J Ind Microbiol Biotechnol (2008) 35:901–906
DOI 10.1007/s10295-008-0363-4

ORIGINAL PAPER

Microbial hydroxylation of *o*-bromophenylacetic acid: synthesis of 4-substituted-2,3-dihydrobenzofurans

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Received: 29 January 2008 / Accepted: 23 April 2008 / Published online: 22 May 2008

Abstract Microbial hydroxylation of *o*-bromophenylacetic acid provided 2-bromo-5-hydroxyphenylacetic acid. This enabled a route to the key intermediate 4-bromo-2,3-dihydrobenzofuran for synthesizing a melatonin receptor agonist and sodium hydrogen exchange compounds. Pd-mediated coupling reactions of 4-bromo-2,3-dihydrobenzofuran provided easy access to the 4-substituted-2,3-dihydrobenzofurans.

Keywords 2,3-Dihydrobenzofuran · 4-Substituted-2,3-dihydrobenzofurans · 2-Bromophenylacetic acid · Microbial hydroxylation

Introduction

The structure of dihydrobenzofuran has been the core of many medicinal chemistry programs [4, 9, 11, 18, 19, 22, 25, 28]. In these programs, several interesting syntheses of dihydrobenzofurans have been reported. During our melatonin receptor agonist [7, 8, 21, 24] and sodium hydrogen exchange (NHE) programs [1, 2], we needed to develop 2,3-dihydrobenzofuran as a core structure which would allow facile access to 4-substituted-2,3-dihydrobenzofurans. We envisioned accomplishing this by microbial hydroxylation [14] of 2-bromophenylacetic acid (**7**), thus providing a suitable substrate (**9**). Substrate **9** could allow us to quickly prepare 4-bromo-2,3-dihydrobenzofuran (**13**) and enable routes to 4-substituted 2,3-dihydrobenzofurans. One such example is hydroxylation of phenylacetic acid, which was well studied by Yoshizako and co-workers [26, 31]. They have shown that various strains of *Aspergillus* and *Penicillum* have the ability to convert phenylacetic acid into 2,6-dihydroxyphenylacetic acid. They have also shown that unlike *T. cutaneum* used by the Dagley's group [5], which produces various mixtures of hydroxylated phenylacetic acid derivatives, strains of *Trichosporon cutaneum* predominantly produce 2,6-dihydroxyphenylacetic acid via 2-hydroxyphenylacetic acid. However, there is only one report of microbial hydroxylation of a halogenated phenylacetic acid [23], i.e., 2-chloro-phenylacetic acid, using *Beauveria bassiana* fungi to give 2-chloro-5-hydroxyphenylacetic acid.

In our program, we investigated the microbial hydroxylation of readily available phenylacetic acid (**1**), 2-hydroxyphenylacetic acid (**2**), 2-bromophenylacetic acid (**7**) and phenylethyl alcohols **4** and **5** to obtain 4-substituted-2,3-dihydrobenzofurans. In this communication, we report hydroxylation of 2-bromophenylacetic acid (**7**), preparation of the 4-bromo-2,3-dihydrobenzofuran (**13**) and its Pd-mediated coupling reactions to give 4-substituted 2,3-dihydrobenzofurans. This is the first report on microbial hydroxylation of 2-bromophenylacetic acid and subsequent conversion of hydroxylated product to 4-substituted 2,3-dihydrobenzofurans, which are the key intermediates required for the synthesis of melatonin receptor agonist and sodium hydrogen exchange compounds.

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Materials and methods

Microbial hydroxylation

For microbial hydroxylation, the *Aspergillus* strains were grown in fungal broth (medium A) consisting of 10 g/L malt extract, 10 g/L yeast extract, 1.0 g/L peptone, and 20 g/L dextrose, pH 7.0. The cultures were incubated at 28 °C with 200-rpm shaking for 2 days. These cultures were transferred to the hydroxylation medium containing 2–10% inoculum. Cultures grown in medium A for 24 h were inoculated (5% inoculum) into 100 mL of medium B consisting of 1% yeast extract, 0.5% K_2HPO_4, 0.1% $CaCl_2{\cdot}2H_2O$, 0.05% $FeSO_4{\cdot}7H_2O$, 0.05% $MgSO_4{\cdot}7H_2O$ with the substrate (2-bromophenylacetic acid, phenylacetic acid or 2-hydroxyphenylacetic acid) concentration at 1 mg/mL. The biotransformation was carried out at 28 °C at a rate of 200 rpm for 2–5 days. Samples of 1 mL were taken every 24 h. These samples were extracted with 2 mL of ethyl acetate. The organic layer was separated, dried, and solubilized in 50% acetonitrile for HPLC and LC/MS analysis.

