Novel entry into 5-decarboxydibenzofurans via Smiles rearrangement of the lichen *para*-depside, erythrin

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Erythrin, isolated in 7.3% yield from the lichen *Roccella montagnei*, was converted via a Smiles rearrangement to a series of diphenyl ethers. Those with a free carboxylic acid at C-1 underwent a novel decarboxylative oxidative cyclisation in the presence of $Pd(OAc)_2$ to produce 5-decarboxydibenzofurans. All compounds were assayed for their antioxidant and β-glucuronidase enzyme inhibitory activity.

Keywords: erythrin, Smiles rearrangement, 5-decarboxydibenzofurans, antioxidant activity, β-glucuronidase activity

Lichens are symbiotic organisms of fungi (mycobionts) and algae (phycobionts) comprising about 18,000 species which have been recorded worldwide.[1] They accumulate large concentrations of secondary metabolites, comprising up to 20% of the thallus' dry weight.[2] The most characteristic of these are depsides, depsidones, diphenylethers and dibenzofurans. Depsides and to some extent depsidones are the major constituents. However, diphenyl ethers and dibenzofurans are rare.[3] Biosynthetically, depsides are considered to be the precursors of dibenzofurans through the intermediacy of diphenyl ethers.[4]

Results and discussion

Following the above biosynthetic hypothesis, the *para* depside erythrin **1** (Fig. 1), isolated in 7.6% yield from *Roccella montagnei* Bel., was successfully converted to its diphenyl ether **2** *via* a Smiles rearrangement.[5] The Smiles rearrangement had been used previously on synthetic *para* depsides leading to a total synthesis of pannaric acid.[6] However, prior to our studies, the Smiles rearrangement had not been tested on naturally occurring *para*-depsides nor on depsides with an unprotected 4-hydroxy group.

Compound **2** underwent transesterification upon refluxing in methanol and base to form the diphenyl ether **3** which, on permethylation with methyl iodide in the presence of NaOH in DMSO, yielded the diphenyl ether **4.**

Interestingly and importantly, palladium (II) acetate mediated oxidative coupling[7] of diphenyl ethers **2** and **3**, containing a free carboxylic acid at C-1, provided the dibenzofurans **5** and **6**, (65% and 77%, respectively). Structural evidence (^{13}C NMR and MS) indicated that the carboxyl group had been lost during the coupling.

Four structural analogues of compounds **5** and **6**, namely hypostrepsilic acid **7**, 6-*O*-methylnorascomatic acid **8**, ascomatic acid **9** and methyl ascomate **10**, have been reported as minor constituents of the lichen *Bundophoron patagonicum*, where, C-1 and C-8 carried CH_3 groups whereas C-3 and C-6 had OH groups (Fig. 1).[8] Tanahasi *et al*.[9] have reported four other decarboxylated dibenzofurans including several chlorinated ones which were isolated from cultured micobionts of the lichen *Lecanora cinereocarnea*. The NMR data and HMBC (Fig. 2) of compounds **5** and **6** confirmed the reversal of the CH_3/OH substitution pattern in compounds **7**–**10**, thus establishing that they are members of a new class of dibenzofurans.

This novel Pd (II) acetate mediated decarboxylation followed by two through-space palladium migrations prior to oxidative coupling is represented in Scheme 1. Following a model proposed recently by Tanaka *et al*.[10] for Pd (II) assisted decarboxylation in simple aromatic systems, we propose that the decarboxylative palladation of the acetato palladium (II) species **I** proceeds via the formation of a four-membered palladacyclic intermediate[10] **II** in which the electrophilic Pd (II) is bonded to the carboxylate oxygen and the *ipso*-carbon of the aromatic ring. Loss of carbon dioxide follows the insertion of palladium onto the aromatic ring, giving intermediate **III** which would then undergo Pd migration from ring A to B at the carbon adjacent to the OH group, possibly through an organopalladium (IV) hydride **IV**[11,12] giving rise to the intermediate **V**. Although such an Pd (IV) intermediate has not been reported, other organopalladium (IV) species are well known.[11] These fairly general through-space migrations of Pd have been reported between vinylic to aryl,[13] aryl to aryl,[14] alkyl to aryl,[15] and vinylic to aryl to allylic.[16] Intermediate **V** then might undergo a second Pd through-space migration back to ring A (at the carbon adjacent to the OH group) giving intermediate **VII** possibly through a second organopalladium hydride **VI**. Loss of HOAc from **VI** would then yield intermediate **VII** where the Pd (II) has been inserted into both ring A and B forming a six-membered palladocycle, displaying a stable association with adjacent OH groups on both rings. Extrusion of Pd (0) from **VII** completes the oxidative cyclisation yielding the dibenzofurans **5** and **6**.

