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# One-Pot Laccase-Catalysed Synthesis of 5,6-Dihydroxylated Benzo[*b*]furans and Catechol Derivatives, and Their Anticancer Activity

**Kevin W. Wellington[1], Tozama Qwebani-Ogunleye[1], Natasha I. Kolesnikova[1], Dean Brady[1], and Charles B. de Koning[2]**

[1] CSIR Biosciences, Pretoria, South Africa
[2] Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand, Wits, South Africa

A commercial laccase, Suberase® from Novozymes, was used to catalyse the synthesis of 5,6-dihydroxylated benzo[*b*]furans and catechol derivatives. The yields were, in some cases, similar to or better than that obtained by other enzymatic, chemical or electrochemical syntheses. The synthesised derivatives were screened against renal (TK10), melanoma (UACC62), breast (MCF7) and cervical (HeLa) cancer cell lines. $GI_{50}$, TGI and $LC_{50}$ are reported for the first time. Anticancer screening showed that the cytostatic effects of the 5,6-dihydroxylated benzo[*b*]furans were most effective against the melanoma (UACC62) cancer cell line with several compounds exhibiting potent growth inhibitory activities ($GI_{50}$ = 0.77–9.76 μM), of which two compounds had better activity than the anticancer agent etoposide ($GI_{50}$ = 0.89 μM). One compound exhibited potent activity ($GI_{50}$ = 9.73 μM) against the renal (TK10) cancer cell line and two exhibited potent activity ($GI_{50}$ = 8.79 and 9.30 μM) against the breast (MCF7) cancer cell line. These results encourage further studies of the 5,6-dihydroxylated benzo[*b*]furans for their potential application in anticancer therapy.

**Keywords:** 1,3-Dicarbonyls / Anticancer / Benzo[*b*]furans / Biocatalysis / Catechols / C–C coupling / Cytostatic effects / Cytotoxic effects / $GI_{50}$ / $LC_{50}$ / Laccase / Michael addition / TGI

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## Introduction

Cancer is a leading cause of death worldwide and accounted for 7.6 million deaths (13% of all deaths) in 2008 (http://www.who.int/mediacentre/factsheets/fs297/en/). Amongst the most deadly cancers are lung, stomach, liver, colon and breast cancer. It is projected that worldwide deaths from cancer will continue to rise to an estimated 13.1 million in 2030 (http://www.who.int/mediacentre/factsheets/fs297/en/).

The primary treatment for many cancers is chemotherapy. The development of multidrug resistance to chemotherapeutic drugs is a main obstacle for the successful treatment of malignant tumours. Overexpression of the ATP-binding cassette (ABC) transporters that actively pump drugs out of tumour cells is one of the best known mechanisms of multidrug resistance [1, 2].

The number of effective drugs available for treating malignant tumours has been reduced by the development of chemoresistance and this has led to a search for therapeutic alternatives through the discovery of new classes of anticancer compounds.

The hydroxylated benzo[*b*]furan moiety has attracted much attention due to its wide range of biological activities [3–7]. This group of compounds act as antifungal agents, antioxidant agents, 5-lipoxygenase inhibitors, cyclooxygenase-2 inhibitors, $Na^+$ and $K^+$-ATPase inhibitors and modulators of the estrogen receptor [8–12]. Examples of benzofuran scaffolds that exhibit anticancer activity are shown in Fig. 1.

Usnic acid **1**, a common and abundant lichen metabolite, showed activity against the wild-type p53 breast cancer cell line MCF7, the breast cancer cell line MDA-MB-231 and the

**Correspondence:** Kevin W. Wellington, CSIR Biosciences, P.O. Box 395, Pretoria, South Africa.
**E-mail:** kwellington@csir.co.za; kwwellington@gmail.com
**Fax:** +2712 841 3388

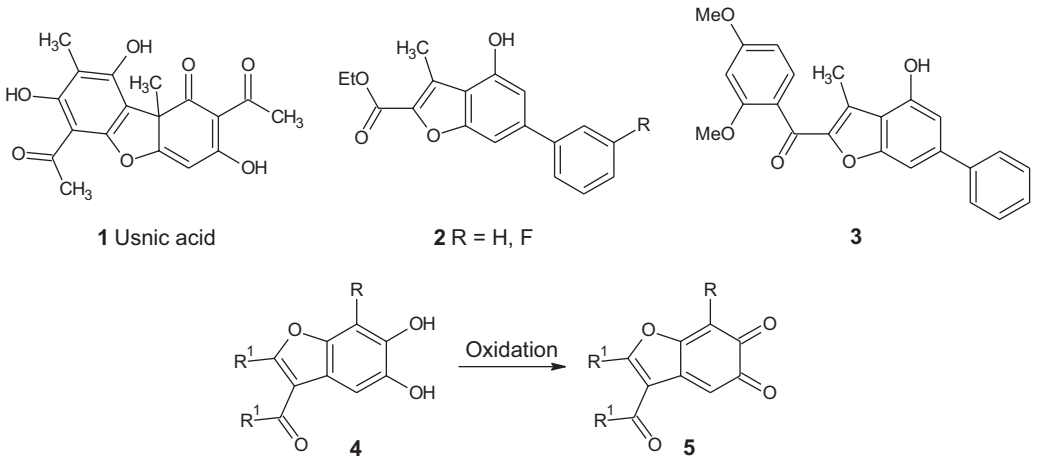



**Figure 1.** Benzofuran scaffolds.

lung cancer cell line H1299 [13]. Benzofuran derivatives **2** and **3** (Fig. 1) were identified as anticancer agents of which derivatives displayed selective cytotoxicity against a tumourigenic cell line [14]. Laccases (EC 1.10.3.1) are enzymes that are widely distributed in plants and fungi. They are characterised by a multinuclear copper-containing active site and have been classified as oxidoreductases. Laccases catalyse the oxidation of a broad range of substrates such as phenols, *o*- and *p*-diphenols, aminophenols, methoxyphenols, aryl thiols, anilines, polyphenols, polyamines and lignin-derivatives [15–17]. In the monoelectronic oxidation of substrates molecular oxygen is used and water is produced as the only by-product (http://www.who.int/mediacentre/factsheets/fs297/en/) [1, 2, 15–17]. Laccases have been successfully applied in organic synthesis which has culminated in several reports in the field of green chemistry [18].

In our laboratories, we have been interested in the use of enzymes for the development of green methods of synthesis, and for accessing compounds that have pharmaceutical value. We have previously reported on the synthesis of diaminobenzoquinones [19a] and aminonaphthoquinones [19b] via C–N bond formation as well as 1,4-naphthoquinone-2,3-*bis*-sulfides [19c] via C–S bond formation using commercial laccases (Denilite II Base on an inert support, and Novozyme 51003) from Novozymes [19]. In this article, we report on the synthesis of 5,6-dihydroxylated benzo[*b*]furan derivatives using another commercial laccase (Suberase® from Novozymes) and on the anticancer activity of the synthesised compounds. We anticipated that the 5,6-dihydroxylated benzo[*b*]furans **4** possessing *ortho* phenolic substituents could be metabolised to the *o*-quinonoid structure **5** in cancer cells (Fig. 1). Since quinonoid compounds display potent biological properties such as antibacterial, anticancer, antifungal and antimalarial activity [20], we postulated that the benzo[*b*]furan **5** could have similar biological activities. This study is, to the best of our knowledge, the first on the synthesis of a variety of 5,6-dihydroxylated benzo[*b*]furans **4** and catechol derivatives using the commercial laccase, Suberase®, as well as the first report on the anticancer activity of the synthesised compounds.

## Results and discussion

### Synthesis

The catechols (**6a–c**) and the 1,3-dicarbonyls (**7a–f**) used in this study are depicted in Fig. 2.

The method used for the synthesis entailed reacting one equivalent of the catechol with one equivalent of the 1,3-dicarbonyl at room temperature using Suberase® in a vessel open to air at pH 7.15 (Scheme 1). A pH of 7.15 was chosen because it would make the reaction medium sufficiently basic to deprotonate the alpha-proton from the 1,3-dicarbonyls and thus facilitate the Michael addition reaction with the *in situ*-generated *o*-quinone.

The first approach, Method A, entailed reacting the catechols **6a–c** with the 1,3-dicarbonyls **7a–f** at room temperature (rt) at pH 7.15 for 24 h. The second approach, Method B, entailed conducting the reaction under the same conditions but for a longer time (44 h) to determine whether a longer reaction time would improve the yield of the product. In the third approach, Method C, a co-solvent, DMF, was added to the reaction mixture to improve the solubility of the organic substrates. The number of equivalents of the 1,3-dicarbonyl was also increased so that a ratio of 1,3-dicarbonyl to catechol was 4:1. These reactions were conducted for 42 h. The results of the investigations with Methods A to C are depicted in Table 1.

