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Biotransformation of *N*-piperidinylacetophenone with *Beauveria bassiana* ATCC-7159

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Abstract

The oxidation of *N*-piperidinylacetophenone by the fungal biocatalyst *Beauveria bassiana* ATCC-7159 was studied under different fermentation conditions. The isolation and product distribution of eight metabolites is reported. *C*-Hydroxylation of unfunctionalized carbon was observed together with Baeyer–Villiger oxidation, hydrolysis, and β-4-*O*-methylglucosidation of aromatic alcohol.

Keywords: Biooxidations; *Beauveria bassiana*; Baeyer–Villiger oxidation; Fungus oxidations; β-4-*O*-methylglucosidation

1. Introduction

We have been particularly interested in the use of microbial transformations which can be of great value in organic synthesis and that are not commonly achievable by conventional chemical methods [1]. One of these reactions is the selective hydroxylation of an unfunctionalized carbon. Various microorganisms containing oxygenases are capable to hydroxylate an unfunctionalized carbon with excellent selectivity and in good yields [2]. *Beauveria bassiana* (ATCC-7159), formerly known as *Sporotrichum sulfurescens* and *Beauveria sulfurescens*, is a filamentous fungus that is becoming very popular among synthetic and natural product chemists because of its ability to hydroxylate a large number of compounds [3]. *B. bassiana* is also known to oxidize sulfanyl to sulfinyl groups [4], insert oxygen in aliphatic ketones (Baeyer–Villiger oxidation) [5], reduce α,β-unsaturated ketones to the corresponding saturated ketones [6], and introduce β-4-*O*-methylglucose in phenolic compounds [1d,7]. Herein, we would like to report our findings on the biotransformation of *N*-piperidinylacetophenone with *B. bassiana*.

A classic substrate for the hydroxylation of an unfunctionalized carbon with *B. bassiana* is the vinylogous amide *N*-piperidinylacetophenone (**1**) (Scheme 1). Johnson initially reported the microbial hydroxylation of vinylogous amide **1** with growing cells of *B. bassiana* in corn steep liquor-dextrose medium furnishing *N*-(4-hydroxypiperidinyl)-acetophenone (**2**) in 20% yield after 3 days [8]. Roberts reported the same hydroxylation to occur in 66% yield when the fermentation was run for 5 days and at a higher dilution of the substrate [9]. Holland also reported the hydroxylation of piperidine **1** to yield *N*-(4-hydroxypiperidinyl)-acetophenone (**2**) in 20–40% yield after 3 days of fermentation [10]. Herein, we would like to report our findings in the hydroxylation of *N*-piperidinylacetophenone with *B. bassiana* under several fermentation conditions, and the production of a series of metabolites that were previously unreported (Scheme 2).

2. Results and discussion

The biotransformation of piperidine **1** with *B. bassiana* was carried out under different fermentation conditions using dextrose and corn steep liquor as the growth medium, Table 1. Corn steep liquor is commonly used as a major component for fermentation medium because of its content of nitrogen and mineral salts, and also its low cost [11]. When a highly diluted amount of substrate (0.1 mg/mL) was subjected to growing cells with an initial pH of 5, we observed after 3 days of biotransformation a 37% yield of alcohol **2**, a 34% yield of recovered starting material, and a small amount of phenolic compound with the piperidine ring untouched (**4**) (entry 1). Phenol **4** is presumably formed by Baeyer-Villigerase activity in *B. bassiana*, followed by hydrol-

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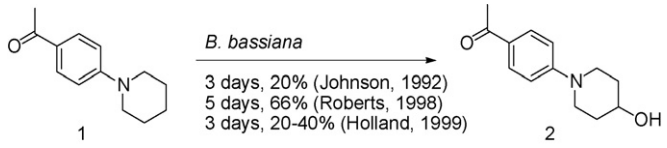
Table 1
Biotransformation of 4-piperidinylacetophenone with *B. bassiana* in corn steep liquor

Entry	Conc. (mg/mL)	pH	Time (days)	**1** (%)[a]	**4** (%)[a]	**5** (%)[a]	**2** (%)[a]	**7** (%)[a]	**8** (%)[a]	**9** (%)[a]
1[b]	0.1	5	3	34	5	0	37	0	0	0
2[b]	0.1	5	5	0	0	2	23	0	0	0
3[b]	1.0	5	5	3	42	4	0	19	0	0
4[b]	1.0	7	5	58	0	0	17	2	4	5
5[c]	1.0	7	5	54	0	0	11	0	1	0
6[c,d]	1.0	7	5	49	10	0	13	2	3	0

[a] Yields reported are of isolated and purified material.
[b] Growing cells of *B. bassiana*.
[c] Resting cells of *B. bassiana*.
[d] Resting cells of *B. bassiana* induced with cyclohexane.