Fermentation studies

The fermentation studies were carried out for the hydroxylation of 2-bromophenylacetic acid with *Aspergillus niger* (SC2164). A 3 L Braun fermentor containing 2 L of medium C (0.5% toasted nutrisoy, 2% glucose, 0.5% yeast extract, 0.5% K_2HPO_4, and 0.5% NaCl, adjusted to pH 7.0 with HCl) was inoculated with 5% inoculum and allowed to grow for 18 h. 2-Bromophenylacetic acid (6 g in 200 mL of 50% ethanol) was added to the fermentor at 18 h after the growth or continuously fed at the rate of 9 mL/h beginning at 18 h after inoculation. After the biotransformation process, the cells were removed by filtration and a resin (SP201, HP20, or XAD16) was added to the filtrate to adsorb the hydroxylated compounds. The hydroxylated compounds and 2-bromophenylacetic acid were adsorbed by XAD16 resins. The hydroxylated compounds were extracted with ethyl acetate from the resin. The extraction with ethyl acetate recovered 80% of the absorbed compounds from the resin.

After the bioconversion of 2-bromophenylacetic acid (5.8 g), XAD16 resin (200 g) was added to the filtrate after removal of cells to adsorb the products. Analysis of cells after ethyl acetate extraction revealed no substrate and >5% product associated with cells. Resin containing 2-bromo-6-hydroxyphenylacetic acid (1.4 g, by HPLC analysis) and 2-bromo-5-hydroxyphenylacetic acid (1.5 g, by HPLC analysis) was isolated and stirred with 400 mL of acetonitrile/water (70/30) and 4 mL of TFA at room temperature for 1 h. The resin was removed by filtration and the filtrate was concentrated (21.82 g crude); further purification was done by passing through a Celite 545 pad with MeOH and a silica gel pad in 5% MeOH:EtOAc. A brown residue of 7.8 g of crude product was obtained, which may contain other materials from microbial cells and medium components which also had been adsorbed on the resin. The product was further purified using preparative column chromatography (YMC C-18 column, gradient method: 20–80% MeOH/water/TFA) to obtain 1.3 g (21% yield) of the desired 2-bromo-6-hydroxyphenylacetic acid (**8**) and 1.38-g (22%) of the undesired 2-bromo-5-hydroxyphenylacetic acid (**9**).

HPLC analysis

The analysis of the products was carried out on an HPLC (1090A Hewlett-Packard) equipped with a C-18 Vydac column (2.6 × 25 cm). The column was equilibrated with 0.1% TFA. Solvent A was 0.1% TFA and solvent B was 70% acetonitrile containing 0.1% TFA. The following conditions were used to monitor product formation. A gradient of 0–100% solvent B was applied in 20 min was applied at a flow rate of 1 mL/min. The detector was set at 215 nm. The retention times for the compounds were as follows: phenylethyl alcohol, 12.47 min; 2-hydroxyphenylethyl alcohol, 7.14 min; 2,6-dihydroxyphenylethyl alcohol, 4.29 min; phenylacetic acid, 12.36 min; 2-hydroxyphenylacetic acid, 6.59 min; 2-bromophenylacetic acid, 10.48 min; 2-bromo-6-hydroxyphenylacetic acid, 7.09 min; 2-bromo-5-hydroxyphenylacetic acid, 5.82 min.

*2-Bromo-6-hydroxyphenylacetic acid (**8**)*

^{1}H NMR (400 MHz, CD_3OD) δ 3.68 (2H, s, $-CH_2Ar$), 6.64 (1H, dd, J = 8.02 Hz, J = 0.6 Hz, H-5), 6.85 (1H, dd, J = 7.9 Hz, J = 7.9 Hz, H-4), 6.91 (1H, d, J = 7.7 Hz, H-3); ^{13}C NMR (400 MHz, CD_3OD) δ 35.97, 114.92, 123.43, 124.25, 127.01, 130.04, 158.15, 174.78; HRMS (EI) calcd for $C_8H_7BrO_3$ (M^+) 229.9578, found 230.