From these results it can be seen that 5,6-dihydroxylated benzo[*b*]furans can be accessed using all three synthesis methods. For Method A the highest yield that was obtained is 98% for **17** (Entry 15, Table 1) and the lowest is 37% for **15**

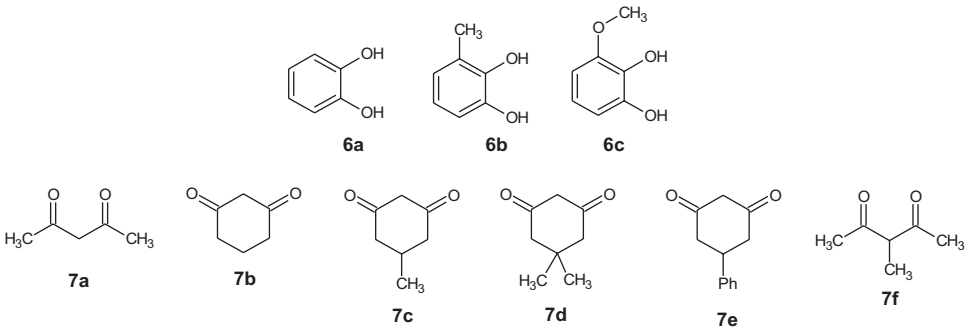


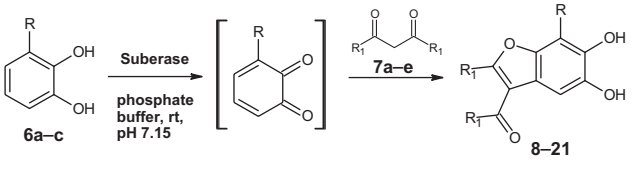

**Figure 2.** The catechols **6a–c** and 1,3-dicarbonyls **7a–f** used in this study.

(Entry 12, Table 1). For Method B the highest yield was 77% for **18** (Entry 18, Table 1) and the lowest 49% for **8** (Entry 2, Table 1). In the case of Method C, the highest yield was 71% for **14** (Entry 11, Table 1) and the lowest 15% for **21** (Entry 23, Table 1).

When comparing the yield of product using Method A to that obtained using Method B, it can be seen that there is not a significant increase in yield even though the reaction time has almost doubled (Entries 1 and 2, 5 and 6, 17 and 18, Table 1). It is therefore concluded that there is a limit to the quantity of product that can be formed in the reaction. The presence of DMF (Method C) may have deactivated the laccase, Suberase®, resulting in a lower yield of the product. A higher volume of organic solvent increases the solubility of a substrate but significantly decreases the overall reaction rate by deactivating the enzyme [21].

The optimum conditions for synthesising these 5,6-dihydroxylated benzo[*b*]furans **4** using Suberase® is thus that used under Method A.

The enzymatic synthesis of 5,6-dihydroxylated benzo[*b*]furans has been reported previously. The first enzymatic synthesis of compound **9** was reported in 2007 by Witayakran et al. [22] using *Trametes villosa* laccase in phosphate buffer (pH 7.0) with a Lewis acid, scandiumtris(trifluoromethanesulfonate), and sodium lauryl sulfate at 20°C and afforded it in 76% yield. In 2009, Witayakran et al. also reported on a laccase–lipase co-catalytic system for the synthesis of compounds **8** and **9** using phosphate buffer (pH 7.0) with lipase [23]. In this reaction, the Michael addition step was enhanced by lipase addition providing improved yields. Compound **8** was obtained in 60% yield using *Candida rugosa* lipase and laccase from *T. villosa* at 23°C. Compound **9** was obtained in 62% yield using *Candida antarctica* (CALB) lipase and laccase from *T. villosa* at 23°C [23]. We have synthesised compounds **8** and **9** without the use of scandiumtris(trifluoromethanesulfonate) or lipase and achieved a 49% yield of compound **8** (Entry 2, Table 1) and a 50% yield of compound **9** (Entry 3, Table 1).




**Scheme 1.** Synthesis of the 5,6-dihydroxylated benzo[*b*]furans **8–21**.

Hajdok et al. [24] was the first to report on the synthesis of compounds **10–21** using laccase initiated oxidative domino reactions. One method entailed using a commercial laccase from *Trametes versicolor* in an acetate buffer (pH 4.38) at room temperature while the other method used laccase from *Agaricus bisporus* in phosphate buffer (pH 5.96) also at rt. The latter method was found to be better since it gave the product in higher yield and purity with yields ranging from 71 to 97%. The yields of compounds **10–21** using our methods are 37–98%. The commercial fungal laccase, Suberase® from *Myceliophthora thermophila*, was overall less effective than the laccase from *A. bisporus* which was used by Hajdok et al. [24].

Compounds **13** and **19** have also been synthesised by employing tyrosinase and laccase from *A. bisporus* and were obtained in 39 and 44% yields, respectively [25]. Our method was higher yielding since compound **13** was obtained in 59% yield and compound **19** in 76% yield (Entries 8 and 19, respectively, Table 1; Fig. 3).

There have also been literature reports on the chemical syntheses of 5,6-dihydroxylated benzo[*b*]furans. The first report of compound **8** was a chemical synthesis which afforded **8** in 47% yield using pyridine and sodium metaiodate in ethanol at 20°C [26]. Our methods afforded similar yields for compound **8** (48 and 49%, Entries 1 and 2, respectively, Table 1). Duthaler and Scherrer [27] reported on the chemical synthesis of compound **10** which was obtained in 22% yield using sodium acetate in water. We were able to obtain compound **10** in 65% yield (Entry 4, Table 1).

Electrochemical syntheses have also been reported for the synthesis of 5,6-dihydroxylated benzo[*b*]furans. The first report on the synthesis of **16** was by Grujić et al. [28] in 1976

**Table 1.** Synthesised benzo[*b*]furans **8–21** (yield in parentheses) at rt in phosphate buffer at pH 7.15.

| Entry | Catechol | Dicarbonyl | Reaction time (h) | Method | Product (%Yield) | Yields of other enzymatic syntheses | Yields of other syntheses |
|---|---|---|---|---|---|---|---|
| 1 | **6a** | **7a** | 24 | A | **8** (48) | 60 [23] | 47 [26] |
| 2 | **6a** | **7a** | 44 | B | **8** (49) | | |
| 3 | **6b** | **7a** | 24 | A | **9** (50) | 76 [22], 62 [23] | |
| 4 | **6a** | **7b** | 24 | A | **10** (65) | 87 [24] | 22 [27] |
| 5 | **6b** | **7b** | 24 | A | **11** (62) | 70 [24] | |
| 6 | **6b** | **7b** | 44 | B | **11** (67) | | |
| 7 | **6c** | **7b** | 24 | A | **12** (70) | 89 [24] | |
| 8 | **6a** | **7c** | 24 | A | **13** (59) | 85 [24], 39 [25] | |
| 9 | **6a** | **7c** | 42 | C | **13** (30) | | |
| 10 | **6b** | **7c** | 24 | A | **14** (78) | 71 [24] | |
| 11 | **6b** | **7c** | 42 | C | **14** (71) | | |
| 12 | **6c** | **7c** | 24 | A | **15** (37) | 97 [24] | |
| 13 | **6a** | **7d** | 24 | A | **16** (58) | 91 [24] | 90 [28], 90 [29], 82 [30], 66 [31] |
| 14 | **6a** | **7d** | 42 | C | **16** (40) | | |
| 15 | **6b** | **7d** | 24 | A | **17** (98) | 92 [24] | 87 [30], 81 [31] |
| 16 | **6b** | **7d** | 42 | C | **17** (59) | | |
| 17 | **6c** | **7d** | 24 | A | **18** (73) | 95 [24] | 93 [30], 80 [31] |
| 18 | **6c** | **7d** | 44 | B | **18** (77) | | |
| 19 | **6a** | **7e** | 24 | A | **19** (76) | 96 [24], 44 [25] | |
| 20 | **6a** | **7e** | 42 | C | **19** (50) | | |
| 21 | **6b** | **7e** | 24 | A | **20** (80) | 91 [24] | |
| 22 | **6c** | **7e** | 24 | A | **21** (43) | 97 [24] | |
| 23 | **6c** | **7e** | 42 | C | **21** (15) | | |

*Method A* – Suberase® (8.0 mL), catechol (2.0 mmol), 1,3-dicarbonyl (2.0 mmol, 1 eq), phosphate buffer (20.0 mL, 0.10 M, pH 7.15), stirring time at rt = 24 h.
*Method B* – Suberase® (8.0 mL), catechol (2.0 mmol), 1,3-dicarbonyl (2.0 mmol, 1 eq), phosphate buffer (20 mL, 0.10 M, pH 7.15), stirring time at rt = 44 h.
*Method C* – Suberase® (4.5 mL), catechol (0.60 mmol), 1,3-dicarbonyl (2.4 mmol, 4 eq), phosphate buffer (4.0 mL, 0.10 M, pH 7.15) and DMF (2.0 mL), stirring time at rt = 42 h.

and afforded it in 90% yield. Later Tabaković et al. [29] also reported on the electrochemical synthesis of **16** in 90% yield in water. Another electrochemical synthesis of **16** using sodium acetate in water by Nematollahi et al. [30] afforded it in 82% yield. The electrochemical synthesis of **16** by Davarani et al. [31] only afforded a 66% yield. We could only obtain a 58% yield for **16** (Entry 13, Table 1). Nematollahi et al. [30] also reported on the electrochemical synthesis of **17** and **18** which were obtained in 87 and 93% yields, respectively, also using sodium acetate in water as a reaction medium. The electrochemical synthesis of **17** and **18** in a sodium acetate solution by Davarani et al. [31] afforded these compounds in slightly lower yields, 81 and 80%, respectively. Compound **17** was obtained in 98% yield (Entry 15, Table 1) which is higher than the yields obtained by electrochemical synthesis, while compound **18** was obtained in 77% yield (Entry 18, Table 1) which was lower than that obtained by electrochemical syntheses.