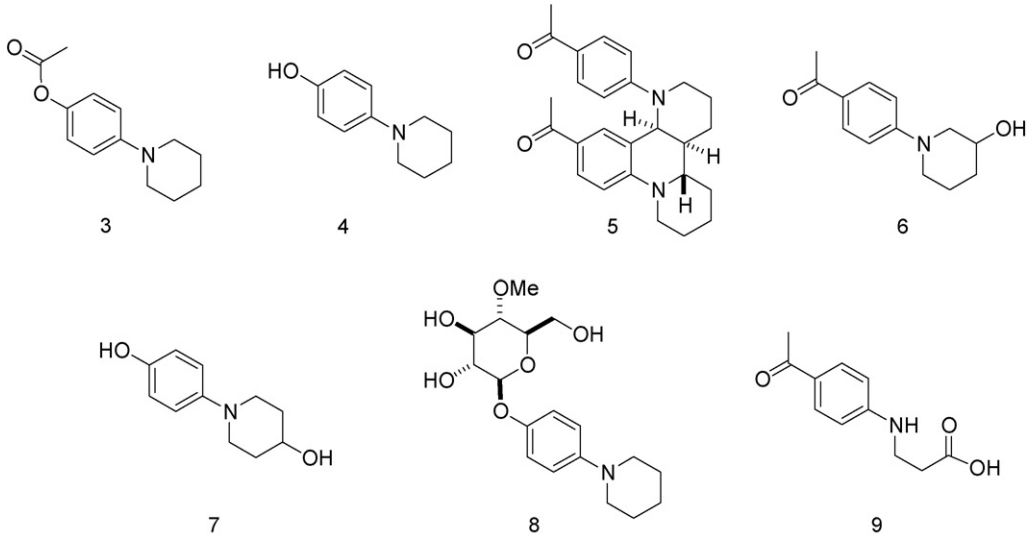
Scheme 1.

ysis of the acetyl group. Although this activity has been seen before in aliphatic ketones with *B. bassiana*, this is the first time to be observed in aromatic ketones [5]. Baeyer-Villigerase activity on acetophenone derivatives has been observed with other fungal strains [12]. When the biotransformation was allowed to run for two more days, the yield of piperidinol **2** dropped and no starting material was recovered, but a small amount of a dimeric compound was isolated (entry 2). NMR spectroscopy indicated the dimer to possess structure **5**, and was later confirmed by X-ray crystallographic analyses (Fig. 1) [13]. Dimer **5** has been previously prepared by chemical oxidation of substrate **1** via Hg(II)-EDTA oxidation [14]. We propose that dimer **5** might be formed by initial α-hydroxylation of the piperidine ring (**10**) forming an iminium salt (**11**) which is in equilibrium with the enamine (**12**). Diels–Alder addition of the iminium salt **11** with the enamine intermediate **12** can furnish dimer **5** (Scheme 3).

α-Hydroxylation of a piperidine ring has been observed in the oxidation of the tubulin-binding agent rhazinilam [15] and also in pyrrolidine rings with *B. bassiana* [16].

We then submitted a larger amount of substrate **1** to growing cells of *B. bassiana* (entries 3 and 4, Table 1). This concentration of substrate has been employed previously in the oxidation of benzhydrylsulfanyl acetic acid with *B. bassiana* [4a]. We observed a large amount of phenol **4** formed (42%), a small amount of dimer **5** (4%), and a new metabolite which was hydroxylated on the piperidine ring, compound **7** in 19% yield (entry 3). By modifying the initial pH of the medium to 7, we observed a different distribution of products (entry 4). This time, we also isolated a phenolic compound which suffered β-4-*O*-methylglucosidation, compound **8**. This activity in *B. bassiana* has been observed previously in phenolic compounds [1d,7]. We also isolated a small amount of metabolite **9**, which presumably resulted from further oxidation of the piperidinol ring and *N*-dealkylation. In order to confirm the structure of aminoacid **9**, we prepared it chemically by Michael addition of 4-aminoacetophenone to acrylic acid in refluxing toluene in 65% yield.