On the other hand, when the diphenyl ether **4**, containing an ester group at C-1,was treated with Pd (II) acetate, it gave a mixture of dibenzofurans **11** and **12** (corresponding to equally facile coupling between C-3 of ring A and C-3' or C-5' of ring B) in a 1:1 ratio. The latter dibenzofuran **12**, obtained in 12% overall yield from erythrin **1** in four steps, has been converted to pannaric acid **13**.[6]

Studies on the biological activity of lichen metabolites are scarce. Amongst the dibenzofurans, usnic acid has been extensively studied. It possesses antibacterial, anti-proliferative, anti-inflammattory, anti-tumour, anti-mutagenic, analgesic, anti-pyretic, plant growth inhibitory activities, and insecticidal activities.[17] Other than this there are no other reports on the bioactivities of dibenzofurans.

Thus, compounds **1–6, 11** and **12** were subjected to antioxidant activity in the super oxide inhibition (SOI) assay and DPPH radical scavenging assay and enzyme inhibitory activity against β-glucuronidase.

Bioassay results showed that erythrin **1,** was a good SOI inhibitor with a IC_{50} value of 127.1 ± 0.1 μM, comparable to the standard propyl gallate (IC_{50} = 106.0 ± 1.70 μM). However,

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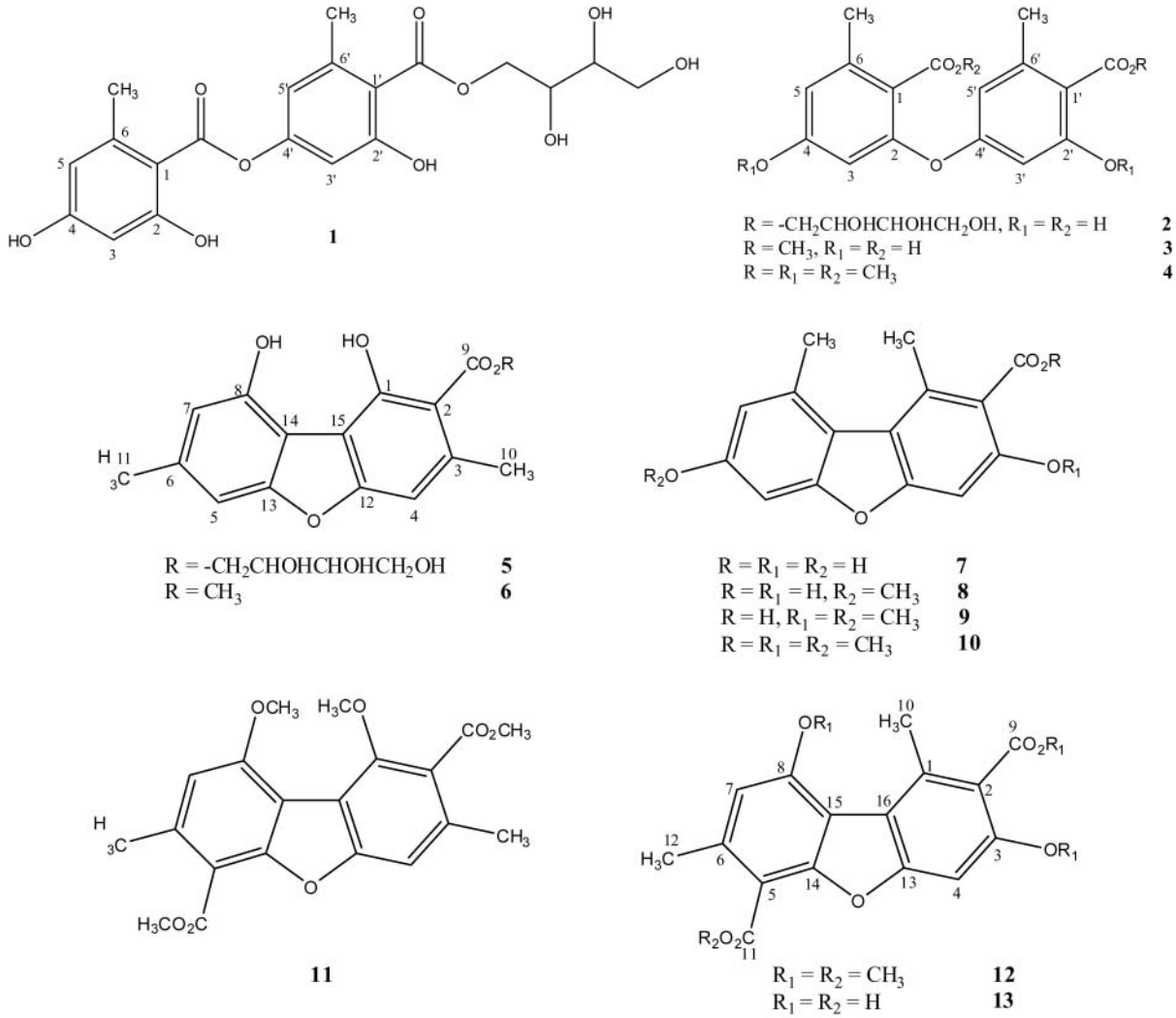