The synthesis of two novel catechol derivatives **22** and **23** was also investigated (Scheme 2).

These reactions were conducted using Method A which was used for the synthesis of the 5,6-dihydroxylated benzo[*b*]-furans **8–21**. In this case, the aim was to achieve only C–C bond formation using the 1,3-dicarbonyl **7f**. The purpose was to determine whether anticancer activity would still be observed without the formation of the furan ring. The results of the investigation are shown in Table 2.

Both reactions proceeded in mediocre yield to afford **22** and **23** (Entries 1 and 2, respectively). A proposed mechanism involving an *o*-quinone intermediate of **22** and **23** is shown in Fig. 4 below.

## Anticancer evaluation

Screening was conducted against renal (TK10), melanoma (UACC62), breast (MCF7) and cervical (HeLa) cancer cell lines using the Sulforhodamine B (SRB) assay to determine the growth inhibitory effects of the compounds [32]. These cell lines have been used routinely at the U.S. National Cancer Institute for screening for new anticancer agents and were derived from tumours that have different sensitivities to chemotherapeutic drugs [33]. Etoposide, an anticancer agent, was used as a positive control. It is known to be an inhibitor of topoisomerase, particularly topoisomerase II, and aids in DNA unwinding which causes the DNA strands to break

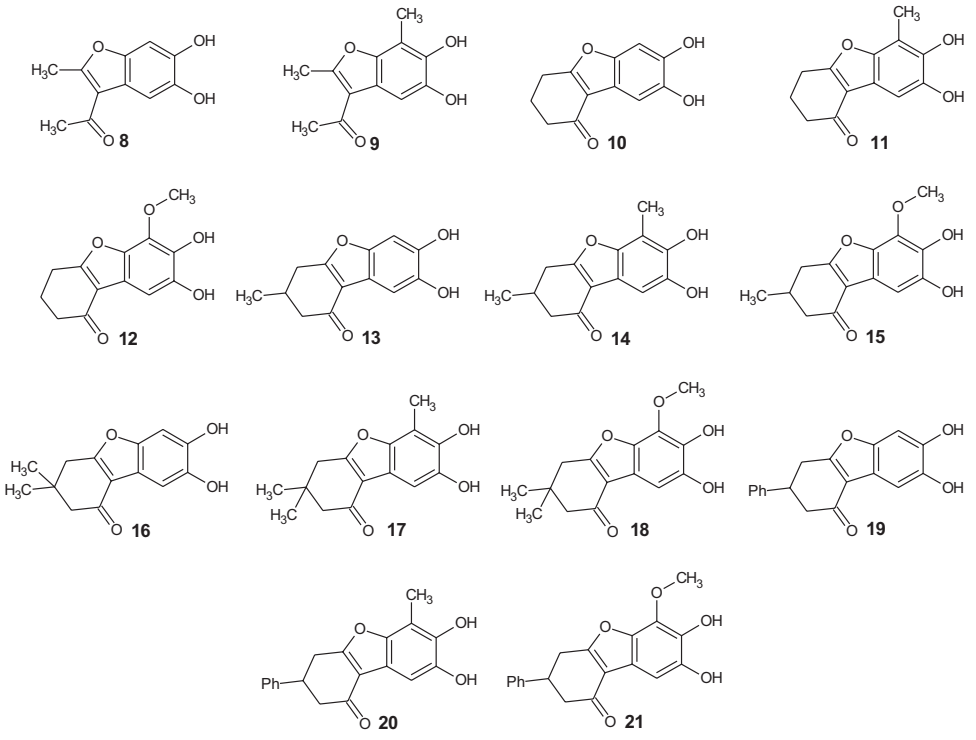
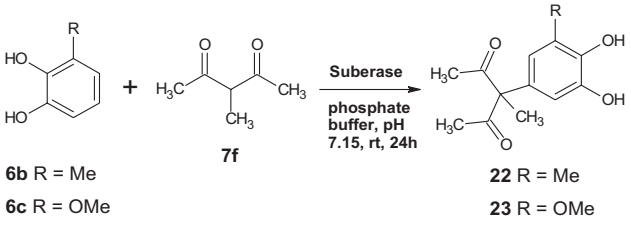



**Figure 3.** Structures of the 5,6-dihydroxylated benzo[*b*]furans **8**–**21** synthesised at rt.

[34]. Three parameters were determined during the screening process: 50% cell growth inhibition ($GI_{50}$), total cell growth inhibition (TGI) and the lethal concentration that kills 50% of cells ($LC_{50}$). The results of this investigation are shown in Table 3 from which it can be seen that several compounds exhibited potent cytostatic effects.

The $GI_{50}$ values of the catechols **6a–c**, the catechol derivative **22** and selected benzo[*b*]furans were compared to that of etoposide. No selection criteria were used for the compounds that were screened. Screening against the TK10 cell line




**Scheme 2.** Synthesis of C-C coupled products **22** and **23**.

**Table 2.** Synthesised catechol derivatives **23** and **24** (yield in parentheses) at rt in phosphate buffer at pH 7.15.

| Entry | Catechol | Dicarbonyl | Reaction time (h) | Product (Yield) |
|---|---|---|---|---|
| 1 | **6b** | **7f** | 24 | 22 (80) |
| 2 | **6c** | **7f** | 24 | 23 (55) |

*Method A* – Suberase® (8.0 mL), catechol (2.0 mmol), 1,3-dicarbonyl (2.0 mmol, 1 eq), phosphate buffer (20.0 mL, 0.10 M, pH 7.15), stirring time at rt = 24 h.

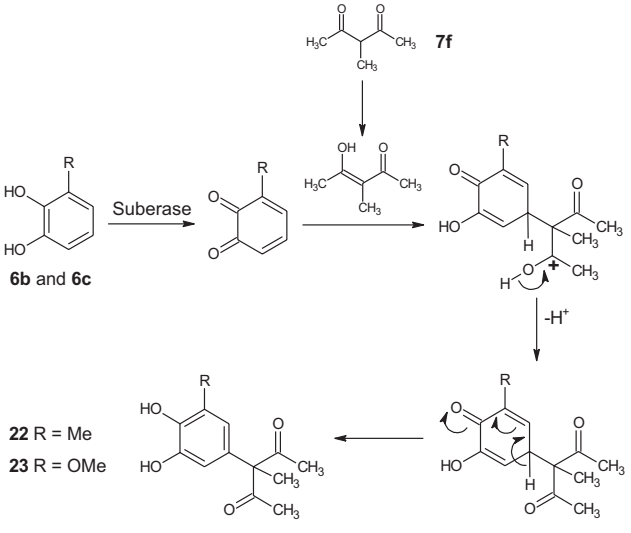



**Figure 4.** A proposed mechanism for C–C bond formation for the catechol derivatives **22** and **23**.

showed that only **21** had potent activity ($GI_{50}$ = 9.73 μM, Entry 12) which was not as good as that of etoposide ($GI_{50}$ = 7.19 μM, Entry 14).

Most of the compounds exhibited potent growth inhibitory activity against the UACC62 cell line. The potent activity exhibited by **15** ($GI_{50}$ = 0.78 μM, Entry 9) and **21** ($GI_{50}$ = 0.77 μM, Entry 12) was slightly better activity than that of etoposide ($GI_{50}$ = 0.89 μM, Entry 14).

Moreover, the same two compounds, **15** ($GI_{50}$ = 8.79 μM, Entry 9) and **21** ($GI_{50}$ = 9.30 μM, Entry 12), also exhibited potent growth inhibitory activity against the MCF7 cell line but this was not as good as that of etoposide ($GI_{50}$ = 0.56 μM, Entry 14).

Most compounds exhibited weak TGI activity and three were inactive against the TK10 cell line. The activities of **18**, **20** and **21** (Entries 10–12, respectively) were slightly better (TGI = 46.14–48.25 μM) than that of etoposide (TGI = 49.74 μM, Entry 14).