*2-Bromo-5-hydroxyphenylacetic acid (**9**)*

^{1}H NMR (400 MHz, CD_3OD) δ 3.7 (2H, s, $-CH_2Ar$), 6.63 (1H, dd, J = 8.72 Hz, J = 2.93 Hz, H-4), 6.80 (1H, d, J = 2.87 Hz, H-6), 7.35 (1H, dd, J = 8.73 Hz, H-3); ^{13}C NMR (400 MHz, CD_3OD) δ 42.71, 115.16, 117.46, 120.14, 134.58, 137.18, 158.61, 174.78; HRMS (EI) calcd for $C_8H_7BrO_3$ (M^+) 229.9578, found 230.

*2-Bromo-3-hydroxyphenylacetic acid (**10**)*

^{1}H NMR (500 MHz, CD_3OD) δ 3.6 (2H, s, $-CH_2Ar$), 6.69 (1H, dd, J = 7.5 Hz, J = 7.5 Hz, H-5), 7.06 (1H, bd, J = 7.5 Hz, H-6), 7.34 (1H, bd, J = 7.5 Hz, H-4); MS (ESI Q3MS LMR up) calcd for $C_8H_7BrO_3$ (M^+) 229.9578, found 229 $(M - H)^-$.

*3-Bromo-2-(2-hydroxyethyl)phenol (**11**)*

To a solution of THF (3 mL) was added to compound **9** (100 mg, 0.432 mmol). The solution was cooled to 0 °C. To this was added $NaBH_4$ (5 mg, 3 eq.) followed by the dropwise addition of $BF_3{\cdot}Et_2O$ (0.164 mL, 3 eq.). The reaction mixture was stirred at 0 °C for 1 h and then it was allowed to warm to room temperature and stirred for additional 3 h. After HPLC analysis, complete disappearance of the starting material was observed, and the reaction was quenched by the addition of acetone (3 mL). The solution was diluted with MTBE (5 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ (3 × 2 mL) and brine (2 × 2 mL). The organic layer was dried ($MgSO_4$) and concentrated. The residue was chromatographed to obtain compound **11** in 88.5% yield (83 mg) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.45 (1H, s, $–CH_2OH$), 3.14 (2H, t, J = 5.32 Hz, $–CH_2$), 4.00 (2H, t, J = 5.3 Hz, $–CH_2$), 6.88 (1H, dd, J = 0.75 Hz, J = 7.3 Hz, Ar-H), 6.99 (1H, t, J = 7.9 Hz, Ar-H), (1H, dd, J = 0.8 Hz, J = 8.0 Hz, Ar-H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 33.51, 64.14, 116.97, 125.40, 125.62, 127.165, 129.24, 156.92; HRMS (EI) calcd for $C_8H_9BrO_2$ (M^+) 215.98, found 216.99 $(M + H)^+$.

*3-Bromo-2-(2-chloroethyl)phenol (**12**)*

To a solution of compound **11** (83 mg, 0.38 mmol) was added (chloromethylene)dimethyliminium chloride (100 mg, 2 eq.) in acetonitrile at −30 °C. The reaction mixture was stirred for 2 h. After complete disappearance of the starting material the reaction was brought to 0 °C. Then the reaction was quenched with sat. $NaHCO_3$ (2 × 3 mL) and brine (3 mL). The separated organic layer was combined, dried ($MgSO_4$), and evaporated to obtain 93 mg of clean phenol **12**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.22 (2H, t, J = 7.20 Hz, $–CH_2$), 4.37 (2H, t, J = 7.11 Hz, $–CH_2$), 6.80 (1H, bd, J = 8.1 Hz, Ar-H), 6.94 (1H, t, J = 8.0 Hz, Ar-H), 7.09 (1H, dd, J = 0.7 Hz, J = 8.0 Hz, Ar-H), 8.0 (1H, s, Ar-OH); ^{13}C NMR (400 MHz, $CDCl_3$) δ 29.57, 62.91, 115.076, 124.17, 124.84, 126.20, 129.14, 163.49; HRMS (EI) calcd for C_8H_8BrClO (M^+) 233.94, found 234.95 $(M + H)^+$.