Another fermentation method is the use of resting cells, growing cells harvested and resuspended in buffer medium. Switching



Scheme 2.

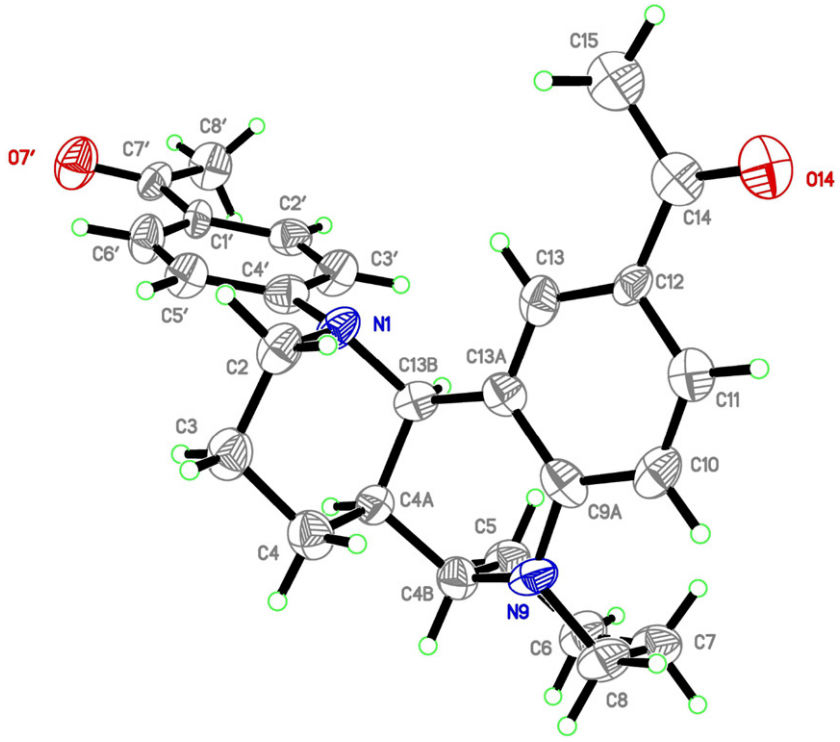
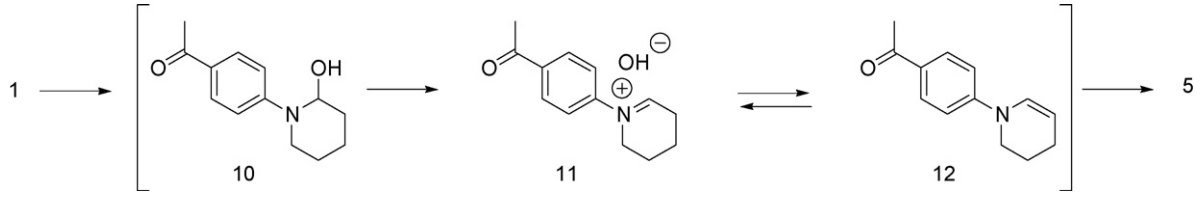


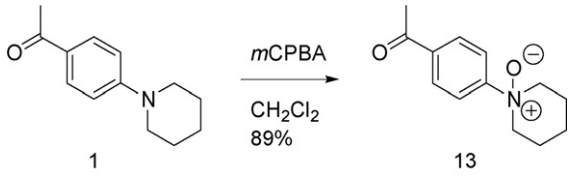
Fig. 1. X-Ray crystal structure of dimer **5**.



Scheme 3.

to this method, isolation of alcohol **2** was achieved in only 11% (entry 5). Previously induced cells with cyclohexane also provided alcohol **2** and a similar amount of phenolic metabolite **4** (entry 6) [17].

