclohexa-2,5-dien-1-ones against cancer cell lines[b]

Compound	HCT116	HT29	MCF-7	MDA 468	A549
1[c]	> 100	83.7	69.4	0.40	NT
2[c]	0.04	0.38	0.35	0.79	2.35
3	0.07	0.26	0.21	0.24	0.86
8a	0.36	0.67	0.70	0.49	2.34
8b	0.26	0.38	0.36	0.44	2.01
8c	0.56	0.78	0.68	0.56	3.36
8d	0.30	0.51	0.50	0.40	2.49
8e	0.26	0.53	0.46	0.42	1.46
8f	0.56	1.48	0.75	1.03	3.95
8g	0.38	0.80	0.62	0.75	3.00
8h	0.18	0.25	0.33	0.35	0.58
8i	0.27	0.40	0.36	0.43	1.24
8j	2.17	4.21	1.97	1.65	4.19
8k	0.24	0.36	0.22	0.23	0.74
8l	0.94	1.83	1.04	1.02	2.52
8m	0.48	1.47	0.37	0.99	3.24
8n	0.48	1.88	0.93	1.10	1.44
10b	51.7	81.6	44.3	37.5	NT
11a	8.16	26.4	10.8	12.0	43.7
11b	14.6	28.2	17.4	11.9	39.1
12	0.74	1.74	0.45	2.57	4.41
14	0.23	0.37	0.30	0.36	1.36

[a] Results are the mean of 3 determinations in MTT assays (see Experimental section). [b] Cancer line cell origin: HCT116 and HT29 (colon); MCF-7 (ER^+ breast); MDA468 (ER^- breast); A549 (lung). [c] Results from ref. 2. NT is not tested.

Certain 1-aryltriazolyl-quinols, **8b,c,f,g** and the 1-(4-methoxybenzyl)triazolyl-quinol **8n**, were also evaluated in the NCI60 *in vitro* cell screen, which involves a 2 day drug exposure.[13] The mean GI_{50} values were in the range 0.5–1.0 μM, whereas the equivalent values for **2** and **3** were 0.23 and 0.016 μM, respectively.[4] These results confirm our earlier SAR analysis of the activities of antitumour quinols: a bicyclic heterocyclic nucleus at the cyclohexadienone 4-position (*e.g.* benzothiazole in **2** and substituted indole in **3**) is preferred to a monocyclic residue.[2,4]

Experimental section

Melting points were recorded on Stuart Scientific SMP3 apparatus, and are uncorrected. IR spectra were recorded on a Shimadzu FT-IR8400S, and NMR spectra were recorded on a Bruker Avance 400 instrument at 400.13 MHz (^{1}H) and 100.62 MHz (^{13}C) in [$^2H_6$]DMSO) or $CDCl_3$; coupling constants are in Hz. Compounds that did not pass CHN Microanalysis were shown to be >95% pure using LC/MS. The LC/MS system consisted of an Agilent Technologies 1200 series LC connected to a 6110 Single Quadrupole MS with ESI source. Merck silica gel 60 (40–60 μm) was used for column chromatography. Azides were prepared according to literature methods.[14,15]

General method for the synthesis of 1,4-disubstituted 1,2,3-triazoles 8a–n, 10a,b (Method A)

To a stirred mixture of the azide (3 mmol) and 4-ethynyl-4-hydroxycyclohexa-2,5-dien-1-one **5** (0.402 g; 3 mmol) in *t*-BuOH (6 mL) and water (6 mL) was added 1 M aqueous sodium ascorbate solution (300 μL; 0.3 mmol), followed by copper sulfate pentahydrate (7.5 mg, 0.03 mmol, in 100 μL of water). The mixture was stirred overnight, diluted with water (50 mL) and filtered. The precipitate was washed with water, and dried under vacuum to afford the 4-substituted-4-hydroxycyclohexa-2,5-dien-1-ones **8a–n**, **10a,b**.