Fig. 1 Structures of compounds **1**–**13**.

its conversion to diphenyl ethers appears to have destroyed this activity as no significant antioxidant activity was exhibited by compounds **2**, **3** and **4**. In the β-glucuronidase enzyme inhibitory assay, only compound **2** showed moderate activity. Interestingly, the novel dibenzofurans **5** and **6**, (which are structural analogues of naturally occurring rare decarboxydibenzofurans **7**–**10)** showed relatively high antioxidant activity in the DPPH assay (**5**: IC_{50} = 201.9 ± 0.9 μM; **6**: IC_{50} = 81.9 ± 3.8 μM) when compared to the standards propyl gallate (IC_{50} = 30.0 ± 0.27 μM) and butylated hydroxyl anisole (IC_{50} = 44.0 ± 2.0 μM). Moreover, compound **5** showed superior β-glucuronidase activity (**5**: IC_{50} = 46.9 ± 0.2 μM) almost the same potency as the standard D-saccharic acid 1,4- lactone (IC_{50} = 48.4 ± 1.3 μM).

This work highlights the usefulness of abundant lichen metabolites in the semi-synthesis of naturally occurring or unknown metabolites and the importance of bioactivity studies of lichen substances which have thus far received scant attention as potential pharmaceuticals.

Experimental

General

The lichen *R. montagnei* was collected from the palm tree *Coccus nucifera* L., in the Kurunegella district, Sri Lanka. ^{1}D and ^{2}D NMR spectra were recorded on a 300 MHz Varian or on a 500 MHz Bruker spectrometer. HRESIMS were recorded on a Micromass LCT spectrometer. Medium pressure liquid chromatography (MPLC), flash chromatography and gravity chromatography were performed on Merck Si gel 60 (230–400 mesh). Thin layer chromatography (TLC) was performed on Merk Si gel 60 F_{254} plates. Bioassays were performed in triplicates in 96-well microplates. The results (change in absorbance per min.) were processed using ELISA (multiple reader Spectra Max Plus 3400 Molecular Device, USA). The IC_{50} values of the compounds were calculated using EZ-Fit enzyme kinetics software program (Perrella Scientific Inc. Amherst, USA).