*4-Bromo-2,3-dihydrobenzofuran (**13**)*

The crude compound **12** (34 mg, 0.14 mmol) was dissolved in acetone (3 mL) and K_2CO_3 powder (70 mg) was added. The reaction mixture was refluxed for 12 h. The reaction mixture was cooled and filtered. The clear filtrate was concentrated and purified by chromatography to obtain compound **13** (20 mg, 72%). Compound **11** was isolated as a side product (5 mg, 16%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.21 (2H, t, J = 8.76 Hz, $–CH_2$), 4.59 (2H, t, J = 8.72 Hz, $–CH_2$), 6.69 (1H, bt, J = 4.3 Hz, Ar-H), 6.97 (1H, bd, Ar-H), 6.98 (1H, bd, Ar-H), ^{13}C NMR (400 MHz, $CDCl_3$) δ 31.68, 70.99, 108.58, 119.59, 123.69, 128.67, 129.81, 160.89; HRMS (EI) calcd for C_8H_7BrO (M^+) 197.97, found 198.98 $(M + H)^+$.

*4-(Pyrrolidine)-2,3-dihydrobenzofuran (**14**)*

The reaction was performed according to Buchwald et al. [28]. To a solution of compound **13** (20 mg, 0.1 mmol) in toluene (2.0 mL) under nitrogen was added pyrrolidine (13.6 mg, 0.192 mmol), sodium *t*-butoxide (16 mg, 0.166 mmol), 2,2′-bi(diphenylphosphino)-1,1′-binaphthyl (BINAP) (1.6 mg, 2%). The reaction mixture was stirred and heated at 80 °C for 3 h, after which HPLC indicated the disappearance of compound **6**. To the reaction mixture MTBE (1 mL) and brine (1 mL) were added, the organic layer was separated, the aqueous layer was back extracted with MTBE (3 × 1 mL), and the combined organic layers were dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was chromatographed (6% EtOAc:hexanes) to give a colorless oil, which crystallized on standing to provide 17.9 mg of **14** (90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.90–1.96 (4H, m,, $2CH_2$), 3.36–3.42 (6H, m, $–3CH_2$), 4.48 (2H, t, J = 8.79 Hz, $–CH_2$), 6.14 (1H, d, J = 7.91 Hz, Ar-H), 6.24 (1H, d, J = 7.91 Hz, Ar-H), 6.98 (1H, t, J = 7.91 Hz, Ar-H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 25.36 (2C), 31.01, 49.30 (2C), 70.42, 99.30, 105.86, 110.20, 128.73, 146.75, 161.43; HRMS (EI) calcd for $C_{12}H_{15}NO$ (M^+) 189.12, found 190.12 $(M + H)^+$.

*Trans-4-(2,3-dihydrobenzofuran)cinnamic acid, methyl ester (**15**) [3]*

The reaction was performed according to Link et al. [14]. To a solution of Cs_2CO_3 (41 mg, 0.125 mmol), compound **13** (23 mg, 0.115 mmol), and Pd_2dba_3 (1.4 mg, 0.0015 mmol) in dioxane (200 µL) was added methyl acrylate (45 µL, 0.50 mmol). Tributyl phosphine (1.2 mg, 0.006 mmol) in dioxane (200 µL) was added. The reaction mixture was stirred and heated at 80 °C; after 4 h the reaction showed slow progress, so additional Pd_2dba_3 (2.8 mg) and tributyl phosphine (∼2.4 mg) were added. The reaction mixture was stirred overnight at 80 °C, and it was cooled, diluted with MTBE (2 mL), and passed through Celite 545. The crude mixture obtained after concentration was chromatographed to obtain 3 mg of starting material, compound **13**, and 10.3 mg of the desired **15** (44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.33 (2H, t, J = 8.79 Hz, $–CH_2$), 3.81 (3H, s, $–OCH_3$), 4.62 (2H, t, J = 8.79 Hz, $–CH_2$), 6.38 (1H, d, J = 16.26 Hz, vinyl-H), 6.81 (1H, d, J = 7.91 Hz, Ar-H), 7.06 (1H, d, J = 7.91 Hz, Ar-H), 7.14 (1H, t, J = 7.69 Hz, Ar-H), 7.68 (1H, d, J = 16.26 Hz, vinyl-H); ^{13}C NMR

(400 MHz, $CDCl_3$) δ 29.27, 51.74, 71.11, 110.86, 119.11, 119.39, 127.08, 128.45, 131.25, 142.51, 160.54, 167.40; HRMS (EI) calcd for $C_{12}H_{12}O_3$ (M^+) 232.07, found 205.09 $(M + H)^+$.