4-Hydroxy-4-(1-phenyl-1*H*-1,2,3-triazol-4-yl)cyclohexa-2,5-dienone 8a

By Method A, to give **8a** as white needles (0.640 g; 83%), mp 145–146 °C. ν_{max}/cm^{-1} 1663, 1624. δ_H (DMSO-d_6) 6.22 (2H, d, *J* 10.1, HC=), 6.63 (1H, s, OH), 7.23 (2H, d, *J* 10.1, HC=), 7.48-7.52 (1H, m, ArH), 7.58-7.62 (2H, m, ArH), 7.90-7.93 (2H, m, ArH), 8.93 (1H, s, CHN). δ_C (DMSO-d_6) 65.7, 120.6, 121.7, 126.8, 129.3, 130.4, 137.0, 149.7, 150.9, 185.6. MS (ESI) *m*/*z* 254 (M+H)$^+$.

4-(1-(4-Bromophenyl)-1*H*-1,2,3-triazol-4-yl)-4-hydroxycyclohexa-2,5-dienone 8b

By Method A, to give **8b** as a beige powder (0.733 g; 74%), mp 176-177 °C. Found C 50.1, H 3.0, N 12.3. Calc. for $C_{14}H_{10}BrN_3O_2$ C 50.6, H 3.0, N 12.7%. ν_{max}/cm^{-1} 1664, 1624, 1495. δ_H (DMSO-d_6) 6.24 (2H, d, *J* 10.1, HC=), 6.68 (1H, s, OH), 7.23 (2H, d, *J* 10.1, HC=), 7.82 (2H, d, *J* 8.9, ArH), 7.92 (2H, d, *J* 8.9, ArH), 8.99 (1H, s, CHN). δ_C (DMSO-d_6) 65.7, 121.8, 121.9, 122.5, 126.9, 133.3, 136.2, 149.8, 150.8, 185.6. MS (ESI) *m*/*z* 332 (M+H)$^+$.

4-(1-(4-Fluorophenyl)-1*H*-1,2,3-triazol-4-yl)-4-hydroxycyclohexa-2,5-dienone 8c

By Method A, to give **8c** as a beige powder (0.600 g; 95%), mp 173-178 °C. Found C 62.0, H 3.6, N 15.3. Calc. for $C_{14}H_{10}FN_3O_2$ C 62.0, H 3.7, N 15.5%. ν_{max}/cm^{-1} 1670, 1629, 1514. δ_H (DMSO-d_6) 3.46 (1H, s, OH), 6.22 (2H, d, *J* 10.1, HC=), 7.22 (2H, d, *J* 10.1, HC=), 7.45 (2H, t, *J* 8.8, ArH), 7.97 (2H, dd, *J* 9.1, 4.7, ArH), 7.90 (1H, s, CHN). δ_C (DMSO-d_6) 65.7, 117.2 (d, *J* 24.2), 122.0, 123.0 (d, *J* 8.8), 126.9, 133.6 (d, *J* 2.8), 149.7, 150.9, 162.2 (d, *J* 345.8), 185.6. MS (ESI) *m*/*z* 272 (M+H)$^+$.

4-Hydroxy-4-(1-(3-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)cyclohexa-2,5-dienone 8d

By Method A, to give **8d** as a beige powder (0.872 g; 91%), mp 165-166 °C. Found C 56.0, H 3.1, N 12.8. Calc. for $C_{15}H_{10}F_3N_3O_2$ C 56.1, H 3.1, N 13.1%. ν_{max}/cm^{-1} 1672, 1630, 1618. δ_H (DMSO-d_6) 6.24 (2H, d, *J* 10.1, HC=), 6.70 (1H, s, OH), 7.23 (2H, d, *J* 10.1, HC=), 7.83-7.89 (2H, m, ArH), 8.28-8.32 (2H, m, ArH), 9.14 (1H, s, CHN). δ_C (DMSO-d_6) 65.7, 117.3 (q, *J* 3.8), 122.1, 124.0 (q, *J* 272.6), 124.5, 125.8 (q, *J* 3.6), 126.9, 131.4 (q, *J* 32.6), 131.8, 137.5, 149.9, 150.7, 185.6. MS (ESI) *m*/*z* 322 (M+H)$^+$.