The Trojan horse for chemical Baeyer–Villiger oxidations is *m*-chloroperoxybenzoic acid (*m*CPBA) [18]. However, when piperidinylacetophenone **1** was oxidized with *m*CPBA, no Baeyer–Villiger oxidized product was observed, but only the corresponding *N*-oxide **13** (Scheme 4). This oxidation has also been reported employing performic acid [19]. Thus, the utility of microbial transformations to obtain products other than the ones obtained with classical chemical reagents can be appreciated in the isolation of metabolite **4**.



Scheme 4.

Another growth media commonly used for biotransformations is the so-called Iowa medium. This media contains (w/v) 2% dextrose, 0.5% yeast extract, 0.5% soybean flour, 0.5% sodium chloride, and 0.5% K_2HPO_4 [20]. Some isoflavones present in soybean are known to induce CYP450, which might result beneficial to hydroxylations by *B. bassiana* [21]. Biotransformations of piperidinylacetophenone **1** with *B. bassiana* in Iowa medium are shown in Table 2. An approximately equal amount of expected alcohol **2** and dimer **5** were obtained together with a large amount of β-aminoacid **9** when the biotransformation was run under high dilution conditions for 3 days (entry 1). Interestingly, the new 3-hydroxylated piperidine **6** was isolated in trace amount [22]. At a higher concentration, also large amounts of carboxylic acid **9**, alcohol **2**, and small amounts of phenol **4**, hydroxyl phenol **7** and dimer **5** were obtained (entry 2). When even higher concentrations of substrate were used, phenol **4** was the major metabolite also in growing and resting cells of *B. bassiana* (entries 5–8). Isolation of a trace amount of Baeyer–Villiger product **3** was possible using resting cells (entry 8). Compound **3** was prepared chemically by acetylation of phenol **4**.

Table 2
Biotransformation of 4-piperidinylacetophenone with *B. bassiana* in Iowa medium

Entry	Conc. (mg/mL)	pH	Time (days)	**3** (%)[a]	**1** (%)[a]	**4** (%)[a]	**5** (%)[a]	**6** (%)[a]	**2** (%)[a]	**7** (%)[a]	**8** (%)[a]	**9** (%)[a]
1[b]	0.1	5	3	0	1	0	14	1	18	0	0	42
2[b]	0.5	7	5	0	7	4	3	0	24	4	0	36
3[b]	1.0	5	5	0	44	0	1	0	5	0	0	0
4[b]	1.0	7	3	0	89	0	5	0	4	0	0	5
5[b]	1.0	7	5	0	2	25	7	0	3	0.2	3	0
6[b]	1.0	7	5	0	2	13	7	0	0	18	0	0
7[c]	1.0	5	5	0	2	29	4	0	0	7	4	0
8[c]	1.0	7	5	1	3	20	2	1	8	0	0	17

[a] Yields reported are of isolated and purified material.
[b] Growing cells of *B. bassiana*.
[c] Resting cells of *B. bassiana*.

3. Conclusions

These results show that several types of oxidative reactions can be observed when piperidinylacetophenone is subjected to biotransformation with *B. bassiana* under different substrate concentration, pH of media, fermentation time and type of growth media. New metabolites were isolated and characterized. These metabolites are oxidation products at different positions on the piperidinyl ring and also oxygen insertion (Baeyer–Villiger oxidation) in an aromatic carbon followed by hydrolysis. These metabolites show the ability of *B. bassiana* to accept substrates and metabolize them in different oxidative transformations. More importantly, these results suggest that this fungus present promising Baeyer–Villiger activity of aromatic ketones.

4. Experimental

4.1. Preparation of corn steep liquor medium

This medium was prepared by mixing corn steep liquor (20 g) and dextrose (10 g) per liter of water. The pH of the brown suspension was adjusted to 5 or 7 with either 2M HCl or 4N NaOH. The medium was distributed in the appropriate De Long fermentation flasks, covered with metal lid and sterilized by autoclaving for 15 min and allowed to cool before inoculation.

4.2. Preparation of Iowa medium

This medium was prepared by placing yeast extract (5 g), soybean flour (5 g), NaCl (5 g), K_2HPO_4 anh. (5 g), and dextrose (20 g) per liter of water. The pH of the beige suspension was adjusted to 5 or 7 with either 2M HCl or 4N NaOH. The medium was distributed in the appropriate De Long fermentation flasks, covered with metal lid and sterilized by autoclaving for 15 min and allowed to cool before inoculation.