The compounds also exhibited moderate to weak activity against the UACC62 cell line with most compounds, **11**, **12**, **14**, **15**, **18** and **21**, exhibiting better activity (TGI = 18.32–51.06 μM) than that of etoposide (TGI = 52.71 μM, Entry 14). The best activity was observed for **15** (TGI = 18.32 μM, Entry 9) which was almost threefold better than that of etoposide.

The test compounds exhibited weak activity against the MCF7 cell line, but the cytostatic effects of these compounds were better than that of etoposide which was inactive (TGI > 100 μM, Entry 14).

The $LC_{50}$ values of the compounds were also compared to that of etoposide to get an idea of the cytotoxic effects of these compounds against the different cell lines. Screening against the TK10 cell line showed that most of the compounds were more lethal than etoposide ($LC_{50}$ > 100 μM) with **18** ($LC_{50}$ = 73.59 μM, Entry 10) being the most lethal.

Again, most of the compounds were more lethal than etoposide ($LC_{50}$ > 100 μM) against the UACC62 cell line and **15** ($LC_{50}$ = 60.32 μM, Entry 9) was the most lethal. Apart from **18**, none of the test compounds were lethal for HeLa cells, indicating a degree of selectivity between cell lines.

The compounds also exhibited more lethal cytotoxic effects against the MCF7 cell line than that of etoposide ($LC_{50}$ > 100 μM, Entry 14) and **21** ($LC_{50}$ = 86.93 μM, Entry 12) was the most lethal. Apart from **18**, none of the test compounds were lethal for HeLa cells indicating a degree of selectivity between cell lines.

The catechols **6a–c** and the catechol derivative **22** (Entry 13) did not exhibit any significant anticancer activity. From the results it can be seen that the $GI_{50}$ concentrations of benzo[*b*]-furan **9** (Entry 4) are almost half of those of **22** (Entry 13) against the renal (TK10), melanoma (UACC62) and breast (MCF7) cancer cell lines. It may thus be concluded that the presence of the furan ring enhances anticancer activity. The benzo[*b*]furans were most effective against the melanoma (UACC62) cell line. Only one benzo[*b*]furan, **21**, exhibited growth inhibitory activity against all three cancer cell lines which may be attributed to the presence of the methoxy group on the benzene ring. When the methoxy group was replaced with a methyl group, as in **20**, growth activity against the renal (TK10) and breast (MCF7) cell lines was diminished. The benzo[*b*]furan **15** exhibited activity against melanoma (UACC62) and breast (MCF7) cell lines. The replace-

**Table 3.** *In vitro* anticancer screening of the compounds against renal (TK10), melanoma (UACC62), breast (MCF7) and HeLa cancer cells expressed as $GI_{50}$, TGI and $LC_{50}$ values (μM).

| Entry | Compound | Renal (TK10) | | | Melanoma (UACC62) | | | Breast (MCF7) | | | Cervical (HeLa) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $GI_{50}$ | TGI | $LC_{50}$ | $GI_{50}$ | TGI | $LC_{50}$ | $GI_{50}$ | TGI | $LC_{50}$ | $GI_{50}$ | TGI | $LC_{50}$ |
| 1 | **6a** | 68.02 | >100 | >100 | 45.00 | >100 | >100 | 52.41 | >100 | >100 | –[a] | –[a] | –[a] |
| 2 | **6b** | 38.14 | 75.72 | >100 | 15.18 | 46.22 | 77.26 | 27.34 | 64.31 | >100 | –[a] | –[a] | –[a] |
| 3 | **6c** | 51.63 | 96.98 | >100 | 23.64 | 50.26 | 76.87 | 27.55 | 60.68 | 93.81 | –[a] | –[a] | –[a] |
| 4 | **9** | 41.05 | 69.94 | 98.82 | 32.74 | 60.84 | 88.94 | 35.10 | 66.12 | 97.14 | 60.70 | >100 | >100 |
| 5 | **10** | 65.00 | >100 | >100 | 37.73 | 68.65 | 99.57 | 35.53 | 64.19 | 92.85 | 49.09 | >100 | >100 |
| 6 | **11** | 24.25 | 54.74 | 85.23 | 23.63 | 51.06 | 78.49 | 25.97 | 61.87 | 97.78 | 44.55 | >100 | >100 |
| 7 | **12** | 52.47 | >100 | >100 | 6.04 | 33.15 | >100 | 15.85 | 54.74 | 93.63 | 56.46 | >100 | >100 |
| 8 | **14** | 16.56 | 55.32 | 94.09 | 6.50 | 33.85 | 74.35 | 25.55 | 68.82 | >100 | 25.93 | >100 | >100 |
| 9 | **15** | 26.54 | 51.65 | 76.75 | 0.78 | 18.32 | 60.32 | 8.79 | 48.79 | 95.11 | 26.68 | 69.67 | >100 |
| 10 | **18** | 18.69 | 46.14 | 73.59 | 6.79 | 28.93 | 66.27 | 28.44 | 60.90 | 93.35 | 34.37 | 62.15 | 89.93 |
| 11 | **20** | 18.60 | 48.25 | 77.90 | 9.76 | >100 | >100 | 17.96 | 60.79 | >100 | 47.28 | >100 | >100 |
| 12 | **21** | 9.73 | 47.15 | 85.35 | 0.77 | 23.60 | 64.58 | 9.30 | 46.93 | 86.93 | 22.32 | 73.19 | >100 |
| 13 | **22** | 86.99 | >100 | >100 | 51.29 | >100 | >100 | 68.03 | >100 | >100 | –[a] | –[a] | –[a] |
| 14 | Etoposide | 7.19 | 49.74 | >100 | 0.89 | 52.71 | >100 | 0.56 | >100 | >100 | 3.56 | 40.18 | 87.54 |

Inactive, i: $GI_{50}$ or TGI > 100 μM; weak activity, w: > 30 μM $GI_{50}$ or TGI < 100 μM; moderate activity, m: < 30 μM $GI_{50}$ or TGI > 10 μM; potent activity, p: $GI_{50}$ or TGI < 10 μM.
[a] Not screened.

ment of the methoxy group with that of a methyl, as in **14**, also resulted in diminished activity and in this case against the breast (MCF7) cancer cell line. When comparing the structure of **21** to that of **15** it appears that the replacement of the methyl group with that of a phenyl specifically affords growth inhibitory activity against the renal (TK10) cancer cell line. It was concluded that the phenyl and methoxy groups are essential for activity against all three cancer cell lines.

## Conclusions

The fungal laccase from *M. thermophila*, commercially available as an inexpensive preparation known as Suberase®, can be used in the catalytic synthesis of 5,6-dihydroxylated benzo[*b*]furan and catechol derivatives under mild and environmentally friendly reaction conditions. The yields are, in some cases, similar to or better than that obtained by other enzymatic, chemical, or electrochemical synthesis. This method has eliminated the use of the Lewis acid, scandiumtris(trifluoromethanesulfonate), and lipase which was used in previous laccase methods.

The 5,6-dihydroxylated benzo[*b*]furans exhibit potent cytostatic effects against the three cancer cell lines but are most effective against UACC62 (melanoma) with two compounds exhibiting better activity than etoposide based on the $GI_{50}$ concentrations. The 5,6-dihydroxylated benzo[*b*]furans generally have better TGI activity than that of etoposide.

These results warrant further studies of the 5,6-dihydroxylated benzo[*b*]furans for their application in anticancer therapy. These studies will entail further synthesis of 5,6-dihydroxylated benzo[*b*]furans and evaluation of their anticancer activity in addition to structural modification of hits to determine whether the anticancer activity can be optimised.

## Experimental

### General

Proton nuclear magnetic resonance ($^1$H NMR) spectra were recorded on a Varian Mercury 400 MHz spectrometer. Carbon-13 nuclear magnetic resonance ($^{13}$C NMR) spectra were recorded on the same instruments at 100 MHz. Chemical shifts are reported in parts per million (ppm) relative to the solvent peaks and coupling constants are given in Hertz (Hz). A Waters UPLC coupled in tandem to a Waters photodiode array (PDA) detector and a SYNAPT G1 HDMS mass spectrometer was used to generate accurate mass data. The PDA detector was used for all purity determinations (Maxplot 200–500 nm). All chemicals for UPLC-MS work were of ultra-pure LC-MS grade and purchased from Fluka (Steinheim, Germany) while ultra-pure solvents were purchased from Honeywell (Burdick & Jackson, Muskegon, USA). Ultra-pure water was generated using a Millipore Elix 5 RO system and Millipore Advantage Milli-Q system (Millipore SAS, Molsheim, France). Reactions were monitored by thin layer chromatography (TLC) on aluminium-backed Merck silica gel 60 F254 plates. Gravity column chromatography was performed using Merck Silica Gel 60 (70–230 mesh). Melting points were determined using a Glassco melting point apparatus and are uncorrected.