4-Hydroxy-4-(1-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)cyclohexa-2,5-dienone 8e

By Method A, to give **8e** as a beige powder (0.820 g; 85%), mp 173-175 °C. Found C 56.1, H 3.1, N 12.9. Calc. for $C_{15}H_{10}F_3N_3O_2$ C 56.1, H 3.1, N 13.1%. ν_{max}/cm^{-1} 1668, 1628. δ_H (DMSO-d_6) 6.24 (2H, d, *J* 10.1, HC=), 6.72 (1H, s, OH), 7.23 (2H, d, *J* 10.1, HC=), 8.00 (2H, d, *J* 8.6, ArH), 8.20 (2H, d, *J* 8.6, ArH), 9.11 (1H, s, CHN). MS (ESI) *m*/*z* 322 (M+H)$^+$.

4-(1-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)-4-hydroxycyclohexa-2,5-dienone 8f

By Method A, to give **8f** as an off-white powder (0.600 g; 72%), mp 136-137 °C. Found C 63.2, H 4.6, N 14.7. Calc. for $C_{15}H_{13}N_3O_3$ C 63.6, H 4.6, N 14.8%. ν_{max}/cm^{-1} 1670, 1512 δ_H ($CDCl_3$) 3.59 (1H, s, OH), 3.89 (3H, s, OMe), 6.32 (2H, d, *J* 10.1, HC=), 7.04 (2H, d, *J* 9.1, ArH), 7.13 (2H, d, *J* 10.1, HC=), 7.62 (2H, d, *J* 9.1, ArH), 7.85 (1H, s, CHN). δ_C (DMSO-d_6) 56.0, 65.7, 115.3, 121.7, 122.3, 126.8, 130.4, 149.4, 150.9, 159.8, 185.6. MS (ESI) *m*/*z* 284 (M+H)$^+$.

4-(1-(4-Acetylphenyl)-1*H*-1,2,3-triazol-4-yl)-4-hydroxycyclohexa-2,5-dienone 8g

By Method A, to give **8g** as a beige powder (0.550 g; 62%), mp 167-170 °C. Found C 64.3, H 4.4, N 14.1. Calc. for $C_{16}H_{13}N_3O_3$ C 65.1, H 4.4, N 14.2%. ν_{max}/cm^{-1} 1678, 1668, 1604. δ_H (DMSO-d_6) 2.65 (3H, s, CH_3), 6.24 (2H, d, *J* 10.1, HC=), 6.72 (1H, s, OH), 7.24 (2H, d, *J* 10.1, HC=), 8.12 (2H, d, *J* 8.9, ArH), 8.18 (2H, d, *J* 8.9, ArH), 9.11 (1H, s, CHN). δ_C (DMSO-d_6) 27.4, 65.7, 120.3, 121.9, 127.0, 130.6, 140.0, 150.0, 150.8, 185.6, 197.4. MS (ESI) *m*/*z* 296 (M+H)$^+$.

4-(1-(4-Butylphenyl)-1*H*-1,2,3-triazol-4-yl)-4-hydroxycyclohexa-2,5-dienone 8h

By Method A, to give **8h** as a beige powder (0.600 g; 95%), mp 162-163 °C. Found C 69.5, H 6.2, N 13.5. Calc. for $C_{18}H_{15}N_3O_2$ C 69.9, H 6.2, N 13.5%. ν_{max}/cm^{-1} 1668, 1628, 1518. δ_H (DMSO-d_6) 0.92 (3H, t, *J* 7.4, CH_3), 1.31-1.36 (2H, m, CH_2), 1.56-1.62 (2H, m, CH_2), 2.67 (2H, t, *J* 7.7, $ArCH_2$-), 6.23 (2H, d, *J* 10.1, HC=), 6.67 (1H, s, OH), 7.25 (2H, d, *J* 10.1, HC=), 7.42 (2H, d, *J* 8.5, ArH), 7.82 (2H, d, *J* 8.5, ArH), 8.90 (1H, s, CHN). δ_C (DMSO-d_6) 14.2, 22.2, 33.4, 34.7, 65.7, 120.5, 121.6, 126.8, 130.1, 134.9, 143.7, 149.5, 150.9, 185.7. MS (ESI) *m/z* 310 $(M+H)^+$.