4.3. Preparation of buffer solution

The buffer solution was prepared by mixing $Na(NH_4)HPO_4{\cdot}4H_2O$ (2.09 g), K_2HPO_4 (1.74 g) per liter of distilled water. The pH was adjusted to 5 or 7 with either 2M HCl or 4N NaOH. The buffer solution was distributed on the corresponding De Long flask, covered with metal lid and sterilized by autoclaving for 15 min. The solution was allowed to cool down and 5 mL of a 2M dextrose solution, sterilized by passing it through 0.45 μm filter, was added.

4.4. Biotransformation procedure with growing cells

A slant of *B. bassiana* ATCC-7159, kept in potato dextrose agar, was transferred to a 25 mL of Iowa medium contained in a 125 mL De Long flask. The flask was incubated at 250 rpm and 28 °C for 3 days (Stage I). Stage I cultures (20 mL) were transferred into 200 mL of Iowa or corn steep liquor medium contained in 1 L De Long flask. The flask was incubated at 250 rpm and 28 °C for 1 day (Stage II). An amount of 1 mL of ethanolic solution of substrate (200 mg/mL) was added over Stage II cultures. The flask was incubated at 250 rpm and 28 °C for the time indicated. The fermented broth was filtered through cheesecloth and the mycelia washed with 20 mL of distilled H_2O. The filtrate was extracted with ethyl acetate (3 × 50 mL), saturated with NaCl and extracted with more ethyl acetate (6 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in rotavapor to give a brown oil. The metabolites were purified by silica gel flash column chromatography (2 cm × 10 cm) eluting with a gradient of petroleum ether–ethyl acetate (30% → 100%) and then eluting with 15% methanol in ethyl acetate.

4.5. Biotransformation procedure with resting cells

A slant of *B. bassiana* ATCC-7159, kept in potato dextrose agar, is transferred to a 25 mL of IM or CSM, contained in a 125 mL De Long flask. The flask was incubated at 250 rpm and 28 °C for 3 days (Stage I). Stage I cultures (20 mL) were transferred into 200 mL of IM or CSM contained in 1 L De Long flask. The flask was incubated at 250 rpm and 28 °C for 1 day (Stage II). The fermented medium was filtered through cheesecloth. The mycelium was washed with buffer solution (3 × 50 mL) and placed on a 1 L De Long flask containing 0.2 L buffer solution (resting cells). An amount of 1 mL of ethanolic solution of substrate (200 mg/mL) was added over resting cells. The flask was incubated at 250 rpm and 28 °C for the time indicated.

The fermented broth was filtered through cheesecloth and the mycelium washed with 20 mL of distilled H_2O. The filtrate was extracted with ethyl acetate (3 × 50 mL), saturated with NaCl and extracted with more ethyl acetate (6 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in rotavapor to give pale-yellow oil. The metabolites were purified by silica gel flash column chromatography (2 cm × 10 cm) eluting with a gradient of petroleum ether–ethyl acetate (30% → 100%) and then eluting with 15% methanol in ethyl acetate.

4.6. 1-[4-(4-Hydroxy-piperidin-1-yl)-phenyl]-ethanone (**2**)

R_f 0.26 (3:7 petroleum ether–ethyl acetate); mp = 125 °C (colorless solid) [lit. 124–126 °C, Ref. [9], 123–125 °C, Ref. [10]]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (2H, dd, J = 8.0, 1.8 Hz), 6.86 (2H, dd, J = 8.0, 1.8 Hz), 3.93 (1H, ddd, J = 13.4, 8.1, 3.9 Hz), 3.75 (2H, ddd, J = 13.6, 5.9, 4.5 Hz), 3.11 (2H, ddd, J = 13.6, 9.6, 3.2 Hz), 2.51 (3H, s), 2.11 (1H, bs), 2.05–1.94 (2H, m), 1.64 (2H, ddd, J = 16.8, 9.0, 3.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.8 (CO), 154.0 (C), 130.7 (2CH), 127.1 (C), 113.6 (2CH), 67.5 (CH), 45.3 ($2CH_2$), 33.7 ($2CH_2$), 26.2 (CH_3).