Smiles rearrangement of erythrin **1** *and synthesis of diphenyl ethers* **2**–**4**

Isolation of erythrin **1:** *R. montagnei* (190 g) was extracted with acetone (2 L × 3), the solvents were evaporated and the crude extract (30 g) was subjected to MPLC (gradient elution 10% hexane: CH_2Cl_2 to 50% CH_2Cl_2: MeOH). Erythrin **1** was found as the major constituent of the fractions which eluted between 5% MeOH: CH_2Cl_2 and 12% MeOH: CH_2Cl_2. These fractions were combined and further subjected to MPLC (CH_2Cl_2 to CH_2Cl_2: MeOH) to obtain erythrin **1** (14.4 g; 7.6%) as white crystals; m.p. 157 °C (lit.[3] 156–157 °C).

Diphenyl ether (**2**): Erythrin (5 g) and K_2CO_3 (1.66 g) were dissolved in DMSO (30 mL) and stirred at 25 °C under anhydrous conditions for 3 hrs. The reaction mixture was acidified and extracted into ethyl acetate and fractionated *via* silica gel MPLC (eluent: CH_2Cl_2 to MeOH) to afford the diphenyl ether (**2**) (2.35 g, 47%) as a white powder; m.p. 162–163 °C: ^{1}H NMR (300 MHz, CD_3OD): δ 2.35 (3H, s), 2.52 (3H, s), 3.63–3.77 (2H, m), 3.76–3.80 (1H, dd, *J*=12.5, 6.5 Hz), 3.89–3.93 (1H, dt, *J*=13.5, 3.0 Hz), 4.38–4.45 (1H, dd, *J*=11.0, 7.0 Hz), 4.58–4.63 (1H, dd, *J*=11.2, 2.7 Hz), 6.25 (1H, d, *J*=2.4 Hz), 6.29 (1H, d, *J*=2.4 Hz), 6.36 (1H, d, *J* = 2.4 Hz), 6.56 (1H, d, *J* = 2.4 Hz), 11.24 (1H, s); ^{13}C NMR (75 MHz, CD_3OD):

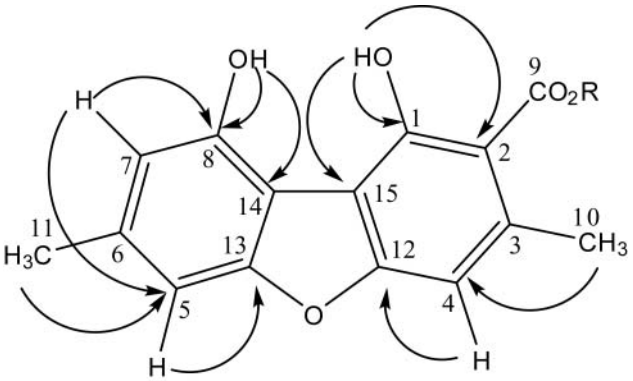


Fig. 2 Selected HMBC interactions in **5** (R = -CH_2CHOHCHOH CH_2OH), and **6** (R = CH_3).

δ 119.9, 154.7, 106.3, 160.5, 114.8, 140.3, 170.6, 20.2, 109.7, 164.4, 103.7, 162.9, 113.2, 144.3, 171.9, 23.9, 68.0, 71.0, 73.6, 64.5. HRESIMS *m/z* Calcd for $C_{20}H_{22}O_{10}Na$ 445.1111. Found 445.1129.