4-(1-(Benzo[*d*]thiazol-6-yl)-1*H*-1,2,3-triazol-4-yl)-4-hydroxycyclohexa-2,5-dienone 8i

By Method A, but the black oil obtained from addition of water was extracted with ethyl acetate, and purified by column chromatography (EtOAc) to give **8i** as a cream powder (0.600 g; 95%), mp 216-220 °C. Found C 57.7, H 3.3, N 17.7. Calc. for $C_{15}H_{10}N_4O_2S$ C 58.1, H 3.3, N 18.1%. ν_{max}/cm^{-1} 1672, 1624, 1485. δ_H (DMSO-d_6) 6.25 (2H, d, *J* 10.0, HC=), 6.72 (1H, s, OH), 7.26 (2H, d, *J* 10.0, HC=), 8.10-8.13 (1H, m, ArH), 8.29-8.31 (1H, m, ArH), 8.82 (1H, d, *J* 2.1, ArH), 9.03 (1H, s), 9.54 (1H, s). δ_C (DMSO-d_6) 65.7, 115.0, 119.5, 122.2, 124.5, 126.9, 134.4, 135.4, 149.8, 150.9, 158.8, 185.6. MS (ESI) *m/z* 311 $(M+H)^+$.

4-Hydroxy-4-(1-(pyridin-4-yl)-1*H*-1,2,3-triazol-4-yl)cyclohexa-2,5-dienone 8j

By Method A, to give **8j** as brown prisms (after allowing to stand for several days) (0.763 g; 53%), mp 149-158 °C. ν_{max}/cm^{-1} 1661, 1597. δ_H (DMSO-d_6) 6.24 (2H, d, *J* 10.1, HC=), 6.72 (1H, s, OH), 7.20 (2H, d, *J* 10.1, HC=), 7.42 (2H, d, *J* 6.3, ArH), 8.01 (2H, d, *J* 6.3, ArH). 8.79 (2H, d, *J* 6.3, ArH), 9.15 (1H, s, CHN) δ_C (DMSO-d_6) 65.8, 114.1, 121.8, 127.0, 143.0, 150.3, 150.6, 152.1, 185.6. MS (ESI) *m/z* 277 $(M+Na)^+$.

4-(1-Decyl-1*H*-1,2,3-triazol-4-yl)-4-hydroxycyclohexa-2,5-dienone 8k

By Method A, to give **8k** as an off-white solid. (0.740; 78%), mp 86-87 °C. Found C 68.0, H 8.5, N 13.1. Calc. for $C_{18}H_{27}N_3O_2$ C 68.1, H 8.6, N 13.2%. ν_{max}/cm^{-1} 2918, 2580, 1670, 1633. δ_H (DMSO-d_6) 0.88 (3H, t, *J* 6.8, CH_3), 1.23-1.29 (14H, m, $7 \times CH_2$), 1.78-1.81 (2H, m, CH_2), 4.33 (2H, t, *J* 7.1, CH_2N), 6.15 (2H, d, *J* 10.1, HC=), 6.45 (1H, s, OH), 7.16 (2H, d, *J* 10.1, HC=), 8.20 (1H, s, CHN). δ_C (DMSO-d_6) 14.4, 22.5, 26.3, 28.8, 29.1, 29.3, 29.3, 30.1, 31.7, 50.0, 65.7, 123.1, 126.5, 148.4, 151.3, 185.7. MS (ESI) *m/z* 318 $(M+H)^+$.