4.7. Acetic acid 4-piperidin-1-yl phenyl ester (**3**)

R_f 0.34 (9:1 petroleum ether–ethyl acetate); mp = 39–40 °C (pale-yellow solid); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.93 (4H, dd, J = 9.3, 2.8 Hz), 3.10 (4H, dd, J = 5.6, 5.3 Hz), 2.25 (3H, s), 1.74–1.66 (4H, m) 1.60–1.51 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1 (CO), 150.4 (C), 143.4 (C), 121.9 (2CH), 117.5 (2CH), 51.2 ($2CH_2$), 26.0 ($2CH_2$), 24.3 (CH_2), 21.2 (CH_3); IR (KBr) υ 3487, 2934, 2818, 1752, 1606, 1512, 1370, 1205, 1125, 1012, 830, 796 cm^{-1}; HRMS (MALDI-TOF) calcd. for $C_{13}H_{17}NO_2$ + H $[M+H]^+$ m/z 220.1338, found: m/z 220.1348.

4.8. 4-Piperidinyl phenol (**4**)

R_f 0.35 (7:3 petroleum ether–ethyl acetate); mp = 155–157 °C (beige solid) [lit. 158–160 °C, Ref. [10]]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.86 (2H, d, J = 8.6 Hz), 6.67 (2H, d, J = 8.6 Hz), 3.48 (1H, s), 2.99 (4H, dd, J = 5.2, 4.9 Hz), 1.76–1.69 (4H, m), 1.57–1.49 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.0 (C), 146.7 (C), 119.5 (2CH), 115.9 (2CH), 52.8 ($2CH_2$), 26.2 ($2CH_2$), 24.3 (CH_2); ^{1}H NMR (300 MHz, acetone-d_6) δ 7.76 (1H, bs), 6.81 (2H, dd, J = 9.0, 2.4 Hz), 6.70 (2H, dd, J = 9.0, 2.4 Hz), 2.94 (4H, dd, J = 5.5, 5.2 Hz), 1.69–1.62 (4H, m), 1.54–1.46 (2H, m); ^{13}C NMR (75 MHz, acetone-d_6) δ 152.0 (C), 147.2 (C), 119.8 (2CH), 116.3 (2CH), 53.1 ($2CH_2$), 27.0 ($2CH_2$), 25.0 (CH_2); IR (KBr) υ 2946, 2816, 1513, 1438, 1205 cm^{-1}; LRMS (ESI) calcd. for $C_{11}H_{15}NO$ $[M^+]$ m/z 177.1154, found: m/z 177.4.

4.9. 1-[1-(4-Acetyl-phenyl)-1,3,4,4a,4b,5,6,7,8,12b-decahydro-2H-1,8a-diaza-triphenylen-11-yl]-ethanone (**5**)

R_f 0.35 (6:4 petroleum ether–ethyl acetate); mp = 161–162 °C (colorless solid) [lit. 157–159 °C, Ref. [14]]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (2H, dd, J = 8.9, 1.8 Hz), 7.78 (1H, dd, J = 8.8, 2.1 Hz), 7.60 (1H, s), 6.93 (2H, dd, J = 8.9, 1.8 Hz), 6.75 (1H, d, J = 8.8 Hz), 5.18 (1H, d, J = 5.0 Hz), 4.14 (1H, d, J = 13.9), 3.80 (1H, d, J = 13.4 Hz), 3.14–2.92 (3H, m), 2.52 (3H, s), 2.34 (3H, s), 2.04–1.91 (2H, m), 1.76–1.39 (9H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.3 (CO), 196.2 (CO), 154.6 (C), 148.9 (C), 131.0 (2CH), 129.7 (CH), 128.5 (CH), 126.6 (C), 125.9 (C), 119.0 (C), 112.7 (2CH), 110.9 (CH), 63.2 (CH), 53.2 (CH), 48.9 (CH_2), 41.9 (CH_2), 38.1 (CH), 32.0 (CH_2), 26.6 (CH_2), 26.2 (CH_3), 25.9 (CH_3), 25.5 (CH_2), 25.0 (CH_2), 24.9 (CH_2); IR (KBr) υ 3436, 2930, 2858, 1672, 1660, 1594, 1550, 1359, 1260, 1193, 983, 807 cm^{-1}; HRMS (MALDI-TOF) calcd. for $C_{26}H_{30}N_2O_2$ + H $[M+H]^+$ m/z 403.2386, found: m/z 403.2370.