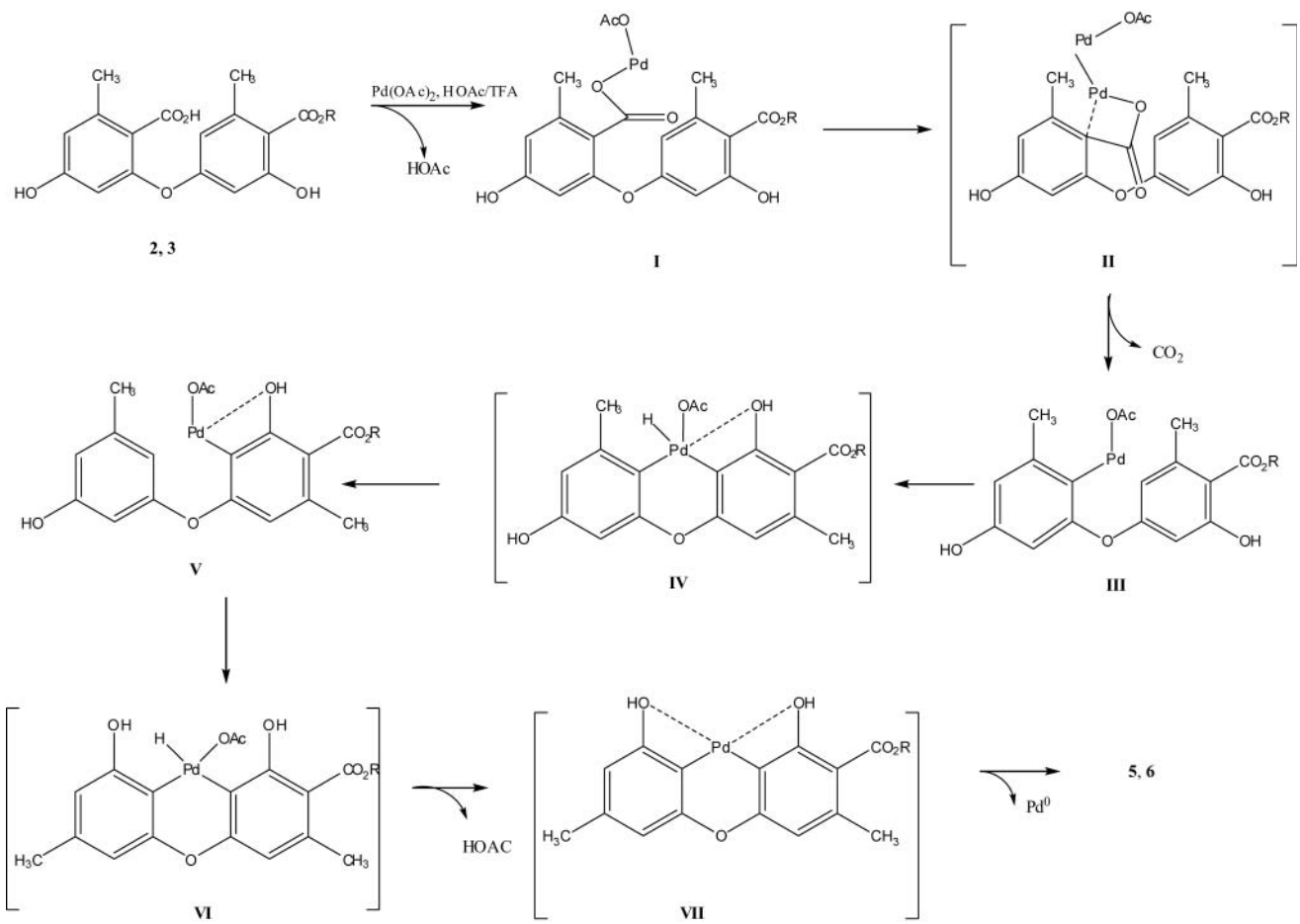
Diphenyl ether (**3**): Diphenyl ether **2** (1.30 g) was refluxed with excess methanolic NaOH for 6–8 h. The methanol was evaporated and the residue was acidified and extracted with ethyl acetate. The crude product was subjected to silica gel gravity column chromatography (eluent: CH_2Cl_2 to CH_2Cl_2: MeOH) to yield the diphenyl ether **3** (0.69 g, 65%) as a white powder; m.p. 182 °C (lit.[6] 182 °C): ^{1}H NMR (300 MHz , CD_3OD): δ 2.35 (3H, s), 2.44 (3H, s), 3.91 (3H, s), 6.26 (2H, d, *J* =1.8 Hz), 6.35 (1H, d, *J* = 1.8 Hz), 6.54 (1H, d, *J* = 1.8 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 121.5, 154.7, 106.3, 160.4, 114.9, 140.2, 171.5, 20.2, 109.7, 164.7, 103.9, 163.3, 113.3, 144.1, 172.8, 22.4, 52.4. HRESIMS *m/z* Calcd for $C_{17}H_{17}O_7$ 333.0974 (MH^+). Found 333.0977.