4-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)-4-hydroxycyclohexa-2,5-dienone 8l

By Method A, to give **8l** as a beige powder. (492 mg; 61%), mp 165-167 °C. Found C 67.2, H 4.9, N 15.5. Calc. for $C_{15}H_{13}N_3O_2$ C 67.4, H 4.9, N 15.7%. ν_{max}/cm^{-1} 1670, 1627. δ_H ($CDCl_3$) 3.48 (1H, s, OH), 5.52 (2H, s, CH_2), 6.23 (2H, d, *J* 10.1, HC=), 7.23 (2H, d, *J* 10.1, HC=), 7.27-7.31 (2H, m, ArH), 7.39 (1H, s, CHN), 7.39-7.42 (3H, m, ArH). δ_C ($CDCl_3$) 54.6, 66.2, 120.4, 127.8, 128.3, 129.1, 129.3, 133.9, 147.7, 148.3, 185.0. MS (ESI) *m/z* 268 $(M+H)^+$.

4-Hydroxy-4-(1-(3-phenylpropyl)-1*H*-1,2,3-triazol-5-yl)cyclohexa-2,5-dienone 8m

By Method A, but the oil obtained from addition of water was extracted with ethyl acetate, and purified by column chromatography (EtOAc) to give **8m** as white flakes (0.440 g; 50%), mp 128-131 °C. ν_{max}/cm^{-1} 1668, 1624. δ_H ($CDCl_3$) 2.23-2.30 (2H, m, CH_2), 2.68 (2H, t, *J* 7.4, CH_2), 4.35 (2H, t, *J* 7.2, CH_2), 6.26 (2H, d, *J* 10.1, HC=), 7.04 (2H, d, *J* 10.1, HC=), 7.15-7.32 (5H, m, ArH), 7.41 (1H, s, CHN). δ_C ($CDCl_3$) 31.5, 32.5, 50.0, 66.2, 120.8, 126.5, 127.7, 128.4, 128.7, 139.8, 147.3, 148.5, 185.0. HRMS (ES) calcd for $C_{17}H_{18}N_3O_2$ $(M+H^+)$ 296.1399 found 296.1336.

4-Hydroxy-4-(1-(4-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)cyclohexa-2,5-dienone 8n

By Method A, but the oil obtained from addition of water was extracted with DCM, the organic layers dried ($Mg SO_4$) and concentrated, and the product crystallised from ethyl acetate–hexane. (0.240 g; 27%), mp 86-88 °C. ν_{max}/cm^{-1} 1672, 1631, 1612, 1518, 1253. δ_H (DMSO-d_6) 3.74 (3H, s, OMe), 5.50 (2H, s, $PhCH_2$), 6.15 (2H, d, *J* 10.0, HC=), 6.48 (1H, s, OH), 6.94 (2H, d, *J* 8.5, ArH), 7.15 (2H, d, *J* 10.0, HC=), 7.32 (2H, d, *J* 8.5, ArH), 8.21 (1H, s, CHN). δ_C (DMSO-d_6) 52.3, 55.6, 65.7, 114.6, 123.1, 126.5, 128.2, 130.3, 148.7, 151.2, 159.7, 185.7. MS (ESI) *m/z* 298 $(M+H)^+$.

4-Hydroxy-4-(1-((2*R*,3*S*,4*R*,5*S*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,3-triazol-5-yl)cyclohexa-2,5-dienone 10a

By Method A, but the aqueous solution was concentrated to give a brown oil, which was dissolved in methanol. Addition of DCM resulted in the precipitation of a small amount of a brown solid, which was filtered. Further addition of DCM, followed by filtration, led to a colourless solution, which was concentrated to form a white solid. On filtration from DCM, the white solid immediately absorbed water, resulting in a colourless oil. δ_H (D_2O) 3.48-3.69 (4H, m) 3.78-3.88 (2H, m), 5.65 (1H, d, *J* 9.2, H-1), 6.25 (2H, d, *J* 10.0, HC=), 7.13 (2H, d, *J* 10.1, HC=), 8.25 (1H, s, CHN). δ_C (H_2O) 60.3, 65.7, 68.8, 72.2, 75.8, 78.8, 87.6, 122.8, 127.2, 147.3, 149.9, 187.8.