4.10. 1-[4-(3-Hydroxy-piperidin-1-yl)-phenyl]-ethanone (**6**)

R_f 0.32 (3:7 petroleum ether–ethyl acetate); mp = 97–98 °C (colorless solid) [lit. 93–95 °C, Ref. [23]]; $[\alpha]^{20}_D$ = +34.8 (c 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (2H, ddd, J = 9.0, 2.8, 2.0 Hz), 6.88 (2H, ddd, J = 9.0, 2.8, 2.0 Hz), 3.89 (1H, m), 3.63 (1H, dd, J = 12.5, 3.4 Hz), 3.43 (1H, m), 3.19 (1H, dd, J = 8.5, 3.4 Hz), 3.13 (1H, dd, J = 12.5, 7.6 Hz), 2.51 (3H, s), 2.08 (1H, bs), 2.00–1.83 (2H, m), 1.74–1.57 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.8 (CO), 154.7 (C), 130.6 (2CH), 127.7 (C), 114.2 (2CH), 66.4 (CH), 55.3 (CH_2), 48.1 (CH_2), 32.8 (CH_2), 26.3 (CH_3), 22.2 (CH_2); IR (KBr) υ 3449, 1643, 1601, 1552, 1516, 1383 cm^{-1}; HRMS (MALDI-TOF) calcd. for $C_{13}H_{17}NO_2$ + H $[M+H]^+$ m/z 220.1338, found: m/z 220.1331.

4.11. 1-(4′-Hydroxyphenyl)-piperidin-4-ol (**7**)

R_f 0.24 (1:9 petroleum ether–ethyl acetate); mp = 163–164 °C (colorless solid); ^{1}H NMR (300 MHz, CD_3OD) δ 6.85 (2H, ddd, J = 9.0, 3.5, 2.3 Hz), 6.66 (2H, ddd, J = 9.0, 3.5, 2.3 Hz), 3.65 (1H, ddd, J = 10.0, 9.4, 3.8, Hz), 3.33–3.25 (2H, m), 2.69 (2H, ddd, J = 12.3, 10.5, 2.9 Hz), 1.99–1.90 (2H, m), 1.63 (2H, ddd, J = 10.0, 9.4, 3.8 Hz); ^{13}C NMR (75 MHz, CD_3OD) δ 153.4 (C), 146.4 (C), 121.2 (2CH), 116.9 (2CH), 68.6 (CH), 51.4 ($2CH_2$), 35.5 ($2CH_2$); IR (KBr) υ 3167, 2932, 2822, 1510, 1463, 1264, 1229, 1070, 908, 830 cm^{-1}; HRMS (MALDI-TOF) calcd. for $C_{11}H_{15}NO_2$ + H $[M+H]^+$ m/z 194.1181, found: m/z 194.1178.

4.12. N-[p-(4′-O-Methyl-β-D-glucopyranosyl)-phenyl]-piperidine (8)

R_f 0.18 (1:9 petroleum ether–ethyl acetate); mp = 159–160 °C (colorless solid); $[\alpha]^{20}_D$ = −50.6 (c 1.0, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 7.00 (2H, ddd, J = 9.2, 2.9, 2.5 Hz), 6.93 (2H, ddd, J = 9.2, 2.9, 2.5 Hz), 4.77 (1H, d, J = 7.6 Hz), 3.82 (1H, dd, J = 12.2, 2.1 Hz), 3.68 (1H, dd, J = 12.2, 4.6 Hz), 3.57 (3H, s), 3.52 (1H, d, J = 8.8 Hz), 3.41 (1H, dd, J = 9.3, 7.6 Hz), 3.35 (1H, ddd, J = 9.6, 4.6, 2.1 Hz), 3.17 (1H, dd, J = 9.6, 8.8 Hz), 3.00 (4H, dd, J = 5.5, 5.2 Hz),

1.76–1.68 (4H, m), 1.60–1.52 (2H, m); ^{13}C NMR (75 MHz, CD_3OD) δ 153.8 (C), 149.5 (C), 120.4 (2CH), 118.9 (2CH), 103.2 (CH), 80.9 (CH), 78.3 (CH), 77.4 (CH), 75.4 (CH), 62.4 (CH_2), 61.2 (CH_3), 54.0 ($2CH_2$), 27.3 ($2CH_2$), 25.4 (CH_2); IR (KBr) υ 3390, 2931, 2856, 1634, 1510, 1451, 1384, 1232, 1080, 822 cm^{-1}; HRMS (MALDI-TOF) calcd. for $C_{18}H_{28}NO_6$ $[M+H]^+$ m/z 354.1917, found: m/z 354.1911.