All chemicals were reagent grade materials. The 1,3-dicarbonyls were purchased from Sigma–Aldrich, South Africa. Suberase® (10757.8 PCU/mL) is a fungal laccase from *M. thermophila* produced by submerged fermentation of a genetically modified *Aspergillus oryzae* strain. The enzymatic preparation is supplied as a brown liquid which is completely miscible with water. Suberase® was obtained from Novozymes in South Africa.

### General methods for the synthesis of the benzo[*b*]furan derivatives

#### *Method A*

The laccase (Suberase®, 2.0 mL) was added to a mixture of the catechol (2.0 mmol), 1,3-dicarbonyl (2.0 mmol) and phosphate buffer (20.0 mL, 0.10 M, pH 7.15) in a 250-mL round-bottom flask stirred under air at rt. More laccase (2.0 mL) was added after 2, 18 and 20 h. The mixture was vigorously stirred under air until the substrates were consumed as judged by TLC. After stirring the reaction mixture was acidified with 32% HCl to pH 4.0. The mixture was extracted with EtOAc and washed with water (20.0 mL). The organic phases were then combined and the solvent evaporated. The residue, a powder, was purified by washing with EtOAc or recrystallising from a combination of MeOH and EtOAc.

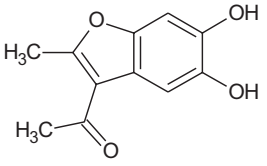
#### *Method B*

Same as Method B except that more laccase (2.0 mL) was added after 4, 24 and 28 h.

#### *Method C*

The laccase (Suberase®, 1.5 mL) was added to a mixture of the catechol (0.60 mmol), 1,3-dicarbonyl (2.40 mmol), phosphate buffer (4.0 mL, 0.10 M, pH 7.15) and DMF (2.0 mL) in a test tube stirred under air at rt. More laccase (1.5 mL) was added after 2 h and then again after 4 h. The mixture was vigorously stirred under air until the substrates had been consumed as judged by TLC. After stirring the reaction mixture was transferred to a separating funnel and the mixture extracted with EtOAc and washed with water (20.0 mL). The organic phases were combined, the solvent evaporated and the residue (a powder) purified by flash chromatography.

#### *1-(5,6-Dihydroxy-2-methyl-1-benzofuran-3-yl)ethanone* ***8***



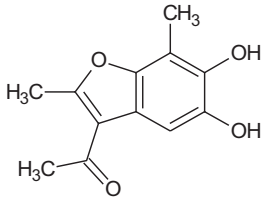

#### *Method A*

Stirring time 24 h. The product was recrystallised from 5% MeOH in EtOAc solution at 35°C to afford a dark-brown powder (198 mg, 48%) (Found: M–H$^+$, 205.0518. $C_{11}H_9O_4$ requires M–H, 205.0501). UPLC 95.5%. $R_f$ 0.40 (EtOAc/hexane, 1:1). mp 200–203°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.51 (3H, s, $CH_3$), 2.68 (3H, s, $CH_3$), 6.91 (1H, s, ArH), 7.33 (1H, s, ArH), 8.94 (1H, br s, OH), 9.06 (2H, br s, OH); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 15.3, 30.8, 97.8, 106.4, 117.2, 117.2, 143.6, 144.2, 147.0, 160.7, 193.9.

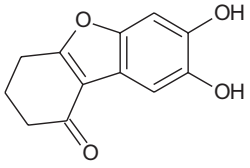
*Method B*
Stirring time 44 h. The product was washed with EtOAc to afford a black powder (202.0 mg, 49%).

### *1-(5,6-Dihydroxy-2,7-dimethyl-1-benzofuran-3-yl)-ethanone* ***9***



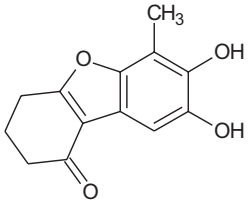
*Method A*
Stirring time 24 h. The product was washed with EtOAc to afford a red-brown powder (220 mg, 50%) (Found: $M-H^+$, 219.0615. $C_{12}H_{11}O_4$ requires M–H, 219.0657). UPLC 97.3%. $R_f$ 0.46 (EtOAc/hexane, 1:1). mp 234°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.19 (3H, s, $CH_3$), 2.45 (3H, s, $CH_3$), 2.64 (3H, s, $CH_3$), 7.17 (1H, s, ArH), 8.38 (1H, br s, OH), 9.25 (1H, br s, OH); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 8.9, 15.4, 30.7, 103.2, 107.1, 116.2, 117.4, 141.8, 143.1, 146.5, 160.5, 193.9.

### *7,8-Dihydroxy-3,4-dihydrodibenzo[b,d]furan-1(2H)-one* ***10***



*Method A*
Stirring time 24 h. The product was recrystallised from 5% MeOH in EtOAc solution at 35°C to afford a brown powder (284 mg, 65%) (Found: $M-H^+$, 217.0458. $C_{12}H_9O_4$ requires M–H, 217.0501). UPLC 96.2%. $R_f$ 0.48 (EtOAc/hexane, 1:1). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.21 (2H, m, $CH_2$), 2.47 (2H, t $J$ 6.4 Hz, $CH_2$) 2.96 (2H, t $J$ 6.4 Hz, $CH_2$), 7.00 (1H, s, ArH), 7.22 (1H, s, ArH), 9.18 (2H, br s, OH); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 22.2, 23.2, 37.3, 98.4, 105.4, 114.4, 115.6, 143.7, 144.4, 147.9, 169.7, 194.4.

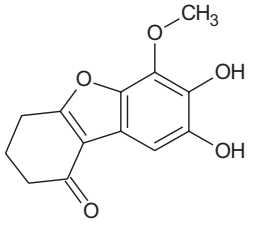
### *7,8-Dihydroxy-6-methyl-3,4-dihydrodibenzo[b,d]furan-1(2H)-one* ***11***



*Method A*
Stirring time 24 h. The product was recrystallised from 5% MeOH in EtOAc solution at 35°C to afford a brown powder (288 mg, 62%) (Found: $M-H^+$, 231.0683. $C_{13}H_{11}O_4$ requires M–H, 231.0657). UPLC 99.7%. $R_f$ 0.48 (EtOAc/hexane, 1:1). mp 250°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.14 (2H, m, $CH_2$), 2.26 (3H, s, $CH_3$), 2.45 (2H, t $J$ 6.1 Hz, $CH_2$), 2.98 (2H, t $J$ 6.1 Hz, $CH_2$), 7.11 (s, 1H, ArH); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 9.0, 22.2, 23.3, 37.9, 102.4, 107.9, 113.5, 115.9, 142.2, 143.4, 147.6, 169.6, 194.6.

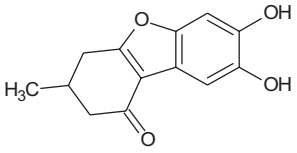
*Method B*
Stirring time 44 h. The product was washed with EtOAc to afford a brown powder (311.0 mg, 67%).

### *7,8-Dihydroxy-6-methoxy-3,4-dihydrodibenzo[b,d]furan-1(2H)-one* ***12***



*Method A*
Stirring time 24 h. The product was recrystallised from 5% MeoH in EtOAc solution at 35°C to afford a brown powder (348 mg, 70%) (Found: $M-H^+$, 247.0557. $C_{13}H_{11}O_5$ requires M–H, 247.0606). UPLC 97.0%. $R_f$ 0.38 (EtOAc/hexane, 2:1). mp 232°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.14 (2H, m, $CH_2$), 2.46 (2H, t $J$ 6.4 Hz, $CH_2$), 3.00 (2H, t $J$ 6.4 Hz, $CH_2$), 3.94 (3H, s, $CH_3$), 7.00 (1H, s, ArH), 8.71 (1H, br s, OH), 9.24 (1H, br s, OH); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 22.1, 23.2, 37.4, 60.4, 99.9, 114.8, 115.6, 133.3, 136.2, 140.3, 145.0, 169.9, 194.5.

### *7,8-Dihydroxy-3-methyl-3,4-dihydrodibenzo[b,d]furan-1(2H)-one* ***13***

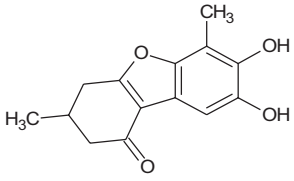


*Method A*
Stirring time 24 h. The product was washed with EtOAc to afford a brown powder (274 mg, 59%) (Found: $M-H^+$, 231.0610. $C_{13}H_{11}O_4$ requires M–H, 231.0657). UPLC 95.3%. $R_f$ 0.38 (EtOAc/hexane, 1:1). mp 263°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 1.11 (3H, d, $J$ 6.0 Hz, $CH_3$), 2.30 (1H, dd, $J$ 4.6, 12.8 Hz, CH), 2.43 (2H, dd $J$ 3.6, 12.8 Hz, $CH_2$), 2.68 (1H, m, CH), 3.01 (1H, dd $J$ 4.6, 12.8 Hz, CH), 6.98 (1H, s, ArH), 7.21 (1H, s, ArH) and 9.15 (2H, br s, OH); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 20.7, 30.3, 40.0, 45.6, 98.5, 105.3, 114.3, 115.3, 143.7, 144.4, 148.2, 169.4 and 194.0.