*4-Vinyl-2,3-dihydrobenzofuran (**16**) [20]*

To a solution of 4-bromo-2,3-dihydrobenzofuran (**13**) (20 mg, 0.10 mmol) and tributylvinyl tin (32 mg, 0.10 mmol) in toluene (3 mL) under nitrogen was added tetrakis(triphenylphosphine) palladium (0) (4 mg, 2%) and the reaction mixture was refluxed for 24 h. The concentrated residue, dissolved in acetonitrile (5 mL) was extracted with hexanes (3 × 1 mL). The organic acetonitrile layer was concentrated and the resulting brown solution was passed through a plug of silica gel to obtain 11 mg (75%) of product **16**. The ^{1}H NMR and ^{13}C NMR were identical to the known product. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.15 (m, 2H), 4.45 (t, 2H), 5.25 (d, 1H), 5.65 (d, 1H), 6.6 (1H, d), 6.7 (d, 1H) 6.95 (d, 1H), 7.05 (t, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 28.84, 70.78, 108.25, 115.33, 117.48, 124.65, 127.87, 134.15, 134.52, 160.09.

Results and discussion

Approximately 40 fungal cultures were screened for hydroxylation of phenylacetic acid and 2-hydroxyphenylacetic acid. The cultures screened were of the genera *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus*. *Aspergillus niger* (SC 2164, SC 2564, SC 2828, SC 9719) and *Aspergillus foetidus* (SC13906) cultures produced 2-hydroxyphenylacetic acid and 2,5-dihydroxyphenylacetic acid from phenylacetic acid based on LC/MS analysis, HPLC retention times and UV spectra. None of these cultures produced the desired 2,6-dihydroxyphenylacetic acid (**3**, Scheme 1). Analytical results (MS) confirmed that hydroxylated products were formed; however, dihydroxylated products of phenylacetic acid were detected only in low concentrations. This may be due to the further metabolism of hydroxylated products.

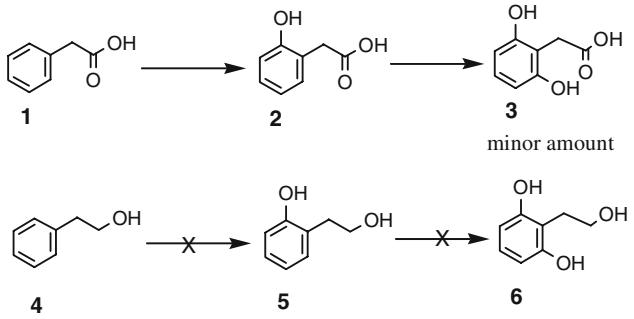
It has been reported that *Aspergillus nidulans* catabolizes phenylacetate (PhAc) and 3-hydroxy-, 4-hydroxy-, and 3,4-dihydroxyphenylacetate through the 2,5-dihydroxyphenylacetate. Using cDNA subtraction techniques, Ferrer–Sevillano and coworkers have isolated a gene, denoted phacB, which is strongly induced by phenylacetate (and its hydroxyderivatives) and encodes a new cytochrome P 450 (CYP450). High-performance liquid chromatography and gas chromatography–mass spectral analyses of in vitro reactions using microsomes from wild-type and several *A. nidulans* mutant strains confirmed that the phacB-encoded CYP450 catalyzes 3-hydroxyphenylacetate and 3,4-dihydroxyphenylacetate 6-hydroxylations to generate 2,5-dihydroxyphenylacetate and 2,4,5-trihydroxyphenylacetate, respectively [12].

Recently, a report was published that an engineered microbial cytochrome P 450 BM-3 (CYP102A subfamily) efficiently catalyzes the α-hydroxylation of phenylacetic acid esters [15].