4.13. 3-(4-Acetyl-phenylamino)-propionic acid (**9**)

R_f 0.15 (9:1 chloroform–methanol); mp = 145–146 °C (beige solid) [lit. 155–157 °C, Ref. [24]]; ^{1}H NMR (300 MHz, acetone-d_6) δ 7.79 (2H, ddd, J = 8.8, 2.5, 1.9 Hz), 6.68 (2H, ddd, J = 8.8, 2.5, 1.9 Hz), 5.82 (1H, bs), 5.07 (1H, bs), 3.50 (2H, t, J = 6.7 Hz), 2.64 (2H, t, J = 6.7 Hz), 2.41 (3H, s); ^{13}C NMR (75 MHz, acetone-d_6) δ 195.2 (CO), 172.9 (C), 153.1 (C), 130.9 (2CH), 126.8 (C), 111.6 (2CH), 39.1 (CH_2), 33.7 (CH_2), 25.6 (CH_3); IR (KBr) υ 3435, 3299, 2929, 2658, 1709, 1698, 1651, 1592, 1276, 1184, 830 cm^{-1}; HRMS (ESI) calcd. for $C_{11}H_{13}NO_3$ $[M^+]$ m/z 207.0895, found: m/z 207.0894.

4.14. Michael addition of p-amino-acetophenone to acrylic acid

p-Amino-acetophenone (135 mg, 1 mmol) was added to a solution of acrylic acid (288 mg, 4 mmol) in toluene (10 mL). The reaction mixture was stirred overnight at 100 °C. The solution was concentrated under vacuum and the residue was purified by flash column chromatography on a silica gel column (2 cm × 7 cm). Elution with 0–10% MeOH in CH_2Cl_2 afforded a light-tan solid (**9**): 134 mg (65% yield).

4.15. Chemical oxidation of N-piperidinylacetophenone

To a solution of *N*-piperidinylacetophenone (203 mg, 1 mmol) in CH_2Cl_2 (50 mL) was added *m*CPBA (173 mg, 1 mmol). The reaction was stirred at room temperature for 1 h. The reaction mixture was treated with sat. $NaHCO_3$ (10 mL) and the aqueous phase was extracted with CH_2Cl_2 (20 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under vacuum to give a brown foam. The crude residue was purified by flash column chromatography on silica gel (2 cm × 8 cm). Elution with 9:1 CH_2Cl_2-methanol gave 15 mg of piperidine **1** and 195 mg of *N*-oxide **13** as a colorless solid (89% yield).

4.16. N-piperidinylacetophenone N-oxide (13)

R_f 0.30 (9:1 CH_2Cl_2-methanol); mp = 149–151 °C (beige solid) [lit. 154–156 °C, Ref. [19]]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (2H, d, J = 8.4 Hz), 7.87 (2H, d, J = 8.4 Hz), 3.63 (2H, bt, J = 11.3 Hz), 3.10 (2H, bd, J = 11.1 Hz), 2.46 (3H, s), 2.45 (3H, m), 1.73 (1H, bd, J = 13.0 Hz), 1.57 (2H, bd, J = 14.0 Hz), 1.36 (1H, qt, J = 13.0, 3.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.7 (CO), 158.5 (C), 137.2 (C), 129.2 (2CH), 120.9 (2CH), 68.5 ($2CH_2$), 26.7 (CH_3), 21.5 (CH_2), 21.0 ($2CH_2$); IR (KBr) υ 3022, 1680, 1599, 1437, 1405, 1269, 962, 838 cm^{-1}; LRMS (ESI) calcd. for $C_{13}H_{17}NO_2 + H$ $[M+H]^+$ m/z 220.1338, found: m/z 220.0.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2007.12.026.

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