*Method C*
Stirring time 42 h. The product was purified by flash chromatography (silica: EtOAc/hexane, 0.5:9.5, 1:9, 2.5:7.5, 1:1) to afford a brown powder (84 mg, 30%).

### *7,8-Dihydroxy-3,6-dimethyl-3,4-dihydrodibenzo[b,d]furan-1(2H)-one* ***14***



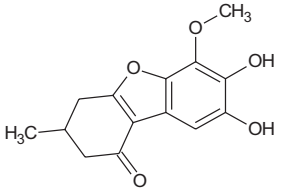
#### *Method A*

Stirring time 24 h. The product was washed with EtOAc to afford a light-brown powder (384 mg, 78%) (Found: M–$H^+$, 245.0817. $C_{14}H_{13}O_4$ requires M–H, 245.0814). UPLC 96.0%. $R_f$ 0.74 (EtOAc/hexane, 1:1). mp 165–168°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 1.11 (3H, d $J$ 6.0 Hz, $CH_3$), 2.25 (3H, s, $CH_3$), 2.29 (1H, dd $J$ 4.7, 12.4 Hz, $CH_2$), 2.43 (2H, dd $J$ 3.4, 12.8 Hz, $CH_2$), 2.68 (1H, m, CH), 3.03 (1H, dd $J$ 4.7, 12.4 Hz, CH), 7.11 (1H, s, ArH), 8.47 (1H, br s, OH), 9.38 (1H, br s, OH); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 9.0, 20.7, 30.3, 31.0, 45.6, 102.3, 107.9, 113.4, 115.5, 142.1, 143.3, 147.8, 169.2, 194.1.

#### *Method C*

Stirring time 42 h. The product was purified by flash chromatography (silica: EtOAc/hexane, 0.5:9.5, 1:9, 1:1; EtOAc) to afford a light-brown powder (209.0 mg, 71%).

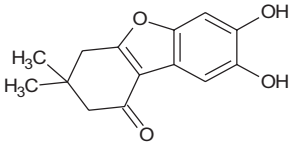
### *7,8-Dihydroxy-6-methoxy-3-methyl-3,4-dihydrodibenzo-[b,d]furan-1(2H)-one* ***15***



#### *Method A*

Stirring time 24 h. The product was washed with EtOAc to afford a light-brown powder (194 mg, 37%) (Found: M–$H^+$, 261.0843. $C_{14}H_{13}O_4$ requires M–H, 261.0763). UPLC 99.6%. $R_f$ 0.50 (EtOAc/hexane, 1:1). mp 179–182°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 1.12 (3H, d $J$ 6.4 Hz, $CH_3$), 2.32 (1H, dd $J$ 4.0, 12.4 Hz, CH) 2.44 (2H, dd $J$ 3.4, 13.2 Hz, $CH_2$), 2.72 (1H, m, CH), 3.06 (1H, dd $J$ 5.0, 12.4 Hz, CH), 3.94 (3H, s, $CH_3$), 6.97 (1H, s, ArH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 20.7, 30.3, 31.0, 45.7, 60.4, 99.8, 114.8, 115.3, 133.4, 136.3, 140.6, 145.1, 169.6, 194.1.

### *7,8-Dihydroxy-3,3-dimethyl-3,4-dihydrodibenzo[b,d]furan-1(2H)-one* ***16***



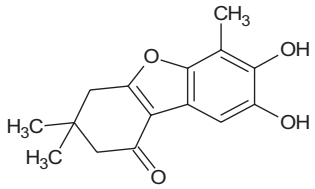
#### *Method A*

Stirring time 24 h. The product was washed with EtOAc to afford a yellow powder (286 mg, 58%). (Found: M–$H^+$, 245.0867. $C_{14}H_{14}O_4$ requires M–H, 245.0814). UPLC 99.8%. $R_f$ 0.64 (EtOAc/hexane, 1:1). mp 278–280°C [lit. [28] 280°C]. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 1.09 (6H, s, 2× $CH_3$), 2.38 (2H, s, $CH_2$), 2.88 (2H, s, $CH_2$), 6.98 (1H, s, ArH), 7.20 (1H, s, ArH), 9.15 (2H, br s, OH); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 28.0 (2× $CH_3$), 34.9, 36.7, 51.5, 98.5, 105.3, 114.2, 114.4, 143.7, 144.3, 148.3, 168.6, 193.7.

#### *Method C*

Stirring time 42 h. The product was purified by flash chromatography (silica: EtOAc/hexane, 0.5:9.5, 1:9, 2.5:7.5, 1:1) to afford a yellow powder (118 mg, 40%).

### *7,8-Dihydroxy-3,3,6-trimethyl-3,4-dihydrodibenzo-[b,d]furan-1(2H)-one* ***17***



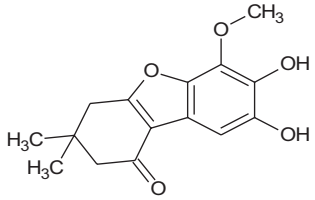
#### *Method A*

Stirring time 24 h. The product was recrystallised from a 5% MeOH in EtOAc solution at 35°C to afford a light-brown powder (510 mg, 98%). (Found: M–$H^+$ 259.0931. $C_{15}H_{15}O_4$ requires M–H, 259.0970). UPLC 96.6%. $R_f$ 0.45 (EtOAc/hexane, 1:2). mp 255°C [lit. [33] 260–262°C]. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 1.09 (6H, s, 2× $CH_3$), 2.26 (3H, s, $CH_3$) 2.37 (2H, s, $CH_2$), 2.90 (2H, s, $CH_2$), 7.10 (1H, s, ArH), 8.41 (1H, br s, OH), 9.32 (1H, br s, OH); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 9.0, 28.0, 34.9, 36.8, 51.5, 102.4, 107.9, 113.3, 114.7, 142.0, 143.3, 168.5, 193.8.

#### *Method C*

Stirring time 42 h. The product was purified by flash chromatography (silica: EtOAc/hexane, 5:95, 1:9, 1:1) to afford a light-brown powder (184 mg, 59%).

### *7,8-Dihydroxy-3,3,6-trimethyl-3,4-dihydrodibenzo-[b,d]furan-1(2H)-one* ***18***



#### *Method A*

Stirring time 24 h. The product was recrystallised from 5% MeOH in EtOAc solution at 35°C to afford a brown powder (403 mg, 73%). (Found: M–$H^+$, 275.0922. $C_{15}H_{15}O_5$ requires M–H, 275.0919). UPLC 94.5%. $R_f$ 0.44 (EtOAc/hexane, 1:1). mp 288–

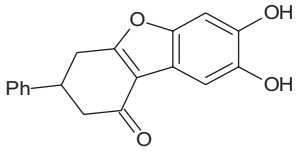
 

291°C [lit. [31] 289–291°C]. $^1$H NMR (400 MHz, MeOH-$d_4$): $\delta$ 1.16 (6H, s, 2× $CH_3$), 2.43 (2H, s, $CH_2$) 2.90 (2H, s, $CH_2$), 4.06 (3H, s, $CH_3$), 7.01 (1H, s, ArH); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 28.1, 35.0, 36.8, 51.2, 60.4, 99.8, 114.5, 114.7, 113.4, 136.2, 140.8, 145.1, 168.9, 193.9.

### *Method B*

Stirring time 44 h. The product was washed with EtOAc to afford a brown powder (425 mg, 77%).

### *7,8-Dihydroxy-3-phenyl-3,4-dihydrodibenzo[b,d]furan-1(2H)-one* ***19***



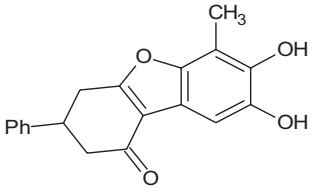

### *Method A*

Stirring time 24 h. The product was washed with EtOAc to afford a white solid (449 mg, 76%). (Found: M–H$^+$, 293.0821. $C_{18}H_{13}O_4$ requires M–H, 293.0814). UPLC 99.8%. $R_f$ 0.50 (EtOAc/hexane, 1:1). mp 240–243°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.58 (1H, dd *J* 3.2, 16.0 Hz, CH), 2.93 (1H, dd *J* 12.4, 16.0 Hz, CH), 3.24 (2H, m, H-11), 3.66 (1H, m, CH), 7.01 (1H, s, ArH), 7.26 (1H, s, ArH), 7.35 (2H, t *J* 7.2, 7.6 Hz, ArH), 7.42 (2H, d *J* 7.6 Hz, ArH), 9.09 (1H, s, CH), 9.13 (1H, s, CH); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 30.7, 44.6, 62.5, 63.4, 68.8, 71.5, 72.3, 73.8, 98.5, 105.4, 114.2, 115.5, 126.9, 128.6, 143.1, 143.9, 144.5, 148.3, 169.0, 193.1.