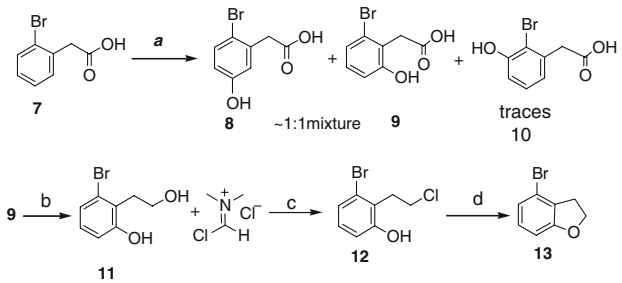
The 3-hydroxyphenylacetate 6-hydroxylase from a *Flavobacterium* sp. hydroxylated 3-hydroxyphenylacetate efficiently, yielding 2,5-dihydroxyphenylacetate as a product. The substrate analogs 3,4-dihydroxyphenylacetate and 4-hydroxyphenylacetate are partially hydroxylated, exclusively at the 6′ (2′) position [27].

A total of 30 *Aspergillus* strains were screened for the conversion of phenylethyl alcohol (**4**, Scheme 1) and 2-hydroxyphenylethyl alcohol (**5**) to obtain 2,6-dihydroxyphenylethyl alcohol (**6**). Although there were hydroxylated compounds (M + 16 mass units by LC/MS analysis) formed during biotransformation by many of the strains, none of the compounds matched with the retention time of the reference standard, 2,6-dihydroxyphenylethyl alcohol.

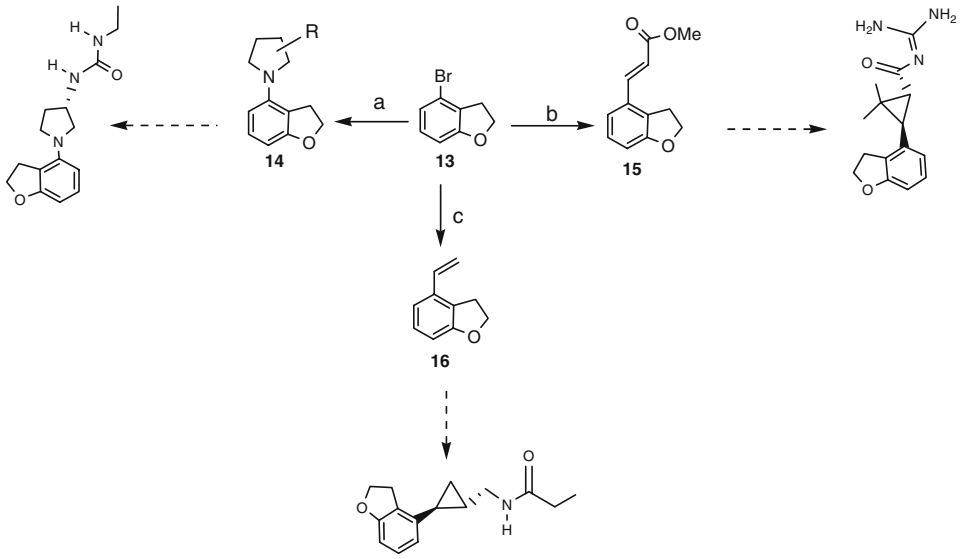
As an alternative, a total of 15 *Aspergillus* species were screened for the hydroxylation of 2-bromophenylacetic acid (**7**, Scheme 2) to obtain 2-bromo-6-hydroxyphenylacetic acid (**9**). Of the cultures screened for the hydroxylation, five *Aspergillus niger* cultures gave products with a molec-



Scheme 1 Enzymatic hydroxylation of phenylacetic acid and phenylethyl alcohol



Scheme 2 Synthesis of 4-bromo-2,3-dihydrobenzofuran; reaction conditions: *a Aspergillus niger*; *b* $NaBH_4$, $BF_3{\cdot}OEt_2$, THF, 0 °C; *c* CH_3CN, −15 °C, warm up to 0 °C; *d* K_2CO_3, acetone, reflux