Diphenyl ether (**4**): The diphenyl ether **3** (2.0 g) was dissolved in DMSO (25 mL) and K_2CO_3 (4.0 g) was added and stirred for 10–15 min at 30 °C. Then, MeI (1.5 mL) was added and stirring was continued for 4 hours. The reaction mixture was acidified and extracted into ethyl acetate. The crude product was subjected to silica gel gravity column chromatography (eluent: 80% hexane: CH_2Cl_2 to 5% MeOH: CH_2Cl_2) to yield the permethylated diphenyl ether **4** (2.1 g, 91%) as a white powder; m.p. 72 °C (lit.[6] 70–72 °C): ^{1}H NMR (500 MHz , CD_3OD): δ 2.36 (3H, s), 2.21 (3H, s), 3.75 (6H, 2s), 3.77 (3H, s), 3.86 (3H, s), 6.37 (1H, d, *J*=1.8 Hz), 6.40 (1H, d, *J*=2 Hz), 6.51 (1H, d, *J*=1.5 Hz), 6.69 (1H, d, *J*=2 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 118.6, 154.6, 103.1, 161.2, 111.5, 139.2, 167.8, 18.6, 118.4, 158.1, 98.9, 159.2, 110.6, 137.8, 168.7, 18.0, 54.5, 55.0, 51.0, 54.5. HRESIMS *m/z* Calcd for $C_{20}H_{22}O_7Na$ 397.1263. Found 397.1272.

Synthesis of dbenzofurans **5**, **6**, **11** *and* **12**

Dibenzofuran (**5**):The diphenyl ether **2** (0.18 g, 0.43 m mol) in acetic acid (5 mL) and palladium II acetate (0.15 g, 0.067 m mol) in trifluroacetic acid (5 mL) were mixed together and stirred at 30 °C for 4 hours. The solvents were evaporated under reduced pressure, and the crude product was subjected to silica gel flash chromatography (eluent:hexane: CH_2Cl_2, 10:90, to 50% CH_2Cl_2: MeOH) to yield the dibenzofuran **5** (0.1 g, 65%) as a white solid; m.p. 276–277 °C: ^{1}H NMR (500 MHz, CD_3OD): δ 2.41 (3H, s), 2.39 (3H, s), 3.36–3.41(2H, m), 3.56–3.57 (1H, dd, *J* = 12.7, 6.6 Hz), 3.67–3.70 (1H, dt, *J* = 13.5, 2.7 Hz), 4.18–4.22 (1H, dd, *J* = 11.0, 7.0 Hz)), 4.45–4.48 (1H, dd, *J* = 11.2, 2.7 Hz), 6.63 (1H, d, *J* = 2.1 Hz), 6.95 (1H, d, *J* = 2.1 Hz), 6.98 (1H, s), 8.30 (1H, s); ^{13}C NMR (125 MHz, CD_3OD): δ 156.0, 115.1, 138.3, 103.7, 103.4, 136.2, 109.7, 149.4, 167.9, 21.3, 20.1, 156.0, 156.2, 109.0, 109.5, 68.3, 71.0, 73.7, 64.5. HRESIMS *m/z* Calcd for $C_{19}H_{20}O_8$, 376.1107. Found 376.1127.