### *Method C*

Stirring time 42 h. The product was purified by flash chromatography (silica: EtOAc/hexane, 5:95, 1:9, 1:1; EtOAc) to afford a white solid (132 mg, 50%).

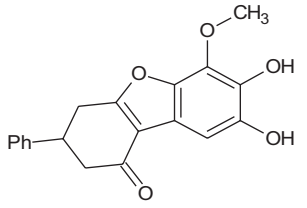
### *7,8-Dihydroxy-6-methyl-3-phenyl-3,4-dihydrodibenzo-[b,d]furan-1(2H)-one* ***20***




### *Method A*

Stirring time 24 h. The product was recrystallised from 5% MeOH in EtOAc solution at 35°C to afford a light-brown powder (279 mg, 80%) (Found: M–H$^+$, 307.0977.1120. $C_{19}H_{15}O_4$ requires M–H$^+$, 307.0970). UPLC 99.0%. $R_f$ 0.45 (EtOAc/hexane, 1:1). mp 250–253°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.28 (3H, s, $CH_3$), 2.59 (1H, dd *J* 4.0, 16.4 Hz, CH), 2.91 (1H, dd *J* 12.2, 16.2 Hz, CH), 3.28 (1H, m, $CH_2$), 3.66 (1H, m, H-11 CH), 7.16 (1H, s, ArH), 7.26 (1H, t *J* 7.0 Hz, ArH), 7.35 (2H, t *J* 7.6 Hz, ArH), 7.42 (2H, d *J* 8.0 Hz, ArH), 8.45 (1H, br s, OH), 9.36 (1H, br s, OH); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 9.1, 30.7, 44.7, 102.4, 108.0, 113.4, 115.8, 126.9, 127.0, 127.1, 128.6, 142.3, 143.2, 143.4, 148.0, 168.8, 193.2.

### *7,8-Dihydroxy-6-methoxy-3-phenyl-3,4-dihydrodibenzo-[b,d]furan-1(2H)-one* ***21***



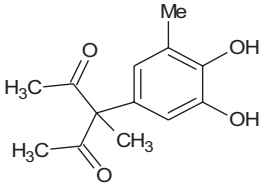

### *Method A*

Stirring time 24 h. The product was washed with EtOAc to afford a dull grey powder (279 mg, 43%). (Found: M–H$^+$, 323.0922. $C_{19}H_{15}O_5$ requires M–H, 323.0919). UPLC 96.8%. $R_f$ 0.38 (EtOAc/hexane, 1:2). mp 165–168°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.57 (1H, dd *J* 4.0, 16.0 Hz, CH), 2.93 (1H, dd *J* 12.0 Hz, 12.4 Hz, CH), 3.26 (2H, m, $CH_2$), 3.67 (1H, m, CH), 3.95 (3H, s, $CH_3$), 7.03 (1H, s, ArH), 7.26 (1H, t *J* 7.2 Hz, ArH), 7.35 (2H, t *J* 7.2, 8.0 Hz, ArH), 7.42 (2H, d *J* 8.0 Hz, ArH), 8.76 (1H, br s, OH) and 9.33 (1H, br s, OH); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 30.6, 44.6, 60.4, 99.8, 114.6, 115.5, 126.8, 127.0, 128.5, 133.4, 136.2, 140.6, 143.0, 145.0, 169.0, 193.0.

### *Method C*

Stirring time 42 h. The product was purified by flash chromatography (silica: EtOAc/hexane, 5:95, 1:9, 1:1; EtOAc) to afford a black powder (58.0 mg, 15%).

### *3-(3,4-Dihydroxy-5-methylphenyl)-3-methylpentane-2,4-dione* ***22***

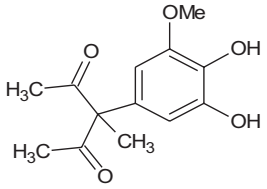



### *Method A*

Stirring time 24 h. The product was washed with EtOAc to afford a dark-brown powder (1.60 g, 80%) (Found: M–H$^+$, 235.0926. $C_{13}H_{14}O_4$ requires M–H, 235.0970. UPLC 98.0%. $R_f$ 0.48 (EtOAc/hexane, 1:1). mp 82–85°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 1.60 (3H, s, $CH_3$), 2.03 (6H, s, $CH_3$), 2.08 (3H, s, $CH_3$), 6.43 (1H, d *J* 2.0 Hz, ArH), 6.46 (1H, d *J* 2.4 Hz, ArH), 8.36 (1H, br s, OH), 9.29 (1H, br s, OH); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 16.2, 19.0, 27.2, 30.7, 68.8, 112.4, 120.1, 124.7, 127.7, 143.0, 144.8, 207.8.

### *3-(3,4-Dihydroxy-5-methoxyphenyl)-3-methylpentane-2,4-dione* ***23***




#### *Method A*

Stirring time 24 h. The product was washed with EtOAc to afford a dark-brown powder (1.10 g, 55%) (Found: M–$H^+$, 251.0933. $C_{13}H_{14}O_5$ requires M–H, 251.0919. $R_f$ 0.48 (EtOAc/hexane, 1:1). mp 108–110°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 1.62 (3H, s, $CH_3$), 2.05 (6H, s, $CH_3$), 3.74 (3H, s, OMe), 6.27 (1H, s, ArH), 6.28 (1H, s, ArH), 8.44 (1H, br s, OH), 9.03 (1H, br s, OH); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 19.1, 27.3, 56.0, 69.0, 103.1, 108.7, 127.7, 133.9, 145.8, 148.5, 207.7.

### *In vitro* anticancer activity evaluation

#### *Assay background*

The growth inhibitory effects of the compounds were tested in a 3-cell line panel consisting of TK10 (renal), UACC62 (melanoma) and MCF7 (breast) cancer cells using the Sulforhodamine B (SRB) assay [32]. The SRB assay was developed by Skehan and colleagues to measure drug-induced cytotoxicity and cell proliferation. Its principle is based on the ability of the protein dye, sulforhodamine B (Acid Red 52), to bind electrostatically in a pH-dependent manner to protein basic amino acid residues of trichloroacetic acid-fixed cells. Under mild acidic conditions it binds to the fixed cellular protein, while under mild basic conditions it can be extracted from cells and solubilised for measurement. The SRB assay is performed at CSIR in accordance with the protocol of the Drug Evaluation Branch, NCI, and the assay has been adopted for this screen.

#### *Materials and method*

The human cell lines TK10, UACC62 and MCF7 were obtained from the NCI in a collaborative research program between the CSIR and the NCI. cell lines were routinely maintained as a monolayer cell culture at 37°C, 5% $CO_2$, 95% air and 100% relative humidity in RPMI containing 5% fetal bovine serum, 2 mM L-glutamine and 50 μg/mL gentamicin.

For the screening experiment the cells (3–19 passages) were inoculated in a 96-well microtitre plate at plating densities of 7–10 000 cells/well and were incubated for 24 h. After 24 h one plate was fixed with TCA to represent a measurement of the cell population for each cell line at the time of drug addition (T0). The other plates with cells were treated with the experimental drugs which were previously dissolved in DMSO and diluted in medium to produce five concentrations (0.01–100 μM). Cells without drug addition served as control. The blank contains complete medium without cells. Etoposide was used as a reference standard.

The plates were incubated for 48 h after addition of the compounds. Viable cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, washed, dried and dyed by SRB. Unbound dye was removed and protein-bound dye was extracted with 10 mM Tris base for optical density determination at a wavelength of 540 nm using a multiwell spectrophotometer. Optical density measurements were used to calculate the net percentage cell growth.

The optical density of the test well after a 48 h period of exposure to test drug is $T_i$, the optical density at time zero is $T_0$, and the control optical density is $C$. Percentage cell growth is calculated as:

$$\left[\frac{(T_i - T_0)}{(C - T_0)}\right] \times 100 \text{ for concentrations at which } T_i \geq T_0$$

$$\left[\frac{(T_i - T_0)}{T_0}\right] \times 100 \text{ for concentrations at which } T_i < T_0.$$

The results of a five dose screening were reported as TGI (total growth inhibition). The TGI is the concentration of test drug where $100 \times (T - T_0)/(C - T_0) = 0$. The TGI signifies a cytostatic effect.

The biological activities were separated into four categories: inactive ($GI_{50}$ or TGI > 100 μM), weak activity (30 μM < $GI_{50}$ or TGI < 100 μM), moderate activity (10 μM < $GI_{50}$ or TGI < 30 μM) and potent activity ($GI_{50}$ or TGI < 10 μM).