Scheme 3 *a* Pyrrolidine, NaO-tBu, BINAP/Tol, 80 °C; *b* Cs_2CO_3, Pd_2dba_3, dioxane, ethyl acrylate/tributyl phosphine in dioxane, 80 °C; *c* tributylvinyl tin/toluene/tetrakis(triphenyl-phosphine) palladium (0), reflux

ular weight of 230, which corresponds to hydroxylated compounds. All these cultures had a similar LC/MS profile, producing three new compounds that had a molecular weight of 230. These compounds were isolated by chromatography and were characterized as 2-bromo-6-hydroxyphenylacetic acid (**9**, desired compound), 2-bromo-5-hydroxyphenylacectic acid (**8**) and 2-bromo-3-hydroxyphenylacectic acid (**10**, minor component) by NMR and LC/MS. The biotransformation conditions and isolation protocols were further optimized to obtain an ~1:1 ratio of compounds **8** and **9**. The desired 2-bromo-6-hydroxyphenylacetic acid (**9**) was isolated from the fermentation broth by preparative HPLC in 21% yield. It should be noted that HPLC analysis of the broth did not show the presence of the starting material, 2-bromophenylacetic acid (**7**) and it did not show any other side products. HPLC analysis of the culture showed that it contained only the desired compound, **8** and its isomer, compound **9**. This could be attributed to some of the 2-bromophenylacetic acid (**7**) (or products **8** and **9**) having been metabolized by the microbial culture.

Intermediate **9** was then converted into 4-bromo-2,3-dihydrobenzofuran (**13**, Scheme 3) in a three-step synthetic process. Acid **9** was first converted into phenylethyl alcohol (**11**) in quantitative yield by in situ generated borane reagent containing sodium borohydride and $BF_3{\cdot}Et_2O$. Our initial attempts to transform compound **11** directly into 4-bromo-2,3-dihydrobenzofuran (**13**) were not successful, as the cyclization process did not progress after initial formation of chloro derivative **12** which we have observed during our previous work [20]. Compound **12**, which was stable and did not undergo elimination, was then isolated and cyclized by refluxing under basic conditions (**13**) in 72% yield. Thus 4-bromo-2,3-dihydrobenzofuran can be obtained in a four-step sequence starting from 2-bromophenylacetic acid.

To explore the general usefulness of compound **13** (Scheme 3) to obtain 4-substituted-2,3-dihydrobenzofurans, we performed palladium-mediated coupling reactions. All three coupling reactions were performed according to literature procedures. The reactions worked well, providing 4-substituted-2,3-dihydrobenzofuran coupling products in high yield. First, we tried a Buchwald/Hartwig palladium-mediated coupling [13, 29, 30] reaction between compound **13** and pyrrolidine, which gave a quantitative yield of the pyrrolidine adduct compound **14**. Then a Pd-catalyzed Heck [6, 10, 16, 17] reaction was performed with methyl acrylate to obtain the 4-substituted methyl acrylate adduct of 2,3-dihydrobenzofuran **15** [3]. The Heck coupling reaction worked very smoothly in >80% yield. In the case of styrene **16**, we were able to achieve Pd-mediated coupling with vinyl tin similar to that of 4-chloro-2,3-dihydrobenzofuran with vinyl tin [32]. Thus, the high reactivity of 4-bromo-2,3-dihydofuran allowed us to perform Pd-mediated coupling to obtain other 4-substituted 2,3-dihydrobenzofurans. During the development of the NHE and melotonin programs, the 4-substituted-dihydrobenzofurans were prepared by routes which required several steps, sometimes with chemistry which was not suitable for large scale development. Now, with the new process, styrene **16** and compound **15** provide a more efficient alternative to drug candidate intermediates.

In summary, microbial hydroxylation of bromophenylacetic acid produces 2-hydroxylated compounds. The desired 2-bromo-6-hydroxyphenylacetic acid can be separated from its regeoisomer via chromatography in 21% yield. However, the selectivity for the microbial hydroxylation needs to be improved. The hydroxylated product is a key intermediate for the synthesis of 4-bromo-2,3-dihydrobenzofuran (**13**), which provides an easy access to various other 4-substituted benzofurans. Compound **13**

easily undergoes Pd-mediated coupling reactions with various substrates in high yield, thus providing access to various 4-substituted-2,3-dihydrobenzofurans, key intermediates for the synthesis of melatonin receptor agonist and a sodium hydrogen exchange compounds.

Acknowledgments We thank Dr. M. Futran for his support during the internship period of Mr. Hamish Christie. We also thank R. Hanson, B. Davis, and D. Brzozowski for their microbiological support.

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