(3*S*,4*R*,5*S*,6*R*)-2-(Acetoxymethyl)-6-(4-(1-hydroxy-4-oxocyclohexa-2,5-dienyl)-1*H*-1,2,3-triazol-1-yl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate 10b

By Method A to give **10b** as a white powder (after allowing to stand for several days) (0.376 g; 49%), mp 91-96 °C. ν_{max}/cm^{-1} 2920, 2852, 1751, 1674, 1633. δ_H ($CDCl_3$) 1.90 (3H, s, CH_3), 2.02 (3H, s, CH_3), 2.06 (3H, s, CH_3), 2.24 (3H, s, CH_3), 4.15-4.24 (3H, m, H-5, H-6), 5.24 (1H, dd, *J* 10.3, 3.3, H-3), 5.46 (1H, dd, *J* 10.3, 9.2, H-2), 5.55 (1H, d, *J* 3.3, H-4), 5.82 (1H, d, *J* 9.2, H-1), 6.27-6.33 (2H, m, HC=) δ_C ($CDCl_3$) 20.3, 20.5, 20.7, 20.7, 61.2, 66.3, 66.7, 68.0, 70.5, 86.5, 119.6, 127.9, 128.2, 147.8, 148.1, 148.2, 169.1, 169.8, 169.9, 170.4, 184.9. MS (ESI) *m/z* 530 $(M+Na)^+$.

General method for the synthesis of arylsulfonyl-substituted 1,2,3-triazoles 11a,b (Method B)

To a stirred mixture of an arylsulfonyl azide (5 mmol), 4-ethynyl-4-hydroxycyclohexa-2,5-diene-1-one **5** (0.80 g; 6 mmol) and copper(I) iodide (95 mg; 0.5 mmol) in chloroform (10 mL) at 0 °C was slowly added 2,6-lutidine (0.7 mL; 6 mmol). The mixture was maintained at 0 °C for 1 h and then stirred at 25 °C for 12 h. The mixture was partitioned between DCM and aqueous ammonium chloride and the combined organic layers were separated, dried ($MgSO_4$), concentrated, and the product purified by column chromatography on silica gel (hexane–ethyl acetate).

4-Hydroxy-4-(1-tosyl-1*H*-1,2,3-triazol-4-yl)cyclohexa-2,5-dienone

By method B, to give **11a** as a beige powder (0.565 g; 34%), mp 100 °C (dec.). Found C 53.7, H 3.8, N 12.5. Calc. for $C_{15}H_{13}N_3O_4S$ C 54.4, H 4.0, N 12.7%. ν_{max}/cm^{-1} 1670, 1393. δ_H ($CDCl_3$) 3.25 (3H, s, OCH_3), 6.30 (2H, d, *J* 10.1, HC=), 7.01 (2H, d, *J* 10.1, HC=), 7.43 (2H, d, *J* 8.4, ArH), 8.04 (2H, d, *J* 8.4, ArH), 8.13 (1H, s, CHN). δ_C ($CDCl_3$) 21.9, 66.1, 120.9, 128.5, 129.0, 130.6, 132.5, 147.2, 147.3, 147.9, 184.6.

4-Hydroxy-4-(1-(4-methoxyphenylsulfonyl)-1*H*-1,2,3-triazol-4-yl)cyclohexa-2,5-dienone

By method B, to give **11b** as a beige powder (0.905 g; 52%) mp 105 °C (dec.). Found C 51.5, H 3.6, N 11.9. Calc. for $C_{15}H_{13}N_3O_5S$ C 51.9, H 3.8, N 12.1%. ν_{max}/cm^{-1} 1688, 1595, 1495, 1397. δ_H ($CDCl_3$) 3.92 (3H, s, OCH_3), 6.29 (2H, d, *J* 10.1, HC=), 7.02 (2H, d, *J* 10.1, HC=), 7.07 (2H, d, *J* 9.1, ArH), 8.08 (2H, d, *J* 9.1, ArH), 8.12 (1H, s, CHN). δ_C ($CDCl_3$) 56.0, 66.1, 115.3, 120.7, 126.3, 128.4, 131.5, 147.1, 147.3, 165.7, 184.6.