Dibenzofuran (**6**): The diphenyl ether **3** (0.1 g, 0.3 m mol) in acetic acid (5 mL), and palladium II acetate (0.067 g, 0.3 m mol) in trifluroacetic acid (5 mL) were mixed and stirred at 30 °C for 4 hours.



Scheme 1 Possible mechanistic pathway of the decarboxylatve oxidative cyclisations of **2** and **3**.

The solvents were evaporated under reduced pressure, and the crude product was subjected to silica gel flash chromatography (eluent: hexane to CH_2Cl_2) to yield the dibenzofuran **6** (0.066 g, 77%) as a slightly off white powder; m.p. 226 °C : ^{1}H NMR (300 MHz, CD_3OD): δ 2.66 (3H, s), 2.45 (3H, s), 4.02 (3H, s), 6.69 (1H, d, *J*=2.4 Hz), 6.87 (1H, d, *J*=2.4 Hz), 6.93 (1H, s), 8.7 (1H, bs), 13.7 (1H, bs); ^{13}C NMR (75 MHz, CD_3OD): δ 156.2, 106.7, 140.7, 108.0, 103.7, 139.8, 111.1, 150.7, 173.2, 25.1, 22.3, 159.0, 157.4, 108.6, 111.2, 52.7. HRESIMS *m/z* Calcd for $C_{16}H_{14}O_5Na$, 309.0739. Found 309.0728.

Dibenzofurans **11** *and* **12**: The diphenylether **4** (0.5 g, 1.34 m mol) in acetic acid (7.5 mL), and palladium II acetate (0.37 g, 1.60 m mol) in trifluroacetic acid (7.5 mL) were mixed together and stirred at 30 °C for 24 hours. The solvents were evaporated under reduced pressure and the crude product was subjected to silica gel gravity column chromatography (eluent: hexane to hexane: EtOAc) to yield the dibenzofuran **11** (0.22 g, 44%) (m.p. 146 °C) and the dibenzofuran **12** (0.20 g, 40%) (m.p. 168 °C, lit.[6] 168 °C) as white powders.

Dibenzofuran (**11**): ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.44 (3H, s), 2.67 (3H, s), 3.94 (3H, s), 3.96 (3H, s), 4.01 (3H, s), 4.06 (3H, s), 6.68 (1H, s), 7.24 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 152.0, 125.1, 135.4, 109.5, 109.5, 141.3, 108.1, 156.3, 169.2, 20.1, 166.5, 22.2, 157.6, 156.7, 111.0, 114.4, 64.8, 56.2, 52.6, 52.3. HRESIMS *m/z* Calcd for $C_{20}H_{20}O_7Na$, 395.1107. Found 395.1110.

Dibenzofuran (**12**): ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.77 (3H, s), 2.62 (3H, s), 3.89 (3H, s), 3.93 (3H, s), 3.98 (3H, s), 3.96 (3H, s), 6.60 (1H, s), 6.99 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 131.7, 121.4, 156.2, 92.8, 109.4, 139.6, 107.8, 155.2, 169.5, 19.5, 166.5, 21.9, 156.5, 156.8, 113.0, 115.8, 56.1, 55.6, 52.5, 52.4. HRESIMS *m/z* Calcd for $C_{20}H_{20}O_7Na$, 395.1107. Found 395.1103.

Bioassays

SOI activities of the test compounds were determined by using the method of Gaulejac *et al.*[18] DPPH free radical scavenging ability were determined by measuring the change in absorbance of DPPH (1,1,-diphenyl-2-picrylhydrazyl radical) at 517 nm by the spectrophotometric method developed by Lee *et al.*[19] β-Glucuronidase inhibitory activity was determined spectrophotometrically by using *p*-nitrophenyl- *β*-D-glucuronide as the substrate.[20]

The authors thank the NSF and NRC Sri Lanka, IFS Sweden for generous funding. A scholarship by the NSF and travel award by the UGC (to visit HEJ research Institute) to VT is gratefully acknowledged.

Received 5 January 2010; accepted 18 February 2010
Paper 100943 doi: 10.3184/030823410X12677394014276
Published online: 23 March 2010

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