For each tested compound, three response parameters, $GI_{50}$ (50% growth inhibition and signifies the growth inhibitory power of the test agent), TGI (which is the drug concentration resulting in total growth inhibition and signifies the cytostatic effect of the test agent) and $LC_{50}$ (50% lethal concentration and signifies the cytotoxic effect of the test agent), were calculated for each cell line.

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## References

[1] M. Dean, A. Rzhetsky, R. Allikmets, *Genome Res.* **2001**, *11*, 1156–1166.

[2] J. P. Gillet, T. Efferth, J. Remacle, *Biochim. Biophys. Acta* **2007**, *1775*, 237–262.

[3] P. Cagniant, D. Carniant, *Adv. Hetercycl. Chem.* **1975**, *18*, 337–360.

[4] D. M. X. Donnelly, M. J. Meegan, in *Comprehensive Heterocyclic Chemistry*, Vol. *4* (Ed.: A. R. Katritzky), Pergamon Press, New York **1984**, Part 3, pp. 657–712.

[5] (a) M. Takasugi, S. Nagao, T. Masamune, A. Shirats, K. Takahashi, *Tetrahedron Lett.* **1978**, *9*, 797–798. (b) R. S. Ward *Nat. Prod. Rep.* **1999**, *16*, 75–96.

[6] M. S. Malamas, J. Sredy, C. Moxham, A. Katz, W. Xu, R. McDevitt, F. O. Adebayo, D. R. Sawicki, L. Seestaller, D. Sullivan, J. R. Taylor, *J. Med. Chem.* **2000**, *43*, 1293–1310.

[7] C. C. Teo, O. L. Kon, K. Y. Sim, S. C. Ng, *J. Med. Chem.* **1992**, *35*, 1330–1339.

[8] S. Maeda, H. Masuda, T. Tokoroyama, *Chem. Pharm. Bull.* **1994**, *42*, 2536–2545.

 

[9] G. D. McAllister, R. C. Hartley, M. J. Dawson, A. R. Knaggs, *J. Chem. Soc. Perkin Trans.* **1998**, *1*, 3453–3458.

[10] J. M. Alcides, M. P. A. da Silva, N. M. V. Silva, F. V. Brito, C. D. Buarque, D. V. de Souza, V. P. Rodrigues, E. S. C. Pocas, F. Noël, E. X. Albuquerque, P. R. R. Costa, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 283–286.

[11] G. D. McCallion, *Curr. Org. Chem.* **1999**, *3*, 67–76.

[12] R. A. Smith, J. Chem, M. M. Mader, I. Muegge, U. Moehler, S. Katti, D. Marrero, W. G. Stirtan, D. R. Weaver, H. Xiao, W. Carley, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2875–2878.

[13] M. Mayer, M. A. O'Neill, K. E. Murray, N. S. Santos-Magalhães, A. M. Carneiro-Leão, A. M. Thompson, V. C. Appleyard, *Anticancer Drugs* **2005**, *16*, 805–809.

[14] (a) I. Hayakawa, R. Shioya, T. Agatsuma, H. Furukawa, S. Naruto, Y. Sugano, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 455–458; (b) I. Hayakawa, R. Shioya, T. Agatsuma, Y. Sugano, *Chem. Pharm. Bull.* **2005**, *53*, 638–640; (c) I. Hayakawa, R. Shioya, T. Agatsuma, H. Furukawa, S. Naruto, Y. Sugano, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4383–4387.

[15] A. Yaropolov, O. Skorobogatko, S. Vartanov, S. Varfolomeyev, *Appl. Biochem. Biotechnol.* **1994**, *49*, 257–280.

[16] C. F. Thurston, *Microbiology* **1994**, *140*, 19–26.

[17] S. Burton, *Curr. Org. Chem.* **2003**, *7*, 1317–1331.

[18] (a) T. H. Niedermeyer, A. Mikolasch, M. Lalk, *J. Org. Chem.* **2005**, *70*, 2002–2008; (b) S. Riva, *Trends Biotechnol.* **2006**, *24*, 219–226; (c) S. Ciecholewski, E. Hammer, K. Manda, G. Bose, V. T. H. Nguyen, P. Langer, F. Schauer, *Tetrahedron* **2005**, *61*, 4615–4619; (d) M. Kurisawa, J. E. Chung, H. Uyama, S. Kobayashi, *Biomacromolecules* **2003**, *4*, 1394–1399; (e) A. Mikolasch, E. Hammer, U. Jonas, K. Popowski, A. Stielow, F. Schauer, *Tetrahedron* **2002**, *58*, 7589–7593; (f) M. Hosny, J. Rosazza, *J. Agric. Food Chem.* **2002**, *50*, 5539–5545; (g) A. Schäfer, M. Specht, A. Hetzheim, W. Francke, F. Schauer, *Tetrahedron* **2001**, *57*, 7693–7699; (h) K. Manda, E. Hammer, A. Mikolasch, T. Niedermeyer, J. Dec, A. D. Jones, A. J. Benesi, F. Schauer, J.-M. Bollag, *J. Mol. Catal. B: Enzym.* **2005**, *35*, 86–92; (i) H. Leutbecher, J. Conrad, I. Klaiber, U. Beifuss, *Synlett* **2005**, *20*, 3126–3130; (j) S. Witayakran, A. J. Ragauskas, *Adv. Synth. Catal.* **2009**, *351*, 1187–1209; (k) A. Kunamneni, S. Camarero, C. García-Burgos, F. J. Plou, A. Ballesteros, M. Alcalde, *Microb. Cell Factory* **2008**, *7*, 32; (l) F. Xu, T. Damhus, S. Danielsen, L. H. Ästergaard, in Modern Biooxidations, *Enzymes, Reactions and Applications* (Eds.: R. D. Schmid, V. Urlacher), Wiley-VCH, Weinheim **2007**, p. 43.

[19] (a) K. W. Wellington, P. Steenkamp, D. Brady, *Bioorg. Med. Chem.* **2010**, *18*, 1406–1414; (b) K. W. Wellington, N. I. Kolesnikova, *Bioorg. Med. Chem.* **2012**, *20*, 4472–4481; (c) K. W. Wellington, R. Bokako, N. Raseroka, P. Steenkamp, *Green Chem.* **2012**, *14*, 2567–2576.

[20] R. W. Middleton, J. Parrick, in *The Chemistry of Quinonoid Compounds* (Eds.: S. Patak, Z. Rappoport), John Wiley & Sons, London **1988**, pp. 1019–1066.

[21] (a) A. N. Semenov, I. V. Lomonsova, V. I. Berezin, M. I. Titov, *Biotechnol. Bioeng.* **1993**, *42*, 1137–1141; (b) J. M. M. Verkade, L. J. C. van Hemert, P. J. L. M. Quaedflieg, H. E. Schoemaker, M. Schürmann, F. L. van Delft, F. P. J. T. Rutjes, *Adv. Synth. Catal.* **2007**, *349*, 1332–1336.

[22] S. Witayakran, L. Gelbaum, A. J. Ragauskas, *Tetrahedron* **2007**, *63*, 10958–10962.

[23] S. Witayakran, A. Ragauskas, *J. Eur. J. Org. Chem.* **2009**, *3*, 358–363.

[24] S. Hajdok, H. Leutbecher, G. Greiner, J. Conrad, U. Beifuss, *Tetrahedron Lett.* **2007**, *48*, 5073–5076.

[25] H. Leutbecher, S. Hajdok, C. Braunberger, M. Neumann, S. Mika, J. Conrad, U. Beifuss, *Green Chem.* **2009**, *11*, 676–679.

[26] L.-X. Pei, Y.-M. Li, X.-Z. Bu, L.-Q. Gu, A. S. C. Chan, *Tetrahedron Lett.* **2006**, *47*, 2615–2618.

[27] R. O. Duthaler, V. Scherrer, *Helv. Chim. Acta* **1984**, *67*, 1767–1775.

[28] Z. Grujić, I. Tabaković, M. Trkovnik, *Tetrahedron Lett.* **1976**, *17*, 4823–4824.

[29] I. Tabaković, Z. Grujić, Z. Bejtović, *J. Heterocycl. Chem.* **1983**, *20*, 635–638.

[30] D. Nematollahi, D. Habibi, M. Rahmati, M. Rafiee, *J. Org. Chem.* **2004**, *69*, 2637–2640.

[31] S. S. H. Davarani, N. M. Najafi, S. Ramyar, L. Masoumi, M. Shamsipur, *Chem. Pharm. Bull.* **2006**, *54*, 959–962.

[32] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.

[33] A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.

[34] C.-C. Wu, T.-K. Li, L. Farh, L.-Y. Lin, T.-S. Lin, Y.-J. Yu, T.-J. Yen, C.-W. Chiang, N.-L. Chan, *Science* **2011**, *333*, 459–462.

 