4-(1-Benzyl-1*H*-1,2,3-triazol-5-yl)-4-hydroxycyclohexa-2,5-dien-1-one 12

To a mixture of benzyl azide (0.4 g; 3 mmol) and 4-ethynyl-4-hydroxycyclohexa-2,5-diene-1-one **5** (0.61 g; 4.55 mmol) in deoxygenated (nitrogen gas) benzene (10 mL) was added $Cp^*RuCl(PPh_3)_2$ (0.025 g).[16] The mixture was stirred at 80 °C for 2 h, then the reaction mixture was concentrated, and the product was purified by column chromatography on silica gel (hexane–diethyl ether) followed by crystallization from hexane–ethyl acetate to afford a beige solid (9%). mp 165-169 °C. Found C 67.1, H 4.9, N 15.5. Calc. for $C_{15}H_{13}N_3O_2$ C 67.4, H 4.9, N 15.7%. δ_H ($CDCl_3$) 3.06 (1H, s, OH), 5.79 (2H, s, $PhCH_2$), 6.12 (2H, d, *J* 10.1, HC=), 6.62 (2H, d, *J* 10.1, HC=), 7.12-7.14 (2H, m, ArH), 7.33-7.35 (3H, m, ArH), 7.56 (1H, s, CHN). δ_C ($CDCl_3$) 53.3, 66.2, 127.3, 128.3, 128.5, 129.0, 132.9, 135.1, 135.5, 145.9. MS (ESI) *m*/*z* 268 $(M+H)^+$.

4-Hydroxy-4-(4-phenyl-1-(phenylsulfonyl)-1*H*-imidazol-2-yl)cyclohexa-2,5-dien-1-one 14

A solution of 1-benzenesulfonyl-4-phenylimidazole **13** (0.369 g, 1.297 mmol) in dry THF (20 mL) was cooled to −78 °C then treated with *n*-butyllithium (2.5 M in hexanes) (0.571 mL, 1.427 mmol) and stirred for 1 h at this temperature. 4,4-Dimethoxycyclohexa-2,5-dienone[17] (0.200 g, 1.297 mmol) was then added dropwise over 5 min and the reaction mixture stirred for a further 2 h. The reaction mixture was warmed to room temperature then treated with a solution of ammonium chloride (0.5 g) in water (10 mL) and stirred for 30 min. The mixture was then treated with 1 M HCl (10 mL) and stirred for 2 h, then diluted with EtOAc (40 mL), and the layers separated. The aqueous phase was extracted with EtOAc (2 × 40 mL) and the combined organic layers were washed with 1 M HCl (2 × 25 mL) and sat. K_2CO_3 solution (4 × 25 mL). The organic layer was dried ($MgSO_4$) and concentrated under vacuum. The resulting solid was suspended in Et_2O, then collected on a filter and dried under vacuum to give 4-(1-benzenesulfonyl-4-phenylimidazol-2-yl)-4-hydroxycyclohexa-2,5-dien-1-one (0.195 g, 38%), mp 135-137 °C. Found C 64.0, H 4.2, N 6.8. Calc. for $C_{21}H_{16}N_2O_4S$ C 64.3, H 4.1, N 7.1. ν_{max}/cm^{-1} 3292, 1659, 1614, 1370, 1175, 1150, 1055, 729 cm^{-1}. δ_H (DMSO-d_6) 6.24 (2H, d, *J* 9.3, HC=), 6.82 (1H, s, OH), 7.29-7.31 (1H, m, ArH), 7.37-7.41 (4H, m), 7.67-7.71 (2H, m, ArH), 7.79-7.82 (3H, m, ArH), 8.15 (2H, d, *J* 8.2, ArH), 8.48 (1H, d, *J* 0.5).

***In vitro* MTT assays**

Compounds were prepared as 10 mM top stocks, in DMSO, and stored at 4 °C. Human-derived cell lines (HCT 116 and HT29 colon carcinoma; MCF-7 and MDA 468 breast carcinoma, and A549 lung adenocarcinoma) were routinely cultivated at 37 °C in an atmosphere of 5% CO_2 in RPMI 1640 medium supplemented with 2 mM L-glutamine and 10% fetal calf serum and subcultured twice weekly to maintain continuous logarithmic growth. Compounds were evaluated against these cell lines according to the method previously described.[2]

References

1 A. J. McCarroll, M. B. Burke, C. A. Laughton and M. F. G. Stevens, *ARKIVOC*, 2007, (11), 172–182.
2 G. Wells, J. M. Berry, T. D. Bradshaw, A. M. Burger, A. Seaton, B. Wang, A. D. Westwell and M. F. G. Stevens, *J. Med. Chem.*, 2003, **46**, 532–541.
3 T. D. Bradshaw, C. S. Matthews, J. Cookson, E.-H. Chew, M. Shah, K. Bailey, A. Monks, E. Harris, A. D. Westwell, G. Wells, C. A. Laughton and M. F. G. Stevens, *Cancer Res.*, 2005, **65**, 3911–3919.
4 J. M. Berry, T. D. Bradshaw, I. Fichtner, R. Ren, C. H. Schwalbe, G. Wells, E.-H. Chew, M. F. G. Stevens and A. D. Westwell, *J. Med. Chem.*, 2005, **48**, 639–644.
5 E.-H. Chew, J. Lu, T. D. Bradshaw and A. Holmgren, *FASEB J.*, 2008, **22**, 2072–2083.
6 (*a*) E. S. Arner and A. Holmgren, *Eur. J. Biochem.*, 2000, **267**, 6102–6109; (*b*) S. Gromer, S. Urig and K. Becker, *Med. Chem. Rev.*, 2004, **24**, 40–89; (*c*) C. H. Lillig and A. Holmgren, *Antioxid. Redox Signaling*, 2007, **9**, 25–47.
7 A. Mukherjee, A. D. Westwell, T. D. Bradshaw, M. F. G. Stevens, J. Carmichael and S. G. Martin, *Br. J. Cancer*, 2005, **92**, 350–358.
8 D. T. Jones, C. W. Pugh, S. Wigfield, M. F. G. Stevens and A. L. Harris, *Clin. Cancer Res.*, 2006, **12**, 5384–5394.
9 A. J. McCarroll, T. D. Bradshaw, A. D. Westwell, C. S. Matthews and M. F. G. Stevens, *J. Med. Chem.*, 2007, **50**, 1707–1710.
10 (*a*) V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596–2599; (*b*) V. D. Bock, H. Hiemstra and J. H. van Maarseveen, *Eur. J. Org. Chem.*, 2006, 51–68; (*c*) M. V. Gil, M. J. Arévalo and Ó. López, *Synthesis*, 2007, 1589–1620.
11 J. Anderson, S. Bolvig and X. Liang, *Synlett*, 2005, 2941–2947.
12 (*a*) L. Zhang, X. Chen, P. Xue, H. H. Y. Sun, I. D. Williams, K. B. Sharpless, V. V. Fokin and G. Jia, *J. Am. Chem. Soc.*, 2005, **127**,

15998–15999; (*b*) B. C. Boren, S. Narayan, L. K. Rasmussen, L. Zhang, H. Zhao, Z. Lin, G. Jia and V. V. Fokin, *J. Am. Chem. Soc.*, 2008, **130**, 8923–8930.
13 M. R. Boyd and K. D. Paull, *Drug Dev. Res.*, 1995, **34**, 91–104.
14 F. B. Mallory, P. A. S. Smith and J. H. Boyer, *Org. Syn. Coll. Vol. 4*, 1963, 74–78.
15 D. R. Buckle and C. J. M. Rockell,, *J. Chem. Soc., Perkin Trans. 1*, 1982, 627–630.
16 M. S. Chinn and D. M. Heinekey, *J. Am. Chem. Soc.*, 1990, **112**, 5166–5175.
17 (*a*) A. Pelter and S. M. A. Elgendy, *J. Chem. Soc., Perkin Trans. 1*, 1993, 1891–1896; (*b*) A. E. Fleck, J. A. Hobart and G. W. Morrow, *Synth. Commun.*, 1992, **22**, 179–187.

